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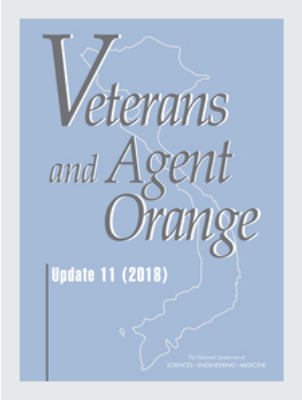

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Veterans and Agent Orange

Update 11 (2018)

Committee to Review the Health Effects in Vietnam Veterans
of Exposure to Herbicides
(Eleventh Biennial Update)

Board on Population Health and Public Health Practice

Health and Medicine Division

A Consensus Study Report of
The National Academies of
SCIENCES • ENGINEERING • MEDICINE

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Reviewers

This Consensus Study Report was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the National Academies of Sciences, Engineering, and Medicine in making each published report as sound as possible and to ensure that it meets the institutional standards for quality, objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process.

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Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations of this report nor did they see the final draft before its release. The review of this report was overseen by **Sandro Galea,** Boston University School of Public Health, and **Martin A. Philbert,** University of Michigan. They were responsible for making certain that an independent examination of this report was carried out in accordance with the standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the authoring committee and the National Academies.

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Acronyms and Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DCP	2,4-dichlorophenol
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4,5-TCP	2,4,5-trichlorophenol
2,4,5-TP	2-(2,4,5-trichlorophenoxy) propionic acid, Silvex
8-OHdG	8-hydroxydeoxyguanosine
ABC-2	Assessment Battery for Children-2
ACC	U.S. Army Chemical Corps
ACS	American Cancer Society
AD	Alzheimer disease
ADM	adrenomedullin
AFHS	Air Force Health Study (also referred to as the "Ranch Hand Study")
AHR	aryl hydrocarbon receptor
AHRE	AHR-responsive element
AHS	U.S. Agricultural Health Study
AL amyloidosis	amyloid light-chain amyloidosis
ALL	acute lymphocytic leukemia
ALS	amyotrophic lateral sclerosis (Lou Gehrig's disease)
ALT	alanine aminotransferase
AML	acute myeloid leukemia (previously called "acute myelogenous leukemia")
ARNT	aryl hydrocarbon nuclear translocator
ASQ	Ages and Stages Questionnaire

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ASRS	Autism Spectrum Rating Scale
B[*a*]P	benzo[*a*]pyrene
BMI	body mass index
BPA	bisphenol A
BRIEF	Behavior Rating Inventory of Executive Function–Preschool version
CDC	U.S. Centers for Disease Control and Prevention
CI	confidence interval
CLL	chronic lymphocytic leukemia (now regarded as same disease as small lymphocytic leukemia)
CML	chronic myeloid leukemia
CNS	central nervous system
COI	chemical of interest to VAO series (TCDD, 2,4,5-T, 2,4-D, picloram, or cacodylic acid)
COPD	chronic obstructive pulmonary disease
CT	computed tomography
CVD	cardiovascular disease
DHA	docosahexaenoic acid
DHEA	dehydroepiandrosterone
DMA	dimethyl arsenic acid
DMBA	dimethylbenzanthracene
$DMMTA^V$	dimethylmonothioarsinic acid
DNA	deoxyribonucleic acid
DoD	Department of Defense
ECG	electrocardiography
EOI	exposure opportunity index
EPA	U.S. Environmental Protection Agency
ER	estrogen receptor
F1, F2, F3	offspring, descendants (later generations: F2 and F3)
GGT	γ-glutamyl transferase
GIS	geographic information system
GSH	glutathione
HbA1c	hemoglobin A1c
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
HCL	hairy-cell leukemia

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HIV	human immunodeficiency virus
HL	Hodgkin lymphoma (previously referred to as Hodgkin's disease in VAO series)
HPV	human papilloma virus
HR	hazard ratio
HSP90	heat shock protein 90
IARC	International Agency for Research on Cancer
ICD	*International Classification of Diseases*
Ig	immunoglobulin antibodies
IgE	immunoglobulin E
IHD	ischemic heart disease
IL	interleukin (a cytokine)
IL-1RA	interleukin-1 receptor antagonist
IOM	Institute of Medicine
KABC-II	Kaufman Assessment Battery for Children, 2nd Edition
KGF	keratinocyte growth factor
LBW	low birth weight
LD_{50}	dose lethal to 50% of exposed animals
LHC	lymphohematopoietic cancer
MCPA	2-methyl-4-chlorophenoxyacetic acid
MCPP	2-(2-methyl-4-chlorophenoxy) propionic acid
MDS	myelodysplastic syndrome
MGUS	monoclonal gammapathy of undertermined significance
MIP-1α	macrophage-inflammatory protein-1 α
MMA	methylarsonous acid
MPN	myeloproliferative neoplasm
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
n	number of study participants
NBDPS	National Birth Defects Prevention Study
NCI	National Cancer Institute
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NK	natural killer cells
NPC	neural progenitor cells
NTIS	National Technical Information Service

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NTP	National Toxicology Program
OC	organochlorine
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ethers
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDD/Fs	polychlorinated dioxins and furans combined
PCDF	polychlorinated dibenzofuran
PCP	pentachlorophenol
PCT	porphyria cutanea tarda
PD	Parkinson disease
PDI	Psychomotor Developmental Index
picloram	4-amino-3,5,6 trichloropicolinic acid
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors
PL	Public Law
PNS	peripheral nervous system
POP	persistent organic pollutant
ppm	parts per million
ppt	parts per trillion (pg/g)
PTSD	posttraumatic stress disorder
RANTES	regulated on activation, normal T cell expressed and secreted
RISC	RNA-induced silencing complex
RITS	RNA-induced initiation of transcriptional silencing
RNA	ribonucleic acid
RR	relative risk
SDQ	Strengths and Difficulties Questionnaire
SEER	Surveillance, Epidemiology, and End Results program (National Cancer Institute)
SHR	standardized hospitalization ratio
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SNP	single-nucleotide polymorphism
STS	soft-tissue sarcoma
SWHS	Seveso Women's Health Study
T3	L-3,5,3'-triiodothyronine
T4	L-3,5,3',5'-tetraiodothyronine
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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TCP	trichlorophenol
TDS	total difficulties score
TEF	toxicity equivalency factor, i.e., potency of a dioxin-like chemical relative to TCDD
TEQ	(total) toxic equivalent
TGF	transforming growth factor
TH	thyroid hormones
TIPA	Triisopropanolamine
TNF	tumor necrosis factor
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
UMHS	Upper Midwest health study
UV	ultraviolet radiation
VA	U.S. Department of Veterans Affairs; previously, Veterans Administration
VAO	Veterans and Agent Orange (refers to series of committees and reports; italicized *VAO* refers to the initial comprehensive review, published in 1994)
VE-HEROeS	Vietnam Era Health Retrospective Observational Study
WHO	World Health Organization

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Summary

From 1962 to 1971, the U.S. military sprayed herbicides over Vietnam to strip the thick jungle canopy that could conceal opposition forces, to destroy crops that those forces might depend on, and to clear tall grasses and bushes from the perimeters of U.S. base camps and outlying fire-support bases. Mixtures of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, and cacodylic acid (collectively, the "chemicals of interest" or COIs) made up the bulk of the herbicides sprayed. The herbicide mixtures used were named according to the colors of identification bands painted on the storage drums. The most-used chemical mixture sprayed was Agent Orange,[1] a 50:50 mixture of 2,4-D and 2,4,5-T. At the time of the spraying, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic form of dioxin, was an unintended contaminant generated during the production of 2,4,5-T and so was present in Agent Orange as well as some of the other formulations sprayed in Vietnam.

Concerns from returning Vietnam veterans about their own health and that of their children combined with emerging toxicologic evidence of the adverse effects of phenoxy herbicides and TCDD exposure from animal studies and some positive findings from epidemiologic studies led Congress to pass Public Law (PL) 102-4, the Agent Orange Act of 1991. This legislation directed the Secretary of Veterans Affairs to ask the National Academies of Sciences, Engineering, and Medicine (the "National Academies") to perform a comprehensive evaluation

[1]Despite loose usage of "Agent Orange" as a collective term for all of the herbicides sprayed by U.S. forces during the Vietnam War—including in the title of this publication—it was only one of the formulations used. The text of the report uses "herbicides" or "COIs" to refer to the full range of herbicide agents, while "Agent Orange" is reserved for that specific formulation.

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of scientific and medical information regarding the health effects of exposure to Agent Orange, other herbicides used in Vietnam, and the various components of those herbicides, including TCDD. The legislation also instructed the Secretary to ask the National Academies to conduct updates every 2 years for 10 years from the date of the first report in order to review newly available literature and draw conclusions from the overall evidence.

In response to the first request, the Institute of Medicine convened a committee whose conclusions were published in 1994 in *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (*VAO*). The work of later committees resulted in a series of biennial updates (*Update 1996*, *Update 1998*, *Update 2000*, *Update 2002*, and *Update 2004*) and focused reports on the scientific evidence regarding type 2 diabetes, acute myeloid leukemia in the children of veterans, and the latent period for respiratory cancers.

Enacted in 2002, PL 107-103, the Veterans Education and Benefits Expansion Act of 2001, mandated a continuation of the *VAO* biennial updates. *Update 2006*, *Update 2008*, *Update 2010*, *Update 2012*, and *Update 2014* were published under that legislation and subsequent extensions to the Department of Veterans Affairs' (VA's) authority to request reports. The current update presents this committee's review of peer-reviewed scientific reports concerning associations between various health outcomes and exposure to TCDD and other chemicals in the herbicides used in Vietnam that were published between September 30, 2014, and December 31, 2017, and the committee's integration of this information with the previously established evidence database.

CHARGE TO THE COMMITTEE

The committee was asked to "determine (to the extent that available scientific data permit meaningful determinations)" the following regarding associations between specific health outcomes and exposure to TCDD and other chemicals present in the herbicides used by the military in Vietnam:

A. whether a statistical association with herbicide exposure exists, taking into account the strength of the scientific evidence and the appropriateness of the statistical and epidemiological methods used to detect the association;
B. the increased risk of disease among those exposed to herbicides during service in the Republic of Vietnam during the Vietnam era; and
C. whether there exists a plausible biological mechanism or other evidence of a causal relationship between herbicide exposure and the disease. [PL 102-4, § 3(d)]

In addition, the committee was asked to address the current research available on possible generational health effects that may be the result of exposures to these

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chemicals, including the biologic plausibility or potential for exposures to lead to an increased risk of birth defects or other adverse conditions in the descendants of male veterans; on myeloproliferative neoplasms (MPN) as part of its consideration of the literature concerning leukemias and related diseases; and on glioblastoma multiforme in its review of brain cancers.

In conducting its work, the committee operated independently of VA and other government agencies. It was not asked to make and did not make judgments regarding specific cases in which individual Vietnam veterans have claimed injury from herbicide exposure or such broader issues as the potential costs of compensation for veterans or policies regarding such compensation.

COMMITTEE'S APPROACH TO ITS CHARGE

Following the pattern established by previous VAO committees, the present committee concentrated its review on epidemiologic studies so as to fulfill its charge of assessing whether specific human health effects are associated with exposure to at least one of the herbicides sprayed in Vietnam or to TCDD. The committee also examined controlled laboratory investigations that provided information on whether a scientifically relevant association between the COIs and a given effect is biologically plausible. Information on dioxins other than TCDD and dioxin-like chemicals was considered because of the common mode of action underlying biologic effects.

The evidence evaluation process presumes neither the presence nor the absence of association for any particular health outcome. Over the sequence of reviews, evidence has accrued of various degrees of association, lack of association, or persistent indeterminacy with respect to a wide array of disease states. For many conditions, however, particularly ones that are very uncommon, any association with the COIs has remained unaddressed in the medical research literature. The committee does not offer a conclusion for these conditions unless the condition is logically subsumed under a broader disease category that has been evaluated, abiding by the maxim that "absence of evidence is not evidence of absence."

In accord with Congress's mandated presumption of herbicide exposure of all Vietnam veterans, VAO committees have treated Vietnam-veteran status as a proxy for herbicide exposure when no more specific exposure information is available. To anticipate the health conditions associated with aging and to obtain additional information potentially relevant to the evaluation of health effects in Vietnam veterans, the committees have reviewed studies of other groups potentially exposed to the constituents present in the herbicide mixtures used in Vietnam.

The original legislation calling for the report series, PL 102-4, did not provide a list of specific diseases and conditions suspected of being associated with herbicide exposure. Instead, a list was developed on the basis of diseases and conditions that had been mentioned in the scientific literature or in other documents identified through the original *VAO*'s extensive literature searches. The *VAO* list

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has since been augmented in response to new scientific findings, requests by VA, and concerns of Vietnam veterans.

The information that the present committee reviewed was identified through a comprehensive search of relevant databases, including databases covering epidemiologic, biologic, medical, toxicologic, chemical, historical, and regulatory information. To determine whether there is a scientifically relevant association between exposure and a health outcome, epidemiologists estimate the magnitude of an appropriate measure (such as the relative risk or the odds ratio) that describes the relationship between exposure and disease in a defined population or group. In evaluating the strength of the evidence linking herbicide exposure with a particular outcome, the committee considered whether such estimates of risk might not be consistent with a causal association (because of confounding, chance, or bias related to errors in selection and measurement) or might be an indication of a true association. Although they are not required, data supporting biologic plausibility can increase the confidence that an association is not spurious, and such data are presented in each of the sections. In this regard, it is important to note that while the biologic plausibility for a particular effect has been considered sufficient evidence of association by other bodies that have reviewed the health effects of environmental exposures, PL 102-4 specifies that the scientific determinations concerning the association between exposures and outcomes be supported by epidemiologic evidence. It has been the practice of all VAO committees to evaluate all studies according to the same criteria and then to weight findings of similar strength and validity equivalently, whether or not the study subjects are Vietnam veterans, when drawing conclusions. The committee recognizes that an absolute conclusion about the absence of association might never be attained, because—as is generally the case in science—studies of health outcomes after an exposure cannot demonstrate that a purported outcome is impossible, only that it is statistically improbable.

Tables on individual health outcomes summarizing the salient results of epidemiologic studies that have been evaluated over the entire series of VAO reports have been compiled and are available in digital form from www.nap.edu/catalog/25137. The results for a particular endpoint are grouped by study population to emphasize and clarify the relationship among successive publications based on the repeated study of particular exposed populations.

EVIDENCE REVIEWED BY THE COMMITTEE

The sections below describe the epidemiologic information that was newly evaluated for this update and illustrate the broad scope and nature of that information. The studies are divided, both here and in the report's health-outcome chapters, into four categories—Vietnam-veteran, occupational, environmental, and case-control—reflecting the committee's judgement of their relative relevance to the exposures of interest. Details of these publications are presented in Chapters 5–11.

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Vietnam-Veterans Studies

There has been an upswing of research interest in studies of the health of veterans of the Vietnam War, with publications addressing both U.S. and other populations. The committee considered analyses of the data and biospecimens collected in the course of the Air Force Health Study—a 20-year study of personnel involved in wartime aerial herbicide spray missions and a matched comparison group—to be particularly valuable because of the large amount of health and other information collected over an extended time period and because of the availability of measured serum dioxin levels. Since *Update 2014*, investigators have published studies of monoclonal gammopathy of undetermined significance (MGUS) and testosterone levels (which influence type 2 diabetes risk) in the cohort. A new study of hypertension in Army Chemical Corps personnel, who managed ground spraying operations, was also reviewed. Researchers using data from VA's Agent Orange Registry, a database containing health information on Vietnam veterans who voluntarily undergo examinations in a VA medical center, produced papers on various health outcomes that relied on surrogates of herbicide exposure such as self-reports. Studies of health outcomes for Australian, Korean, and New Zealand Vietnam veterans were also released.

Occupational Studies

In contrast with *Update 2014*, which found few studies of health outcomes in workers occupationally exposed to the COIs, there were several new publications available for the current committee to consider. The subjects of these included chemical manufacturing workers in the United States, New Zealand agrochemical production personnel, waste incineration workers in Japan, and employees of an electric arc furnace facility in Italy, a transformer and capacitor recycling plant in Germany, and five factories in the United Kingdom manufacturing or formulating phenoxy herbicides.

The Agricultural Health Study—a longstanding examination how agricultural, lifestyle, and genetic factors affect the health of U.S. farming populations being conducted by the National Institutes of Health (NIH)—has yielded numerous papers reviewed in the VAO series of reports. New studies of asthma, body mass index (a risk factor for type 2 diabetes), end-stage renal disease, lung cancer, prostate cancer, and rheumatoid arthritis were reviewed by this committee.

Environmental Studies

The committee reviewed a considerable number of studies of the effects of environmental exposures to the COIs. Most involved measurements of compounds with dioxin-like activity in blood samples and their association with a diverse set of health outcomes. The U.S. efforts included analyses of data

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from Centers for Disease Control and Prevention's (CDC's) National Health and Nutrition Examination Surveys and NIH's Longitudinal Investigation of Fertility and the Environment study cohorts. The large body of international work examined included the Danish Fetal Origins 1988–1989 Cohort, Duisburg (Germany) Birth Cohort Study, Hokkaido Study on Environment and Children's Health and its Sapporo companion study, the (Chapaevsk) Russian Children's Study, the Seveso (Italy) Woman's Health Study, and publications concerning populations in Belgium, Brazil, Canada, China, Finland, France, Greece, Hong Kong, Italy, Korea, Nicaragua, Norway, Spain, and Taiwan. Ten newly published studies of birth and other health outcomes in the Vietnamese population were also identified and reviewed.

Case-Control Studies

Several new publications using case-control methodology from the CDC's National Birth Defects Prevention Study were identified for the current update. These included studies of parental exposure to the COIs and spina bifida, congenital heart defects, gastroschisis, and a series of defects including anotia/microtia, anorectal atresia/stenosis, transverse limb deficiency, craniosynostosis, and diaphragmatic hernia in offspring.

A number of case-control studies in various other populations that examined forms of cancer (including cutaneous melanoma, female breast cancer, hepatocellular carcinoma, infiltrating ductal carcinomas, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer, soft tissue sarcoma, and testicular cancer) and other health outcomes including Parkinson disease, amyotrophic lateral sclerosis, and kidney and urinary disorders were also reviewed.

THE COMMITTEE'S CONCLUSIONS

General Observations Regarding Findings

VAO committees classify the evidence regarding exposure to the COIs and health outcomes into four categories: sufficient, limited or suggestive, inadequate or insufficient, and no association. Table S-1 sets forth the criteria for assigning categorizations and summarizes the committee's conclusions, with the changes in classification made since the previous volume (*Update 2014*) indicated in boldface. The classifications are based on the committee's evaluation of the epidemiologic literature and reflect the committee members' judgement of the relative certainty of the association between the outcome and exposure to the herbicides that were used in Vietnam or to any of their components or contaminants (with no intention of specifying particular chemicals).

The changes and the decisions not to modify other findings from earlier VAO committees were made after the present committee weighed the strengths and

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TABLE S-1 Summary of the *Eleventh Biennial Update* Findings on Vietnam-Veteran, Occupational, and Environmental Studies Regarding Scientifically Relevant Associations Between Exposure to Herbicides and Specific Health Outcomes[a]

Sufficient Evidence of an Association

Epidemiologic evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between exposure to herbicides and the outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.[b] For example, if several small studies that are free of bias and confounding show an association that is consistent in magnitude and direction, there could be sufficient evidence of an association. There is sufficient evidence of an association between exposure to the chemicals of interest and the following health outcomes:

Soft-tissue sarcoma (including heart)
* Non-Hodgkin lymphoma
* Chronic lymphocytic leukemia (including hairy cell leukemia and other chronic B-cell leukemias)
* Hodgkin lymphoma
Chloracne
Hypertension (category change from Limited or Suggestive in *Update 2014*)
Monoclonal gammopathy of undetermined significance (MGUS) (newly considered condition)

The committee did not reach consensus on whether the evidence regarding type 2 diabetes (mellitus) was more properly classified as *Sufficient* or *Limited or Suggestive*.

Limited or Suggestive Evidence of an Association

Epidemiologic evidence suggests an association between exposure to herbicides and the outcome, but a firm conclusion is limited because chance, bias, and confounding could not be ruled out with confidence.[b] For example, a well-conducted study with strong findings in accord with less compelling results from studies of populations with similar exposures could constitute such evidence. There is limited or suggestive evidence of an association between exposure to the chemicals of interest and the following health outcomes:

Laryngeal cancer
Cancer of the lung, bronchus, or trachea
Prostate cancer
Cancer of the urinary bladder
* Multiple myeloma
* AL amyloidosis
Early-onset peripheral neuropathy
Parkinson disease (including Parkinsonism and Parkinson-like syndromes)
Porphyria cutanea tarda
Ischemic heart disease
Stroke
Hypothyroidism

The committee did not reach consensus on whether the evidence regarding type 2 diabetes (mellitus) was more properly classified as *Sufficient* or *Limited or Suggestive*.

continued

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TABLE S-1 Continued

Inadequate or Insufficient Evidence to Determine an Association

The available epidemiologic studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association. For example, studies fail to control for confounding, have inadequate exposure assessment, or fail to address latency. There is inadequate or insufficient evidence to determine association between exposure to the chemicals of interest and the following health outcomes that were explicitly reviewed:

Cancers of the oral cavity (including lips and tongue), pharynx (including tonsils), or nasal cavity (including ears and sinuses)
Cancers of the pleura, mediastinum, and other unspecified sites in the respiratory system and intrathoracic organs
Esophageal cancer
Stomach cancer
Colorectal cancer (including small intestine and anus)
Hepatobiliary cancers (liver, gallbladder, and bile ducts)
Pancreatic cancer
Bone and joint cancers
Melanoma
Non-melanoma skin cancer (basal-cell and squamous-cell)
Breast cancer
Cancers of reproductive organs (cervix, uterus, ovary, testes, and penis; excluding prostate)
Renal cancer (kidney and renal pelvis)
Cancers of brain and nervous system (including eye)
Endocrine cancers (thyroid, thymus, and other endocrine organs)
Leukemia (other than chronic lymphocytic leukemia, including hairy-cell leukemia and other chronic B-cell leukemias)
Other myeloid diseases (including myeloproliferative neoplasms)
Cancers at other and unspecified sites
Infertility
Spontaneous abortion (other than after paternal exposure to TCDD, which appears not to be associated)
Neonatal or infant death and stillbirth in offspring of exposed people
Low birth weight in offspring of exposed people
Birth defects in offspring of exposed people, including spina bifida
Childhood cancer (including acute myeloid leukemia) or other adverse health outcomes in offspring of exposed people
Neurobehavioral disorders (cognitive and neuropsychiatric)
Neurodegenerative diseases, excluding Parkinson disease
Chronic peripheral nervous system disorders
Hearing loss
Respiratory disorders (wheeze or asthma, chronic obstructive pulmonary disease, and farmer's lung)
Gastrointestinal, metabolic, and digestive disorders (changes in hepatic enzymes, liver disorders including cirrhosis, lipid abnormalities, and ulcers)
Immune system disorders (immune suppression, allergy, and autoimmunity)
Circulatory disorders (other than hypertension, ischemic heart disease, and stroke)
Endometriosis

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TABLE S-1 Continued

Disruption of thyroid homeostasis (other than hypothyroidism)
Eye problems
Bone conditions
Kidney and urinary disorders (including chronic kidney disorder, differences in kidney function, nephropathy, and end stage renal disorder)
Chronic skin disorders (including skin infections and changes in skin pigmentation)

The committee used a classification that spans the full array of cancers. However, reviews for non-malignant conditions were conducted only if they were found to have been the subjects of epidemiologic investigation or at the request of the Department of Veterans Affairs. By default, any health outcome on which no epidemiologic information has been found falls into this category.

Limited or Suggestive Evidence of No Association

Several adequate studies, which cover the full range of human exposure, are consistent in not showing a positive association between any magnitude of exposure to a component of the herbicides of interest and the outcome. A conclusion of "no association" is inevitably limited to the conditions, exposures, and length of observation covered by the available studies. In addition, the possibility of a very small increase in risk at the exposure studied can never be excluded. There is limited or suggestive evidence of no association between exposure to the herbicide components of interest and the following health outcome:

Spontaneous abortion after paternal exposure to TCDD

[a]*Herbicides* indicates the following chemicals of interest: 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin), cacodylic acid, and picloram. The evidence regarding association was drawn from veteran, occupational, and environmental cohort studies in which people were exposed to the herbicides used in Vietnam, to their components, or to their contaminants.

[b]Evidence of an association is strengthened by experimental data supporting biologic plausibility, but its absence would not detract from the epidemiologic evidence.

*The committee notes the consistency of these findings with the biologic understanding of the clonal derivation of lymphohematopoietic cancers that is the basis of the World Health Organization classification system (Campo et al., 2011; see table here: www.ncbi.nlm.nih.gov/pmc/articles/PMC3109529/table/T1, accessed May 17, 2018).

limitations of the epidemiologic evidence reviewed in this report and in previous VAO reports. Although the studies published since *Update 2014* are the subject of detailed evaluation in this report, the committee drew its conclusions in the context of the entire body of literature, and the committee did not weigh new findings more heavily than past research.

As mandated by PL 102-4, the distinctions among categories are based on statistical association and not on strict causality. The committee was directed to review the scientific data, not to recommend VA policy; therefore, the conclusions reported in Table S-1 are not intended to imply or suggest policy decisions. The conclusions are related to the associations between exposure and outcomes in

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human populations, not to the likelihood that any individual's health problem is associated with or caused by the herbicides in question.

Hypertension

The committee concluded that the information now assembled constitutes sufficient evidence of an association between exposure to at least one of the COIs and hypertension. The decision to change the classification from limited or suggestive evidence of an association was motivated in large part by the work of Cypel and colleagues (2016). These investigators conducted a study on the population of interest, U.S. Vietnam veterans (specifically, the Army Chemical Corps), that was characterized by a large sample size, appropriate controls, and validated health endpoints. The statistical analyses conducted were robust, used state-of-the-art methods, and adjusted for relevant confounders. The study clearly showed that self-reported hypertension rates were the highest among those military personnel with the greatest opportunity for exposure to the COIs. Among Vietnam-deployed veterans, there was a statistically significantly elevated association between the odds of hypertension for sprayers versus nonsprayers that remained after an adjustment for potential confounders. Similarly, for those veterans who did not deploy to Vietnam, self-reported hypertension was significantly elevated among sprayers compared with nonsprayers. Earlier studies reviewed in previous updates consistently reported increased hypertension with increasing levels of serum dioxin in Vietnam veterans as well as increased prevalence in veterans with higher presumed exposure to the COIs. When considered in light of other new research and earlier studies that demonstrated a consistency in the direction and magnitude of this effect, the committee found that this body of literature constitutes sufficient evidence of an association.

Monoclonal Gammopathy of Undetermined Significance (MGUS)

The committee also concluded that there was sufficient evidence of an association between exposure to at least one of the COIs and MGUS. MGUS is a precursor to multiple myeloma, although only an estimated 1% of MGUS cases progress to multiple myeloma each year. It is a clinically silent condition defined by the presence of a monoclonal antibody, antibody heavy chain, or antibody light chain in the blood or urine of a person lacking symptoms or signs of a more serious plasma cell dyscrasia. The foundation of this finding was a well-conducted study by Landgren and colleagues (2015) that examined data and biospecimens from a population of veterans that included participants with known exposure to herbicides in Vietnam: the Air Force Health Study cohort. The study used previously measured serum levels of TCDD and performed a new assay of serum samples to detect MGUS. Known confounders including age, race, body mass index, smoking and drinking history, and a history of radiation therapy or

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chemotherapy were considered. The investigators found a statistically significant higher prevalence of MGUS in veterans involved in herbicide spray operations than in comparison veterans. Although no previous study directly addressed MGUS incidence, the direct relevance of the exposure and exposed population and the high quality of the study and underlying database were persuasive in finding sufficient evidence of an association.

Type 2 Diabetes

Finally, the committee—after extensive deliberations—could not come to a consensus on whether the available evidence regarding exposure to the COIs and type 2 diabetes continued to be limited or suggestive or merited elevation to sufficient. Both newly reviewed and previously reviewed studies quite consistently show a relationship between well-characterized exposures to dioxin and dioxin-like chemicals and measures of diabetes health outcomes in diverse cohorts, including Vietnam veteran populations. However, the lack of exposure specificity and the potential for uncontrolled confounding that characterized many of these studies complicates any attribution of the outcome to the COIs. Thus, a case can be made that the body of literature regarding the exposure to the COIs and diabetes meets the criteria for sufficient evidence of an association, but it was not clear to the committee as a whole whether a category change was appropriate, given its limitations.

Findings on Health Outcomes Identified for Special Focus by VA

As noted, VA asked the committee to specifically address three health outcomes: possible generational health effects that may be the result of herbicide exposure among male Vietnam veterans, myeloproliferative neoplasms, and glioblastoma multiforme.

Research on the effects of paternal chemical exposures on their descendants is burgeoning. Few studies address Vietnam veterans, however, and almost all of them that were conducted on other populations have weaknesses—prominently, different exposures than those experienced by veterans and poor exposure characterization—that limit their usefulness when assessing the risks for veterans. Some find associations between exposures and various outcomes, but there are no circumstances where there is a consistent and compelling body of evidence that would lead the committee to conclude that there might be limited or suggestive or sufficient evidence of an association between an exposure to a COI and a particular outcome. Transgenerational effects—those that might occur in descendants when gestational exposure did not take place—are of great interest to veterans, but no literature exists to evaluate whether the COIs might have an influence on outcomes. Given these gaps in the knowledge base, the committee strongly believes that more work in this area is warranted. It concurs with the

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Update 2014 committee that there is a critical need for this research to include animal studies in order to elucidate whether and which mechanisms for intergenerational and transgenerational effects might exist. It is in principle possible to do studies on the health of children and grandchildren of veterans, but it must be understood up front that such complex studies will need to be carefully planned and conducted if they are to yield meaningful results. Voluntary participation surveys and registries relying on self-reported information will not be helpful.

Myeloproliferative neoplasms (MPNs) and myelodysplastic syndromes are diseases of the blood cells and bone marrow. VA asked that MPNs be explicitly examined as part of the consideration of the literature concerning leukemias and related diseases. However, after conducting a targeted search of science and medical databases, the committee was able to identify only one relevant paper, which assessed cancer incidence among Korean veterans who had served in Vietnam during 1964–1973 and was evaluated by the Update 2014 committee. The paper's authors reported non-significant and imprecise increased risks of myeloproliferative disease and of myelodysplastic syndromes in internal comparisons of high- and low-exposure groups. The committee observes that, in general, those studies that have looked at the correlation between exposure to the COIs and hematological outcomes have generated much more compelling results for abnormalities of lymphoid development and immune function, such as non-Hodgkin lymphoma, chronic lymphocytic leukemia, multiple myeloma, and MGUS, than have those that examined myeloid neoplasms of granulocytic lineage such as MPN. Given the absence of new studies, the paucity of epidemiologic studies in general, and the lack of information on the biologic plausibility of a connection between exposures to the COIs and abnormalities of hematopoietic cells, the committee concluded there was inadequate or insufficient evidence of an association between exposure to the COIs and MPN. Because the outcome has not been subject to previous research attention and is of interest to veterans, **the committee recommends that investigators examine existing databases on myeloid diseases to determine whether there are data available that would allow for an evaluation of MPN in Vietnam veterans and others who have been exposed to dioxin and the other COIs.**

The scientific literature regarding exposure to the COIs and brain and other nervous system cancers, including glioblastoma, has been examined since the first *VAO* report. The body of evidence that has been developed has not found statistically significant associations between exposure and any relevant outcome in studies performed on Vietnam-veteran, occupational, or environmental cohorts. These studies have by and large been underpowered because of the relative rarity of these cancers. Given the limited epidemiologic data available on glioblastoma, the committee heard invited presentations from two experts on the disease. While their presentations to the committee were helpful and impressive, demonstrating that the biological understanding of glioblastoma in particular is rapidly advancing, they reinforced the absence of clear data suggesting that the COIs are associated with the occurrence of brain cancers. Information on

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glioblastomas in Vietnam veterans submitted for the committee's consideration by the Sierra Valley Cancer Registry Services, Inc., was, in part, anecdotal and without documented levels of exposure and was therefore of limited usefulness for the purpose of drawing conclusions. Furthermore, the committee did not identify any animal studies that have reported an association between exposure to the COIs and any brain cancer. While some studies have put forward mechanisms that might explain why dioxin exposure would be associated with glioblastoma, the information reviewed by the committee along with the presentations it received from experts in the field were not sufficient to alter the conclusion of previous reports that the evidence is inadequate or insufficient to determine whether there is an association between exposure to the COIs and brain or other nervous system cancers. The committee believes it is appropriate for VA to be mindful of the concerns raised about the possible association between Vietnam service and glioblastoma, but it observes that the outcome is so rare and the information concerning herbicide exposures so imprecise that it is doubtful that any logistically and economically feasible epidemiologic study of veterans—no matter how well designed or executed—would produce meaningful results. **The committee therefore recommends that epidemiologic studies of glioblastoma in Vietnam veterans should not be pursued and that VA should instead focus on fostering advancements in other areas that may be used to inform improved treatment options.**

Risk in Vietnam Veterans

Part of the committee's charge was to determine, to the extent permitted by available scientific data, the increased risk of disease among veterans exposed to herbicides or the contaminant TCDD during service in Vietnam. Estimating the magnitude of risk of each particular health outcome among herbicide-exposed Vietnam veterans requires quantitative information about the dose–time–response relationship for the health outcome, information on the extent of herbicide exposure among Vietnam veterans, and estimates of individual exposure. Vietnam veterans were exposed to other agents and stresses—such as tobacco smoke, insecticides, therapeutics, drugs, diesel fumes, alcohol, hot and humid conditions, and combat—that may have independent effects or increase or decrease the ability of chemicals in herbicides to produce a particular adverse health outcome. Few, if any, studies either in humans or in experimental animals have examined those interactions. The committees that produced the first VAO report and the updates found that the body of evidence was sufficient for reaching conclusions about statistical associations between herbicide exposures and health outcomes but that the lack of adequate data on Vietnam veterans themselves complicated the consideration of this part of the charge. The committees responsible for the VAO report series have therefore concluded that, in general, it is impossible to quantify the risk posed to veterans by their exposure to herbicides in Vietnam.

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COMMITTEE RECOMMENDATIONS

As part of their charge, all VAO committees have been asked to offer recommendations concerning the need for additional scientific studies and research to resolve areas of continuing scientific uncertainty concerning the health effects of the COIs. The previous (tenth; *Update 2014*) update of the VAO series was originally understood to be the last of the reports mandated by Congress. The committee responsible for that update thus considered it appropriate to compile the recommendations made by prior VAO committees and, in light of the lessons learned in this process, to consider what would be the most important activities to undertake in the future. That committee produced a compendium of the recommendations of prior committees condensed and sorted into topic areas, with comments on what response these recommendations had received from VA, Department of Defense (DoD), and other parties along with a summary of the future activities that the committee considered most important for monitoring and evaluating the health issues of Vietnam veterans and other veterans who might experience service-related health problems long after discharge.

Generally speaking, the recommendations of previous VAO committees fell into four primary areas: better management of veterans' health information; additional epidemiologic studies; improvement of exposure estimation; and priority areas for toxicologic research. Suggested future activities included these areas plus initiatives related to the collection and analysis of additional information on Vietnam veterans' service, exposures, and health.

While there have been a few laudable exceptions—notably, the initiation of additional epidemiologic studies on Vietnam veterans, the development of a herbicide exposure assessment model for use in studies of Vietnam veterans, and the fostering of additional research on the data and biospecimens collected in the course of the Air Force Health Study[2]—there has been no known follow-up to the vast majority of recommendations that have been offered. The current committee did not choose to revisit this issue in general, concluding that the Update 2014 committee had effectively covered it. It does observe, though, that the very first *VAO* (1994) indicated that "carefully conducted epidemiologic studies—with adequate sample size to detect elevated associations—of the reproductive history of individuals with occupational or environmental exposure to herbicides and dioxin are . . . needed" (p. 731). Several subsequent volumes have echoed and expanded on this. **The current committee** is in agreement with these sentiments and therefore **recommends further specific study of the health of offspring of male Vietnam veterans.**

The Update 2014 committee also offered suggestions for research activities that should follow the end of the *VAO* report series. Several of these addressed

[2]The Institute of Medicine publications *Disposition of the Air Force Health Study* (2006) and *The Air Force Health Study Assets Research Program* (2015) provide details of this work.

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reproductive outcomes. As that committee noted, although progress has been made in understanding the health effects of exposure to the COIs and the mechanisms underlying these effects, significant gaps in our knowledge remain. Many additional opportunities for progress via continuing and new toxicologic, mechanistic, and epidemiologic research exist. Such work should include efforts to gain new knowledge through the integration of existing DoD and VA databases. While they were mentioned in previous VAO updates, that committee restated them to emphasize their conviction that more progress should be made in the fields noted. This committee concurs in this assessment and endorses the recommendations offered in Table 12-3, noting that research in the rapidly advancing field of epigenetics appears to hold particular promise.

FINAL OBSERVATIONS

In the course of carrying out its Statement of Task, the committee has offered myriad criticisms of the conduct of studies of Vietnam veterans' health, pointing out specific weaknesses and shortcomings in particular papers and widespread (although not universal) issues such as poor exposure characterization, failure to fully control for confounding influences on outcomes, and sample sizes that are inadequate to draw statistically meaningful results. It wishes to make clear, though, that the difficulty in conducting research on Vietnam veteran health issues should not act as a barrier to carrying out such work. There are many questions regarding veterans' health that cannot be adequately answered by examining superficially analogous exposures and outcomes in other populations. It is only through research on veterans themselves that the totality of the military service experience can be properly accounted for.

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Introduction

The Agent Orange Act of 1991—Public Law (PL) 102-4, enacted February 6, 1991, and codified as Section 1116 of Title 38 of the United States Code—directed the Secretary of Veterans Affairs to ask the National Academies of Sciences, Engineering, and Medicine ("the National Academies") to conduct an independent comprehensive review and evaluation of scientific and medical information regarding the health effects of exposure to herbicides used during military operations in Vietnam. The act specified that the herbicides picloram and cacodylic acid were to be addressed, as were chemicals in various formulations that contain the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Agent Orange refers specifically to a 50:50 formulation of 2,4-D and 2,4,5-T, which was stored in barrels identified by an orange band, but the term has come to often be used more generically to refer to all the herbicides sprayed by the U.S. military in Vietnam.[1] 2,4,5-T contained the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, commonly referred to as "dioxin," which is referred to in this report as TCDD, to represent a single—and the most toxic—congener of the tetrachlorodibenzo-*p*-dioxins. It should be noted that TCDD and Agent Orange are not synonymous. The National Academies was also asked to recommend, as appropriate, additional studies needed to resolve continuing scientific uncertainties related to health effects and herbicide exposures and to comment

[1]Despite loose usage of "Agent Orange" by many people, in numerous publications, and even in the title of this series, this committee uses "herbicides" to refer to the full range of herbicide exposures experienced in Vietnam, while "Agent Orange" is reserved for a specific one of the mixtures sprayed in Vietnam.

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on particular programs mandated in the law. The original legislation called for biennial reviews of newly available information for a period of 10 years, which was subsequently extended to October 1, 2014, by the Veterans Education and Benefits Expansion Act of 2001 (PL 107-103). Subsequently, through the Department of Veterans Affairs Expiring Authorities Act, the Agent Orange Act of 1991 was extended annually for 4 years (PL 113-175, PL 114-58, PL 114-228, and PL 115-62), with a final termination date of September 30, 2018. Although the previous Veterans and Agent Orange (VAO) update was thought to be the final update in the series mandated by PL 107-103, the Department of Veterans Affairs (VA) interpreted the extension act to require an additional study.

PREVIOUS VETERANS AND AGENT ORANGE REPORTS

In response to the request from VA, the Institute of Medicine (IOM)[2] of the National Academies convened the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. The results of the original committee's work were published in 1994 as *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam*, hereafter referred to as *VAO* (IOM, 1994). Successor committees formed to fulfil the requirement for updated reviews produced *Veterans and Agent Orange: Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), *Update 2000* (IOM, 2001), *Update 2002* (IOM, 2003c), *Update 2004* (IOM, 2005), *Update 2006* (IOM, 2007), *Update 2008* (IOM, 2009), *Update 2010* (IOM, 2011a), *Update 2012* (IOM, 2014), and *Update 2014* (NASEM, 2016a).

Intermittently between biennial updates, VA has requested targeted reviews of specific health outcomes to determine whether they are associated with exposure to any of the chemicals of interest (COIs). These separate and focused topics have included type 2 diabetes (IOM, 2000b), childhood acute myelogenous leukemia (now generally referred to as acute myeloid leukemia) associated with parental exposure to any of the COIs (IOM, 2002), and respiratory cancers (IOM, 2004). The respiratory cancer review was included in PL 107-103, which was passed by Congress in 2001 and which directed the Secretary of Veterans Affairs to ask the National Academies to review "available scientific literature on the effects of exposure to an herbicide agent containing dioxin on the development of respiratory cancers in humans" and to address "whether it is possible to identify a period of time after exposure to herbicides after which a presumption of service-connection" of the disease would not be warranted.

Each report in the VAO series contains detailed reviews of the scientific studies evaluated by the committees and their implications for cancers, reproductive and developmental effects, neurologic disorders, and other health effects. VA has specified particular areas of focus for each update, for example, the Update

[2]Since March 2016, the Health and Medicine Division of the National Academies has continued the consensus studies and convening activities previously undertaken by the Institute of Medicine (IOM).

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2014 committee was asked to specifically address whether all neurodegenerative diseases with Parkinson-like symptoms should be considered service-related under the association identified between Parkinson disease and herbicide exposure by the committee for *Update 2006*. Each committee used the compilation of evidence presented in the previous reports as a starting point for updating the evidence base concerning the associations of health outcomes with exposure to any of the COIs. As such, each committee operated independently of prior committees, chose how to present the new and existing information, and determined its own conclusions regarding the strength of the evidence and each health outcome.

In conducting their work, the committees responsible for the updates and the targeted reviews operated independently of VA and other government agencies. They were not asked to and did not make judgments regarding specific cases in which individual Vietnam veterans claimed injury from herbicide exposure. The reports are intended to provide evidence-based assessments of the scientific information available for the Secretary of Veterans Affairs to consider as VA exercises its responsibilities to Vietnam veterans. This report and all previous VAO reports are freely accessible online at the National Academies Press's website (http://www.nap.edu).

CHARGE TO THE COMMITTEE

Box 1-1 shows the committee's Statement of Task. A VA representative delivered the charge to the committee during the open session of the committee's first meeting. In addition to the standard language of assessing associations between health outcomes and exposures to chemicals present in the herbicides used by the military in Vietnam that all VAO update committees are charged with reviewing, the committee for this final update was asked to assess three health outcomes in particular: possible generational effects on the descendants of male Vietnam veterans that may be the result of exposure to any of the COIs, myeloproliferative neoplasms, and glioblastoma multiforme. In addition, the committee was asked to offer recommendations as appropriate, such as which areas to prioritize for future research, a request that has been made of each of the VAO committees.

Both the committee's congressional mandate and the Statement of Task instruct that the evaluation be focused on the "association" between exposure and health outcomes, although biologic mechanisms and causal relationships are also mentioned as part of the evaluation in Article C. The criteria for causation do not themselves constitute a set checklist, but they are more stringent than those for association. The unique mandate of VAO committees to evaluate association rather than causation means that the rigor of the evidence required to support a finding of statistical association is weaker than what is required to support causality. Positive findings on any of the indicators for causality would strengthen a conclusion that an observed statistical association is valid. In accordance with its

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BOX 1-1
Committee's Statement of Task

In accordance with PL 102-4, the committee was asked to "determine (to the extent that available scientific data permit meaningful determinations)" the following regarding associations between specific health outcomes and exposure to TCDD and other chemicals present in the herbicides used by the military in Vietnam:

A) whether a statistical association with herbicide exposure exists, taking into account the strength of the scientific evidence and the appropriateness of the statistical and epidemiological methods used to detect the association;
B) the increased risk of the disease among those exposed to herbicides during service in the Republic of Vietnam during the Vietnam era; and
C) whether there exists a plausible biological mechanism or other evidence of a causal relationship between herbicide exposure and the disease. [PL 102-4, Section 3(d)]

It will also assess the current research available on possible generational health effects that may be the result of exposures to these chemicals—including the biologic plausibility or potential for an exposure to lead to an increased risk of birth defects or other adverse conditions in the descendants of male veterans—and will address myeloproliferative neoplasms (MPNs) as part of its consideration of the literature concerning leukemias and related diseases. The review of brain cancer will address, in particular, glioblastoma multiforme. The report will offer findings, conclusions, and recommendations as appropriate.

charge, the committee examined a variety of indicators appropriate for the task, including factors commonly used to evaluate statistical associations, such as the adequacy of control for bias and confounding and the likelihood that an observed association could be explained by chance, and it assessed evidence concerning biologic plausibility derived from laboratory findings in cell culture or animal model systems. As such, a full array of indicators was used to categorize the strength of the evidence. In particular, associations supported by multiple indicators were interpreted as having stronger scientific support.

INFORMATION GATHERING

The committee convened by the National Academies included experts in epidemiology, biostatistics, environmental health, exposure assessment, military and veteran's health, genetics and epigenetics, toxicology, oncology, and reproductive health. It comprised 12 members who met in person 7 times over 14 months. Between in-person meetings, small groups of committee members would

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hold conference calls related to reviewing the specific studies or to discuss the evidence base on a particular topic.

Several activities were undertaken to develop the scientific foundation for the report's findings, conclusions, and recommendations. As has been the practice of previous VAO committees, the committee held three open sessions not only to gather additional information from people who have particular expertise on topics and subjects that arise during deliberations (such as VA researchers, experts in glioblastoma multiforme, and specialists in environmental exposures and heritable health effects), but also especially to listen to individual Vietnam veterans and others, such as spouses and other family members and veterans service organization advocates, who are concerned about aspects of health that may be service-related. Open sessions were held during meetings 1, 3, and 4, the agendas and presentation topics of which are presented in Appendix A. The comments and information provided by the public at the open meetings and over the course of the study were used to identify information gaps in the literature regarding specific health outcomes of concern to Vietnam veterans.

In addition to information provided from invited speakers and public attendees at open sessions, the committee made information requests to VA to follow up on issues raised during presentations and on sources of data on Vietnam veterans who use VA health care. All presentations, responses to information requests, and written comments are available in the public access file for the project.

The principal source of information on health effects from potential exposure to the COIs for the committee to consider and deliberate on came from detailed searches of the peer-reviewed literature published since *VAO: Update 2014* (NASEM, 2016a). The literature search strategy and process for reviewing all results is discussed in detail in Chapter 3: Evaluating the Evidence Base. This was supplemented by examining other pertinent published literature, government documents and reports, and testimony presented to Congress; attending professional meetings and educational events; and consulting relevant National Academies reports. The committee also received data from VA, veterans' advocacy organizations, and other sources including the Sierra Valley Cancer Registry.

ORGANIZATION OF THE REPORT

The remainder of this report is organized into 11 chapters, 2 appendixes, and an online supplement of studies reviewed in the course of the VAO series for each health outcome that has an associated conclusion. Chapter 2 presents background information about the population of Vietnam veterans and the military herbicides used in the conflict and addresses exposure-assessment issues. Chapter 3 briefly describes the considerations that guided the committee's review and evaluation of the scientific evidence. Chapter 4 summarizes the toxicology data on the effects of 2,4-D, 2,4,5-T and its contaminant TCDD, cacodylic acid, and picloram; these data contribute to the committee's consideration of the

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biologic plausibility of health effects in human populations. Chapter 5 offers a selected overview of the study populations that have generated findings (in some instances presented in dozens of separate publications) reviewed in the VAO report series as well as those studies that have presented multiple health outcomes, which are presented and discussed in several chapters. In addition to showing where the new literature fits into the compendium of previous publications on Vietnam veterans, occupational cohorts, environmentally exposed groups, and case-control study populations, that chapter includes a description and critical appraisal of the approaches used in the design, exposure assessment, and analysis in these studies.

The committee's evaluation of the epidemiologic literature and its conclusions regarding the associations between exposures and the particular health outcomes that might be manifested long after exposure to the COIs are presented in Chapters 6–11. Because many individual outcomes are included in each chapter, a summary of the findings for each health outcome reviewed in a particular chapter is presented at the beginning of the chapter.

Chapter 6, the first of the chapters evaluating epidemiologic evidence concerning particular health outcomes, addresses immunologic effects and discusses the reasons for what might be perceived as a discrepancy between a clear demonstration of immunotoxicity in animal studies and a paucity of human epidemiologic studies with similar findings. Its placement in the report reflects the committee's belief that immunologic changes may constitute an intermediate step in the generation of distinct clinical conditions, as discussed in subsequent chapters.

Chapter 7 discusses issues related to the possible overall carcinogenic potential of the COIs, particularly TCDD, and then assesses, in order of their codes in the *International Classification of Diseases*, the available epidemiologic evidence on specific types of cancer, which are regarded as individual disease states that might be found to be service-related. Two of the conditions specified in the committee's charge—glioblastoma multiforme and myeloproliferative neoplasms—are covered in this chapter.

Chapter 8 addresses reproductive outcomes that may have been manifested in the veterans themselves, such as reduced fertility and pregnancy loss. It then covers gestational issues, including low birth weight and preterm delivery. This is followed by problems that might be manifested in veterans' children at birth (traditionally defined as birth defects) or later in their lives (childhood cancers, plus a broad spectrum of conditions for which impacts from parental exposures have been posited) or even in later generations.

Chapter 9 addresses neurologic disorders and diseases of the nervous system. Chapter 10 covers conditions related to cardiovascular and metabolic effects (including diabetes) on the basis of their apparent interrelationship in the emerging medical phenomenon known as "metabolic syndrome." Chapter 11 contains information covering the residual "other chronic health outcomes" about which

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epidemiologic results related to the COIs have been encountered in the course of this series of VAO reports and in the current literature search: respiratory disorders, gastrointestinal problems and liver toxicity, kidney and urinary bladder disease, thyroid homeostasis and other endocrine disorders, chronic skin conditions (new to this report), eye problems, and bone conditions.

A summary of the committee's findings and its research recommendations are presented in Chapter 12. In the previous report, which was intended to be the final report of the series as mandated by PL 102-4 and PL 107-103 (and extended by PL 113-175, PL 114-58, PL 114-228, and PL 115-62), the committee reported on the status of recommendations made throughout the VAO series.

In the interest of minimizing unnecessary repetition, the citations for all chapters have been merged into a single reference list that follows all of the chapters. Appendix A provides a list of open meeting agendas and invited presentation topics. Committee and staff biographies can be found in Appendix B. Compendium tables summarizing new results identified for this current update as well as those reviewed by prior committees are available in digital form only and can be accessed from www.nap.edu/catalog/25137.

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2

Background

This chapter provides background information on the current population of Vietnam veterans, the military use of herbicides during the Vietnam War, how different groups of veterans were exposed to the herbicides and how that exposure can be characterized, and the determination of risks due to that exposure. It condenses and updates some of the information presented in chapters 2 (Evaluating the Evidence) and 3 (Exposure to the Herbicides Used in Vietnam) of *Veterans and Agent Orange*: *Update 2014* (NASEM, 2016a), which contain more detailed descriptions of the topics under discussion.

THE CURRENT POPULATION OF VIETNAM VETERANS

Although the conflict ended more than 40 years ago, there are a substantial number of Vietnam veterans who are still living and who, because of herbicide exposure in Vietnam, may have a higher risk than the general public for various negative health effects. The exact number of U.S. military personnel who served in Vietnam is unknown because deployment to the theater was not specifically recorded in military records. Estimates range from 2.6 million to 4.3 million (see Table 2-1), depending on which dates are used to define the Vietnam era and which areas are included in the Vietnam theater of operations (that is, Laos, Cambodia, Vietnam, and the surrounding waters). Approximately 7,500 American women are thought to have served in Vietnam (VA, 2017a). The lack of a comprehensive or official roster of U.S. Vietnam veterans has made it much more difficult to conduct epidemiology studies of health trends in this group of

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TABLE 2-1 Estimates (in millions) of the Vietnam Veteran Population (adapted from IOM, 1994)

Reference	Definition of Service	Time Period	N (millions)
DoD (1976)	Vietnam	1/1/65 – 3/31/73	2.6
Fischer et al. (1980)	Vietnam	8/64 – 6/75	3.8
VA (1985)	Vietnam theater	8/4/64 – 5/7/75	2.7
Kulka et al. (1988)	Vietnam theater	8/64 – 5/75	3.1
DoD (1976)	Vietnam theater	1/1/65 – 3/31/73	3.4
BLS (1990)	Vietnam theater	8/64 – 5/75 (males)	3.7
Fischer et al. (1980)	Vietnam theater	8/64 – 6/75	4.3
BLS (1990)	Vietnam era	8/64 – 5/75 (males)	7.9
VA (1985)	Vietnam era	8/4/64 – 5/7/75	8.3
DoD (1976)	Vietnam era	8/4/64 – 1/27/73	8.7

NOTE: BLS, Bureau of Labor Statistics; DoD, Department of Defense; VA, Department of Veterans Affairs.

military personnel or to track their survival. However, Australian, New Zealand, and South Korean militaries did keep registries of personnel who were deployed to Vietnam.

Beginning in 1990, the Bureau of Labor Statistics used its Current Population Surveys to generate several estimates of the number and age distributions of deployed and non-deployed male Vietnam-era veterans in the civilian population. The 1990 Survey estimated that the number of surviving deployed Vietnam veterans was 29.0% of living male Vietnam-era veterans and 32.7% of surviving non-deployed veterans (Cohany, 1992; NASEM, 2016a).

In 2018, VA estimated that approximately 5,978,000 Vietnam-era veterans (deployed and non-deployed, defined as dates of service from August 1964 to April 1975) were living (VA, 2018c). The committee notes that current information on overall mortality in U.S. Vietnam veterans themselves has been elusive. The most recent reliable information was obtained in the 30-year update of mortality (through 2000) based on the Vietnam Experience Study (Boehmer et al., 2004). The study reported that mortality among the deployed veterans was approximately 9% higher than among the non-deployed veterans. A follow-up study of a random sample of 1,000 Australian Vietnam veterans selected from Australia's comprehensive roster of 57,643 service members deployed to Vietnam found that mortality among Vietnam veterans was 11.7% through 2004 (O'Toole et al., 2010). This estimate of mortality among Australian veterans is slightly higher than but comparable with what was reported among Americans in the Vietnam Experience Study. The most recent update on mortality among female U.S. Vietnam veterans stated that at the end of 2010, 20.2% of the deployed women in the cohort had died compared with 24.6% of those who remained in the United States (Kang et al., 2014a). However, there are considerable differences

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in mortality profiles between men and women, and the information provided by Kang and colleagues may not necessarily apply to the majority of American Vietnam veterans who are male. VA informed the committee that an updated mortality study was underway as of 2017 (Davey, 2017), but no results were available at the time the committee completed its work.

MILITARY USE OF HERBICIDES IN VIETNAM

Military use of herbicides in Vietnam took place from 1962 through 1971. Specific herbicides were selected based on tests conducted in the United States and elsewhere that were designed to evaluate defoliation efficacy (IOM, 1994; Young and Newton, 2004). Four compounds were used in the herbicide formulations in Vietnam: 2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); 4-amino-3,5,6-trichloropicolinic acid (picloram); and dimethylarsinic acid (DMA, or cacodylic acid). These herbicides were used to defoliate inland hardwood forests, coastal mangrove forests, cultivated lands, and zones around military bases. Whereas the chlorinated phenoxy acids 2,4-D and 2,4,5-T persist in soil for only a few weeks, picloram is much more stable and can persist in soil for years, and cacodylic acid is nonvolatile and stable in sunlight (NRC, 1974). More details on the herbicides used are presented in Chapter 4.

However, other toxic compounds were also present in these herbicide formulations. Specifically, polychlorinated dibenzo-*p*-dioxins (PCDDs), which includes 75 different congeners that vary by the number and placement of the chlorine atoms, can be formed during the manufacture of 2,4,5-T and the half-lives of these in subsurface soil may exceed 100 years (Sinkkonen and Paasivirta, 2000). One contaminant of particular concern is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This compound is an unintentional byproduct of the production of 2,4,5-trichlorophenol (NRC,1974). The structures of the chemicals of interest (COIs) identified above are shown in Figure 2-1.

Herbicides were identified by the color of a band on 55-gallon shipping containers and were called Agent Pink, Agent Green, Agent Purple, Agent Orange, Agent White, and Agent Blue. Table 2-2 shows the herbicides used in Vietnam by color code name and summarizes the chemical constituents, concentration of active ingredients, years used, and estimated amount sprayed, based on original and revised estimates. Two different formulations of Agent Orange were used in the course of military operations in Vietnam. All agents were liquid except Agent Blue, which was used in powder form in 1962–1964 and as a liquid in 1964–1971. Agent Pink, Agent Green, Agent Purple, Agent Orange, and Agent Orange II all contained 2,4,5-T and were contaminated to some extent with TCDD. Agent White contained 2,4-D and picloram. Agent Blue (powder and liquid) contained cacodylic acid.

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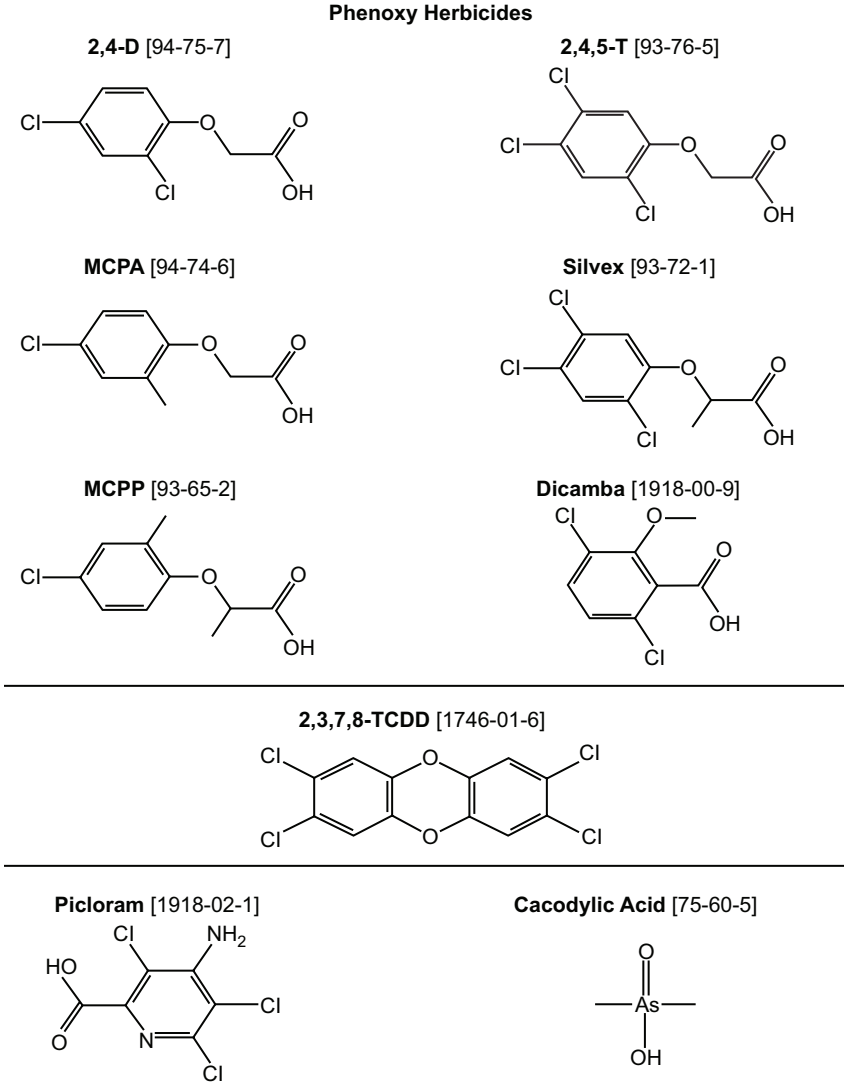

FIGURE 2-1 Chemical structures and Chemical Abstracts Service (CAS) numbers for the specific chemicals of interest.

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TABLE 2-2 Military Use of Herbicides in Vietnam (1961–1971)

Code Name	Chemical Constituents[a]	Concentration of Active Ingredient[a]	Years Used[a]	Amount Sprayed	
				VAO Estimate[b]	Revised Estimate[a]
Pink	60% n-butyl ester, 40% isobutyl ester of 2,4,5-T	961–1,081 g/L acid equivalent	1961, 1965	464,817 L (122,792 gal)	50,312 L sprayed; 413,852 L additional on procurement records
Green	n-butyl ester of 2,4,5-T	—	1961, 1965	31,071 L (8,208 gal)	31,026 L on procurement records
Purple	50% n-butyl ester of 2,4-D, 30% n-butyl ester of 2,4,5-T, 20% isobutyl ester of 2,4,5-T	1,033 g/L acid equivalent	1962–1965	548,883 L (145,000 gal)	1,892,733 L
Orange	50% n-butyl ester of 2,4-D, 50% n-butyl ester of 2,4,5-T	1,033 g/L acid equivalent	1965–1970	42,629,013 L (11,261,429 gal)	45,677,937 L (could include Agent Orange II)
Orange II	50% n-butyl ester of 2,4-D, 50% isooctyl ester of 2,4,5-T	910 g/L acid equivalent	After 1968	—	Unknown; at least 3,591,000 L shipped
White	Acid weight basis: 21.2% triisopropanolamine salts of 2,4-D, 5.7% picloram	By acid weight, 240 g/L 2,4-D, 65 g/L picloram	1966–1971	19,860,108 L (5,246,502 gal)	20,556,525 L
Blue, powder	Cacodylic acid (dimethylarsinic acid) sodium cacodylate	Acid, 65% active ingredient; salt, 70% active ingredient	1962–1964	—	25,650 L
Blue, aqueous solution	21% sodium cacodylate + cacodylic acid to yield at least 26% total acid equivalent by weight	Acid weight, 360 g/L	1964–1971	4,255,952 L (1,124,307 gal)	4,715,731 L
Total, all formulations	—	—	—	67,789,844 L (17,908,238 gal)	76,954,766 L (including procured)

[a]Based on Stellman et al., 2003a.
[b]IOM, 1994, based on data from MRI, 1967; NRC, 1974; Young and Reggiani, 1988.

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Estimates of TCDD Concentrations and Amounts of Herbicides Sprayed

TCDD concentrations in individual herbicide shipments were known to vary between batches and between manufacturers, but the concentrations were not recorded (FAO/UNEP, 2009; Young et al., 1976). In 1974, domestic manufacturing standards for 2,4,5-T required that TCDD not be present at more than 0.05 parts per million (ppm) (NRC, 1974). Tests of TCDD concentrations were conducted in stocks of Agent Orange that remained after the conflict that had been returned from South Vietnam or had been procured but not shipped. The concentration of TCDD in these samples ranged from less than 0.05 ppm to almost 50 ppm, and averaged 2–3 ppm in two sets of samples (NRC, 1974; Young et al., 1978). Later work by Stellman et al. (2003a) resulted in substantial revisions of the earlier estimates of TCDD contamination in the herbicide formulations. The researchers concluded that the mean TCDD concentration in Agent Orange was closer to 13 ppm than to the earlier estimate of 3 ppm. Using their revised estimate, the researchers proposed that 366 kilograms of TCDD was likely applied in Vietnam between 1961 and 1971.

Several studies have attempted to estimate the amount of herbicides sprayed in Vietnam. Through the National Academies of Sciences, Engineering, and Medicine ("the National Academies"), one committee used records gathered from August 1965 through February 1971 and calculated that about 18 million gallons (about 69 million liters) of herbicides were sprayed from helicopters and other aircraft over an area of about 3.6 million acres in Vietnam over that time period (NRC, 1974). This estimate does not include the amount of herbicides sprayed on the ground to defoliate the perimeters of base camps and fire bases or the amount sprayed by Navy boats along river banks.

A revised analysis of spray activities and of the exposure potential of troops emerged from a study overseen by a committee of the Institute of Medicine (IOM, 1997, 2003a,b). That work yielded new estimates of the amounts of military herbicides used in Vietnam from 1961 through 1971 (J. M. Stellman et al., 2003a). The investigators reanalyzed the original data sources used to develop herbicide-use estimates in the 1970s and identified errors that had inappropriately removed spraying missions from the dataset. They also added new data on spraying missions that took place from 1961 to 1965. Finally, a comparison of procurement records with spraying records found errors that suggested that additional spraying had taken place but had gone unrecorded at the time. The new analyses led to a revision of the estimates of the amounts of the agents applied, as indicated in Table 2-2, as well as of the concentration of TCDD-containing herbicides. The new research effort estimated that about 77 million liters were applied, which is about 9 million liters more than the previous estimate.

EXPOSURE OF DIFFERENT GROUPS OF VIETNAM VETERANS

The lack of accurate estimates of chemical exposures experienced by U.S. military personnel who served in Vietnam has significantly hindered

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the epidemiological study of the health effects associated with herbicides and TCDD. For example, some military personnel stationed in cities or on large bases may have received little or no herbicide exposure, whereas troops who moved through defoliated areas soon after treatment may have been exposed through many pathways, including skin contact (directly or indirectly through clothing), direct (through contaminated foodstuffs) or indirect (through hand-to-mouth contact) ingestion, or via contaminated water (drinking, bathing, or immersion during operations). The lack of records regarding individual behaviors and locations combined with the lack of contemporaneous chemical measurements and the lack of a full understanding of the movement and behavior of the defoliants in the environment greatly reduces the ability to conduct the exposure assessments that are necessary for ascertaining the association between the COIs and health effects among Vietnam veterans.

Consequently, most studies have focused on populations that had well-defined tasks that brought them into contact with the agents. These populations primarily include Air Force personnel involved in fixed-wing aircraft spraying activities (often referred to as Operation Ranch Hand) and members of the U.S. Army Chemical Corps. The exposures of ground troops are difficult to define, so this group has not been studied as intensively. In accordance with Congress's mandated presumption of herbicide exposure of all Vietnam veterans, Veterans and Agent Orange (VAO) committees have treated Vietnam-veteran status as a proxy for herbicide exposure when more specific exposure information is not available.

Herbicide Handlers

Military personnel who came into direct contact with the herbicidal chemicals through mixing, loading, spraying, and clean-up activities had relatively high chemical exposures. The precise number of U.S. military personnel who handled herbicides directly is not known. However, two groups have been identified as high-risk subpopulations among veterans: Air Force personnel involved in Operation Ranch Hand and members of the Army Chemical Corps. Additional units and individuals handled or sprayed herbicides around bases or communication lines. For example, Navy river patrols were reported to have used herbicides to clear inland waterways, and engineering personnel used herbicides to remove underbrush and dense growth when constructing fire-support bases. At the time, the herbicides used in Vietnam were not thought to present an important human health hazard, so few precautions were taken to prevent the exposure of personnel (GAO, 1978, 1979). Thus, military personnel did not typically use chemical-protective gloves, coveralls, or protective aprons, and dermal exposure almost certainly occurred in these populations in addition to exposure by inhalation and other routes.

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Operation Ranch Hand

Air Force personnel who participated in Operation Ranch Hand were the first Vietnam-veteran population to receive special attention with regard to herbicide exposure. The Air Force Health Study (AFHS) was a longitudinal, prospective epidemiologic study initiated by the Air Force in 1979. Its purpose was to determine whether Air Force personnel who had participated in Operation Ranch Hand had experienced adverse health outcomes as a result of their service. The study protocol had three components: a retrospective mortality study, a retrospective morbidity study, and a 20-year prospective follow-up study.

The 20-year prospective follow-up study identified 1,242 Ranch Hands who were individually matched on age, race, and military occupation to comparison subjects who served in the Air Force between 1962 and 1971 and who were stationed in and flew cargo operations elsewhere in Southeast Asia during the Vietnam conflict but had not been exposed to tactical herbicides. The comparison group was assumed to be similar to the Ranch Hands regarding lifestyle, training profiles, and socioeconomic factors. The study itself consisted of six comprehensive exams that began with a baseline exam in 1982 and occurred thereafter in years 3, 5, 10, 15, and 20 of the study. A comprehensive set of clinical measurements and observations were made, and biological specimens (serum, whole blood, urine, semen, and adipose tissue) were obtained and preserved during the exams. In all, 2,758 individuals participated in at least one exam cycle. Data collection and analysis for the formal study was completed in 2006 (IOM, 2015).

Exposure among Ranch Hands and the comparison group was evaluated by objective measurements of TCDD in serum samples drawn in 1987 or later, and serum TCDD concentrations were often used as the primary exposure metric for epidemiologic classification in the AFHS (AFHS, 1991a; Kern et al., 2004; Michalek et al., 2001a, 2003; Pavuk et al., 2003). Pavuk et al. (2014) examined the serum concentrations of several dioxins and dioxin-like chemicals in serum samples gathered in 2002 from 777 Ranch Hands and 1,173 AFHS comparison subjects. While the median serum TCDD levels were more than twice as high in the Ranch Hands as in the comparison veterans (5.0 and 2.2 pg/g, respectively), no substantial differences were found between these groups for the other COIs. Additionally, the authors compared serum dioxin levels in AFHS participants with data collected by the National Health and Nutrition Examination Survey (NHANES) during 2001–2002 in U.S. civilian men of the same age range. The Ranch Hands had serum concentrations of dioxins that were similar to those found in the civilians except for TCDD, where the Ranch Hand mean level of 9.5 pg/g lipid was more than three times the NHANES mean level of 2.5 pg/g lipid. This demonstrates the specificity of the dioxin exposure experienced from contact in Vietnam with military herbicides.

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Additionally, several methods for estimating the herbicide exposure of members of the cohort were developed based on questionnaires that focused on such factors as the number of days of skin exposure, the percentage of skin area exposed, and the concentration of TCDD in the different herbicidal formulations (Michalek et al., 1995). Overall, serum TCDD was generally elevated among airmen whose jobs involved more frequent handling of herbicides, but there was no clear demarcation between the distributions of serum TCDD concentrations in the Ranch Hands and those in the comparison group (AFHS, 1991a). A more detailed discussion of the study design and exposure measurement methods used in the AFHS can be found in Chapter 5.

As illustrated by Figure 2-2, the median TCDD levels in veterans who had worked in Operation Ranch Hand were higher than those measured in their own comparison group or in ground troops. The TCDD concentrations found in the controls were of the magnitude of a few parts per trillion (ppt), which are in the range of contemporaneous background levels. Herbicide production workers, by contrast, have serum TCDD levels that are about an order of magnitude higher, and individuals who resided near the site of the industrial explosion in Seveso, Italy, had serum TCDD levels that are about two orders of magnitude higher (Pirkle et al., 1995).

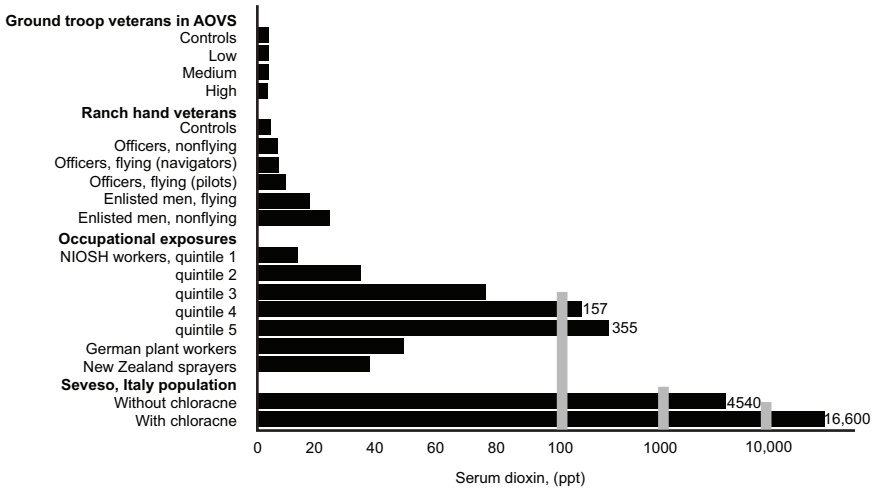

FIGURE 2-2 Median serum TCDD levels in various study populations.
NOTE: AOVS = Centers for Disease Control and Prevention's Agent Orange Validation Study (CDC, 1989a); NIOSH = National Institute for Occupational Safety and Health.
SOURCE: Pirkle et al., 1995.

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Army Chemical Corps

Members of the Army Chemical Corps used hand-operated equipment and helicopters to conduct smaller-scale operations, including defoliation around special-forces camps; clearing of the perimeters of airfields, depots, and other bases; and small-scale crop destruction (NRC, 1980; Thomas and Kang, 1990; Warren, 1968). Studies of health effects related to herbicide exposure in this population were first conducted in the late 1980s (Thomas and Kang, 1990). A small feasibility study of deployed and non-deployed Vietnam-era veterans from within the Army Chemical Corps found an association between veterans reporting having sprayed herbicides and higher serum TCDD concentrations (Kang et al., 2001); this finding was confirmed in a follow-up study of a larger fraction of the cohort (Kang et al., 2006). Modeling efforts (Ross et al., 2015a,b) have also found that higher exposures were probably experienced by those involved with mixer, loader, and applicator activities than by bystanders because the former were generally in closer proximity to and had more frequent contact with the herbicides.

Other Groups of Herbicide Handlers

Other veteran populations also handled herbicides during their service in Vietnam, although probably to a small degree. For example, as part of Operation PACER IVY, Air Force personnel who were separate from, but who were also assisted by, the Ranch Hands and Army Chemical Corps were responsible for removing stocks of Agent Orange from Vietnam to Johnston Island in the central Pacific Ocean (Young, 2009). As part of the operation, procedures included the identification of unused herbicides, the transport of the identified herbicides to a central location in Vietnam for relabeling, and re-drumming for about half of the barrels before shipment. Most of the relabeling, repackaging, and handling of Agent Orange during PACER IVY was overseen and conducted by contractors from China, local residents, and the Vietnam military, so exposure to U.S. military personnel was likely low. However, there were spills of Agent Orange in the re-drumming and storage areas, which contaminated surrounding soils and asphalt (Young, 2009), and these have been suggested as possible sources of exposure.

Other possible points of contamination for Vietnam-era veterans include defoliation tests conducted in South Vietnam as part of Project AGILE; ports in the United States that served as embarkation points for shipping of Agent Orange to Vietnam; storage locations on Johnston Island, where contamination could have occurred from the re-drumming and maintenance of drums that contained Agent Orange; and at-sea incineration of Agent Orange as part of Operation PACER HO (Young, 2009). Because the Army of the Republic of Vietnam was responsible for the handling, transport, and storage of herbicides from the time it was delivered to Vietnam until it was loaded onto Ranch Hand aircraft, Young

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asserts that the herbicide exposures of Allied troops during these procedures may have been negligible.

Ground Troops

Given the widespread, long-term use of herbicides in Vietnam, it is reasonable to assume that many military personnel were inadvertently exposed to the COIs. In contrast with government reports and veterans' testimony about exposure (CDC, 1989b; GAO, 1979), Young et al. (2004a,b) used data from unpublished military records and environmental-fate studies to argue that ground troops had little direct contact with herbicide sprays and that TCDD residues in Vietnam had low bioavailability. They also argued that direct exposures of ground troops were relatively low because herbicide-spraying missions were carefully planned and because spraying occurred only when friendly forces were not in the target area.

To resolve the issue, numerous attempts were made in the 1980s to characterize the herbicide exposures of ground troops in Vietnam (CDC, 1988a; Erickson et al., 1984a; NRC, 1982; S. D. Stellman and J. M. Stellman, 1986; S. D. Stellman et al., 1988a). Those efforts combined self-reports of contact with herbicides or military service records with aerial-spray data to produce a measure of the opportunity for exposure (discussed in greater detail later in this chapter). The search for a way to validate this method led to the development of exposure biomarkers in veterans. Initial studies measured concentrations of dioxin in the adipose tissue of veterans (Gross et al., 1984; Schecter et al., 1987), and dioxin concentrations in adipose tissue were then linked to dioxin concentrations in blood (Kahn et al., 1988). The Centers for Disease Control and Prevention's (CDC's) Agent Orange Validation Study measured TCDD concentrations in blood serum from a relatively large sample of Vietnam veterans and other Vietnam-era veterans (CDC, 1989a) but did not find a statistically significant difference in mean serum TCDD concentrations between the groups. The mean values in each group were about 4 ppt (CDC, 1988a). However, the long lag time between exposure and the serum measurements (about 20 years) leads to questions regarding the accuracy of exposure classification based on serum concentrations. The first VAO committee cautioned that serum TCDD measurements that are collected years after the potential exposure occurred should not be regarded as a "gold standard" or as a fully accurate measure of herbicide exposure because each COI has a different biological half-life. More details on the metabolism of the COIs are presented later in this chapter.

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Brown Water Navy

Navy riverine units (or the "Brown Water Navy") are known to have used herbicides while patrolling inland waterways (IOM, 1994).[1] It is generally acknowledged that estuarine waters became contaminated with herbicides and dioxin as a result of shoreline spraying and runoff from spraying on land, particularly in heavily sprayed areas that experienced frequent flooding. Thus, military personnel who did not serve on land could have been among those exposed to the chemicals during their service in Vietnam.

Blue Water Navy

Dioxin exposure among personnel who served offshore but within the territorial limits of the Republic of Vietnam has also been of concern. It has been hypothesized that in addition to possibly experiencing drift from herbicide-spray missions, personnel on ships that converted seawater by distillation may have been exposed via drinking water. Those concerns were heightened by the findings of an Australian study that showed that TCDD could be enriched in a simulation of the potable-water distillation process that was used on U.S. Navy and Royal Australian Navy ships during the Vietnam War era (Muller et al., 2002). The National Academies convened the Blue Water Navy Vietnam Veterans and Agent Orange Exposure Committee to address that specific issue; its report found that there was inadequate information to determine the extent of exposure experienced by Blue Water Navy personnel, but that there were possible routes of exposure (IOM, 2011b).

Vietnam Military and Civilians

Although studies of the residents of Vietnam are not directly relevant to the committee's task, a summary is provided because these studies may inform possible routes of exposure in U.S. military forces, future epidemiologic studies, and the development of risk-mitigation policies. Researchers have attempted to characterize the herbicide exposure of residents of Vietnam in the process of trying to assess adverse reproductive and other outcomes (Constable and Hatch, 1985). Some researchers compared residents of South Vietnam with residents of unsprayed North Vietnam, others compared South Vietnam residents who lived in sprayed and unsprayed villages as determined by observed defoliation, and other researchers compared women from North Vietnam married to veterans who had served in South Vietnam with women whose husbands had not in order to evaluate reproductive and pregnancy outcomes. Few studies have provided information

[1]E. R. Zumwalt, Jr. 1993. Letter to the Institute of Medicine Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides regarding draft version of the IOM chapter on the U.S. military and the herbicide program in Vietnam, May 20.

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on TCDD concentrations in Vietnamese civilians who were exposed to the COIs during the conflict (Schecter et al., 1986, 2002, 2006).

Armitage et al. (2015) indirectly estimated dermal exposure from direct overspray and long-term dietary exposures in South Vietnam using an aerial dispersion model coupled with a chemical fate and transport model and additional models. The investigators concluded that people in the upland forests of South Vietnam did not commonly experience highly elevated exposures. Dwernychuk et al. (2002) evaluated dioxin contamination around former air bases in Vietnam. The researchers collected environmental and food samples, human blood, and breast milk from residents of the Aluoi Valley of central Vietnam and identified locations where relatively high dioxin concentrations existed in soil and water systems. Soil dioxin concentrations were particularly high in areas (termed "hot spots") around former airfields and military bases where herbicides were handled. Other hot spots included depots of chemical defoliants, air bases used for defoliant spray missions, and areas where chemical defoliants were used extensively. People have since inhabited the areas in and around many former air bases and depots, which have become the focus of studies of environmental contamination and bioaccumulation. For example, studies of poultry raised by Vietnam residents currently living in hot spots have found that dioxin total toxic equivalent (TEQ) levels in poultry eggs are more than twice the adult exposure guideline set by the World Health Organization (WHO) and more than five times the child guideline (Hoang et al., 2014) and that the dioxin content per unit of fat mass in the muscle and liver of the poultry also exceeded the WHO guidelines (Banout et al., 2014).

Publications reviewed in earlier updates have reported environmental concentrations and human body burdens of dioxins in various areas throughout Vietnam (Brodsky et al., 2009; Feshin et al., 2008; Hatfield Consultants, 2009a,b; Nhu et al., 2009; Saito et al., 2010; Tai et al., 2011) and have found pervasive exposure to dioxins more than 50 years after the Vietnam War. Dioxin concentrations in breast milk reflect the residence location of the mothers, with levels and TEQs being elevated in areas where herbicides were sprayed or stored during the war.[2] Another study compared men who had lived in and around the Phu Cat airbase for 50 years or more with men who lived in the unsprayed Kim Bang district of Ha Nam Province in North Vietnam (Manh et al., 2014). Men currently living in the vicinity of the Phu Cat air base have a greater body burden of dioxin than those who spent time in areas that had been the target of herbicide spraying (a geometric mean TEQ of 41.7 pg/g lipid in men who spent time closest to the air base, versus ~29 pg/g lipid for those in two nearby sprayed areas).

[2]For example, Tai et al. (2011, pp. 6629–6630) reported that "[t]he total toxic equivalents of 2,3,7,8-substituted PCDDs/PCDFs in breast milk of mothers living in the hot spots, and the sprayed and unsprayed areas were 14.10 pg/g lipid, 10.89 pg/g lipid, and 4.09 pg/g lipid for primiparae and 11.48 pg/g lipid, 7.56 pg/g lipid, and 2.84 pg/g lipid for multiparae, respectively, with significant differences in the values among the three areas."

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International Forces

Military personnel of the Republic of Korea served in Vietnam during 1964–1973. The Korean Veterans Health Study is a large epidemiological study of more than 114,000 South Korean veterans who served in Vietnam. Several publications from it, including one on how exposure metrics were derived, have been reviewed by prior VAO committees. A more detailed discussion of exposure measurement methods used with this cohort is found in Chapter 5.

Other nations also sent military personnel to assist the South Vietnam military. Reports on the morbidity and mortality of Australian Vietnam veterans have been published on different groups of service members who deployed to Vietnam. These groups include military and some nonmilitary personnel of both sexes who served on land or in the waters of Vietnam from May 23, 1962, to July 1, 1973. However, the comprehensive studies are limited to male members of the military, and most of the analyses focus on men who served in the defense forces—the Army (41,084), the Navy (13,538), and the Air Force (4,570). Objective measures of exposure were not collected, and deployment to Vietnam is generally considered a surrogate of exposure.

A total of 3,394 men and women from New Zealand served in Vietnam between 1962 and 1971, 36 of whom were killed in action or died of wounds or accidents. A cohort of 2,783 living male veterans has been followed prospectively using the New Zealand Veterans Affairs and National Health Index. The 23 women who served in Vietnam were excluded because analyses by sex would not have sufficient statistical power to rule out chance findings. As with the Australian cohort, objective measures of exposure were not collected, and deployment to Vietnam is considered a surrogate of exposure.

Previous volumes of the VAO series and later chapters of this report provide citations to research on these populations as appropriate.

CHARACTERIZING EXPOSURE

The development of a means of characterizing the exposure of individual Vietnam veterans has long been a prime objective of researchers who are interested in refining epidemiologic investigations of health outcomes in this population. Serum TCDD levels might have been a very useful proxy for harmful exposures to all the components of the herbicides used by the U.S. military in Vietnam had they been measured both before and after deployment; however, these data were not collected. Since over time metabolic processes would have reduced the initial chemical concentrations by many half-lives, collecting new samples would not provide valuable information about exposures that occurred during the Vietnam War even among individuals who were likely highly exposed, such as some of the Ranch Hands. For example, serum TCDD measurements taken in 2002 from the Ranch Hands were still sufficiently elevated to distinguish exposed and unexposed

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veterans at the group level. However, TCDD has a half-life of about 7.6 years, and the half-lives of the other COIs are considerably less. With the passage of several decades, serum concentrations decrease exponentially, and newly drawn serum samples are thus unlikely to be useful metrics for assessing health outcomes in surviving Vietnam veterans, occupational cohorts, or Seveso residents. The factors influencing TCDD's half-life are discussed in Chapter 4.

The consideration of records detailing the herbicide spray missions has provided another approach to deriving individual-specific exposure estimates. Two models—a proximity-based Exposure Opportunity Index (EOI) model and an aerial spray distribution model (explained and contrasted below)—have been proposed for estimating the exposure of Vietnam veterans. However, to date only a few studies (which are addressed in detail later in the chapter) have used these exposure assessment methodologies to study the health of Vietnam veterans.

Other National Academies reports have addressed the issue of exposure assessment in military environments, pointing out both the challenges of gathering good contemporaneous information and the desirability of such data for assessing service members' and veterans' health and well-being (IOM, 2000a; NRC, 2000a,b).

Methodologic Issues and Considerations in Exposure Assessment

The focus of this section is on three key methodological issues that complicate the development of accurate exposure estimates in the Vietnam-veteran population and the other study populations discussed in this report: the latent period between exposure and disease, exposure misclassification, and exposure specificity.

Latency

The temporal relationship between exposure and disease is complex and often difficult to define in studies of human populations. Many diseases do not appear immediately after exposure. Cancers, for example, might not appear for many years after exposure. The latency period refers to the amount of time between an initiative event, such as a toxic exposure, and the manifestation of the clinical disease. For many disease processes the latency period is not known. If the latency period is underestimated, the effect of the exposure of interest on health outcomes will not be captured by epidemiological methods. Exposures can be brief (acute) or occur over a longer period of time (chronic). At one extreme, an exposure can be the result of a single event, as in an accidental poisoning. At the other extreme, a person exposed to a chemical that is stored in the body may continue to experience "internal exposure" for years even if exposure from the environment has ceased.

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Exposure Misclassification

Exposure misclassification in epidemiologic studies can affect estimates of risk. In a case-control study, this would be a situation in which the reported measurement of exposure in either the cases or the controls (or sometimes in both cases and controls) is incorrect (classifying a person who was not exposed as having been exposed, for example). Non-differential exposure misclassification occurs if the probability of exposure misclassification is the same in both cases and controls. If this happens, then the estimated association between disease and exposure is biased toward the null value. In other words, one would expect the true association, if it exists, to be stronger than the observed association. Differential exposure misclassification occurs if the probability of misclassification is different between cases and controls. If this occurs, then the estimated association can be biased in either direction, either toward the null value or away from the null value. Then the true association, if it exists, might be stronger or weaker than the observed association.

When exposure is classified into more than two levels, the slope of a dose–response trend is not necessarily attenuated toward the null value even if the probability of misclassification is the same in the two groups of subjects being compared. Therefore, the observed trend in disease risk among the several levels of exposure may be either an overestimate or an underestimate of the true trend (Dosemeci et al., 1990). Greenland and Gustafson (2006) discussed the effects of exposure misclassification on the statistical significance of the result and demonstrated that if one adjusts for exposure misclassification when the exposure is represented as a binary variable, the resulting association is not necessarily more significant than in the unadjusted estimate. That result remains true even though the observed magnitude of the association might be increased.

The Update 2014 committee was concerned about the strong degree of misclassification associated with publications concerning various health outcomes authored by researchers who categorized exposure based on a variable in a patient's electronic medical record indicating whether the individual was "exposed to Agent Orange" (Ansbaugh et al., 2013; Q. Li et al., 2013). Likewise, several studies of VA health care–user populations have been identified that continue to define exposure on the basis of that indicator in the medical record. That variable conveys a degree of authenticity that this and the prior committee strongly suspected is unmerited because there is little documentation about the source of information used to make this classification—deployment status, entry on the Agent Orange Registry, the veteran's self-report, a physician's observation that the patient has a condition presumed to be service-related, results of serum TCDD measurements performed on some patients, or perhaps some other criterion. Regardless, none of these approaches offers a reliable method of determining whether an individual was truly exposed to herbicides (above some unspecified level) or yields a method that can be applied uniformly to all veterans using the VA medical system, who themselves are a self-selected subset of veterans. The current committee concurs

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with the Update 2014 committee's concern that these publications do not adequately discuss the reliability of their exposure metric.

Specificity

The evaluation of the findings of studies of persons exposed to components of the herbicides sprayed in Vietnam requires some decisions about their relative contributions to the VAO project's evidentiary database. Only a few herbicidal chemicals were used as defoliants during the Vietnam conflict: esters and salts of 2,4-D and 2,4,5-T, cacodylic acid, and picloram in various formulations. TCDD as a contaminant of 2,4,5-T is also of import. The committee recognizes that in real-world conditions, exposure to TCDD virtually never occurs in isolation and that there are hundreds of similar compounds to which humans might be exposed, including other PCDDS, polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Human exposure to TCDD is almost always accompanied by an exposure to one or more of the other compounds, but the exposure of Vietnam veterans to substantial amounts of the other chemicals relative to the exposure to TCDD has not been documented. Studies that analyzed for dioxin-like PCDF and PCB congeners and expressed the results in terms of TEQs have also been considered since *Update 1998*.

Among the various chemical classes of herbicides that have been identified in published studies reviewed by the committee, phenoxy herbicides, particularly 2,4-D and 2,4,5-T, are directly relevant to the exposures experienced by U.S. military forces in Vietnam. On the basis of the assumption that compounds with similar chemical structure may have analogous biologic activity, information on the effects of other chemicals in the phenoxy herbicide class—such as 2-(2,4,5-trichlorophenoxy) propionic acid (fenoprop or Silvex), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2-(2-methyl-4-chlorophenoxy) propionic acid (mecoprop, MCPP), and 3,6-dichloro-2-methoxybenzoic acid (dicamba)—has been factored into the committee's deliberations with somewhat less weight (see Figure 2-1).

Many scientific studies reviewed by the current and prior committees report exposures to broad categories of chemicals rather than to those specific chemicals. Many studies have examined the relationship between exposure to "pesticides" and adverse health outcomes, while others have used the category of "herbicides" without identifying specific chemicals. A careful reading of a publication often reveals that none of the COIs contributed to the exposures of the study population, so such studies could be excluded from consideration. Chapter 3, Evaluation of the Evidence Base, contains a detailed discussion of inclusion and exclusion criteria for the studies reviewed in the current update.

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Modeling Herbicide Exposure in Vietnam

Two models have been used to estimate exposure to herbicides experienced by ground personnel in Vietnam: the EOI and AgDRIFT®. The models have different purposes; the EOI model was specifically developed to estimate herbicide exposure potential to persons on the ground on the basis of data on aerial spray history and troop locations, whereas AgDRIFT® is a modification of a model developed by the U.S. Forest Service that predicts agricultural pesticide ground concentrations based on variables related to dispersal, drift, and deposition. The AgDRIFT® model thus does not consider troop-location data, nor is it specific to exposures in Vietnam.

Exposure Opportunity Index Model

Following from recommendations contained in the first VAO report (IOM, 1994), VA asked the National Academies to issue a request for proposals seeking individuals and organizations to develop historical exposure-reconstruction approaches suitable for epidemiologic studies of the herbicide exposure of U.S. veterans during the Vietnam War (IOM, 1997). A team of researchers in Columbia University's Mailman School of Public Health was awarded the contract for the Characterizing Exposure of Veterans to Agent Orange and Other Herbicides in Vietnam project. Several sources of information concerning spraying activities and information on the locations of military units assigned to Vietnam were integrated into a database. The resulting EOI model (J. M. Stellman and S. D. Stellman, 2003) generates individualized estimates (EOI scores) of the exposure potential of troops who served in Vietnam.

Mobility factor analysis, a technique used for studying troop movement, was developed for use in reconstructing herbicide-exposure histories. The analysis is a three-part classification system for characterizing the location and movement of military units in Vietnam. It comprises a mobility designation (stable or mobile), a distance designation (usually in kilometers) to indicate how far a unit might travel in a day, and a notation of the modes of travel available to the unit (by air, by water, or on the ground by truck, tank, or armored personnel carrier). A mobility factor was assigned to every unit that served in Vietnam.

The data were combined into a geographic information system (GIS) for Vietnam. Herbicide-spraying records were integrated into the GIS and linked with data on military-unit locations to derive individual EOI scores. The results are the subject of reports by the contractor (J. M. Stellman and S. D. Stellman, 2003) and the Committee on the Assessment of Wartime Exposure to Herbicides in Vietnam (IOM, 2003a,b). A summary of the findings on the extent and pattern of herbicide spraying (Stellman et al., 2003a), a description of the GIS for characterizing exposure to Agent Orange and other herbicides in Vietnam (Stellman et al.,

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2003b), and an explanation of the EOI model based on that work (S. D. Stellman and J. M. Stellman, 2004) have been published in peer-reviewed journals. In those publications the researchers argued that it is feasible to conduct epidemiologic investigations of veterans who served as ground troops during the Vietnam War. The National Academies later issued a report that examined the feasibility of using the EOI model (IOM, 2008). The report concluded that "despite the shortcomings of the exposure assessment model in its current form and the inherent limitations in the approach, the committee agreed that the model holds promise for supporting informative epidemiologic studies of herbicides and health among Vietnam veterans and that it should be used to conduct studies" (p. 2). This model has since been used in analyses of the Korean Veterans Health Study (Yi and Ohrr, 2014; Yi et al., 2014b).

AgDRIFT®-Based Model

Ginevan et al. (2009a,b, 2014, 2015) proposed the use of the AgDRIFT® Tier III forestry model for estimating the deposition of herbicides via aerial spraying as alternative to the EOI model. They suggested that dermal exposure through both direct deposition and post-application transfer from foliage could be derived from application information such as aircraft speed and altitude, from nozzle characteristics, and from droplet evaporation and environmental parameters such as canopy density, canopy roughness, and crosswind speed. The authors did not consider exposures resulting from contact with soil and dust or through inhalation because they considered these routes to be negligible (Ginevan et al., 2009a; citing Driver et al., 1989; Gunther et al., 1977; Nadal et al., 2004). However, subsequent reviews of the methodology underlying the authors' analyses (S. D. Stellman and J. M. Stellman, 2014, 2015) found several weaknesses that call the results by Ginevan et al. into serious question.

DETERMINING INCREASED RISK IN VIETNAM VETERANS

Part of the committee's charge—derived from the text of Public Law 102-4—was to determine, to the extent permitted by the available scientific data, the increased risk of disease among veterans exposed to herbicides or the contaminant TCDD during service in Vietnam. Estimating the magnitude of risk of each particular health outcome among herbicide-exposed Vietnam veterans requires quantitative information about the dose–time response relationship for the health outcome in humans, information on the extent of herbicide exposure among Vietnam veterans, and estimates of individual exposure. Vietnam veterans were exposed to other agents and stressors—such as tobacco smoke, insecticides, therapeutics, drugs, diesel fumes, alcohol, hot and humid conditions, and combat—that may increase or decrease the ability of chemicals in herbicides to

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produce a particular adverse health outcome. Few, if any, studies either in humans or in experimental animals have examined those interactions.

As mentioned earlier in this chapter, most health studies of Vietnam veterans were hampered by relatively poor measures of exposure to herbicides or TCDD and by other methodological problems. Most of the evidence on which the findings regarding associations are based, therefore, comes from studies of people exposed to TCDD or herbicides in occupational and environmental settings rather than from studies of Vietnam veterans. The committees that produced the first VAO report and the updates found that the body of evidence was sufficient for reaching conclusions about statistical associations between herbicide exposures and health outcomes but that the lack of adequate data on Vietnam veterans themselves complicated the consideration of this part of the charge.

Although some groups had well-documented high exposures of herbicides (such as participants in Operation Ranch Hand and Army Chemical Corps personnel), most Vietnam veterans had lower exposures to herbicides and TCDD than did the subjects of many occupational and environmental studies (see Figure 2-2 from Pirkle et al., 1995). The committees responsible for the first VAO report and the updates have concluded that in general it is impossible to quantify the risk posed to veterans by their exposure to herbicides in Vietnam.

After decades of research, the challenge of estimating the magnitude of potential risk posed by exposure to the COIs remains intractable. The requisite information is still not available despite concerted efforts to use modeling to reconstruct likely exposure from records of troop movements and spraying missions (J. M. Stellman and S. D. Stellman, 2003; S. D. Stellman and J. M. Stellman, 2004; J. M. Stellman et al., 2003a,b), to extrapolate from agricultural models of drift associated with spraying (Ginevan et al., 2009a; Teske et al., 2002), to measure serum TCDD in individual veterans (Kang et al., 2006; Michalek et al., 1995), and to model the pharmacokinetics of TCDD clearance (Aylward et al., 2005a,b; H. L. Chen et al., 2006; Emond et al., 2004, 2005, 2006). Additionally, there is still uncertainty about the toxicity and health effects of the specific COIs. Prior committees have thought it unlikely that additional information or more sophisticated methods would permit any sort of quantitative assessment of Vietnam veterans' increased risks of particular adverse health outcomes that are attributable to exposure to the chemicals associated with herbicide spraying in Vietnam. Even if one accepts an individual veteran's serum TCDD concentration as the best available surrogate for overall exposure to Agent Orange and the other herbicide mixtures sprayed in Vietnam, not only is it nontrivial to make this measurement, but the hurdle of accounting for biologic clearance and extrapolating to the proper timeframe remains. Accordingly, the lack of exposure estimations for Vietnam veterans will likely remain a hurdle to epidemiologic studies, and unless this issue is resolved, the potential for additional epidemiologic studies to yield improved information regarding the specific question of whether an association exists between herbicide exposure and health outcomes will remain limited.

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3

Evaluation of the Evidence Base

This chapter describes the approach and methods that the committee used to identify and evaluate the scientific and medical literature on exposures to herbicides that occurred in U.S. military personnel during the Vietnam War as well as the process that the committee used to reach conclusions on the association between exposures to the chemicals of interest (COIs)—2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); picloram; dimethylarsinic acid (DMA or cacodylic acid); and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)—and a given health outcome. The committee's process entailed the following steps: a literature search, screening of abstracts, a full text review of studies flagged in the abstract screening, and the evaluation of a final set of studies identified as relevant after the full text review. The first part of this chapter details the methodology used to identify and screen the literature. The second part of the chapter details the evaluation criteria used to review the relevant studies, including the types of studies considered, the health outcomes considered, and the categories of association used to draw conclusions about the strength of the evidence of possible health effects resulting from herbicide exposure. The committee also describes some of the issues it encountered when reviewing the literature on Vietnam War exposures and health outcomes, such as multiple exposures and individual variability. Because the current committee closely followed the approach used by prior Veterans and Agent Orange (VAO) and Update committees, much of the following information was previously described in those volumes as well, particularly *Update 2014* (NASEM, 2016a).

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IDENTIFICATION AND SCREENING OF THE LITERATURE

Literature Identification

Much as was the case with previous VAO committees, this committee was tasked with comprehensively reviewing, evaluating, and summarizing the scientific literature that has appeared since the previous biennial update regarding associations between TCDD and other chemicals present in the herbicides used by the U.S. military in Vietnam and health outcomes and then adding this new information to the existing compendium of evidence to draw conclusions on the strength of associations between exposure and health outcomes. To begin, the committee oversaw extensive searches of the scientific literature using a strategy adapted from prior committees' literature search methodology (see Box 3-1). This committee's search included additional terms to evaluate specific conditions called out in the Statement of Task: possible generational health effects, myeloproliferative neoplasms, and brain cancer, in particular, glioblastoma multiforme (see Chapter 1 for the full Statement of Task).

For this update, electronic searches of the medical and scientific literature were carried out on four databases: Web of Science, Scopus, Medline, and Embase. In the previous VAO updates, only Medline and Embase were used. The four searchable databases index biological, chemical, medical, and toxicological publications. The full texts of the articles were searched so that if any of the search terms was included in the title or abstract or indexed in the key words or text of the article (excluding the cited references section), the article would be included in the results of the search. Search terms included full and abbreviated chemical names, common and manufacturer trade names, the Chemical Abstracts Service numbers, and MeSH[1] descriptors for each of the COIs and other similar chemicals on an assumption that compounds with similar chemical structure may have analogous biologic activity. The aryl hydrocarbon receptor (AHR) and related MeSH descriptors were also included as primary search terms since this protein appears to mediate essentially all of the toxicity of TCDD.

Using the search terms in Box 3-1, the databases were searched in two phases, with the searches spanning over timeframes that were extended from those used in prior updates. In the spring of 2017, the databases were searched for articles published between January 1, 2014, and March 31, 2017. Then in early February 2018 the databases were again searched for any articles with the relevant search terms published between March 1, 2017, and December 31, 2017. The timeframes for those searches overlapped each other and also overlapped with the Update 2014 committee's search (October 1, 2012–September 30, 2014). The overlapping dates made possible the capture of articles that might

[1]MeSH descriptors are sets of terms naming descriptors in a hierarchical structure that permits searching at various levels of specificity. For example, MeSH terms for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin include "TCDD" and "dioxin," without those terms having to be specified individually.

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BOX 3-1
Search Terms

TERMS INCLUDED IN PREVIOUS VAO SEARCHES

Herbicide Formulations2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), and dimethylarsinic acid (DMA or cacodylic acid) and the contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

Other Similar Chemicals

2-(2,4,5-trichlorophenoxy) propionic acid (Silvex), 2-methyl-4-chlorophenoxyacetic acid, 2-(2-methyl-4-chlorophenoxy) propionic acid (Mecoprop), 3,6-dichloro-2-methoxybenzoic acid (dicamba), and polycholorinated biphenyls (PCBs). Full and abbreviated chemical names, common and manufacturer trade names, the Chemical Abstracts Service (CAS) numbers (that serve as a unique identifier for every chemical), and MeSH descriptors for each of the chemicals of interest.

TCDD Toxicity Mediator

Aryl hydrocarbon receptor (AHR) and related MeSH descriptors

Generic Terms

Vietnam, veteran, Vietnam veteran, Agent Orange, dioxin, herbicide, and phenoxy

SEARCH TERMS FOR CONDITIONS SPECIFIED IN THE STATEMENT OF TASK

Generational Health Effects

Reproductive OR Reproduction OR Gonads OR Fertility OR Infertility OR Fertilization OR Sperm* OR Testes OR Testicular OR Pregnancy OR Birth OR Embryo OR Fetus OR Fetal OR Spermatozoon (MeSH) OR Infertility, male (MeSH) OR Infertility, female (MeSH) OR Testis (MeSH) OR Testicle OR Sperm–ovum interactions (MeSH) OR Semen OR Semen quality OR Ova OR Ovum OR Oocyte OR Egg OR Parturition (MeSH) OR Embryonic structures (MeSH) OR Fetal development OR Embryonic and fetal development (MeSH) OR Pregnancy complications (MeSH C13.703) OR Abortion, spontaneous (MeSH) OR Miscarriage OR Premature birth OR Fetal death OR Pregnancy loss OR Preconception injuries (MeSH C21.676) OR Germline OR Microbiome OR DNA methylation OR Histone modification OR Histone* OR SNP* OR Polymorphism* OR Non-coding RNAs OR ncRNA* OR Small RNA* OR MicroRNA* OR miRNA OR Imprinted gene* OR Gene–environment OR Gene OR Epigenetic processes OR Epigenetic OR Epigenomic OR Transgeneration* OR Trans-generation* OR Multigeneration* OR Multi-generation* OR Intergeneration* OR Inter-generation* OR Generation* OR Multigenerational effect* OR Multi-generational effect* OR Multigenerational inheritance OR Multi-generational inheritance OR Multigenerational epigenetic inheritance OR Multi-generational epigenetic inheritance OR F1 OR “F 1” OR

continued

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BOX 3-1 Continued

"F(1)" OR F2 OR "F 2" OR "F(2)" OR F3 OR "F 3" OR "F(3)" OR F4 OR "F 4" OR "F(4)" OR Offspring OR Progeny OR One generation OR Two generation* OR Second generation* OR Three generation* OR Third generation* OR Four generation* OR Fourth generation* OR Child* OR Parent* OR Father* OR Son* OR Daughter* OR Grandparent* OR Grand parent* OR Grandfather* OR Grand father OR Grandmother OR Grand mother OR Grandchild* OR Grandson* OR Grand son* OR Granddaughter* OR Grand daughter* OR Greatgrandparent* OR Great-grandparent* OR Greatgrandmother* OR Great-grandmother* OR Great-grandchild* OR Great-grandchild* OR Greatgrandson* OR Great-grandson* OR Greatgranddaughter* OR Great-granddaughter*

Myeloproliferative Neoplasms

Myeloproliferative disorder* OR Myeloproliferative neoplasm* OR Aryl hydrocarbon receptor OR Myeloproliferative syndromes OR Myeloproliferative disorders OR "P vera" OR Polycythemia vera OR "ET" essential thrombocytosis OR Essential thrombocythemia OR Myeloid metasplasia OR Agnogenic myeloid metaplasia OR Myelofibrosis OR Primary myelofibrosis OR Chronic idiopathic myelofibrosis OR "CML" OR Chronic myelogenous leukemia OR Jak2 mutation OR Janus kinase 2 OR bcr-abl OR Philadelphia chromosome OR "CALR" mutation OR Calreticulin mutation OR MPL mutation OR Thombopoietin mutations OR "XPD polymorphism" OR LNK (Src homology 2B3) OR CBL (Casitas B lineage lymphoma)

Glioblastomas

"TET" OR ASXL2 OR IDH OR IKZF1 OR EZH2 OR DNMT3A OR Glioblastoma OR Glioblastoma cell line OR Brain neoplasm OR Glioblastoma multiforme OR Astrocytoma grade IV OR Multifocal glioblastoma OR Glioma OR Neuroepithelial neoplasm OR Optic glioblastoma OR T98G glioblastoma multiforme cell line OR brain tumor (MeSH)

have been published but not indexed at the time of the first search. Other than dates, no limitations (such as language, populations, or species) were put on the search. In addition, potentially relevant articles were also identified by searching the reference lists of relevant review and research articles, books, and reports. Exact duplicate articles and those that had been summarized and referenced in *Update 2014* were deleted. The committee became aware of a few studies that reported updated findings on relevant exposed populations (such as the Seveso, Italy, cohort and New Zealand phenoxy herbicide producers) published following the December 31, 2017, search cutoff and reviewed these studies as well.

Literature Screening

The search strategy was devised to ensure that abstracts of all potentially relevant articles were subjected to closer screening, but it also resulted in the

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identification of a large number of non-relevant studies. The first search produced in excess of 12,000 "hits," and the second search identified more than 1,600 articles of potential relevance. The articles were generally in English, but VAO committees have traditionally obtained translations for crucial ones that were not in English. Article titles and abstracts were screened for relevance by committee members and the Health and Medicine Division staff to determine which studies should be considered for full-text retrieval using the criteria in Box 3-2.

In the VAO series, neurologic deficits associated with Vietnam service are distinguished from psychiatric/psychologic conditions—such as posttraumatic stress disorder (PTSD), depression, and anxiety—and their sequelae. While, as past VAO committees have noted, the increased risks of these types of mental health conditions among veterans of all U.S. conflicts are of scientific and public health concern, military service alone, including deployment and service in Vietnam, is known to confer a range of exposures to potentially traumatic events that may be expected to increase the risk of developing PTSD and related psychologic conditions. For example, compelling evidence has established that the prevalence of PTSD is more than twice as high for operational infantry units exposed to direct combat than it is in the general population (Kok et al., 2012). Previous committees further drew the conclusion that it would be infeasible to disentangle potential adverse effects from exposure to the COIs on mental health outcomes that may occur independently of psychological effects accrued through military service. The current committee expands upon that perspective by placing it in a framework that underscores the relevance of the concepts of multifactorial causation, the literature on which has recently begun to mature and offer new insights. Both independent and interacting risk factors may play a role. An example of multiple independent effects can be seen in a recent study of Vietnam veterans in Australia, which found that both sons and daughters of veteran fathers diagnosed with PTSD had an increased risk for PTSD even after controlling for PTSD in the mother and for other diagnoses (O'Toole et al., 2017). The statistical interactions of risk factors, which can have synergist or antagonistic effects, can result in effects of combined exposures that would not have been predicted based on their independent impacts. An example of a synergistic interaction is the association with lung cancer from combined exposures to workplace arsenic and smoking: in this case, the risks from arsenic are much higher among smokers than among non-smokers (Hertz-Picciotto et al., 1992).

Disentangling the separate effects of combined exposures or risk factors in relation to a particular outcome does raise serious challenges, however, and it may indeed be infeasible when the correlations among those exposures are exceedingly high, to the point of inseparability, or when sufficiently large studies cannot be conducted. For example, service-related emotional trauma, such as occurs in combat, and exposures to the COIs in Vietnam are associated (J. M. Stellman et al., 1988), but whether they are separable depends on the extent to which those experiencing service-related trauma also had high exposures to the

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BOX 3-2
Criteria for Excluding Scientific Articles from Further Consideration

- Study does not include the chemicals used as tactical herbicides in military operations in Vietnam. Examples include exposure to herbicides containing organophosphates (not otherwise specified), atrazine, paraquat, glyphosate, metamifop, rotenone, clarityon, and diuron; and exposure to pesticides and insecticides (e.g., chlorpyrifos, imidacloprid, thiacloprid, DDT, DDE, etc.), fungicides, rodenticides, and non-dioxin-like PCBs.
- Descriptions of ecosystem or environmental monitoring or exposure surveillance.
- Evaluations of chemical toxicities in environmental samples (e.g., plants, water, soils).
- Therapeutic or treatment studies following intentional or unintentional exposure to one of the chemicals of interest (COIs).
- Descriptions of intentional acute chemical exposure or poisoning (including suicide).
- Case studies or case series (while such reports may be interesting and provide unusual information, it is not appropriate to extrapolate findings from a single case to an entire population).
- Conference abstracts or proceedings, commentaries, or letters to the editor.
- Stated narrative or historical accounts of the Vietnam era.
- Studies that did not relate exposure to a COI and a health outcome.
- Exposures experienced as children or adolescents (if the study subjects were exposed prenatally and followed, the article was further considered).
- Studies of lactation or ingestion of breast milk as the sole outcome of interest without associated outcomes.
- Studies about occupational exposure in greenhouse workers (consistent with previous updates).
- Described mental health or psychological conditions as a result of herbicide or pesticide exposure, because it would be impossible to disentangle the potential adverse effects on mental health outcomes caused by exposure to the COIs that may occur independently of psychological effects accrued through military service.
- Epidemiologic literature on the health effects of inorganic arsenic. Inorganic arsenic and benzene were not considered as relevant service-related exposures among Vietnam veterans and were not evaluated in relation to their potential risk of adverse health outcomes. VAO committees have considered and reviewed toxicologic studies in which animals were directly exposed to dimethylarsinic acid (DMA), but the extensive literature on the health effects of exposure to inorganic arsenic (including the epidemiologic research, animal experiments, and mechanistic studies) has been regarded as not primarily pertinent to DMA exposure and has not been considered.

COIs and, conversely, the extent to which those with high exposures to the COIs invariably had a high degree of service-related trauma.

Current epidemiologic thinking recognizes that multiple risk factors may operate not only independently, but also synergistically, and that sometimes the

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combined impacts may have both an independent and synergistic or antagonistic component. Thus, a nuanced and comprehensive approach to combined exposures is critical to understanding causation. Underlying susceptibility is not always genetic, but can instead be a prior or concomitant exposure, and thus the possibility of multifactorial causation requires paying attention to confounding as well as to interactions. Few studies have rigorously addressed the combination of combat duty and the COIs through an assessment of interactions. In addition, a review of the vast toxicology literature that relates to the COIs reveals that there is a dearth of reports that address the potential associations and mechanistic explanations relating to how exposure to the COIs may influence the risk of developing mental health conditions. This applies specifically to an overall absence of published evidence as to how TCDD exposure could be etiologically implicated in the development of PTSD and related psychological comorbidities. Animal models will not be informative for studying potential associations between exposure to the COIs and development of PTSD. Because the literature appears to be quite limited, it does not provide the opportunity to address effects on PTSD or other mental health conditions that may occur as a result of the combined effect of military service/combat duty and the COIs. Thus, at this time the committee was not able to determine whether the COIs might magnify the impact of traumatic events.

Although exposure to 2,4-D, 2,4,5-T, TCDD, cacodylic acid, and picloram are most germane to the committee's charge and given the most weight in the review of the evidence on a particular health outcome, chemicals that are structurally similar to the herbicides and contaminants found in the tactical herbicides used in Vietnam are assumed to have similar biologic activity and thus were also included in the committee's review of the literature, consistent with prior VAO Update committees. These related chemicals include other phenoxy herbicides—such as 2-(2,4,5-trichlorophenoxy) propionic acid (Silvex), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2-(2-methyl-4-chlorophenoxy) propionic acid (Mecoprop or MCPP), and 3,6-dichloro-2-methoxybenzoic acid (dicamba)—as well as dioxin-like chemicals such as those found in mono-ortho and non-ortho-substituted polychlorinated biphenyls (PCBs) and also metabolites of organic arsenic (DMA^V). Very few epidemiologic studies on exposure to picloram or cacodylic acid have been published, which is another reason for the committee to consider metabolites of these compounds.

Examining the structural representation of the COIs shown in Figure 2-1, one can readily see the basis of an assertion heard repeatedly from individual Vietnam veterans that "benzene is contained in TCDD." Indeed, the two rings at the ends of the three-ring structure constituting the basic structure of dioxin compounds, to which chlorine molecules or other chemical radicals can be attached, do have the molecular structure of a single benzene molecule, and the "dibenzo-dioxin" in TCDD's chemical name does mean that the molecule is a benzene-substituted dioxane. The benzene ring structure is a basic building block of a vast number of organic compounds, both industrial (such as polyaromatic

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hydrocarbons, the phenoxy herbicides, picloram, and PCBs) and natural (such as estradiol, a hormone present in both men and women). However, the biologically active compound benzene does not emerge from dioxin, whose three-ring structure is extremely stable and resistant to metabolism.

An interaction or synergism among the COIs or in conjunction with other agents is another theoretical concern. The committee was not charged with attributing effects to specific COIs, and joint effects among them should be adequately identified by the committee's approach. The combinations of the chemicals with other agents that might lead to problems are virtually infinite, and hence, not feasible for systematic and comprehensive evaluation. Real-life experience, as investigated with epidemiologic studies, effectively integrates any results of exposure to a target substance in combination with other substances that may be etiologically relevant.

Thus, in aggregate, the primary COIs evaluated by the committee with respect to potential associations with adverse health outcomes among Vietnam veterans are 2,4-D, 2,4,5-T, picloram, cacodylic acid, and TCDD. As explained, inorganic arsenic and benzene were not considered as relevant service-related exposures among Vietnam veterans and thus were not evaluated in terms of their risk for adverse health outcomes.

The committee recognizes that in real-world conditions, human exposure to TCDD virtually never occurs in isolation and that there are hundreds of similar compounds to which humans might be exposed, including other polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDFs), and PCBs which have dioxin-like biologic activity. The literature on the other compounds, particularly PCBs, has not been reviewed systematically by the committee except for those reports in which TCDD was identified as an important component of the exposure or when the risks of health effects were evaluated in relation to exposures to dioxin-like chemicals. In many cases, when dioxin-like chemicals are present the exposure is expressed in terms of toxic equivalencies (TEQs), which are the sums of toxicity equivalence factors (TEFs) for individual dioxin-like chemicals as measured by activity with AHR. Studies that report TEQs based only on mono-ortho PCBs (which are PCBs 105, 114, 118, 123, 156, 157, 167, and 189) are considered even though their TEQs are several orders of magnitude lower than those of the non-ortho PCBs (77, 81, 126, and 169), based on the revised World Health Organization (WHO) 2005 TEF values (La Rocca et al., 2008; van den Berg et al., 2006). This is because the lower TEQs of the mono-ortho PCBs may be counterbalanced by their abundance, which is generally many orders of magnitude higher than the non-ortho PCBs (H.-Y. Park et al., 2010). For example, PCB congeners 118 and 156 are present in numerous human populations (United States, Europe, and Japan), at levels that are 4 to 5 orders of magnitude greater than the levels of TCDD itself (Bake et al., 2007; Becher et al., 1995; Lorber et al., 2009, Tsuchiya et al., 2003), with the levels of mono-ortho TEQs closely approximating those of TCDD or in some cases exceeding them (H.-Y. Park et al., 2010).

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Exposure to non-dioxin-like PCBs only has not been considered by VAO committees for two reasons. First, Vietnam veterans may have been exposed to substantial amounts of the other chemicals, and the amount of exposure to those chemicals relative to TCDD has not been documented. Second, the most important mechanism for TCDD toxicity involves its ability to bind to and activate the AHR. Many of the other chemicals act by different or multiple mechanisms, so it is difficult to attribute toxic effects after such exposures specifically to TCDD. Furthermore, an individual's exposures to dioxin-like chemicals and their non-dioxin-like counterparts tend to be correlated, which means that it is difficult for epidemiologic studies to attribute any observed association to a particular chemical configuration (Longnecker and Michalek, 2000); carrying out analyses in terms of TEQs somewhat circumvents this problem. The interaction or synergism among the COIs or in combination with other agents is a concern, but the combinations of the COIs with other toxicants, or physical, biological stressors is outside of the committee's charge.

In cases when the title or abstract mentioned only broad chemical categories or used non-specific terms such as "herbicide" or "pesticide," the full text was reviewed for mention of the specific COIs. It was evident from most of the abstracts that the article did not address health effects in association with exposure to the specific COIs. The committee only deleted studies from further consideration if it verified that exposure information specifically did not include at least one of the COIs or if the study was not at all relevant, such as studies that evaluated the efficacy of the COIs in killing specific plants or that measured concentrations of the COIs in environmental samples, studies that merely provided a description of manufacturing processes, or studies that did not include a health outcome. All studies that discussed health effects or changes in pathophysiology or cell signaling were considered if the text indicated that any of the herbicides of interest (or any of their components) may have been investigated. The committee only included literature that had undergone peer review or government reports and invited presentations that were provided to the committee, under the assumption that they have been carefully reviewed. The process of peer review by fellow professionals increases the likelihood that high-quality studies will appear in the literature, but it does not guarantee the validity of any particular study or the ability to generalize its findings.

Because the use of the general search terms "pesticides" or "pesticide," "defoliants," "defoliant agent," and "herbicide" or "herbicides" resulted in more than 57,000 "hits," the committee focused its search on the specific COIs as well as related chemical classes. Even using the more targeted terms for the COI identified many studies that examined the relationship between exposure to "pesticides" or "herbicides" and adverse health outcomes without identifying specific chemicals. After carefully reading the full text of an identified article, if none of the COIs were specified as contributing to the exposures of the study population, the committee generally excluded such a study from further consideration and

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did not summarize it. An exception to this practice was made for studies that indicated exposure to herbicides but did not characterize exposure with sufficient specificity for their results to meet the committee's criteria for inclusion in the evidentiary database. For example, numerous case-control studies characterized exposure to pesticides or herbicides on the basis of job titles, farm residence, or longest-worked industry. Consistent with prior *VAO* reports, such studies were not given full evidentiary weight because their results are not regarded as fully relevant for the purpose of this review, but they are presented more briefly under the heading of "Other Identified Studies." However, given that the exposure was not adequately described for these studies, their results are not included in supplementary summary tables.

Articles using the generic term "herbicide" were kept if a published report specified that the COIs were among the pesticides or herbicides used by the study population or the COIs are used commonly for the crops identified in the study or the COIs are used commonly for a specific purpose, such as the removal of weeds and shrubs along highways. For instance, this rubric would apply to any published articles from the Agricultural Health Study because 2,4-D was one of the most frequently used pesticides in this large prospective cohort, but some results have lumped all herbicide exposure together. In general, if the COIs were not presented separately in the results of a study, the study was considered to be of limited relevance and acknowledged as an "other identified study." Exposures to dioxin-like chemicals, such as mono-ortho and non-ortho substituted PCBs, PCDFs, and dioxins other than 2,3,7,8-TCDD, were also considered relevant to the committee's charge and were included as long as the results were expressed in terms of total or individual congener toxic equivalents. The most weight was given to studies that used quantitative measures (such as TEQs) and specified exposure.

Studies with original data collection and analyses were preferred over studies that were re-analyses of a population (without the incorporation of additional information), pooled analyses or meta-analyses, reviews, and so on, and the former are the type of evidence that the committee preferentially considered when assessing the strength of association between herbicide exposure and a health outcome when drawing its conclusions. While studies of the latter type may be informative and may be discussed in conjunction with primary results or in synthesis sections on a given health outcome, they are not themselves part of the evidence dataset and therefore were not considered in the final count of new literature considered in this volume.

EVALUATION PROCESS

This section details the methods used by the committee for evaluating and synthesizing the relevant studies identified by the literature search and screening and for making its conclusions on the relationships between the COIs and health

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outcomes among Vietnam veterans. The quantitative and qualitative procedures underlying the committee's literature evaluation have been made as explicit as possible, but ultimately the conclusions about associations expressed in this report are based on the committee's collective judgment. The committee has endeavored to express its judgments as clearly and precisely as the data allow.

A total of 14,750 abstracts were retrieved from the two literature searches. Full text was then obtained for any articles that were considered potentially relevant based on their titles and abstracts and after applying the inclusion and exclusion criteria. Full-text articles were distributed among the committee members based on their areas of expertise, with at least two committee members reviewing each paper. As with all VAO committees, the committee began its assessment of the literature by assuming neither the presence nor the absence of an association between exposure and any particular health outcome. Because of the variability in the descriptions and diagnoses of the health conditions considered in this report, the committee made no a priori assumptions about the usefulness of any article or report for a health outcome. Each study was reviewed and objectively evaluated for each health outcome it presented. If a study examined more than one health outcome, it was considered separately for each of those outcomes. After reading, if the full text revealed that the study met one of the exclusion criteria (see Box 3-2), it was excluded from further consideration.

After review of the full text of the identified articles, studies that were considered relevant (165 epidemiologic studies and nearly 100 toxicologic studies) were discussed and evaluated thoroughly, and are included in this report. New evidence on each health outcome was reviewed in detail, but the committee's conclusions are based on the accumulated evidence of all VAO reports, not just on recently published studies.

The responsible committee members then presented the information from each new relevant study—including the methods used for selecting the study populations and conducting the research (i.e., design, measures of exposure and health outcomes, statistical analyses used, adjustment factors, etc.), the results, and an assessment of the strengths, limitations, and potential biases—to the full committee for discussion. Based on the details of exposure and the description of how exposure was measured, an epidemiologic study was classified either as a primary article, in which case it was given full evidentiary weight, or as a secondary article, in which case it was reviewed and more briefly described under the heading of "Other Identified Studies." In a secondary study, the exposure was either not adequately described or considered not to be specific enough for its findings to contribute evidentiary weight. An epidemiologic study was also classified as secondary if the outcome was a biologic marker of effect as opposed to a recognized condition or disease. Studies that measured well-defined functional outcomes indicative of mild impairments and likely of worsening function that have not yet progressed to frank clinical diagnoses, such as cognitive function and loss of grip strength, as evaluated in Chapter 9: Neurologic Disorders, were

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considered primary evidence. Mechanistic and toxicologic studies contributed to the evidence for biologic plausibility but were not considered primary studies, so that based on those studies alone, their weight would not be enough to change the level of evidence of an association.

Following the discussion of each individual study for a specific health outcome, the lead committee member for an outcome then reviewed and summarized for the full committee the epidemiologic evidence reported in prior VAO updates and the most recent conclusion from *Update 2014* on that health outcome. The toxicologists on the committee provided a summary of previous and new mechanistic or toxicologic studies for that health outcome. Although both primary and secondary studies contributed to the committee's conclusion regarding the evidence of the COIs to be associated with a particular health condition or outcome, primary studies were given more weight. Using all of the available information, the full committee then came to a consensus regarding the conclusion and assigned a category of association (discussed later in this chapter) on the strength of the evidence of exposure to at least one of the COIs and the health outcome under scrutiny. When drafting language for a conclusion, the committee considered the nature of the exposures, the nature of the specific health outcome, the populations exposed, and the quality of the evidence examined.

The draft text was reviewed and discussed in further plenary sessions until all committee members reached a consensus on the description of the studies and the conclusion for each health outcome. The committee did not use a formulaic approach to determining the number of primary or supporting studies that would be necessary to assign a specific category of association. Rather the committee's review required a thoughtful and nuanced consideration of all the studies as well as expert judgment, and this could not be accomplished by adherence to a narrowly prescribed formula of what data would be required for each category of association or for a particular health outcome.

The supplementary tables to this report (available online at www.nap.edu/catalog/25137) were revised to include only the new primary epidemiologic studies; secondary studies, including mechanistic and toxicologic studies, were not included in the compendium evidence tables for each outcome. If no new primary studies for a health outcome were identified, the evidence table from *Update 2014* was included. Effect estimates, data, and units of measure are presented as reported in the cited studies, except where otherwise noted. The committee did not collect original data, nor did it perform any secondary data analyses, such as meta-analyses.

Types of Studies Considered

The committee focused on epidemiologic studies because epidemiology deals with the determinants, frequency, and distribution of disease in human populations rather than in individuals and animal models, which have several

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limitations as discussed below. Epidemiologic studies effectively integrate any results of exposure to a target substance in combination with other substances that may be etiologically relevant. Several types of epidemiologic studies were evaluated, including cohort, case-control, and cross-sectional designs. Other reports of health outcomes among veteran populations have described these major types of epidemiologic studies and chronicled the limitations inherent in conducting them and in using them to make conclusions based on associations between deployment-related factors and health conditions (IOM, 1994; NASEM, 2016a).

The committee weighed the importance of the epidemiologic studies in the following order: Vietnam veterans, occupationally exposed workers, and people who were exposed environmentally. Exposures gleaned from case-control designs were considered separately. Vietnam veterans (U.S. veterans and those from allied countries) are presumed to have been exposed to all the COIs; the limitations of such an assumption are discussed in Chapter 2. Although Vietnam veterans constitute the source population of interest, the committee has taken into account the potential for more precise quantification and evaluation of the risks of adverse health outcomes associated with the COIs in better characterized cohorts (for example, occupational and environmental cohorts). Including these more highly exposed populations had the additional advantage that epidemiologic studies of them were likely to have greater statistical power to detect any adverse effects that might occur with exposure. Within the occupational-study populations, due to the substantial differences in the nature and intensity of their exposures, workers in the production of herbicides and other industrial products contaminated with TCDD were prioritized, followed by those involved in occupational use of the herbicides of interest (such as agricultural workers). The committee for *VAO* and the first several updates gave more weight to results that were based on job title (for example, "farmer" with no additional information) than have the committees since *Update 2006* (where such studies are now considered secondary and given less weight). Those first committees also entirely excluded findings from the Yusho and Yucheng PCDF and PCB poisonings, whereas recent committees have considered these and other environmental studies that analyzed for dioxin-like PCDF and PCB congeners and expressed the results in terms of TEQs. Toxicologic studies, particularly in animal models, are included to inform the understanding of biologic plausibility through the toxicology of the chemicals and their exposure pathways.

Vietnam Veterans

Because Vietnam veterans are the target population of the charge to the VAO committees, studies of these veterans (whether American or otherwise) have always been accorded considerable weight in the committees' deliberations and are presented first in the literature for each health outcome. The committee reviewed all identified studies of U.S. and international Vietnam veterans

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published since *Update 2014*. In general, few studies include objective measures of TCDD or other chemical concentrations; those that are available were performed in small subsets of Vietnam veterans, such as those from the Air Force Health Study and Army Chemical Corps. Instead, having served in Vietnam or participating in the Agent Orange Registry is often considered a proxy of herbicide exposure. Therefore, it is difficult to quantify the risk of specific health outcomes when the exposures of the total at-risk population have not been measured or estimated. In the absence of actual measures of exposure, comparisons between deployed and non-deployed Vietnam-era veterans are considered the next most relevant comparison. Moreover, in many studies of Vietnam veterans, not all health outcomes of interest were reported (in some cases there were too few cases to report, only specific health outcomes were of interest, or the veteran population was too young for a particular manifestation). Consequently, several other groups known or thought to have potentially higher and better-characterized exposure to TCDD or phenoxy herbicides than the Vietnam veterans themselves were considered.

Human Studies Among Non-Veterans

Whereas exposure estimation and characterization of the COIs has been lacking in many (especially early) studies of Vietnam veterans, studies of occupational exposure (for example, among chemical-production, paper and pulp, railroad, agricultural, and forestry workers) and of environmental exposure (for example, residents of dioxin-contaminated sites such as Seveso, Italy, and the areas around some former U.S. military installations in Vietnam) to TCDD and the other COIs provide evidence of exposure and health outcomes to supplement the studies of Vietnam veterans. Some occupational and environmental cohorts that received exceptionally high exposures have produced many informative results and provide stronger evidence about health outcomes than studies of Vietnam veterans because the exposures were better characterized and measured sooner relative to the exposure. Moreover, in several studies of chemical-production plant workers, the magnitude and duration of exposure to the chemicals were measured and generally greater than among Vietnam veterans, so the likelihood that any possible health consequences would be manifested was greater. Some of the populations used in these types of studies were large enough to examine dose–response relationships. Other populations, such as the Agricultural Health Study, a prospective cohort study of U.S. agricultural workers with specific information on the COIs, continue to contribute new evidence of association with health outcomes.

As the information on populations that had established exposure to the COIs has grown, VAO committees have become less dependent on data from studies that had non-specific exposure information (e.g., exposure to non-specified herbicides) and have been able to focus more on the findings of studies that had refined

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exposure specificity. For each health outcome, occupational and environmental studies are presented after the studies of Vietnam veterans.

Animal and Mechanistic Studies

The committee used studies of toxicology data to determine whether there is a plausible biologic mechanism or other evidence of a causal relationship between herbicide exposure and a health effect. A positive statistical association between an exposure and an outcome does not necessarily mean that the exposure is the cause of that outcome. Data from toxicology studies may support or conflict with a hypothesis that a specific chemical can contribute to the occurrence of a particular disease. Insights about biologic processes inform whether an observed pattern of statistical association might be interpreted as the product of more than error, bias, confounding, or chance. Discussions on biologic plausibility are presented after new epidemiologic evidence and before the synthesis of all the evidence. The degree of biologic plausibility itself influences whether the committee perceives positive findings to be indicative of a pattern or the product of statistical fluctuations. Ultimately, the results of the toxicology studies should be consistent with what is known about the human disease process if they are to support a conclusion that the development of the disease was influenced by an exposure.

Aryl Hydrocarbon Receptor Many of the available toxicologic and mechanistic studies involve the AHR because it has been found that essentially all of the toxic effects of TCDD involve interaction with this protein (receptor). The AHR can modulate transcription by binding TCDD and other aromatic hydrocarbons with high affinity. The formation of an active complex that involves the intracellular receptor, the ligand (the TCDD molecule), and other proteins is followed by an interaction of the activated complex with specific sites on DNA, which can alter the expression of specific genes involved in the regulation of cellular processes. The affinity of TCDD for the AHR is species- and strain-specific, and responses to the binding of the receptor vary among cell types and developmental stages.

Although studying AHR biology in transformed human cell lines minimizes the inherent error associated with species extrapolations, it is still not clear to what extent toxicity is affected by the transformation itself or by the conditions under which cell lines are cultured in vitro. Genetically based differences in the properties of the AHR are known to exist in human populations (Zhou et al., 2010), as they are in laboratory animals, so there are genetically based differences in people's responses to TCDD, which leads to some people having an intrinsically greater risk of toxic effects from the same TCDD exposure, while others have less risk. Therefore, as humans have AHR with differing affinities for dioxin, a single transformed human cell line will not accurately reflect the spectrum of responses observed in the entire human population. As a result, the

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TEQ approach should be seen as qualitatively generalizable, but cannot be definitively quantitative when discussing differences between tissues or individual humans.

Limitations of Animal Models Animal models are the basis for many of the toxicologic and mechanistic studies, although cell lines and in vitro cell cultures (human or animal) are also used. Studies that use isolated cells in culture also can elucidate how a chemical alters cellular processes. The objectives of those toxicology studies are to determine what toxic effects are observed at different exposure levels and to identify the mechanisms by which the effects are produced.

To be considered an acceptable surrogate for the study of a human disease, an animal model must reproduce, with some degree of fidelity, the manifestations of the disease in humans. However, a given effect of an exposure in an animal species does not necessarily establish its occurrence in humans, nor does the apparent absence of a particular effect in animals mean that the effect could not occur in humans. In addition to possible species differences, many factors affect the ability to extrapolate results from animal studies to health effects in humans. For example, animals used in experimental studies are most often exposed to purified chemicals, not to mixtures. Even if herbicide formulations or mixtures are used, the conditions of exposure might not realistically reproduce the human exposures that occur in the field. Other variables, including the amount and duration of exposure, can be controlled precisely in laboratory settings.

TCDD is thought to be responsible for many of the toxic effects of the herbicides used in Vietnam. Attempts to establish correlations between the effects of TCDD on experimental systems and their effects on humans are particularly difficult because there are well-known species-, sex-, and outcome-specific differences in susceptibility to TCDD toxicity. Even in humans, the data on TCDD's toxic effects are not consistent (DeVito and Birnbaum, 1995; Ema et al., 1994; Moriguchi et al., 2003). Differences in vulnerability may also be affected by variations in metabolism and by the rate at which TCDD is eliminated from the body. Although the degree of susceptibility is generally thought to be an inherent biological response, it can be influenced by life stage, past history, and co-exposures.

Many factors may contribute to differences between the results of controlled animal studies and the effects observed in humans. The following, which are elaborated on in Chapter 4, are among the most important:

- **Physiologic differences.** Laboratory animals are not miniature humans. Depending on the biologic process under investigation, a particular test species may match the human system more closely and so be a better experimental model than others.

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- **Magnitude of exposure.** As is often the case for toxicologic studies of any chemical, the TCDD exposure used for animal studies has been many orders of magnitude higher than Vietnam veterans are likely to have received during military service, although the ultimate body burdens may not be as different.
- **Duration of exposure.** Although TCDD is a persistent organic pollutant, animal studies seldom examine the chronic low-level exposure that occurs over a period of years or even many months.
- **Timing of exposure.** It is well known that many organ systems are highly susceptible to xenobiotic exposure during critical stages of development, such as gestation; the response of some systems (such as the immune system) may also depend on the timing of the exposure to antigens relative to the timing of the exposure to xenobiotics such as TCDD.
- **The route of exposure.** The route of exposure by which an exogenous agent enters an organism may influence the nature of any toxic response elicited. The outcomes of animal studies may be perturbed by the delivery of treatment doses by "unnatural" routes of exposure, such as a bolus by gavage or intraperitoneal injection, but the route of exposure does not seem to be a major reason that the results of epidemiology studies may not agree with the findings of controlled studies for the COIs considered in the VAO series.
- **Genetic constitution and expression.** The etiologies of most diseases in humans and in animals are likely to be influenced by numerous genes and to involve complex gene–environment interactions, and preliminary evidence suggests that TCDD can induce epigenetic modifications of an organism's DNA and alter expression of its genes.
- **Sex differences.** There are well-known differences between male and female animals (including humans) in susceptibility to xenobiotic exposures, some of which are modified by sex steroids.
- **Prior and recurring exposures to multiple sources.** Humans are exposed to xenobiotics from multiple sources throughout their lifetimes.
- **Complex mixtures.** Most xenobiotic exposures occur in complex mixtures; the makeup of these mixtures can heavily influence the ultimate toxic effects. In addition to the dietary modulation of responses to other exposures of both humans and animals, including dietary supplements in humans, prescription and over-the-counter pharmaceuticals, and other factors (such as cigarette smoking and ambient pollution) may have effects.
- **Stress.** Stress—of known or unknown origin—is a well-known modifier of human disease responses (such as immune responses); stress is an ever-present variable that is difficult to assess or control for in epidemiologic studies because there is substantial individual variation in response to it.

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Selection of Health Outcomes

The committee was charged with summarizing the strength of the scientific evidence concerning associations between exposure to various herbicides and contaminants during service in the Vietnam War and individual diseases or other health outcomes. The list of outcomes was developed on the basis of diseases and conditions identified from searches of the scientific literature, as first done by the original VAO committee and amended and expanded as needed by VAO Update committees. For example, the current committee included chronic skin conditions, which had not specifically been addressed by prior committees. Some health outcomes in the series have been added in response to requests from the Department of Veterans Affairs (VA) and various Veterans Service Organizations and in response to concerns of Vietnam veterans and their families. Comments received at public hearings and in written submissions from veterans and other interested persons have been valuable in identifying issues to be pursued to greater depth in the scientific literature. The current committee defined a health outcome as any recognized diagnosis (based on ICD-9 or ICD-10[2]). Studies of exposure to the COIs that examined changes in pathophysiology, cell signaling, or other biologic markers of effect, such as hormone levels and blood counts, are more briefly mentioned as "Other Identified Studies" in applicable chapters, but because of the uncertainty of their relevance on the actual health outcome of interest, they are not considered further.

In aggregate, the health outcomes that the committee has focused on include cancers of all types, cardiovascular and metabolic outcomes such as diabetes, immune system disorders, and neurologic disorders. Other chronic health outcomes have also been considered, including respiratory disorders, gastrointestinal disorders, endocrine disorders, and bone conditions. Generational effects were specifically included in the committee's charge and are addressed along with reproductive outcomes in a combined chapter. Although for most health outcomes the primary focus of the evaluation was on adverse outcomes in the veterans themselves, to examine potential effects, the children of Vietnam veterans and also later generations were included in the evaluation of the literature.

After reviewing the updated literature, the committee agreed that some reorganization of the health conditions was warranted for this volume. For example, it was more appropriate to group monoclonal gammopathy of undetermined significance, multiple myeloma, and amyloid lightchain amyloidosis under the heading "Plasma Cell Dyscrasias." Whereas reproductive outcomes and effects on descendants were presented in a single chapter from *VAO* through *Update 2010*, the committees for Update 2012 and Update 2014 divided these outcomes into two chapters. The current committee believed that it made more sense to

[2]ICD refers to the *International Classification of Diseases*, which is maintained by the World Health Organization. Its current version, ICD-10, is the 10th revision.

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present the reproductive outcomes and effects on descendants as a continuum in a single chapter.

Because any effect of the herbicides or contaminants in individuals or groups of veterans is evaluated in terms of disease or medical outcome, the committee paid particular attention to disease diagnosis and classification as it assembled pertinent data from various investigations related to a particular outcome in preparation for integrating the information. Pathologists, clinicians, and epidemiologists use several classification systems, including the *International Classification of Diseases* (ICD), which has been updated over time and with regard to topic. ICD codes are a hierarchic system for indicating the type of disease and site. For example, whereas many medical insurance and research investigators preferentially use the ninth revision of ICD (ICD-9) or its clinical modification (ICD-9-CM) to classify diagnoses or clinical outcomes, the tenth revision (ICD-10) is used to classify mortality information. Although outcome misclassification is still a possibility when recording a diagnosis with a specific ICD code into a record, the ICD system has been refined over many decades and is virtually universally used and understood, in addition to being exhaustive and explicit. Therefore, this and previous VAO committees have opted to use the ICD system as an organizing tool.

Many of the epidemiologic studies reviewed by VAO committees have not used the ICD approach to classification of disease and have relied instead on clinical impression alone. Self-reported diagnoses obtained from survey questionnaires often have inaccuracies due to recall bias and misinterpretations of the questions being asked. For example, a patient may report having been treated for stomach cancer when the correct diagnosis was gastric adenocarcinoma, gastric lymphoma, or peritoneal cancer. Furthermore, many epidemiologic studies report disease outcome by organ system. Sometimes this is done because there are few cases of a specific outcome and the study is lacking the necessary statistical power to make valid statistical associations using such specific diagnoses. However, such grouping into broader outcome categories can be problematic (and the same is true when categorizing potential exposure). For instance, the term "digestive system" may be used for conditions that are benign or malignant and that affect the esophagus, stomach, liver, pancreas, small intestine, large intestine, or rectum. Therefore, if a report indicated that a cohort has an increased incidence of digestive system cancers, then it would be unclear whether the association was attributable to excess cases of any single organ or type or to some combination thereof. Additionally, such generalization is complicated by the fact that the cause of cancer may differ among anatomic sites. For instance, there are strong associations between smoking and squamous cell carcinoma of the esophagus and between chronic hepatitis B infection and hepatic cancer. Furthermore, a single site may experience a carcinogenic response to multiple agents, while the same agent may cause cancer at multiple sites.

Several of the identified studies examined the association of exposure to at least one of the COIs (or a mixture containing it) with an individual health

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outcome. However, some of the primary studies (for example, Collins et al., 2016; Cox et al., 2015) reported multiple health outcomes, and individuals might have been counted in more than one category. This can also be an issue in mortality studies when more than the primary cause of death is used. Designing studies to analyze concurrent health outcomes is much more difficult, and valid methods that can be applied with confidence to identify patterns among multiple health outcomes associated with a single exposure have not yet been developed. If such methods were developed, studies of multiple health outcomes could potentially provide more insight into whether the COIs cause multiple health effects, into competing risks among various health outcomes, and into the interactive effects of health outcomes.

Defining Statistical Association

Box 3-3 provides brief definitions of some of the most common terms used in the epidemiologic studies considered by this committee. The strength of an association between exposure and a condition is generally estimated quantitatively by using relative risks, odds ratios, correlation coefficients, or hazard ratios, depending on the epidemiologic design used. A ratio greater than 1.0 indicates that the outcome variable has occurred more frequently in the exposed group, and a ratio less than 1.0 indicates that it has occurred less frequently. Ratios are typically reported with a confidence interval (CI) to quantify random error. Statistical significance may be represented by a CI or a p-value. If the 95% CI for a risk estimate (such as a risk ratio or odds ratio) includes 1.0, the association is not considered to be statistically significant; however, if the interval does not include 1.0, the association is said to be statistically significant with a type I alpha error (likelihood that the association is due to chance) of less than 5% (that is, $p < 0.05$).

Determining whether an estimated association between an exposure and an outcome represents a real relationship requires careful scrutiny because there can be more than one explanation for an estimate. Bias is a general term for a distortion of the measure of association. There are several types of biases, and each type may affect the estimate differently. For example, misclassification bias may result in exaggerated or underestimated estimates, whereas self-selection bias affects the representativeness of the study population and can limit the applicability of the results to the larger population of interest. Another type of bias that may potentially affect studies of Vietnam veterans is detection bias, in which veterans who are encouraged to and who choose to participate in screening programs or registries, such as the Agent Orange Registry (discussed in Chapter 5) may have additional tests or follow-up exams that could potentially detect disease or a condition earlier or because more thorough assessments were conducted. This type of bias may occur disproportionately for Vietnam veterans with known or who report herbicide-exposure compared with Vietnam-era veterans who were not

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BOX 3-3
Statistical Terms Used in This Report

Confidence interval (CI) is a range of values for the estimate within which the true value is thought to lie, with a (usually specified) 95% level of confidence.

Hazard ratio (HR) is a ratio of the chance of an event occurring in one group compared with the chance in another group.

Incidence is the number of new cases of illness during a given period of time in a specified population divided by the total population.

Odds ratio (OR) represents the odds that an outcome will occur, given a particular exposure, compared with the odds of the outcome occurring in the absence of exposure. The measure approximates the relative risk when a disease is rare.

Prevalence is the number of existing cases of an illness or disease in a given population at a specific time or within a specified time period.

Relative risk (RR) denotes the risk of a disease or outcome in a population with a given exposure compared to a population without the exposure. Relative risk may be described by a risk ratio, rate ratio, or odds ratio.

Standardized hospitalization ratio (SHR) is the ratio of hospitalizations among a population for a specific condition by person-years of follow-up divided by the national age- and sex-specific hospitalization rates for a health outcome using the annual hospitalization numbers and annual mean resident population estimates.

Standardized incidence ratio (SIR) is the ratio of observed new cases of an outcome in the exposed cohort to the expected number of new cases in the general population. The term "standardized" refers to adjustments made for age and sex differences between the study population and the general population.

Standardized mortality ratio (SMR) is the ratio of the number of deaths observed in a study population to the number of deaths expected if the study population had the same mortality rate as the general population. The term "standardized" refers to adjustments made for age and sex differences between the study population and the general population.

known to be exposed to herbicides or who were deployed elsewhere. Detection bias may lead to an overestimate or underestimate of the true effect size.

Confounding is a common type of bias in epidemiologic studies that occurs when a risk factor for the disease is also related to the exposure and creates a spurious exposure–disease association. Potentially, if a confounder is known, there are methods that can be used to adjust for its effects; however, not all confounders are always known or identified, and unknown confounders may affect the estimate of association. Effect modifiers differ from confounders in that the

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former are associated with the outcome but not the exposure. Effect modification occurs when an exposure has a different effect among different subgroups or strata. Chance is the degree to which an estimated association might vary randomly among different samples of the population studied.

Integrating New Information into the Evidence Base

Because relatively few studies of exposure to herbicides or TCDD (especially with objective measures) and health effects have been conducted among cohorts of Vietnam veterans, much of the evidence that VAO committees have considered has been from studies of well-documented populations that received occupational or environmental exposures to TCDD or specific herbicides. In many other studies, TCDD exposure was combined with exposures to an array of "dioxin-like" chemicals, and the herbicides were often analyzed as members of a functional class. Such studies are less informative for the committee's purposes than individual results on a specific chemical but are still considered as part of the evidence base. In the case of epidemiologic studies, exposure to multiple, possibly toxic, chemicals is common in some industries, such as agriculture, and those exposures cannot be controlled for in the same way that laboratory experiments can. In its examination of these epidemiologic studies, the committee looked for evidence of health effects that are associated with the specific compounds in the herbicides used in Vietnam and sought consideration of and adjustment for other possibly confounding exposures. When all the available epidemiologic evidence has been evaluated, Vietnam veterans are presumed to be at increased risk for a specific health outcome if there is evidence of a positive association between one or more of the COIs and the outcome.

The quality of exposure information in the scientific literature reviewed by this and previous VAO committees varies widely. Some studies relied on interviews or questionnaires to determine the extent and frequency of exposure. Such self-reported information, which has the potential for recall bias, generally carries less weight than do more objective measures of exposure, such as levels of a contaminant as measured in serum or other biospecimens. The strength of questionnaire-based information as evidence of exposure is enhanced to the extent that the information can be corroborated or validated by other sources. Similarly, greater weight is given for studies that use more objective measures of health outcome assessments (such as clinical diagnosis).

In drawing conclusions, the committee examined the most thoroughly adjusted quantitative estimates of association, judged whether an adjustment for any crucial confounders was lacking, and evaluated the potential influences of bias and chance. In integrating the findings of various studies, the committee considered the degree of statistical significance associated with every estimated risk (a reflection of the magnitude of the observed effect and the power of the study designs) and took note of whether dose–response relationships were evident with increasing

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exposure rather than simply tallying the "significant" and "non-significant" outcomes as dichotomous items of evidence. The committee also considered whether controlled laboratory investigations provide information consistent with the COIs being associated with a given effect and perhaps causally linked to it.

Categories of Association

As was done in previous volumes, the current committee used four categories of association to rate health outcomes based on the strength of the scientific evidence. The criteria for each category express a degree of confidence based on the extent to which bias and other sources of error could be reduced. The coherence of the full body of epidemiologic information, including biologic plausibility, is considered when the committee reaches a judgment about association for a given outcome. As was the case with the past three update committees, this committee did not use the Bradford Hill criteria for causality (Hill, 1965) as a checklist for its strength-of-association assessments. The committee discussed the evidence and reached consensus on the categorization of the evidence for each health outcome, which appears in the Conclusion section for each health outcome. Those categories of association have gained wide acceptance by Congress, VA, researchers, and veterans groups.

Sufficient Evidence of an Association

For effects in this category, a positive association between herbicides and the outcome must be observed in studies in which chance, bias, and confounding can be ruled out with reasonable confidence. For example, the committee might regard evidence from several small studies that are free of bias and confounding and that show an association that is consistent in magnitude and direction to be sufficient evidence of an association. Experimental data supporting biologic plausibility strengthen the evidence of an association but are not a prerequisite and are not enough to establish an association without corresponding epidemiologic findings.

Limited or Suggestive Evidence of an Association

In the category of "limited or suggestive evidence of an association," the evidence must suggest an association between exposure to herbicide compounds or COIs and the outcome in studies of humans, but the evidence can be limited by an inability to confidently rule out chance, bias, or confounding. Typically, at least one high-quality study indicates a positive association, but the results of other studies could be inconsistent. Because the VAO series has a number of agents of concern whose toxicity profiles are not expected to be uniform—specifically, four herbicides and TCDD—apparent inconsistencies can be expected among

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study populations that have experienced different exposures. Even for a single exposure, a spectrum of results would be expected, depending on the power of the studies, inherent biological relationships, and other study design factors.

Inadequate or Insufficient Evidence to Determine an Association

By default, any health outcome is placed in the category of "inadequate or insufficient evidence to determine an association" before enough reliable scientific data have accumulated to promote it to the category of sufficient evidence or limited or suggestive evidence of an association or to move it to the category of limited or suggestive evidence of no association. In this category, the available human studies may have inconsistent findings or be of insufficient quality, validity, consistency, or statistical power to support a conclusion regarding the presence of an association. Such studies might have failed to control for confounding factors or might have had inadequate assessment of exposure. Several health effects have been moved into or out of this category since the original VAO committee reviewed the evidence then available.

For nonmalignant conditions, the diversity of disease processes involved makes the use of broad ICD ranges less useful, but because VAO committees could not possibly address every rare nonmalignant disease, they do not draw explicit conclusions about diseases that are not discussed, and thus, this category is the default or starting point for any health outcome. If a condition or outcome is not addressed specifically, then it will be in this category.

Limited or Suggestive Evidence of No Association

The original VAO committee defined the category "limited or suggestive evidence of no association" for health outcomes for which several adequate studies covering the "full range of human exposure" were consistent in showing *no* association with exposure to herbicides at any concentration and had relatively narrow confidence intervals. A conclusion of "no association" is inevitably limited to the conditions, exposures, and observation periods covered by the available studies, and the possibility of a small increase in risk related to the magnitude of exposure studied can never be excluded. However, a change in classification from inadequate or insufficient evidence of an association to limited or suggestive evidence of *no* association would require new studies that correct for the methodologic problems of previous studies and that have samples large enough to limit the possible study results attributable to chance.

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4

Biologic Mechanisms

The committee reviewed all the relevant experimental studies of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), dimethylarsinic acid (DMA, also called cacodylic acid), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) that have been published since *Update 2014* (NASEM, 2016a). The findings have been incorporated into this chapter when it is appropriate and into the biologic-plausibility sections of Chapters 6–11 when they are of consequence for particular health outcomes. For each substance, this chapter includes a review of its toxicokinetic properties, a brief summary of the toxic outcomes investigated in animal experiments, and a discussion of underlying mechanisms of action as illuminated by in vitro studies. The final section of this chapter discusses factors that complicate the extrapolation of findings from laboratory experimentation to humans. Additionally, information about three emerging subjects in molecular and biologic science—epigenetics, developmental immunotoxicology, and oxidative stress—are discussed because they provide insights into the potential mechanisms that could explain biologic responses associated with exposure to the herbicides sprayed in Vietnam.

The establishment of biologic plausibility through laboratory studies strengthens the case for a cause–effect relationship between herbicide exposure and health effects that has been reported in epidemiologic studies. Such information supports the existence of the less stringent relationship of association, which is the target of this committee's work. Experimental studies of laboratory animals or cultured cells make it possible to observe the effects of herbicide exposure under controlled conditions, which is difficult or impossible to do in epidemiologic studies. The conditions that can be controlled in a laboratory animal study

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include genetic differences, the frequency and magnitude of exposure, exposure to other chemicals, dietary intake, preexisting and confounding health conditions, stressors, and the tissues or cells under examination. The limitations of extrapolating results of laboratory studies to human responses is discussed later in this chapter.

Once a chemical contacts the body, it becomes subject to the processes of absorption, distribution, metabolism, and excretion. The combination of those four biologic processes determines the concentration of the chemical in the various tissues and organs in the body and how long each organ or tissue is exposed to the chemical and thus influences its pharmacologic and possibly toxic activity (Lehman-McKeeman, 2013).

The absorption of a substance in an organism can occur through different routes. If ingested, it normally is taken up into the bloodstream from mucous surfaces, such as the intestinal walls of the digestive tract. If inhaled, the substance enters the bloodstream through the alveoli in the lungs. Some compounds can also be absorbed across the skin (dermal adsorption). Animal studies may involve additional routes of exposure that are not ordinarily encountered by humans, such as intravenous or intraperitoneal injection, when a chemical is injected into, respectively, the bloodstream or the abdominal cavity. The route of exposure and other factors influence how much of a chemical dose is absorbed by the organism. For example, the hydrophobicity of a chemical and its solubility in fat influence how much of that chemical is absorbed. Thus, absorption is a critical determinant of a chemical's bioavailability—that is, the fraction of the chemical that reaches systemic circulation.

Once a chemical is absorbed, it is affected by distribution. This refers to the movement of a substance from the site of entry to the different tissues and organs in the organism. Distribution takes place most commonly via the bloodstream. As the chemical is moved through the body, it may enter a target tissue where it may have its ultimate toxic effect, or it may enter into tissues that sequester it. As a chemical is distributed in the organism, it will also begin to undergo metabolism.

Biotransformation or metabolism is the process by which a foreign substance is chemically modified when it enters an organism. For many environmental toxicants, this process takes place largely in the liver via the action of enzymes, including cytochromes P450, which catalyze the oxidative metabolism of many chemicals. As metabolism occurs, the parent chemical is converted into new chemicals called metabolites, which are often more water-soluble (polar) and thus more readily excreted. When the resulting metabolites are pharmacologically or toxicologically inert, metabolism has deactivated the administered dose of the parent chemical and thus reduced its effects on the body. Metabolism may, however, generate a chemical that is more potent or more toxic than the parent compound. This process is often referred to as bioactivation. A chemical's structure influences the organism's ability to metabolize (structurally transform) the

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chemical, and this ability ultimately determines whether it persists in the body or is excreted.

Excretion is the removal of substances or their metabolites from the body, most commonly in urine or feces. This is different from elimination, which refers to the disappearance of the parent molecule from the bloodstream. The rate of excretion of a chemical from the body is often limited by the rate of metabolism of the parent chemical into more water-soluble, readily excreted metabolites. Excretion is often incomplete, especially in the case of chemicals that resist biotransformation. Incomplete excretion results in the accumulation of foreign substances that can adversely affect biologic functions.

Elimination is referred to as "first-order" when its rate is directly proportional to the amount of chemical in the body, in which case the chemical's half-life is independent of dose. A half-life is defined as the time required for the plasma concentration or the amount of a chemical in the body to be reduced by half. The half-life of TCDD in humans is estimated to vary from 0.4 to more than 10 years, based on measurements of TCDD in serum samples; the half-life depends on a number of factors, including a person's body mass index (BMI), age, sex, and exposure (Aylward et al., 2005a; Emond et al., 2005; Flesch-Janys et al., 1996; Geusau et al., 2002; Kumagai and Koda, 2005; Leung et al., 2006; Michalek et al., 2002; Milbrath et al., 2009; Needham et al., 1994; Pirkle et al., 1989; Sorg et al., 2009). Shorter half-lives were observed in humans during the first months after exposure or in severely contaminated persons, which is consistent with the nonlinear elimination predicted by physiologically based pharmacokinetic models. Several studies of the half-life of TCDD have been conducted in monkeys, mice, and rats, but the estimates are generally shorter than those found in humans, ranging from 8 to 74 days, and are generally proportional to body weight (DeVito and Birnbaum, 1995; Emond et al., 2006; Gasiewicz et al., 1983; Hurst et al., 1998; Koshakji et al., 1984; Minero et al., 2001; Neubert et al., 1990; Viluksela et al., 1996).

Collectively, the routes and rates of absorption, distribution, biotransformation or metabolism, and excretion of a toxic substance make up the toxicokinetics (or the pharmacokinetics for chemicals used as pharmaceutical agents) of the substance. Those processes determine the amount of a particular substance or metabolite that will reach specific organs or cells and the amount of a particular substance that persists in the body. Understanding the toxicokinetics of a chemical is useful for assembling a valid reconstruction of a human exposure. It is also important in assessing the concentration of the active chemical in target tissues, which influences the risk of disease. The basic principles involved in toxicokinetics are similar from chemical to chemical, but the precise way in which principles are applied will depend on the structure and other inherent properties of the particular chemical under consideration. For example, the lipophilicity or hydrophobicity of a chemical influences how long that chemical will persist in the body because those factors influence how much of the chemical is absorbed into

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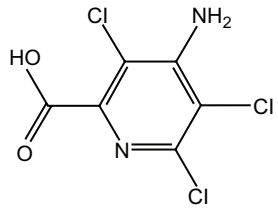
fat cells, metabolized, and excreted. The degree to which different toxicokinetic processes influence the toxic potential of a chemical depends on the metabolic pathways, which often differ among species. Animal and cell culture studies are often conducted at higher exposures and for shorter durations than are typical in human exposures, which can influence biotransformation. For that reason, attempts to extrapolate from experimental animal studies to human exposures must be done extremely carefully.

Many chemicals were used by the U.S. armed forces in Vietnam. The nature of the substances themselves was discussed in detail in Chapter 4 of the original *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam* (*VAO*) report (IOM, 1994). Four herbicides documented in military records were of particular concern in that report and are examined here: 2,4-D; 2,4,5-T; picloram; and cacodylic acid. This chapter also examines TCDD, the most toxic congener of the tetrachlorodibenzo-*p*-dioxins, also commonly referred to as dioxin, which is a contaminant of 2,4,5-T. Considerably more information is available on TCDD than on the herbicides themselves. Other contaminants present in 2,4-D and 2,4,5-T are of less concern. Except as noted, the laboratory studies of the chemicals of concern used pure compounds or formulations; the epidemiologic studies discussed in later chapters often tracked exposures to mixtures.

PICLORAM

Chemistry

Picloram (Chemical Abstracts Service Number [CAS No.] 1918-02-1; see chemical structure in Figure 4-1) was used with 2,4-D in the herbicide formulation Agent White, which was sprayed in Vietnam. It is also used commonly in Australia in a formulation that has the trade name Tordon 75D®. Tordon 75D contains several chemicals, including 2,4-D; picloram; a surfactant, diethyleneglycolmonoethyl ether; and a silicone defoamer. A number of studies of picloram used such mixtures as Tordon formulations or other mixtures of 2,4-D and picloram that are similar to Agent White.



4-amino-3,5,6-trichloropicolinic acid

FIGURE 4-1 Chemical structure of picloram.

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Toxicokinetics

The original VAO committee reviewed studies of the toxicokinetics of picloram (IOM, 1994). Studies of animals showed a rapid absorption through the gastrointestinal tract and a rapid elimination of picloram in unaltered form in urine. In humans, Nolan et al. (1984) examined the toxicokinetics of picloram in six healthy male volunteers who were given a single oral dose of either 0.5 or 5.0 mg/kg or a dermal dose of 2.0 mg/kg. In the oral study picloram was rapidly absorbed and rapidly excreted unchanged in urine. More than 75% of the dose was excreted within 6 hours, and the remainder with an average half-life of 27 hours. On the basis of the quantity of picloram excreted in urine in the dermal study, the authors concluded that only 0.2% of the picloram applied to the skin was absorbed. Because of its rapid excretion, picloram has low potential to accumulate in humans.

In general, the literature on picloram toxicity continues to be sparse. Studies of humans and animals indicate that picloram is rapidly eliminated as the parent chemical. Studies of animals indicate that picloram has low toxicity even at high doses (IARC, 1991).

Toxicity Profile

The original VAO committee reviewed studies of the carcinogenicity, genotoxicity, acute toxicity, chronic systemic toxicity, reproductive and developmental toxicity, and immunotoxicity of picloram (IOM, 1994). In general, there is some evidence of benign liver carcinogenicity in female Osborne-Mendel rats but picloram was not found to be carcinogenic for either male or female Osborne-Mendel rats or B6C3F1 mice (NCI, 1978). Because of some concern that contaminants in picloram (in particular, hexachlorobenzene) might be responsible for the carcinogenicity, picloram itself has not been established as a chemical carcinogen. Furthermore, studies conducted by the Environmental Protection Agency (EPA, 1988) yielded no evidence that picloram is a genotoxic agent.

Acute Toxicity

Picloram is considered a mild irritant. At high doses, it has produced erythema in rabbits. Some neurologic effects—including hyperactivity, ataxia, and tremors—were reported in pregnant rats exposed to picloram at 750 or 1,000 mg/kg (Thompson et al., 1972). However, the available information on the acute toxicity of picloram is very limited.

Chronic Systemic Toxicity

There is some evidence from chronic experimental animal studies that ingesting high doses of picloram affects the liver. Several studies have reported various effects of technical-grade picloram on the livers of rats. In the carcinogenicity

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bioassay conducted by Stott et al. (1990), treatment-related hepatomegaly, hepatocellular swelling, and altered tinctorial properties were noted in the central regions of the liver lobules in groups of rats exposed at 60 and 200 mg/kg per day. Gorzinski et al. (1987) reported a dose-related increase in hepatocellular hypertrophy and changes in centrilobular tinctorial properties in male and female F344 rats exposed for 13 weeks to picloram at 150 mg/kg per day and higher in the diet. In a 90-day study, cloudy swelling in the liver cells and bile duct epithelium occurred in male and female F344 rats given 0.3% or 1.0% technical picloram in the diet (EPA, 1988). No other toxic effects of chronic exposure to picloram have been reported.

Reproductive and Developmental Toxicity

The reproductive toxicity of picloram was evaluated in a two-generation study. No toxicity was detected at the highest dose tested, which was 150 mg/kg per day; however, only a few animals were evaluated (EPA, 1988). Some developmental toxicity was produced in the offspring of pregnant rabbits exposed to picloram by gavage at 400 mg/kg per day on gestation days 6–18. Fetal abnormalities were forelimb flexure, fused ribs, hypoplastic tail, and omphalocele, each occurring in a single litter (John-Greene et al., 1985). Some maternal toxicity was observed at that dose, however, and EPA concluded on the basis of the sporadic nature of the findings that the malformations were not treatment related (EPA, 1988). No teratogenic effects were produced in the offspring of rats given picloram by gavage at up to 1,000 mg/kg per day on gestation days 6–15, but the occurrence of bilateral accessory ribs was significantly increased (Thompson et al., 1972).

Immunotoxicity

Studies of the potential immunotoxicity of picloram have included dermal sensitization in humans and rodent immunoassays. In one study, 53 volunteers received nine 24-hour applications of 0.5 mL of a 2% potassium picloram solution on the skin of both upper arms. Each volunteer received challenge doses 17–24 days later. The particular formulation of picloram (its potassium salt) was not a skin sensitizer or an irritant (EPA, 1988). In a similar study, a 5% solution of picloram (M-2439, Tordon 101 formulation) produced a slight dermal irritation and a sensitization response in 6 of the 69 volunteers exposed. When the individual components of M-2439—picloram, triisopropanolamine (TIPA) salt, and 2,4-D TIPA salt—were tested separately, no sensitization reaction occurred (EPA, 1988). Tordon K+, but not technical-grade picloram, was also found to be a skin sensitizer in guinea pigs (EPA, 1988). CD1 mice exposed to Tordon 202C (94% 2,4-D and 6% picloram) had no consistent adverse effects on antibody responses (Blakley, 1997), but the lack of a consistent response may be due to the fact that CD1 mice are outbred.

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Mechanisms

No well-characterized mechanisms of toxicity for picloram are known.

CACODYLIC ACID

Chemistry

Arsenic (As) is a naturally occurring element. It exists in inorganic forms that can be trivalent (As^{+3} or As^{III}) or pentavalent (As^{+5} or As^{V}). It can also exist in organic forms that are often methylated. See Figure 4-2 for the chemical structures of selected arsenic-containing compounds. Sodium arsenite, which contains As^{III}, is generally considered to be the most toxic of these arsenic compounds. Inorganic arsenic is commonly present in drinking-water sources that are associated with volcanic soils and can reach high concentrations (more than 50 parts per billion). Numerous human health effects have been attributed to drinking water contaminated with inorganic arsenic, particularly bladder, skin, kidney, and lung cancers; developmental effects on learning and memory; reproductive endpoints; skin lesions; and diabetes and vascular diseases (NRC, 2001; WHO, 2018). Inorganic arsenic is readily metabolized in humans and other species into organic forms of arsenic. Although organic forms of arsenic can be converted into inorganic forms by microorganisms in the soil, there is no evidence that this can occur in humans or other vertebrate species (Cohen et al., 2006).

The arsenic in cacodylic acid (CAS No. 75-60-5) is an organic form of arsenic and has a valence of +5. Cacodylic acid (also known by its more standard chemical name, dimethylarsinic acid [DMA^{V}]) was the form of arsenic used in Agent Blue, one of the mixtures used for defoliation in Vietnam. DMA^{V} made up about 30% of Agent Blue. Agent Blue was chemically and toxicologically unrelated to Agent Orange, which consisted of phenoxy herbicides contaminated with dioxin-like compounds. Potential cacodylic acid exposure of Vietnam veterans would have involved direct exposure to exogenous DMA^{V}, rather than exposure to inorganic arsenic, which would have led to the endogenous formation of MMA^{V} and MMA^{III} and then DMA^{V}. The old hypothesis that methylation of inorganic arsenic was a detoxifying mechanism has been dispelled by newer studies. The direct treatment of laboratory animals with these metabolic products has demonstrated the products to be linked to an increased incidence of cancers and non-cancer health outcomes. However, it is still the trivalent species (As^{III} and MMA^{III}) that appear to be the most toxic. No studies of health effects in humans following direct exposure to DMA have been identified by the VAO committees. If there were, such studies could provide epidemiologic evidence of an association between DMA and health effects. However, most studies have focused on exposure to inorganic arsenic, which human metabolism turns into DMA. Consequently, VAO committees have excluded studies where the source

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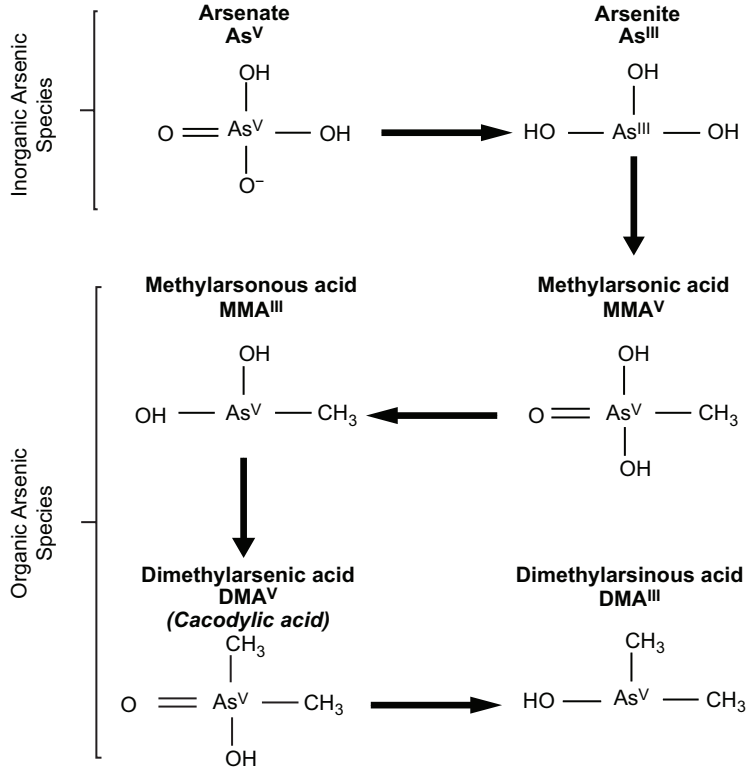

FIGURE 4-2 Chemical structures of selected arsenic-containing compounds.
NOTES: Inorganic arsenic (As^{V} and As^{III}) is commonly found in the environment and is metabolized by humans to form methylated organic arsenic species (MMA and DMA). The methylation process is incomplete in humans, and both inorganic and organic arsenic species are excreted in urine when approximately 10% is in inorganic forms (As^{V} and As^{III}), 10–20% is MMA, and 70–80% is DMA. Cacodylic acid, which is the type of arsenic found in Agent Blue, is the same as DMA^{V} and is excreted in urine as DMA.

of exposure was inorganic arsenic and have only considered and reviewed toxicologic studies in which the animals were directly exposed to DMA. Information about effects of inorganic arsenic exposure may be found in reviews by the International Agency for Research on Cancer (IARC, 2012a) and the National Research Council (NRC, 2013).

Toxicokinetics

In humans, metabolism studies have generally found DMA^{V} to be rapidly excreted and mostly unchanged in the urine after an exposure has occurred (Cohen

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et al., 2006; S. Suzuki et al., 2010). However, rats differ from most other mammals (including humans) in that a larger percentage of DMA^V binds to hemoglobin in their red blood cells, which leads to a considerably longer half-life in blood (Cui et al., 2004; K. T. Suzuki et al., 2004). The binding of DMA^V to hemoglobin is 10 times higher in rats than in humans (M. Lu et al., 2004). Chronic exposure of normal rat hepatocytes to DMA^V results in decreased uptake and increased excretion, so that over time the rats developed resistance to the cytotoxic effects of DMA^V (Kojima et al., 2006). This tolerance is mediated by the induction of glutathioneS-transferase activity and of multiple-drug-resistant protein expression. Recent work by Banerjee et al. (2014, 2016) found that multiple-drug-resistant protein 4 on renal proximal tubules transports DMA^V, which facilitates urinary excretion, and that polymorphic variants of this protein can alter the transport activity and change local cellular responses. Rats also appear to biotransform DMA^V to DMA^{III}. Adair et al. (2007) examined the tissue distribution of DMA in F344 rats after they were exposed to DMA for 14 days through drinking water. They found that DMA^V was extensively metabolized to trimethylated forms that may play a role in toxicity. In a study of DMA treatment of Wistar rats for 10 weeks, the metabolism to trimethylated forms was far less apparent, and the tissue distribution of DMA^V and trimethylated metabolites was strikingly different (S. Liu et al., 2015). Thus, DMA distribution and metabolism may differ according to exposure duration or the strain of rat, or both.

A physiologically based pharmacokinetic model of intravenous and ingested DMA^V has been developed on the basis of mouse data (Evans et al., 2008). Similar models have been developed for humans on the basis of exposure to inorganic arsenic (El-Masri and Kenyon, 2008), but these models have limited relevance for assessing potential harm to Vietnam veterans, who are presumed to have been directly exposed to DMA^V.

Although epidemiologic studies of direct exposure to DMA^V are not available, investigations into the relationship between health outcomes and the metabolic profiles of humans exposed to inorganic arsenic provide some insight into the roles of the individual metabolites in producing adverse outcomes. An increased incidence of urothelial cancer (cancer of the bladder, kidney, renal pelvis, ureter, and urethra) among people exposed to high levels of inorganic arsenic in drinking water was found in those who generate more MMA and less DMA endogenously (S. K. Huang et al., 2008). Also, a lower risk of arsenical skin lesions was associated with evidence of higher arsenic methylation capacity in people in areas of high arsenic exposure via the drinking water (Q. Zhang et al., 2014a,b) as well as in smelter workers (Wen et al., 2012). These results could suggest that elevated cumulative levels of urinary MMA may be causally associated with an increased risk of inorganic arsenic-induced adverse health outcomes, but they could also imply that complete methylation of inorganic arsenic to DMA and resulting enhanced excretion are relatively protective.

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Toxicity Profile

This section discusses the toxicity associated with organic forms of arsenic, most notably DMA^V because it is the active ingredient in Agent Blue. The toxicity of inorganic arsenic is not considered to be relevant to veteran exposures to Agent Blue.

Neurotoxicity

Kruger et al. (2006) found that both DMA^{III} and DMA^V significantly attenuated neuronal ion currents through N-methyl-D–aspartate receptor ion channels, whereas only DMA^V inhibited ion currents through α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. The data suggest that DMA may have neurotoxic potential.

Immunotoxicity

Previous studies have shown that a low concentration of DMA^V (10^{-7} M) could increase the proliferation of human peripheral blood monocytes after their stimulation with phytohemagglutinin, whereas it took a high concentration (10^{-4} M) to inhibit the release of interferon-γ. This suggested that different concentrations of DMA^V may produce various immunomodulatory effects (Di Giampaolo et al., 2004).

Skin Toxicity

In an evaluation of the effects of topical exposure of pregnant mice to DMA (valence not stated) on the skin of the dams and offspring (E. Kim et al., 2012), no effects were observed in offspring, but the exposure did increase skin thickness in the area of application and alter the expression of multiple apoptosis-related genes (*Bcl-2*, *Bad*, *Casp-12*). The results suggested that transient DMA exposure can be a skin irritant and produce dermatitis.

Genotoxicity and Carcinogenicity

DMA^{III} and DMA^V are genotoxic and increase oxidative stress and cause DNA damage, particularly aneuploidy, but they are poor mutagens (Rossman and Klein, 2011). Gómez et al. (2005) demonstrated that DMA^{III} induces a dose-related increase in DNA damage and oxidative stress in Jurkat cells. DMA^{III} was considerably more potent than DMA^V in inducing DNA damage in Chinese hamster ovary cells (Dopp et al., 2004), which was associated with a greater uptake of DMA^{III} into the cells. Similarly, an analysis of arsenical dimethylated metabolites in human bladder cancer cells found dimethylmonothioarsinic acid

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($DMMTA^V$) and DMA^{III} to be more toxic and DMA^V to be less toxic in terms of DNA damage (Naranmandura et al., 2011). DNA damage from $DMMTA^V$ was shown to be related to the accumulation of reactive oxygen species and to the down-regulation of p53 and p21 (DNA repair proteins); these processes were mediated in part through the intracellular conversion of $DMMTA^V$ to DMA^V and DMA^{III} (Naranmandura et al., 2011). Thus, although extracellular DMA^V has little toxic effect in cells because of its low uptake, intracellular DMA^V can be highly toxic. Gene-expression profiling of bladder urothelium after chronic exposure to DMA^V in drinking water showed significant increases in genes that regulate oxidative stress (Sen et al., 2005). Additionally, hepatic gene-expression profiling showed that DMA^V exposure induced changes consistent with oxidative stress (Xie et al., 2004). In vivo, DMA^V-induced proliferation of the urinary bladder epithelium could be attenuated with the antioxidant N-acetylcysteine (M. Wei et al., 2005). Arsenicals, including DMA, also interfere with certain DNA repair mechanisms (both base- and nucleotide-excision repair) and may thereby act as co-carcinogens enhancing the effect of other genotoxic carcinogens (Rossman and Klein, 2011). In fact, MMA^{III} is a stronger inhibitor of nucleotide-excision repair than inorganic arsenic (Shen et al., 2008).

Toxicological data suggest DMA^{III} and DMA^V are carcinogenic. Cancers have been induced in the urinary bladder, kidneys, liver, thyroid glands, and lungs of laboratory animals exposed to high concentrations of DMA. In a 2-year bioassay of F344/Crl rats fed a diet containing 40 or 100 parts per million (ppm) DMA^V, the females consuming the highest dose (100 ppm) developed urothelial carcinomas and papillomas in the bladder, and males and females at both dose levels (40 and 100 ppm) developed hyperplastic non-neoplastic changes in the bladder (Arnold et al., 2006). M. Wei et al. (2002) exposed male F344/DuCrj rats to DMA (valence not stated) via their drinking water and found statistically significant incidences of bladder hyperplasia and transitional cell papillomas and carcinomas at doses of 50 and 100 ppm. Similarly, A. Wang et al. (2009) found that exposure of F344 rats to DMA^V in drinking water at 1, 4, 40, or 100 ppm resulted in a change in the urinary bladder epithelium, but there were no changes in DNA repair capacity. In another study, Cohen et al. (2007b) exposed F344 rats to DMA^V in the diet for 2 years and found an increase in bladder tumors in those receiving 100 ppm. As noted above, at least some strains of rats can reduce DMA^V to DMA^{III}, and thus these researchers proposed that that trimethylated forms of arsenic (MMA^{III} and DMA^{III}) may be responsible for bladder cancer in rats. Direct intravesical administration of 90 mg/kg DMA^V to female adult rats resulted in increased bromodeoxyuridine labeling in urothelial cells, indicating DNA damage; weak neutrophil infiltration; and the proliferation of urothelial epithelium mediated through modest increases in oxidative-stress indexes (Takahashi et al., 2011). Increased urothelial cell proliferation was also found following DMA (valence not stated) exposure via the drinking water (M. Wei et al., 2002). It is noteworthy that co-treatment with an antioxidant, N-acetylcysteine, worsened

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the DMA^V-induced bladder injury rather than ameliorating it as expected. This suggests that the carcinogenic mechanism of DMA^V is more complicated than a simple production of oxidative stress. In the mouse lung, DMA^V acts as a tumor initiator (Yamanaka et al., 2009) and as a tumor promoter (Mizoi et al., 2005). DMA^V can also act as a complete carcinogen, inducing lung tumors in susceptible strains of mice, including those with deficient DNA-repair activity (Hayashi et al., 1998; Kinoshita et al., 2007). In F344/DuCrj rats treated with a mixture of carcinogens for 4 weeks, subsequent exposure to DMA (no indication of whether this was DMA^{III} or DMA^V) via their drinking water for 24 weeks caused tumor promotion in the urinary bladder, kidney, liver, and thyroid gland but inhibited the induction of tumors of the nasal passages (S. Yamamoto et al., 1995). In a similarly designed experiment, DMA (valence not stated) was found to be a bladder tumor promotor after treatment with the bladder carcinogen N′-butyl-N′-(4-hydroxybutyl) nitrosamine (Wanibuchi et al., 1996). Yamanaka et al. (2009) suggested that DMA^{III} can act as a tumor promoter through the formation of a DMA^{III} radical after the reduction of DMA^V. Studies have also found that an oral exposure of adult mice to 200 ppm DMA^V in addition to fetal inorganic arsenic exposure can act as a promoter of renal and hepatocellular carcinoma, markedly increasing tumor incidence beyond that produced by the fetal arsenic exposure alone (Tokar et al., 2012). These findings emphasize how multiple life events can contribute to an adverse health outcome in which adult DMA^V exposure triggered an otherwise dormant disease.

Mechanisms

It is believed that arsenic exerts its toxicity through several different mechanisms. Anwar-Mohamed et al. (2014) found that DMA^V and other pentavalent arsenic metabolites induce the carcinogen-activating enzyme CYP1A1. There is also considerable evidence that arsenic induces oxidative stress, which can induce cancers in animals. Ebert et al. (2016) found that thio-DMA^V exposure upregulated DNA repair and damage response proteins in human urothelial cells, and studies have shown that mice that are deficient in enzymes associated with the repair of oxidative DNA damage are highly susceptible to the induction of tumors, particularly lung tumors, by DMA^V (Kinoshita et al., 2007). Some studies have also suggested that methylated arsenicals (MMA^{III} and DMA^{III}) can induce aneuploidy in mammalian cells at concentrations below those required to produce oxidative stress after in vitro exposure (Kligerman and Tennant, 2007; Rossman and Klein, 2011). The chemical reaction of arsenicals with thiol groups in sensitive target tissues, such as red blood cells and kidneys, may also be a mechanism by which organic arsenicals act (Naranmandura and Suzuki, 2008).

Cohen et al. (2007b) postulated that regenerative cell proliferation in response to cytotoxicity in the urothelium is a major factor in the carcinogenicity of DMA to the rat bladder, and, indeed, urothelial cell proliferation has been

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shown to increase following DMA exposure (Takahashi et al., 2011; M. Wei et al., 2002). There may also be epigenetic effects involved in DMA carcinogenicity, as suggested by a study in humans in which there was a significant association between global DNA methylation and urinary DMA levels (Tellez-Plaza et al., 2014). Unterberg et al. (2014) observed DNA hypomethylation in urothelial cells that had been incubated for 21-days with inorganic As^{III} or thio-DMA^{V}.

The variation in the susceptibility of various animal species to tumor formation caused by inorganic and organic arsenic is thought to arise in large part from differences in metabolism and distribution. Thus, genetic differences may play an important role. Numerous investigators have examined potential human susceptibility factors and gene polymorphisms that may increase a person's risk of cancer and other diseases induced by arsenicals (Aposhian and Aposhian, 2006; Hernandez et al., 2008; S. K. Huang et al., 2008; Y. K. Huang et al., 2008; McCarty et al., 2007; Meza et al., 2007; Steinmaus et al., 2007, 2010), but no polymorphisms that may contribute to a person's susceptibility to DMA-induced cancers or tissue injury have yet been identified.

Summary

DMA is genotoxic and carcinogenic in certain animal models and in vitro assays, including studies in human cells. It is believed to exert a direct toxic effect by generating oxidative stress, inducing epigenetic dysregulation, binding to thiols, and interfering with DNA repair mechanisms. These mechanisms of action may be involved in gene damage and carcinogenesis. However, it is not clear whether these effects would also occur in humans directly exposed to DMA^{V}.

PHENOXY HERBICIDES: 2,4-DICHLOROPHENOXY ACID AND 2,4,5,-TRICHLOROPHENOXYACETIC ACID

Chemistry

2,4-D (CAS No. 94-75-7) is an odorless crystalline powder that, when pure, is white in color (see Figure 4-3); it may appear yellow when phenolic impurities

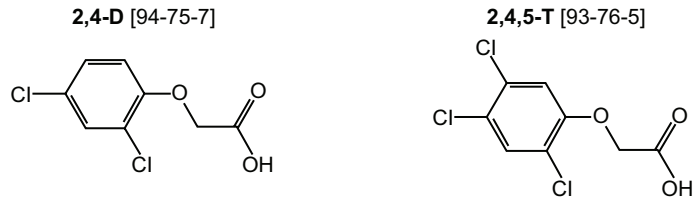

FIGURE 4-3 Chemical structures of 2,4-D and 2,4,5-T.

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are present. The melting point of 2,4-D is 138° C, and the free acid is corrosive to metals. It is soluble in water and in a variety of organic solvents (such as acetone, alcohols, ketones, ether, and toluene). 2,4,5-T (CAS No. 93-76-5) is an odorless, white to light-tan solid with a melting point of 158° C. 2,4,5-T is noncorrosive and is soluble in alcohol and water. It reacts with organic and inorganic bases to form salts and with alcohols to form esters.

Uses of 2,4-D and 2,4,5-T

2,4-D has been used commercially in the United States since World War II to control the growth of broadleaf plants and weeds on range lands, lawns, golf courses, forests, roadways, parks, and agricultural land; it remains a widely used herbicide that is approved for use by both the European Union and EPA. Formulations include 2,4-D amine and alkali salts and esters, which are mobile in soil and readily absorbed through the leaves and roots of many plants. Like 2,4-D, 2,4,5-T was developed and marketed as a herbicide during World War II. However, the registration for 2,4,5-T was canceled by EPA in 1978 when it became clear that it was contaminated with TCDD during the manufacturing process. It is recognized that the production of 2,4-D also involves the generation of some contaminants with dioxin-like activity, but the fraction of TCDD represented in the toxic equivalent (TEQ) is comparatively very small.

The herbicidal properties of 2,4-D and 2,4,5-T are related to the chemical's ability to mimic the plant growth hormone indole acetic acid. They are selective herbicides in that they affect the growth of only broadleaf dicots (which include most weeds) and do not affect monocots, such as wheat, corn, and rice.

Toxicokinetics

Several studies have examined the absorption, distribution, metabolism, and excretion of 2,4-D and 2,4,5-T in animals and humans. Data on both compounds are consistent among species and support the conclusion that the absorption of oral or inhaled doses is rapid and complete. One study indicates that 2,4-D can bind to innate intestinal, intracellular lipid-binding proteins, which may be how these compounds move through columnar absorptive epithelial cells from the intestines to systemic distribution (Carbone and Velkov, 2013). Absorption through the skin is much lower but may be increased with the use of sunscreens or alcohol (Brand et al., 2002; Pont et al., 2004). After absorption, 2,4-D and 2,4,5-T are distributed widely in the body but are eliminated quickly, predominantly in an unmetabolized form in urine (Sauerhoff et al., 1977), but 2,4,5-trichlorophenol and 2,4-dichlorophenol have been identified as trace metabolites in urine. The half-life of single doses of 2,4-D or 2,4,5-T in humans has been estimated to be about 18–23 hours and is highly dependent on urinary pH (Gehring et al., 1973; Kohli et al., 1974; Sauerhoff et al., 1977;

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WHO, 1984). Hines et al. (2003) found that concentrations of 2,4-D and its metabolites in the urine of herbicide applicators—that is, those who apply the herbicides—were consistent with 2,4-D urinary half-life estimates of 13–40 hours in humans.

Toxicity Profile

The toxicity database on 2,4-D is extensive, whereas the available data on the toxicity of purified 2,4,5-T, independent of its contamination by TCDD, are sparse. TCDD is much more toxic than 2,4,5-T, and much of the toxicity that had been attributed to 2,4,5-T in early studies was later shown to be caused by the TCDD contaminant. The following summary therefore focuses on 2,4-D toxicity, and information on pure 2,4,5-T is added when it is available.

A 2018 monograph from the IARC classified 2,4-D as possibly carcinogenic to humans. This statement originated from limited evidence available on the carcinogenic effects in animals and inadequate evidence in humans (IARC, 2018). When reviewing animal literature, studies show that after a single oral dose, 2,4-D is considered to produce moderate acute toxicity with a dose lethal to 50% of exposed animals (LD_{50}) of 375 mg/kg in rats, 370 mg/kg in mice, and from less than 320 to 1,000 mg/kg in guinea pigs. Rats and rabbits have dermal LD_{50}s of 1,500 mg/kg and 1,400 mg/kg, respectively. 2,4,5-T itself also produces moderate acute toxicity, with oral LD_{50}s of 389 mg/kg in mice and 500 mg/kg in rats. Death from acute poisoning with 2,4-D or 2,4,5-T has been attributed to the ability of the chemicals to uncouple oxidative phosphorylation, a vital process used by almost all cells in the body as the primary means of generating energy. After an animal is exposed to a high dose, death due to multiple organ failure can occur rapidly. Studies in rats, cats, and dogs indicate that the central nervous system is the principal target organ for acute 2,4-D toxicity in mammals and suggest that the primary site of action is the cerebral cortex or the reticular formation (Arnold et al., 1991; Dési et al., 1962a,b). Based on case reports, in humans the predominant effect of acute inhalation and oral exposure to 2,4-D is neurotoxicity; symptoms include stiffness of the arms and legs, lack of coordination, lethargy, anorexia, stupor, and coma. 2,4-D is also an irritant of the gastrointestinal tract that causes nausea, vomiting, and diarrhea.

Chronic exposure to 2,4-D at relatively high concentrations has been shown to produce a variety of toxic effects, including hepatic and renal toxicity, neurotoxicity, and hematologic changes. A no-observed-effect level of 2,4-D of 1 mg/kg was identified for renal toxicity in rats (Hazleton Laboratories America, 1986). Exposure to 2,4-D was associated with reduced survival and decreased growth rates in offspring of mothers fed high doses during pregnancy; these doses were also associated with maternal toxicity (Munro et al., 1992). Charles et al. (2001) found that 2,4-D did not affect fertility or produce teratogenic effects in the offspring of rats or rabbits given doses lower than 90 mg/kg/day by gavage, which did, however, cause overt

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maternal toxicity. A one-generation study in which rats were fed diets containing 2,4-D (females: up to 40 mg/kg/day; males: up to 45 mg/kg/day) from 4 weeks before breeding through 3 weeks of lactation confirmed these results and furthermore found that even at the highest exposure there is no evidence of interaction with the androgen, estrogen, or steroidogenesis pathways in the pups (Marty et al., 2013). Other studies, however, suggest that exposure to 2,4-D does have an impact on the male reproductive system (Alves et al., 2013; Joshi et al., 2012). Alves et al. (2013) found that the exposure of rat Serotoli cells in culture at 10 µM 2,4-D resulted in alterations in cellular metabolism linked to effective spermatogenesis, which could be a mechanism that would reduce sperm density. Mazhar et al. (2014) treated pregnant rats by gavage on gestation days 1–19 with 100 mg/kg/day of 2,4-D alone or administered with 100 mg/kg/day of the antioxidant vitamin E. Morphological and skeletal defects and low birth weight were observed in the fetuses of dams treated with only 2,4-D, but not in those whose mothers were also treated with vitamin E, thereby suggesting that 2,4-D exposure elicits fetotoxicity through inducing oxidative stress. In vitro exposure of human erythrocytes to 2,4-D caused changes in antioxidant enzyme activity as well as increased protein carbonyls, indicating induction of oxidative stress (Bukowska, 2003). Similar changes were observed in the liver of exposed rats (Tayeb et al., 2010, 2013). The immunotoxicity of 2,4-D has been reported in a small number of studies, including a few studies of 2,4-D applicators showing both immunosuppression (Faustini et al., 1996) and immunostimulation (Figgs et al., 2000; Holland et al., 2002). At high doses that produced clinical toxicity in experimental animals, a suppression of the antibody response was observed, whereas other measures of immune function were normal. The immunotoxicity of 2,4,5-T has not been evaluated in laboratory animals.

The carcinogenicity of 2,4-D and 2,4,5-T has been studied in rats, mice, and dogs after exposure in their food, direct placement in their stomachs, or exposure on their skin. Early studies in mice (NTIS, 1968) and rats (W. H. Hansen et al., 1971) found little to suggest tumor induction when animals were treated with 2,4-D by gavage or subcutaneously. Hazelton Laboratories of America (1986, 1987) conducted a series of studies in rats and mice, which all had negative results except one that found an increased incidence of brain tumors in male rats—but not female rats—that received the highest dose of 45 mg/kg/day 2,4-D in their feed. The occurrence of malignant lymphomas in dogs kept as pets was reported to be higher when owners reported that they used 2,4-D on their lawns than when they did not (Hayes et al., 1991, 1995), but a detailed reanalysis did not confirm this finding (Kaneene and Miller, 1999). A controlled study that used dogs exposed to 2,4-D in the laboratory had negative results. Timchalk (2004) suggested that dogs are not relevant for comparative evaluation of human health risk attributable to 2,4-D exposure because they excrete 2,4-D less efficiently than rats or humans. Given the degree of variability observed in humans (Hines et al., 2003), however, the canine information might be applicable for some people. 2,4-D is not metabolized to reactive intermediates capable of interacting with

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DNA, and the evidence supports the conclusion that 2,4-D is not a genotoxic carcinogen. However, Sandal and Yilmaz (2011) found that lymphocytes from smokers show genotoxic damage after exposure to 2,4-D, whereas lymphocytes from non-smokers do not. In the Agricultural Health Study (AHS), Andreotti et al. (2015) found that a cumulative metric of 2,4-D exposure in pesticide applicators was associated with shorter relative telomere length in leukocytes. Although 2,4-D may not be a carcinogen, it may have some role influencing the activity of known carcinogens or potentially carcinogenic changes within a cell.

Genetic Studies

In recent years, a number of investigators have used telomere length as a sensitive marker of exposure to a variety of chemical pollutants. Telomere length generally decreases with age and the assumption is that the activation of many different biochemical pathways affects telomere maintenance and repair. The alterations in these pathways can in turn either reduce or increase telomere length, depending on which factors they affect. Since *Update 2014*, two large studies of telomere length have examined the effects of exposure to the chemicals of interest (COIs).

Andreotti et al. (2015) studied 568 cancer-free male subjects from the AHS who were between 31 and 94 years, taking blood samples from them between 2006 and 2008. Relative telomere length in DNA from blood was examined for associations with self-reported cumulative pesticide use of 57 types, with exposure data collected at enrollment (1993–1997) and in two follow-up telephone questionnaires (1998–2003, 2005–2008). The researchers used three metrics of pesticide use (ever use, lifetime days, and intensity-weighted lifetime days), divided into tertiles and applied multivariable linear regression analysis to examine associations, adjusting for age at blood-draw and the use of other pesticides. Further adjustments were made for potential confounding from the use of other pesticides. The analysis showed an association between blood telomere shortening, independent of age, and the cumulative use of 2,4-D in lifetime days ($p < 0.01$) for ever versus never used, but is borderline significant when using lifetime intensity-weighted days ($p < 0.05$). Similar effects had previously been observed for buccal relative telomere length in a subset of 40 AHS subjects, but their sample buccal and blood relative telomere length values were uncorrelated. A significant trend ($p < 0.001$) was shown for categorical 2,4-D lifetime use (low, medium, high) (Andreotti et al., 2015); however, the 2,4-D never used category had substantially longer telomeres than any other group in the study and some of the other chemicals were shown to lead to longer telomeres and this could confound analysis. Thus, relative telomere length might show an effect with cumulative 2,4-D exposure in blood leukocytes, but the mechanisms of action and consequences for disease are unknown.

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2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN

Chemistry

TCDDs are polychlorinated dibenzo-*p*-dioxins that have a triple-ring structure consisting of two benzene rings connected by an oxygenated ring with four attached chlorine atoms; in the case of the dioxin congener of greatest concern, 2,3,7,8-TCDD (commonly called simply TCDD), the chlorine atoms are attached at the 2, 3, 7, and 8 positions of the benzene rings (see Figure 4-4). The chemical properties of TCDD include a molecular weight of 322, a melting point of 305–306°C, a boiling point of 445.5°C, and a log octanol–water partition coefficient of 6.8 (National Toxicology Program substance profile). It is very lipophilic, or fat soluble; is virtually insoluble in water (19.3 ng/L); and is soluble in organic solvents, such as benzene and acetone. It has been suggested that the volatilization of dioxin from water may be an important mechanism of transfer from the aqueous to the atmospheric phase (EPA, 2004); however, because of its very low water solubility, most TCDD is bound to sediments and particulate matter.

Toxicokinetics

The disposition of TCDD (which includes its absorption, distribution, biotransformation, and excretion) has been extensively studied in humans and a number of other animal models over the past 30 years. Given the plethora of data, this section highlights and summarizes only key findings. TCDD is absorbed into the body rapidly but is eliminated slowly. Because it is very lipophilic, resistant to biotransformation, and slowly eliminated, the concentration of TCDD in the lipid fraction of blood serum is thought to be in dynamic equilibrium with that in the lipid fraction in other tissue compartments. Thus, the lipid-adjusted blood serum concentration of TCDD is used to estimate total body burdens; however, at high TCDD concentrations, the liver sequesters some of the dioxin, so a lipid adjustment that ignores the hepatic fraction would underestimate the total body burden. The exposure of humans to TCDD is thought to occur primarily via the mouth, skin, and lungs. In laboratory animals, oral administration of TCDD has been shown to result in absorption of 50–93% of the administered dose (Nolan et al., 1979; Rose et al., 1976). Similarly, a study performed in a 42-year-old man found that 87% of the oral dose was absorbed (Poiger and Schlatter, 1986). Dermal absorption appears to be dose-dependent: lower absorption occurs at

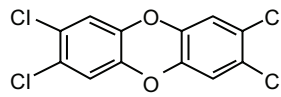

FIGURE 4-4 Chemical structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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higher doses (Banks and Birnbaum, 1991). In vitro studies of tissues isolated from humans indicate that intact human skin may not be readily penetrable (Weber et al., 1991). The varied and complex environmental matrices make environmental exposures difficult to quantify. Animal studies have demonstrated that the presence of soil or lipophilic agents dramatically reduces dermal absorption of TCDD: application in an activated carbon–water paste essentially eliminates absorption in contrast with the absorption of the pure compound dissolved in solvents. The oral bioavailability of TCDD and related compounds also depends on the matrix: contaminated breast milk and food products have much higher bioavailability than soil-bound or sediment-bound TCDD, and activated carbon essentially blocks oral bioavailability (Olson, 2012).

After ingestion and gastrointestinal absorption, TCDD associates primarily with the lipoprotein fraction of the blood and later partitions into the cellular membranes and tissues (Henderson and Patterson, 1988). TCDD is distributed to all compartments of the body; the amounts differ from organ to organ, but most studies indicate that the primary distribution of TCDD is in the liver and adipose tissues. For example, in a human volunteer it was found that 135 days after ingestion, 90% of TCDD was in fat (Poiger and Schlatter, 1986), and TCDD persists in adipose tissue in the rhesus monkey (Bowman et al., 1989). The specific details of the distribution and elimination of TCDD depend on the tissue examined, the time that has elapsed since exposure, total exposure, and other factors. For example, the concentration of cytochrome P450 1A2 (CYP1A2) in the liver is increased by TCDD (Poland et al., 1989). Direct binding of TCDD to CYP1A2 is thought to result in the sequestration of TCDD in the liver and to inhibit its distribution to other tissues. The importance of CYP1A2 concentrations for the toxic actions of TCDD has also been demonstrated in several laboratory situations; for instance, *CYP1A2*-knockout mice were more susceptible than wild-type mice to TCDD immunotoxicity (Smialowicz et al., 2008), and maternal hepatic CYP1A2 was found to sequester TCDD and protect mouse fetuses against TCDD-induced teratogenesis (Dragin et al., 2006). In addition, the distribution of TCDD is age dependent, as shown by studies in which young animals displayed the highest concentration of TCDD in the liver and aged animals the highest concentrations in kidneys, skin, and muscle (Pegram et al., 1995). Finally, the rate of elimination of TCDD, particularly after low exposures, depends heavily on the amount of adipose tissue mass (Aylward et al., 2005a; Emond et al., 2005, 2006).

In laboratory animals, TCDD is metabolized slowly. It is eliminated primarily in feces as both the parent chemical and its more polar metabolites. However, elimination appears to be dose dependent; at low doses, about 35% of the administered dose of TCDD was detected in the feces, while at higher doses, about 46% was observed (Diliberto et al., 2001). The dose-dependent occurrence of TCDD metabolites in the feces is thought to be due to the increased expression of metabolizing enzymes at higher doses and to hepatic sequestration, which makes dioxins more available for metabolism. Milbrath et al. (2009) conducted

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a comprehensive review of studies that reported the congener-specific elimination rates of TCDD and related compounds and analyzed the relationships between the apparent half-lives of the compounds as a function of age, body fat, smoking status, and breastfeeding. In infants (less than 2 years old), the compounds have a reported half-life of 0.4 year (Leung et al., 2006), and in adults a half-life of 7.2 years, based on the regression method used by Flesch-Janys et al. (1996) (Milbrath et al., 2009). Aging results in an increase in and redistribution of body fat and lipophilic chemicals that alters their rate of elimination (Van der Molen et al., 1996). Human studies of the Ranch Hand cohort have consistently found a similar relationship between an increasing half-life of TCDD and an increasing BMI (Michalek and Tripathi, 1999; Michalek et al., 1992, 1996). Smoking and breastfeeding are associated with promoting the elimination of TCDD and, in the case of breastfeeding, exposing infants through breast milk. Polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke are capable of inducing CYP1A1, 1A2, and 1B1, which in turn may increase the rate of metabolism and subsequent elimination of TCDD. Smoking has been associated with a 30% decrease in TCDD plasma half-life (Flesch-Janys et al., 1996). *Update 2014* (NASEM, 2016a) includes a table (4-1; pp. 95–96) summarizing the results of studies of TCDD half-life in humans and animals.

Special Case of the Poisoning of Victor Yushchenko

In 2004 Victor Yushchenko, a candidate for the presidency of the Ukraine, was poisoned with TCDD. It led to severe chloracne and a blood serum TCDD concentration of 108,000 ppt (pg/g lipid), which was about 50,000 times as great as that in the general population. The incident provided an unfortunate opportunity to assess the toxicokinetics of TCDD after a single large exposure. Serum and fat analysis of TCDD supports the first-order elimination half-life of 15.4 months in Yushchenko, and the similarity in the decay curves of serum lipids and subcutaneous fat confirmed that TCDD was in equilibrium between the two (Sorg et al., 2009). The elimination half-life of 15.4 months is much shorter than the 7.2-year reference half-life reported by Milbrath et al. (2009) and supports a dose-dependent elimination of TCDD, which is associated with the induction of potential TCDD-metabolizing enzymes (CYP1A1, 1A2, and 1B1) in very high TCDD exposures. Two metabolites of TCDD (2,3,7-trichloro-8-hydroxydibenzo-*p*-dioxin and 1,3,7,8-tetrachloro-2-hydroxydibenzo-*p*-dioxin) were detected in Yushchenko's feces, serum, and urine but not in his fat or skin. Over a 12-month period, about 38% of the TCDD-derived material was eliminated as metabolites (95% in feces, 5% in urine) and 62% as parent chemical. The metabolite-to-TCDD ratio in the blood serum was about one-fiftieth of that in the feces; this supports the conclusion that the metabolites were not originally ingested with TCDD (Sorg et al., 2009). The very slow metabolism of TCDD has been previously reported in laboratory animal models (Gasiewicz et al., 1983; Olson, 1986;

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Olson et al., 1980; Poiger and Schlatter, 1979) and in humans (Wendling et al., 1990). It is also noteworthy that the structures of the human metabolites are the same as previously reported in the rat and dog (Poiger et al., 1982; Sawahata et al., 1982). A continued analysis of Yushchenko's condition has revealed putative metabolomic and transcriptomic biomarkers that may prove useful for predicting health effects in populations with significant TCDD exposures (Jeanneret et al., 2014; Saurat et al., 2012).

In light of the variables discussed above and the effect of differences in physiologic states and metabolic processes, which can affect the mobilization of lipids and possibly of the compounds stored in them, complex physiologically based pharmacokinetic models have been developed to integrate exposure dose with organ mass, blood flow, metabolism, and lipid content in order to predict the movement of toxicants into and out of each organ. A number of modeling studies have been performed in an effort to understand the relevance of animal experimental studies to the exposures that occur in human populations (Aylward et al., 2005a,b; Beaudouin et al., 2010; Emond et al., 2005).

Toxicity Profile

Effects on Tissues and Organs of Laboratory Animals

The effects of TCDD in laboratory animals have been observed in several species (rats, mice, guinea pigs, hamsters, monkeys, cows, and rabbits) after the administration of a variety of doses and after periods that represent acute exposures (less than 24 hours), sub-chronic exposures (1 day–3 months), and chronic exposure (more than 3 months). Some differences have been observed between species, particularly with respect to the degree of sensitivity, but in general the effects observed are qualitatively similar. Some differences in sensitivity have been observed within rodent species, and has been found to be related to species-specific aryl hydrocarbon receptor (Ahr)[1] AHR polymorphisms (Ema et al., 1994; Pohjanvirta et al., 1998). Relatively high exposures of TCDD affect a variety of organs and result in organ dysfunction and death. The lethal toxicity of TCDD varies widely among animal species; the oral LD_{50} of the chemical varies from 1 µg/kg in guinea pigs to 5,000 µg/kg in hamsters. The developing fetus, however, is especially vulnerable to TCDD exposure, and there is only about a 10-fold variability in fetal lethal potency among these species (Kransler et al., 2007; Peterson et al., 1993; Poland and Knutson, 1982). One characteristic of TCDD exposure is a wasting syndrome that includes the loss of adipose and muscle tissue and severe weight loss, but the specific mechanisms of lethality remain unknown. Hwang et al. (2016) suggested that mitochondrial dysfunction caused by Ahr-mediated, TCDD-induced toxicity contributes to wasting syndrome and metabolic

[1]The aryl hydrocarbon receptor is denoted as AHR when in human cells and Ahr in rodent models.

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dysfunction. In most rodents, exposure to TCDD leads to hepatic enlargement, the presence of hepatic lesions, and impaired hepatic function (Harrill et al., 2016); work by X. Cheng et al. (2014) suggest a role for TCDD-AHR acting on fibroblast growth factor 21 as a contributor to liver effects. The thymus is also sensitive. Recent work by Diani-Moore et al. (2017) in a chick-embryo model found poly(ADP-ribose) polymerase activation and resulting NAD+ loss offers a possible unifying mechanism for the effects of TCDD exposure such as wasting, hepatosteatosis, and thymus atrophy. Finally, in both humans and nonhuman primates, TCDD exposure results in chloracne and associated dermatologic changes. As will be discussed in more detail in Chapters 6–11, studies performed in animal models have indicated that exposure to TCDD adversely affects the heart, the skin, and the immune, endocrine, and reproductive systems and increases the incidence of cancers of the liver, skin, thyroid, adrenal cortex, hard palate, nasal turbinates, tongue, and respiratory and lymphatic systems (ATSDR, 1998; Barouki et al., 2012; Birnbaum, 1994; Huff et al., 1994; Knerr and Schrenk, 2006; Tian et al., 2015). When TCDD has been administered to pregnant animals, birth defects—such as cleft palate, malformations of the reproductive organs of male and female progeny, and abnormalities in the cardiovascular, pulmonary, endocrine, skeletal, and nervous systems—have been observed. Of course, effects arising from perinatal exposure are not in question for Vietnam veterans themselves, but this activity is of concern with respect to their offspring. The developmental origins of health and disease are discussed in more detail in Chapter 8.

Effects on Enzymes, Hormones, and Receptors in Laboratory Animals and Cultured Cells

In addition to adversely affecting the ability of specific organs to fulfill their normal physiologic roles, TCDD has been found to alter the function and expression of essential proteins, particularly a number of enzymes. The enzymes that are most affected by TCDD are ones that act on or metabolize xenobiotics and hormones, often by changing the chemicals' polarity (water solubility) and thus promoting the elimination of the metabolites. Among the enzymes affected by TCDD, the best studied is CYP1A1, which metabolizes some xenobiotics. In laboratory animals, exposure to TCDD commonly results in an increase in CYP1A1 in most tissues; CYP1A1 therefore is often used as a marker of TCDD exposure. Related enzymes whose levels are also increased with TCDD exposure include CYP1B1 and CYP1A2, which together with CYP1A1 are capable of biotransforming some procarcinogens to potentially mutagenic and carcinogenic metabolites.

In addition to CYP1A1 and CYP1A2, TCDD can affect other enzymes that metabolize hormones, such as thyroid hormones, retinoic acid, testosterone, estrogens, and adrenal steroids (reviewed in Kung et al., 2009, and Safe et al., 2000). Those hormones transmit their signals by interacting with specific proteins

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called receptors and in this manner initiate a chain of events in many tissues of the body. For example, the binding of the primary female sex hormone, estrogen, to the estrogen receptor promotes the formation of breasts and the thickening of the endometrium, regulates the menstrual cycle, and influences brain development. Exposure to TCDD can increase the metabolism of estrogen and thus lead to a decrease in the amount of estrogen available for binding and activating the estrogen receptor. The ultimate effect of TCDD is an interference with all the bodily functions that are regulated by estrogens. Similarly, the actions of TCDD on the adrenal steroids can adversely affect their ability to regulate glucose tolerance, insulin sensitivity, lipid metabolism, body weight, vascular function, and cardiac remodeling. In addition to changing the amount of hormone present, TCDD has been found to interfere with the ability of receptors to fulfill their role in transmitting hormone signals. Those actions of TCDD on enzymes and hormone receptors are thought to underlie, in part, the observed developmental and reproductive effects and cancers that are hormone responsive.

Effects on Paths of Cellular Differentiation

The broad spectrum of TCDD effects on hormone and growth factor systems, cytokines, and other signal-transducer pathways indicates that TCDD is an extremely powerful growth dysregulator (Birnbaum, 1994). Research performed primarily in cultured cells has shown that TCDD can affect the ability of cells to undergo such processes as proliferation, differentiation, and apoptosis (Duan et al., 2014; Marlowe and Puga, 2005). During the proliferation process, cells grow and divide. When cells are differentiating, they are undergoing a change from less specialized to more specialized. Cellular differentiation is essential for an organism to mature from a fetal to an adult state. In the adult, proper differentiation is required for the normal functioning of the body—for example, in maintaining a normally responsive immune system. The processes of controlled cell death, such as apoptosis, are similarly important during the development of the fetus and are necessary for normal physiologic functions in the adult. Apoptosis is a way for the body to eliminate damaged or unnecessary cells. The ability of a cell to undergo proliferation, differentiation, and apoptosis is tightly controlled by an intricate network of signaling molecules that allows the body to maintain the appropriate size and number of all the specialized cells that form the fabric of complex tissues and organs. Any disruption of the network that alters the delicate balance of cell fate can have severe consequences, including impairment of the function of the organ because of the absence of specialized cells. Alternatively, the presence of an excess of some kinds of cells can result in the formation and development of tumors. Thus, the ability of TCDD to disrupt the normal course of a specific cell to proliferate, differentiate, or undergo apoptosis is thought to underlie (at least in part) its adverse effects on the immune system and the

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developing fetus and its ability to promote the formation of some cancers (Kolluri et al., 2017).

Mechanisms

TCDD binds and activates AHR in the cells of virtually every tissue in the body. The ability of TCDD to bind to AHR with high affinity is necessary—but not sufficient—to produce most of the adverse effects associated with TCDD exposure, including those from direct TCDD binding to and activation of the AHR and later alterations in the expression of TCDD-regulated genes as well as changes to those signaling pathways altered through interactions with the canonical AHR pathway (Poland and Knutson, 1982; Safe, 1990; Schmidt and Bradfield, 1996; Whitlock, 1990).

In the canonical pathway, AHR functions as a ligand-activated nuclear transcription factor. Upon binding of agonists (ligands), such as TCDD, AHR forms a heterodimer with a structurally related protein called AHR nuclear translocator (ARNT). The dimeric complex (structural model proposed by Corrada et al., 2017) binds to core DNA sequences called xenobiotic-responsive elements or dioxin-responsive elements in the promoter region of responsive genes and enhances the transcription of those genes. Many of the AHR-regulated genes encode drug-metabolizing enzymes, such as CYP1A1, CYP1A2, CYP1B1, and a variety of Phase II conjugating enzymes. Although the up-regulation of these enzymes is a sensitive biomarker of exposure to TCDD and may contribute mechanistically to some of the adverse effects of TCDD, the tissue-, species-, time-, and dose-specific modulation (increase or decrease) of many genes is thought to contribute to the wide array of toxic responses to TCDD exposure (Black et al., 2012; Boverhof et al., 2006; Y. Chen et al., 2017; Ovando et al., 2006, 2010; Perdew, 2008; Puga et al., 2009; Schneider et al., 2014). Many of these effects may be the result of activation of non-canonical cytoplasmic AHR pathways that lead to SRC activation, calcium release, and binding to other transcription factors, and their downstream effects as reviewed in Larigot et al. (2018).

AHR Signaling Pathways

The primary and most intensely studied pathway by which TCDD elicits biologic responses is depicted in Figure 4-5. In the absence of a bound ligand, the inactive AHR is retained in the cytoplasm of the cell in a complex consisting of two molecules of the heat-shock protein HSP90, one molecule of prostaglandin E synthase 3 (p23) (Kazlauskas et al., 1999), and one molecule of the immunophilin-like protein hepatitis B virus X-associated protein 2 (XAP2) (Petrulis et al., 2003), previously identified as either AHR-interacting protein (Ma and Whitlock, 1997) or AHR-associated protein 9 (Carver and Bradfield, 1997). The HSP90 dimer–p23 complex plays multiple roles in the protection of the AHR

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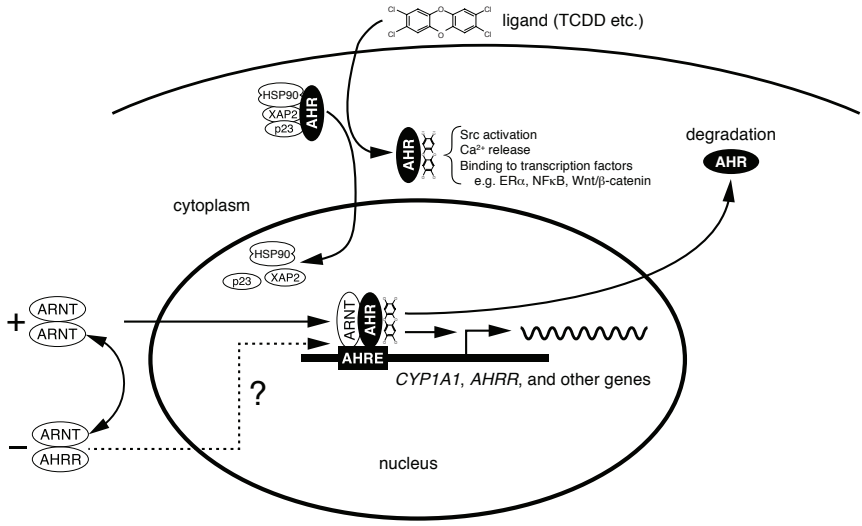

FIGURE 4-5 Mechanisms of gene regulation by the AHR following activation by TCDD. NOTES: In the canonical pathway, activation of the AHR though binding TCDD or other ligands, the AHR undergoes conformational changes that result in the dissociation of the AHR/HSP90 complex and its translocation to the nucleus. Once in the nucleus, the AHR establishes protein–protein interactions with the AHR nuclear translocator (ARNT) and additional coactivators and transcription factors. These complexes bind to AHRE (aryl hydrocarbon response elements) to control the transcriptional activity of target genes (e.g., *CYP1A1*, *AHR repressor* [*AHRR*]). In the non-canonical pathway, activation of the AHR through binding TCDD, or other ligands, results in cytoplasmic interactions leading to rapid changes in intracellular calcium, activation of signaling pathways, and direct binding to other transcription factors.

from proteolysis, maintaining it in a conformation that makes it accessible to ligand binding at the same time that it prevents the premature binding of ARNT (Carver et al., 1994; Pongratz et al., 1992; Whitelaw et al., 1993). XAP2 interacts with the carboxyl terminus of HSP90 and with the AHR nuclear-localization signal, a short amino acid domain that targets the receptor for interaction with nuclear-transport proteins. The binding of XAP2 blocks such an interaction, preventing the inappropriate trafficking of the receptor into the nucleus (Petrulis et al., 2003).

The binding of ligands (such as TCDD) induces the release of XAP2 and the exposure of the nuclear localization signal and leads to the binding of nuclear-import proteins and the translocation of the cytosolic complex into the nucleus (Davarinos and Pollenz, 1999; Song and Pollenz, 2002). Once the cytosolic complex is in the nucleus, HSP90, p23, and XAP2 dissociate from the AHR, which allows the binding of ARNT (Hoffman et al., 1991; Probst et al., 1993).

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The activated AHR–ARNT heterodimeric complex is then capable of directly or indirectly interacting with DNA by binding to recognition sequences in the regulatory region of responsive genes (Dolwick et al., 1993; Probst et al., 1993). DeGroot and Denison (2014) found that the nucleotide specificity of DNA binding by the activated AHR-ARNT is not affected by structurally different ligands.

The canonical DNA recognition motif of the AHR–ARNT complex is referred to as the AHR-responsive element (AHRE, also referred to as the dioxin- or xenobiotic-response element, respectively). This element is found in the promoter region of AHR-responsive genes and contains the core sequence 5′-GCGTG-3′ (Shen and Whitlock, 1992), which is part of a more extensive consensus-binding sequence, 5′-T/GNGCGTGA/CG/CA-3′ (Lusska et al., 1993; Yao and Denison, 1992). The AHR–ARNT complex binds to the AHRE core sequence in such a manner that ARNT binds to 5′-GTG-3′ and AHR binds to 5′-TC/TGC-3′ (Bacsi et al., 1995; Swanson et al., 1995). A second type of element, termed AHRE-II, 5′-CATG(N6)C[T/A]TG-3′, has been shown to be capable of acting indirectly with the AHR–ARNT complex (Boutros et al., 2004; Sogawa et al., 2004). The end result of the process is the recruitment of the transcriptional machinery associated with RNA polymerase II and the initiation of differential changes in the expression of the genes bearing the AHR–ARNT recognition motif. Many of the genes code for proteins responsible for detoxification reactions directed at the elimination of the ligand. Research suggests that posttranslational modifications in histone proteins may modify the response (Hestermann and Brown, 2003; J. B. Kim et al., 2017; Schnekenburger et al., 2007).

In the canonical pathway, the actions of TCDD are mediated by the binding of the activated AHR–ARNT dimer to AHREs on DNA, which results in altered gene expression (see Figure 4-5). More recently, studies suggest that a non-canonical pathway within the cytoplasm also contributes to the toxic effects of TCDD, as first reviewed by Matsumura (2009). The TCDD-mediated activation of AHR within the cytoplasm does not involve binding to ARNT or DNA and appears to contribute to the rapid inflammatory responses associated with TCDD (Sciullo et al., 2008). In several cell lines, the activation of protein kinase C and the later activation of the serine phosphorylated form of cytosolic phospholipase A2 (cPLA2) takes place within 15 minutes of TCDD exposure (Dong and Matsumura, 2008; S. Park et al., 2007). It is proposed that within the cytoplasm, the TCDD-mediated activation of AHR leads to a rapid increase in intracellular Ca2+, plus activation of cPLA2, protein kinases, and pro-inflammatory proteins, such as cyclooxygenase (COX-2) (Matsumura, 2009). This pathway and other alternative mechanisms of TCDD-mediated AHR activation have also been reviewed by Denison et al. (2011), Larigot et al. (2018), Perdew (2008), and Wright et al. (2017).

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AHR Physiology

The vertebrate AHR is presumed to have evolved from its counterpart in invertebrates, in which it serves a ligand-independent role in normal development processes. The ancestral function of the AHR appears to be the regulation of specific aspects of embryonic development, it having acquired the ability to bind xenobiotic compounds only during vertebrate evolution (Hahn, 2001; Hahn et al., 2017). The invertebrate AHR also functions as a transcription factor and binds to the same dimerization partner (ARNT) and DNA-response elements as the vertebrate protein, but it does not respond to any of the environmental ligands recognized by the vertebrate receptor. Instead, it regulates diverse developmental processes that are independent of exogenous ligand exposure, such as neuronal differentiation during worm development in *Caenorhabditis elegans* (X. Huang et al., 2004; Qin and Powell-Coffman, 2004) or the normal morphogenesis of legs, antennae, and bristles in Drosophila melanogaster (Adachi-Yamada et al., 2005; Céspedes et al., 2010). In developing vertebrates, AHR seems to play a role in cellular proliferation and differentiation and, in keeping with this role in invertebrates, also has a developmental role in craniofacial, muscle, pulmonary, renal, cardiovascular, and reproductive tract morphogenesis and blood cell differentiation (Birnbaum et al., 1989; C. Chiu et al., 2014; Fernandez-Salguero et al., 1997; Lahvis et al., 2005). Other potential functional roles of AHR include reproduction, innate immunity, tumor suppression, and blood-pressure regulation (Fujii-Kuriyama and Kawajiri, 2010). Dietrich (2016) reviewed antioxidant functions of the AHR.

The clearest adaptive physiologic response to AHR activation is the induction of xenobiotic-metabolizing enzymes involved in the detoxification of toxic ligands. Evidence of that response, which was described above, was first observed in conjunction with the induction of CYP1A1, which resulted from exposure to PAHs or TCDD and was directly related to the activation of the AHR signaling pathway (Israel and Whitlock, 1983, 1984). Because of the presence of the AHRE motif in their gene promoters, other metabolizing genes were tested and found to be induced by AHR ligands, which led to the identification of a so-called AHR gene battery of phase I and phase II detoxification genes that code for the drug-metabolizing enzymes CYP1A1, CYP1A2, CYP1B1, NQO1, ALHD3A1, UGT1A2, and GSTA1 (Nebert et al., 2000). Presumably, vertebrates have evolved those enzymes to detect a wide array of foreign, potentially toxic chemicals, represented in the wide variety of substrates that the AHR is able to bind to and whose biotransformation and elimination it is able to facilitate.

A potential complication of the adaptive responses elicited by AHR activation is the induction of a toxic response. Toxicity may result from the adaptive response itself if the induction of metabolizing enzymes results in the production of toxic metabolites. For example, the PAH benzo[*a*]pyrene, an AHR ligand, induces its own metabolism and detoxification by the AHR-dependent signaling

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mechanism described earlier, but paradoxically it becomes bioactivated to a toxic metabolite in several tissues by a metabolism that depends on CYP1A1 and CYP1B1 activity (Harrigan et al., 2004). A second potential source of AHR-mediated toxicity may be aberrant changes in global gene expression beyond those observed in the AHR gene battery. The global changes in gene expression may lead to deleterious changes in cellular processes and physiology. Microarray and other transcriptomic analyses have proved invaluable in understanding and characterizing that response (Boverhof et al., 2006; Martinez et al., 2002; Ovando et al., 2006, 2010; Puga et al., 2000, 2004; Takeda et al., 2012; Vezina et al., 2004).

Endogenous AHR functions likely involve interaction with endogenous ligands that activate specific physiological processes. Several chemicals and chemical classes have been identified as putative endogenous ligands, including equilenin, indigoids, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester, leukotrienes, heme metabolites, arachidonic acid metabolites, tryptophan metabolites, and ultraviolet (UV) photoproducts of tryptophan (Guyot et al., 2013; Nguyen and Bradfield, 2008). The tryptophan catabolite and AHR ligand kynurenine has been identified as a tumor promoter that suppresses anti-tumor immune responses and promotes tumor-cell survival and motility (Opitz et al., 2011). The tryptophan UV photoproduct 6-formylindolo[3,2-b] carbazole (FICZ) is a high-affinity AHR ligand, an inducer of CYP1A1 (Y.-D. Wei et al., 1998), and a substrate for CYP1A1 (Wincent et al., 2012). This autoregulatory loop maintains endogenous low levels of FICZ, which influence circadian rhythms, responses to UV light, homeostasis associated with pro- and anti-inflammatory processes, and genomic stability (Wincent et al., 2012). FICZ has been shown to reduce the inflammatory response in skin inflammation models (Di Meglio et al., 2014) and to enhance natural killer cell control of tumors (Shin et al., 2013).

It is clear that AHR is an essential component of the toxicity of dioxin and of dioxin-like chemicals. Studies of certain genetic polymorphisms in AHR in human cells suggest an association with differential susceptibility to dioxins (Kovalova et al., 2016; G. Liu et al., 2015), and experimental alteration of human AHR can suppress ARNT function (J. H. Xie et al., 2014). The homozygous deletion of *Ahr* in mice leads to a phenotype that is resistant to the toxic effects of TCDD and to the carcinogenic effects of benzo[*a*]pyrene (Fernandez-Salguero et al., 1996; Lahvis and Bradfield, 1998; Schmidt and Bradfield, 1996). The *Ahr* knockout mice, however, have other phenotypic effects, including reduced liver size, hepatic fibrosis, and cardiovascular abnormalities. *Ahr* knockout rats also demonstrate resistance to the toxic effects of TCDD and, in contrast to mice, display pathological alterations to the urinary tract in the absence of TCDD (Harrill et al., 2013). Hence, it is likely that dioxin has effects that are due to the disruption of endogenous AHR functions and that are unrelated to the intrinsic toxicity of some of its ligands.

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Definition of Dioxin-Like Compounds, Toxic Equivalence Factor, and Toxic Equivalents

TCDD has the highest affinity for the AHR, but many other chemicals have dioxin-like properties: they have similar chemical structures, have similar physiochemical properties, and cause a common battery of toxic responses because of their relatively high affinity for the AHR. Because of their hydrophobic nature and resistance to metabolism, these chemicals persist and bioaccumulate in the fatty tissues of animals and humans. Although there are several hundred polychlorinated, polybrominated, and mixed polychlorinated–polybrominated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls, only a relatively small number of congeners of these chemical classes display dioxin-like activity. Only 17 polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) with chlorine at the 2, 3, 7, and 8 positions and a few of the coplanar polychlorinated biphenyls (PCBs) that are often measured in environmental samples are recognized as being dioxin-like chemicals.

In the context of risk assessment, these polychlorinated–polybrominated dibenzo-*p*-dioxin, polychlorinated dibenzofuran, and biphenyl dioxin-like chemicals are commonly found as complex mixtures when detected in environmental media and biologic tissues or when measured as environmental releases from specific sources. That complicates the human health risk assessment that may be associated with exposures to varied mixtures of dioxin-like chemicals. To address the problem, the concept of toxic equivalence has been elaborated by the scientific community, and the toxic equivalence factor (TEF) has been developed and introduced to facilitate the risk assessment of exposures to those chemical mixtures (reviewed in Tavakoly Sany et al., 2015; van den Berg et al., 2006). On the most basic level, TEFs compare the potential toxicity of each dioxin-like chemical found in a mixture with the toxicity of TCDD, the most toxic member of the group. The procedure involves assigning individual TEFs to the dioxin-like chemicals on the basis of in vivo and in vitro potency relative to TCDD, which is assigned a TEF of 1.0. The dioxin-like chemicals have been assigned TEFs ranging from 0.00001 to 1.0 by the World Health Organization (WHO) (van den Berg et al., 2006, as summarized in Table 4-1). This approach has significant limitations due to the lack of in vivo human data and the difficulty of extrapolating cell line and rodent data to humans.

Larsson et al. (2015) used 17 in vitro assays from multiple species and in silico models to develop consensus toxicity factors that were then compared to internationally recognized WHO TEF values. The vast majority of the consensus toxicity factors for PCDDs and PCDFs were generally lower than the WHO TEFs but within an order of magnitude. The greatest differences between the human (but not rodent) consensus toxicity factor and TEQ values were in the effects of PCBs, which were inactive in the human cell line assays. The computational tools used by Larsson et al. (2015) could be quite useful in future discussion of TEF values, however, in vivo data must have more weight.

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TABLE 4-1 World Health Organization Toxicity Equivalence Factors (TEFs) for Dioxin-Like Chemicals (values revised in 2005)

Chemical	TEF
Chlorinated dibenzo-*p*-dioxins	
2,3,7,8-TCDD	1.0
1,2,3,7,8-PeCDD	1.0
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OctoCDD	0.0003
Chlorinated dibenzofurans	
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.03
2,3,4,7,8-PeCDF	0.3
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
2,3,4,7,8,9-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OctoCDF	0.0003
Non-*ortho*-substituted PCBs	
PCB 77—3,3′,4,4′-tetraCB	0.0001
PCB 81—3,4,4′,5-tetraCB	0.0003
PCB 126—3,3′,4,4′,5-pentaCB	0.1
PCB 169—3,3′,4,4′,5,5′-hexaCB	0.03
Mono-*ortho*-substituted PCBs	
PCB 105—2,3,3′,4,4′-pentaCB	0.00003
PCB 114—2,3,4,4′,5-pentaCB	0.00003
PCB 118—2,3′,4,4′,5-pentaCB	0.00003
PCB 123—2′,3,4,4′,5-pentaCB	0.00003
PCB 156—2,3,3′,4,4′,5-hexaCB	0.00003
PCB 157—2,3,3′,4,4′,5′-hexaCB	0.00003
PCB 167—2,3′,4,4′,5,5′-hexaCB	0.00003
PCB 189—2,3,3′,4,4′,5,5′-heptaCB	0.00003

NOTE: CB, chlorinated biphenyl; CDD, chlorinated dibenzo-*p*-dioxin; CDF, chlorinated dibenzofuran; PCB, polychlorinated biphenyl; TEF, toxicity equivalence factor.
SOURCE: Adapted from van den Berg et al., 2006.

Interim TEF values have been established for brominated congeners by the 2011 joint WHO–UN Environment Programme meeting to evaluate the WHO TEF scheme. The recommendation is to use the TEF of the corresponding chlorinated congener as an interim TEF value for brominated congeners for human risk assessment (van den Berg et al., 2013).

When several chemicals are present in a mixture, the toxicity of the mixture is estimated by multiplying the TEF of each dioxin-like chemical in the mixture

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by its mass concentration and summing the products to yield the TCDD TEQs of the mixture. In that approach to assessing the dioxin-like activity of a complex real-world mixture of dioxin-like chemicals, an environmental or biologic specimen with a 100-ppt (100-pg/g) TEQ is toxicologically equivalent to 100-ppt TCDD. There are two accepted specialized methods for assessing the dioxin-like chemicals in a complex biologic or environmental specimen: one involves analytic chemistry that quantifies specific dioxin-like chemicals (high-resolution gas chromatography–mass spectroscopy), and the other is a reporter-gene biologic screen that assesses dioxin-like activity due to binding to AHR in a transformed cell line (CALUX, EPA method 4435). Epidemiologic studies discussed in this and other updates assess exposure by reporting the specific concentration of TCDD in a specimen or by expressing dioxin-like activity in a complex mixture in units of TEQs.

Carcinogenic Classification

EPA and IARC have developed criteria to classify the potential carcinogenicity of chemicals on the basis of the weight of scientific evidence from animal, human, epidemiologic, mechanistic, and mode-of-action studies. EPA classified TCDD as a "probable human carcinogen" in 1985 and as "carcinogenic to humans" in a 2003 reassessment. In 1998 an IARC panel of experts concluded that the weight of scientific evidence supported the classification of dioxin as a class I carcinogen, that is, as "carcinogenic to humans." Four years later the U.S. National Toxicology Program upgraded its classification to "known to be a human carcinogen." In 2006 a panel of experts convened by the National Research Council to evaluate the EPA reassessment concluded that TCDD was "likely to be carcinogenic to humans"; this designation reflected the revised EPA *Guidelines for Carcinogen Risk Assessment* made public in 2005.

Genotoxicity

Genotoxicity refers to a deleterious action that affects the integrity of a cell's DNA. Genotoxic substances are known to be potentially mutagenic or carcinogenic. Although TCDD is carcinogenic in humans and laboratory animals, it is generally classified as nongenotoxic and nonmutagenic (Wassom et al., 1977). There is no evidence of covalent binding of TCDD or its metabolites to DNA (Poland and Glover, 1979). TCDD does interact with DNA through a receptor-mediated pathway that involves the initial binding of TCDD to AHR, binding of the activated receptor complex to dioxin responsive elements on DNA and later alterations in the expression of TCDD-regulated genes, and altered signaling of the biologic pathways that interact with the AHR signal-transduction mechanism (Poland and Knutson, 1982; Safe, 1990; Schmidt and Bradfield, 1996; Whitlock,

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1990). TCDD, 2,4,5-T, and 2,4-D were not mutagenic in *Salmonella typhimurium* with or without the addition of liver metabolic-activation enzymes (Blevins, 1991; Mortelmans et al., 1984). TCDD-induced cytogenetic damage in laboratory mice showed no increase in the frequencies of sister-chromatid exchanges, chromosomal aberrations, or micronuclei in the bone marrow cells of either C57Bl/6J or DBA/2J mice after the administration of a single high dose of TCDD—up to 150 µg/kg (Meyne et al., 1985). TCDD did not alter the frequency or the spectrum of mutations in male and female Big Blue transgenic rats (Thornton et al., 2001). There is one report of a positive result with TCDD in a test that measured the induction of chromosomal deletions resulting from intrachromosomal recombination in mouse embryos in vivo (Schiestl et al., 1997). More recently, Scinicariello and Buser (2015) found associations of dioxin-like PCBs and dioxins (other than TCDD) with longer leukocyte telomere length. In that study, leukocyte telomere length asays were used to assess the effects of exposure to dioxin-like PCBs (126, 169), PCDDs, and 1,2,3,4,6,7,8-heptachlorodibenzofuran. The study sample consisted of 2,413 adults from the 1999–2002 cycle of National Health and Nutrition Examination Survey (NHANES). Lipid-adjusted PCBs, PCDDs, and PCDFs were measured for a randomly selected one-third of all NHANES subjects. Both individual values and TEQs were estimated. Multivariate linear regression was used to study associations between natural log-transformed leukocyte telomere length and persistent organic pollutant quartiles. Individuals in the 3rd and 4th quartiles of the sum of PCBs were associated with, respectively, 8.33% (95% confidence interval [CI] 4.08–13.88) and 11.63% (95% CI 6.18–17.35) longer leukocyte telomere length, compared with the lowest quartile, with an evidence of a dose–response relationship (p-trend < 0.01). Most of these effects were stronger in the non-dioxin-like congeners than in the dioxin-like congeners, which brings into question the direct relevance to the committee's charge. No significant associations were observed between leukocyte telomere length and the sum of PCDD; however, the congener 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin was associated with a longer leukocyte telomere length. By TEQs, the highest quartiles of dioxin-like and the TEQ PCBs were associated with 11.63% (95% CI 4.08–18.53) and 6.18 (95% CI 1.01–11.63) longer leukocyte telomere length; both demonstrated a dose–response relationship ($p < 0.01$). This study suggests that telomere length may be influenced by exposure to dioxin and dioxin-like chemicals, however, the association between telomere length and disease is not well understood.

In summary, although TCDD does have some genotoxic activity, the vast majority of studies did not detect mutagenic activity of TCDD in a variety of in vitro and in vivo short-term tests.

Epigenetic Activity

Chromosomes contain the genetic material of an organism and are composed of both DNA and proteins called histones. Interactions between DNA

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and histones regulate the accessibility of DNA to binding factors that activate or suppress gene transcription. This interaction is controlled by chemical modifications of the DNA (generally methylation of cytosine bases in cytosine–guanine dinucleotides or CpG sites) and histones (such as methylation, acetylation, sulfation, and ubiquitination), which are maintained by enzymatic processes. These modifications are considered "epigenetic" because they control the function of genes without changing the coding sequence. TCDD has been associated with epigenetic marks on the chromosomes, indicating that it could potentially be altering the function of numerous genes. Below is a brief summary of the epigenetic effects of TCDD. More detailed information about epigenetic mechanisms in general can be found later in this chapter, particularly concerning somatic modifications in an individual, and again in Chapter 8 with respect to effects that may affect offspring of an exposed organism.

The exposure of rodents to TCDD has been shown to result in the methylation of DNA in the adult rodents' tissues—sperm, mammary tissue, liver, and a number of other solid tissues (Amenya et al., 2016; Papoutsis et al., 2013; Somm et al., 2013). The effects on DNA methylation were found to persist in rats for three generations following maternal exposure to 100 ng/kg TCDD and so could essentially be considered permanent and heritable (Manikkam et al., 2012a). As Ahr activation is generally considered necessary, but not sufficient, to induce adverse biological responses, and the dose–response relationship for Ahr activation is generally quite steep, the relevance of high-dose rodent studies such as these to human "low-dose exposures" is not always clear. When Olsvik et al. (2014) fed female zebrafish TCDD in their diet prior to breeding, they found no change in the level of DNA methylation throughout the genomes of fish or their embryos, but altered methylation was identified by probes for the promoter regions of a number of specific genes, with corresponding marked elevations in the expression of *CYP1A1* and *CYP1B1* in the mothers and embryos (Olsvik et al., 2014). To date, however, there have not been publications reporting on the persistence of methylation or other epigenetic modifications in the offspring of males exposed to TCDD as adults. In vitro studies in cell lines have identified altered DNA methylation and expression in genes related to adipocyte differentiation following TCDD exposure (van den Dungen et al., 2017) and in *CYP1A1* following PCB 126 exposure (Vorrink et al., 2014).

Other Toxic Outcomes

Chloracne is a signature effect of high exposure to TCDD and dioxin-like chemicals in some species and in humans who are sensitive. There is an extensive body of evidence from experimental studies in animal model systems that TCDD, other dioxins, and several dioxin-like chemicals are immunotoxic (Kerkvliet, 2009, 2012; Kreitinger et al., 2016). Although the available evidence on dioxin immunotoxicity in humans is scant, mechanistic considerations support

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the suggestion that chemical alterations of immune function would cause adverse health outcomes because of the critical role that the immune system plays in general protection—fighting off infection and eliminating cancer cells at early stages. Because of those considerations, the chemicals are potential immunotoxicants.

Similarly, reproduction and embryonic development clearly are targets of TCDD (Chen and Chan, 2016), other dioxins, and dioxin-like chemicals; it is a consistent finding that the adverse effects are more prevalent during fetal development than in the adult. Data on the developmental effects of dioxin-like chemicals in humans have begun to emerge over the past 10 years (Mocarelli et al., 2008). Human and animal studies have revealed other potential health outcomes, including cardiovascular disease, hepatic disease, thyroid dysfunction, lipid disorders, neurotoxicity, and metabolic disorders such as diabetes.

A number of effects of TCDD exposure in vitro appear to be independent of AHR-mediated transcription and, in at least one instance, perhaps independent of AHR itself. Guo et al. (2004) showed that TCDD induced expression of transforming growth factor α and other genes involved in extracellular matrix deposition in cells from mice that had homozygous ablation of the *Ahr* gene. Studies have shown that TCDD can mobilize calcium from intracellular sources and increase calcium imported from the culture medium (Puga et al., 1995). Mitochondrial oxidative stress has been shown to be induced when calcium is mobilized (Senft et al., 2002). Calcium mobilization by TCDD may have an important effect on signal-transduction mechanisms that control gene expression, inasmuch as several proto-oncogenes, such as *c-fos*, are activated by calcium changes.

Earlier *VAO Update* reports address the other toxic effects of TCDD in greater detail, including the relationship between human and animal-model sensitivity to exposure.

Summary of Biologic Plausibility That TCDD Induces Adverse Effects in Humans

Mechanistic studies in vitro and in laboratory animals have characterized the biochemical pathways and types of biologic events that contribute to the adverse effects of exposure to TCDD. For example, much evidence indicates that TCDD, acting via AHR in partnership with ARNT, alters gene expression. Receptor binding may result in the release of other cytoplasmic proteins that alter the expression or activity of other cell-regulatory proteins. Mechanistic studies also indicate that many other cellular-component proteins contribute to the gene-regulatory effect and that the response to TCDD exposure involves a complex interplay between genetic and environmental factors. Comparative data from animal and human cells in vitro and from tissues suggest a strong qualitative similarity among species in their response to TCDD, and this further supports the applicability to humans of the generalized model of initial events in response to dioxin exposure.

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Biochemical and biologic responses to TCDD exposure are considered adaptive or simply reflective of exposure and not adverse in themselves if they take place within the normal homeostatic ranges of an organism; the exception to this is during development. However, they may exceed normal physiologic boundaries or constitute early events in a pathway that leads to damage in sensitive members of the population. In the latter case, the response is toxic and would be expected to cause an adverse health effect. Those qualitative generalizations about dose–response and individual variability are central to establishing biologic plausibility, which in the case of TCDD largely relies on extrapolation from animal studies to human risks. However, there are significant quantitative differences between responses in humans and rodents. Evidence from highly exposed human populations is an important means for corroborating biological plausibility.

OVERARCHING TOXICOLOGIC ISSUES RELATED TO THE CHEMICALS OF INTEREST

Limitations of Extrapolating Results of Laboratory Studies to Human Responses

In some instances, the toxic responses identified in laboratory-animal and cell-culture studies are not detected in epidemiologic studies with human exposure to the same chemicals. Although animal and cell-culture studies provide important links to understanding the biochemical and molecular mechanisms associated with toxicity induced by xenobiotics, many factors must be considered in extrapolating their results to human disease and disease progression. The following are key factors that might limit the ability of laboratory studies to predict human responses completely and accurately.

- **Magnitude and duration of exposure** In many instances, animal and cell-culture studies are conducted at higher exposures and for shorter durations than are typical in human exposures. For example, the concentrations of TCDD used in animal studies can be many times higher than was typical in the TCDD exposures of Vietnam veterans during their military service. In addition, TCDD is a persistent organic pollutant, and this results in human exposure that occurs over a lifetime, whereas animal studies seldom examine chronic low-level exposures that occur over a period of many months or years, except those that evaluate chronic toxicity or carcinogenicity. Animal studies that establish a measurement of body burden over a specific period provide the best potential for extrapolation to humans.
- **Toxicokinetics** The toxicokinetics—absorption, distribution, metabolism, and excretion—of xenobiotics can differ sharply between laboratory animals and humans. As described previously, the biologic half-life of TCDD

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varies from 8 to 29 days in rats and mice, while it is about 7 years in humans even though the drug-metabolizing enzymes—including cytochrome P450 1A1, 1A2, and 1B1—are up-regulated or induced via TCDD-mediated activation of the AHR in both rat and human liver (Black et al., 2012).

- **Timing of exposure** Many organ systems are more susceptible to xenobiotic exposure during critical stages of development, differentiation, or function—such as during gestation or in the face of another external challenge (for example, antigens, smoking, dietary salt, and fat)—than at other times. Therefore, the response of some systems (such as the immune or cardiovascular systems) may depend on the timing of exposure relative to the other challenges.
- **Exposure composition** Most animal and cell-culture studies involve exposure to single chemicals or to a well-defined mixture, but most human exposures are to complex mixtures from multiple sources, so it is difficult to definitively attribute any observed effects to a particular component of the environment.
- **Difference in AHR affinity** The binding affinity of AHR for TCDD differs between species (discussed in Okey et al., 2005). Many of the strains of mice used for toxicologic studies harbor a high-affinity *Ahr* allele (Ahr^b), and these mice exhibit greater sensitivity to hepatic CYP1A induction, immunosuppression, birth defects, and other responses than do strains that carry the low-affinity allele (Ahr^d). Such a simple allelic difference in AHR affinity has not been observed in humans, and the TCDD-binding affinity of the AHR found in most humans more closely resembles the low-affinity mouse Ahr^d allele. Nonetheless, Nebert et al. (2004) reported that some people have TCDD-binding affinity that is 12 times higher than that found in others. Thus, although humans are generally considered to be less sensitive on the basis of an AHR that has low TCDD-binding affinity, this assumption may not apply to everyone.
- **Complex disease etiology and environment** The etiology of human diseases is highly influenced by genetics, environmental factors, and gene–environment interactions; these factors can be protective as well as deleterious. In addition to the COIs, the environmental factors that commonly influence human responses include diet, prescription and over-the-counter pharmaceuticals, cigarette smoking, alcohol consumption, physical activity, the microbiome, and stress. Stress (not to be confused with oxidative stress) produced via known or unknown sources is a well-known modifier of human disease responses (for example, immune and cardiovascular responses). Furthermore, stress is an ever-present factor that is difficult to assess or control for in epidemiologic studies because there is substantial individual variation in response to it (Cohen et al., 2007a). In contrast, laboratory studies are often conducted with inbred strains of animals and under tightly controlled experimental conditions, thus possibly underestimating or overestimating the potential

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contribution of a single chemical exposure to disease development. On the other hand, direct cause-and-effect relationships are more easily established in animal studies because of their standardization.

- **Sex differences** There are well-known differences in susceptibility to xenobiotic exposures between male and female animals, some of which are modified by sex steroids. For example, female Sprague Dawley rats are significantly more responsive to the hepatotoxic (neoplastic and non-neoplastic) effects of TCDD than are males of the same strain (Kociba et al., 1978).

Epigenetics

Epigenetics, as previously noted, is the term used to describe the mechanisms that regulate gene expression and genomic stability but that involve no changes in DNA sequence. The epigenetic marks on DNA and bound histones are mitotically stable because they are maintained every time a cell divides. The totality of epigenetics marks in each cell, termed the *epigenome*, creates and maintains the identity and function of the cell type (Christensen and Marsit, 2011; Cortessis et al., 2012; Skinner et al., 2010).

Conrad Waddington used the term "epigenetics" in the 1940s to describe environment–gene interactions that alter biologic traits (Waddington, 1940, 1953, 1956). It was not until the 1970s, however, that the first molecular epigenetic factor was described: DNA methylation, the chemical addition of a methyl group to DNA (Holliday and Pugh, 1975). In the 1980s, the role of DNA methylation in modifying gene expression—turning genes on and off—was established (Chen and Riggs, 2005). In the 1990s, the chemical modification of histone proteins associated with DNA was shown to also modify gene expression, thus establishing a second molecular epigenetic mechanism (Turner, 1998). In the early 2000s, various small noncoding RNA molecules were shown to regulate DNA activity (Sato et al., 2011). Around 2005, the first mapping of the yeast epigenome was conducted (Pokholok et al., 2005). Since that time, the mapping of cell-specific human epigenomes has accelerated under the National Institutes of Health's Epigenomics Roadmap and ENCODE Project, and more than 100 human cell lines and tissues have been epigenetically characterized (Kundaje et al., 2015). The studies show that epigenetic marks act together in an exquisitely choreographed fashion to control cellular differentiation and the cellular ability to interact with, process, and initiate events and to respond to the signals and needs of the individual and local tissue environment. New National Institutes of Health efforts are under way within the Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription Program to use mouse models of human relevant exposures to address the role of the environment in disease susceptibility as a function of changes to the epigenome, including TCDD and arsenic (T. Wang et al., 2018).

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Today, the processes recognized as epigenetic mechanisms are DNA methylation (Chen and Riggs, 2005; Holliday and Pugh, 1975), histone modification (Turner, 1998), alterations in chromatin structure (Murr, 2010), and modulation of expression by some small and long non-coding RNA molecules (Valeri et al., 2009). DNA methylation is the addition of a methyl group onto specific nucleotides. In mammals it occurs mostly at cytosine nucleotides that are adjacent to guanine nucleotides (CpG sites), but it can also occur at cytosine nucleotides followed by other bases in embryonic cells and brain cells (Lister et al., 2013). Methylation of DNA in the promoter region of the gene can reduce the expression of the adjacent gene. Other modulations of DNA include hydroxymethylation (which is prominent in stem cells and brain cells), formylcytosine, and carboxylcytosine (X. Cheng et al., 2014; Song et al., 2013). Histones are the proteins that bind and form complex structures with DNA called nucleosomes in which DNA is wrapped around the histone core. The combination of DNA and histones is called chromatin. Chemical modifications of histones, such as methylation and acetylation, can alter the histone structure and modify gene expression by attracting protein complexes that can stimulate or repress transcription, in part by changing nucleosome spacing (Reid et al., 2009; Zhang and Pradhan, 2014). The influence of regulatory non-coding RNA including small interfering RNA and piwi-interacting RNA, as well as long non-coding RNA, on gene transcription is another field of epigenetic gene regulation that is now emerging. For example, recent findings suggest that micro-RNAs may be important regulators of cytokines involved in T-cell polarization and the allergic response (Tay et al., 2014).

Small RNAs can elicit both epigenetic and non-epigenetic responses depending on whether they are inhibiting transcription via the RNA-induced initiation of transcriptional silencing (RITS) pathway (epigenetic) or degrading mRNA through RNA-induced silencing complex (RISC) complex (not epigenetic). For example, short antisense RNA transcripts are produced within the nucleus by the action of the enzyme Dicer, which cleaves double-stranded RNA precursors into 21–26 nucleotide long RNA species (Matzke and Birchler, 2005; Verdel et al., 2005). These then associate with silencing–effector complexes, such as RISC, which directs cleavage of cognate mRNA or causes translational repression and RITS, which mediates heterochromatin formation at target loci and abrogates gene expression (Verdel et al., 2005). Thus, regulation mediated by small non-coding RNAs occurs both at the posttranscriptional and transcriptional levels. The latter transcriptional regulation is referred to as "epigenetic silencing," and is mediated either by covalent modifications of chromatin (such as H3 methylation at Lys9) or by DNA methylation (Verdel and Moazed, 2005).

The interaction of all those epigenetic processes creates the epigenome, which has a critical role in regulating gene expression (Christensen and Marsit, 2011; Cortessis et al., 2012; Skinner et al., 2010). The variation that is possible in the epigenome is remarkable: The histone proteins that control chromatin configurations have dozens of possible modifications, and there are upwards of

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50 million nucleotides in the DNA where methylation can occur and participate in regulating the cellular state. That implies that trillions of configurations of the epigenome are possible, although typically only about half of all genes are expressed in any given cell. Epigenetic marks are erased and re-established at two times during the life cycle—shortly after fertilization and during gametogenesis—to allow gamete-specific epigenomes to be converted to cell-specific epigenomes and vice versa (Dean, 2014).

Environmental epigenetics is the study of how environmental factors such as nutrition, toxicants, and stress alter epigenetic programming. In particular, epigenetics provides a molecular mechanism—other than mutations in the DNA itself—by which environmental factors can influence disease etiology (Jirtle and Skinner, 2007; Szyf, 2007). Epigenetics has been shown to have a role in the disease etiology of cancers and a number of other diseases (Christensen and Marsit, 2011; Cortessis et al., 2012; Skinner et al., 2010). In addition, exposure to environmental factors at critical times of development when epigenomes are shifting has the ability to alter epigenetic programming and cause changes in gene expression because these are times when epigenomes are evolving rapidly as stems cells differentiate into more mature cell types (Skinner et al., 2010). Hence, immune responses, fetal development, and gamete formation are important examples of physiological processes whose functioning can be affected by environmentally induced epigenetic changes.

New investigative tools and a more refined understanding of the epigenetic process have given rise to active research on the nature of the relationship between environmental exposure to epigenetically active agents and the occurrence of diverse disease states, including cancers, reproductive-developmental problems, immune dysregulation, diabetes, obesity, and psychiatric illnesses (Brookes and Shi, 2014). The committee sought to review data on the potential relationship of the exposures of interest with adverse epigenetic effects in directly exposed veterans in an attempt to find evidence linking the exposures to disease processes that might have been mediated epigenetically. The committee also sought to review relevant data on female veterans and male veterans separately inasmuch as the epigenetic consequences of exposures could be different, particularly in the case of adverse reproductive outcomes. Of note, possibly the greatest limitation to environmental epigenetic studies in human cohorts is access to the target tissue of interest. Researchers rely on more accessible proxy tissues such as blood leukocytes, saliva, buccal cells, or placenta. A second consideration is that DNA methylation levels change as a function of age in both humans and animal models and that these age-related changes in methylation are gene- and tissue-specific, can be either random or predictable, and are thought to play a role in chronic disease development.

A relevant example within the COIs is that the in vitro exposure of preimplantation embryos to TCDD alters the DNA methylation of imprinted genes (Q. Wu et al., 2004). Imprinted genes are a unique category of genes in which only

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one copy is expressed in a parent-of-origin specific manner, and imprinted genes are important for growth and development. Similar results to those of Q. Wu et al. were obtained more recently when several solid tissues and sperm DNA were analyzed in adult male mice exposed to TCDD in utero (Somm et al., 2013). In utero exposure to TCDD was also associated with altered DNA methylation and reduced expression of the BRCA1 (breast cancer) gene in mammary tissue in adult female offspring (Papoutsis et al., 2013). More generally, studies of the developmental origins of health and disease have shown that early-life exposures or environmental influences can be associated with the onset of disease much later in life (Barker et al., 2010). These early developmental alterations in the epigenome provide a molecular mechanism by which environmental exposures of female veterans can have effects on their children into adulthood. In humans, using data from the AHS, Rusiecki et al. (2017) evaluated high pesticide exposure events and altered DNA methylation in the blood of male pesticide applicators (n = 695). A candidate gene approach was used to investigate three gene promoters as well as the repetitive element LINE-1. Ever having had a high pesticide exposure event was associated with increased DNA methylation at the *GSTp1* promoter, an effect that was more pronounced among applicators older than 59 years. High pesticide exposure events were also associated with decreased DNA methylation at the *MGMT* promoter among applicators older than 59 and in applicators with less than 16.6 ng/mL plasma folate.

Of note, there are almost no toxicological studies with animal models that have evaluated more than a single, and at times very high dose, of the COIs. The potential for epigenetic changes induced via TCDD in Vietnam veterans and their descendants remains uncertain until more dose–response studies are completed to determine if epigenetic effects are secondary to AHR activation or separate from AHR activation. If epigenetic responses are the result of downstream events following extensive and prolonged AHR activation, they may be irrelevant to exposure scenarios that are inadequate to extensively induce AHR. On the other hand, if evidence demonstrates that TCDD can induce epigenetic changes at doses that cause no or minimal induction of AHR, it could well be the most important toxicological outcome to potentially occur in humans. There is precedent within the endocrine disrupting chemical literature for epigenetic alterations to have low-dose and non-monotonic effects, which are not necessarily linked to blocking or mimicking hormones, but may occur through other mechanisms such as oxidative stress or direct interactions with any of the many epigenetic enzymes and co-factors necessary for epigenetic gene regulation (Tapia-Orozco et al., 2017).

Most epigenetic modifications occur in somatic cells and are heritable only within the altered cell line, thereby having the potential to generate effects in the exposed individual but not in that individual's offspring. Other studies have found direct effects of exposures on the germline (e.g., sperm or oocytes), which can be multi-generationally transmitted to the next generation. The possibility exists, however, of epigenetic transgenerational inheritance, which involves the

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environment promoting a stable alteration in the germline that is transmitted to later unexposed generations (Schmidt, 2013; Skinner, 2014; Skinner et al., 2010; Y. Wei et al., 2015). Manikkam et al. (2012b) showed that the exposure of pregnant female rats to TCDD at critical times of development of the germline (when epigenetic programming is being established) can lead to abnormalities in the third-generation offspring, including kidney disease and changes in the ovaries and sperm of the offspring. There are few data on possible male-mediated heritable effects of TCDD or other environmental compounds. The sparse data include the results of studies of lead, a known developmental and neurologic toxicant. Lead exposure can alter semen quality in males (Alexander et al., 1996) and has been shown to induce paternally mediated developmental toxicity in rats (Anjum et al., 2011); this demonstrates that male-mediated effects on reproduction can be induced by reproductive toxicants. It has been suggested that environmental exposures can result in reduced fertility (Guerrero-Bosagna and Skinner, 2014; Paoloni-Giacobino, 2014). However, a serious need exists for additional study of the question, particularly of the effects of the COIs to this committee.

In summary, the ability of epigenetic mechanisms to regulate gene expression coupled with the interaction of the epigenome and the environment, including multi- and trans-generational effects, might underlie the ability of xenobiotic exposure to contribute to disease development and the potential for offspring to inherit the effects of the disrupted epigenetic processes.

Developmental Immunotoxicity

A second emerging field in the biologic sciences that may provide insight into the mechanism of xenobiotic-induced disease is developmental immunotoxicity, the study of the disruption of the developing immune system by xenobiotic exposure. The developing immune system is among the most sensitive physiologic targets of prenatal and childhood environmental insult. The sensitivity is due, in part, to the novel processes of gene rearrangement, somatic-cell selection, and immune-cell distribution that are required to produce a security system that can effectively protect not only the child but also the aging adult from disease. To produce that security system, the immune system, as it matures, must coordinate steps that result in highly specialized immune cells that are capable of self-versus-non-self recognition and that are tailored to the specialized functional environments of different tissues and organs (such as brain, lungs, skin, liver, gastrointestinal tract, and reproductive tract). A disruption of immune development can place the integrity of the organism at risk.

Among the known risk factors for developmental immunotoxicity are various chemicals including heavy metals, some pesticides, TCDD, industrial solvents such as trichloroethylene, and PCBs. The adverse outcomes of developmental immunotoxicity may become apparent soon after exposure or can emerge much later in life (Gascon et al., 2013). Often, childhood or adult infections can

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trigger the appearance of developmental immunotoxicity-associated immune problems that were established earlier in life (Dietert, 2009). Developmental immunotoxicity-induced alterations can also contribute to myriad health problems related to dysfunction or pathologic conditions in virtually any tissue or organ. Chemicals, drugs, infectious agents, and physical and emotional stressors can act synergistically and increase the risk of developmental immunotoxicity. Not everyone is at identical risk for developmental immunotoxicity. People who have particular genotypes may be at increased risk for specific chemical-induced developmental immunotoxicity on the basis of heritable factors that affect metabolism or immune vulnerability.

The heightened sensitivity of the developing immune system is due to the existence of critical developmental windows of vulnerability during which environmental interference with key steps of immune maturation can change the entire course of immune development and result in later-life immune dysfunction and an increased risk of disease. The events programmed for these critical developmental windows have several basic features:

- They are necessary, usually one-time events of early development, with no equivalents in adults.
- They lock in building blocks on which additional maturational events rely.
- If they do not occur both on time and efficiently, the ramifications are usually profound, prolonged, and irreversible.

Examples of critical windows of immune vulnerability and the chemicals that can cause disruptions have been described in several reviews (R. Dietert and J. Dietert, 2008; R. Dietert and Piepenbrink, 2006; R. Dietert et al., 2000; Hessel et al., 2015; Holsapple et al., 2003; Landreth, 2002) and include

- The process of the seeding of immune cells in tissues where they grow into resident populations.
- The selection process of thymocytes in the thymus to distinguish nonself from self during the development of acquired immunity.
- The maturation of macrophage populations in the lung, in the brain, and elsewhere.
- The maturation of dendritic cells to provide balanced immune responses.
- The initial development, expansion, and seeding to the periphery of regulatory cell populations.
- Comparison with current tolerable daily intakes and their underlying overall no observed adverse effect levels showed that for some of the compounds, the tolerable daily intakes may need reconsideration based on developmental immune parameters.

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The increased sensitivity of the fetal, neonatal, and juvenile immune systems compared with the immune system of an adult can manifest itself as a sensitivity to lower doses of chemical exposure than the doses that affect the adult, a greater persistence of the immune problems that follow exposure than are seen in adults, a broader array of immune problems than are experienced by adults, and a greater likelihood that a second later-life chemical exposure or environmental stressor will trigger an unexpected immune problem (Rooney et al., 2008). It is also important to note, similar to epigenetic mechanisms discussed above, the relevance of single or high-dose animal model studies remains uncertain until more dose–response studies are completed to determine if developmental immunotoxicity effects are secondary to AHR activation or separate from AHR activation.

Disruption of immune maturation is not the only route for developmental immunotoxicity. Early-life chemical exposure may affect the status of genes (the epigenome) in such a way that their pattern of expression in later life is affected and thereby alters immune functional capacity (J. Xu et al., 2016a). Such changes in gene status that affect immune status could occur in the exposed generation (for people exposed in utero or during childhood), or they could carry through one or more additional generations as a result of epigenetic alterations.

MEDIATORS OF HEALTH OUTCOMES

Identifying and assessing the mediators of health outcomes, which are effectors in the pathway of exposure to disease and may contribute to disease progression, is essential for characterizing the impact of toxicant exposures on health outcomes. Such mediators include oxidative stress, the cytogenetic status of cells, and factors that affect normal function and the development of the blood–brain barrier.

Recently, the study of oxidative stress has emerged as a mechanism for investigating toxicity. Oxidative stress is classically defined as an imbalance in oxidant and antioxidant species within a system in which oxidant species are predominant (Lushchak et al., 2014). Although oxidative stress and exposure to toxicants have been associated with many of the same health effects, they are rarely studied in parallel. For example, both oxidative stress and toxicant exposures have been associated with metabolic syndrome, insulin resistance, diabetes, obesity, and cardiovascular complications (Bonomini et al., 2015; Lang et al., 2008).

Using a longitudinal study of non-smoking Iowa corn farmers (n = 30) and non-farming controls (n = 10), Lerro et al. (2017) evaluated the effects of atrazine and 2,4-D exposures on three markers of oxidative stress in 225 samples collected during five agricultural periods (pre-planting, planting, growing, harvest, and off-season). Farmers exhibited higher urinary levels of atrazine and 2,4-D than controls. 2,4-D exposure (but not atrazine exposure) was associated with elevated levels of 8-OHdG (a marker of oxidative damage) and 8-isoPGF (a marker of lipoprotein peroxidation). No effects on the oxidative stress biomarker

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malondialdehyde were observed in either atrazine or 2,4-D. Thus, 2,4-D may induce oxidative stress and contribute to the pathogenesis of cancer and other chronic diseases.

Using the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort, Kumar et al. (2014a) evaluated serum persistent organic pollutants levels and several biomarkers of oxidative stress in 992 male and female subjects 70 years and older. Measurements included PCBs, organochlorine pesticides, octachlorinated dibenzo-*p*-dioxin, and polybrominated diphenyl ethers, and TEQ values were calculated for dioxin and dioxin-like mono-ortho PCBs via the van den Berg method. Oxidative stress markers consisted of protein stress markers (e.g., homocysteine, GSH, GSSG), lipid stress (e.g., conjugated dienes), and total antioxidant capacity. Several PCBs showed a significant positive association with oxidized low density lipoproteins (PCB 99, 138, 153, 156, 170, 180, 194, 206, and 209). In multivariable-adjusted analyses, the sum of PCBs was positively associated with oxidized low density lipoproteins and negatively associated with glutathione-related markers. Other summary measures did not show any significant associations with oxidative stress markers.

Sycheva et al. (2016) studied the cytogenetic status of cells isolated from the buccal and nasal epithelium from a sample of cases (n = 26) and controls (n = 35) from a rural population in Vietnam. The cases were individuals living in dioxin-contaminated areas (TCDD in soil 2.6 ng/kg), and the controls were villagers living in an area with negligible contamination (TCDD in soil 0.18 ng/kg). A variety of cellular damage parameters were examined, and a small increase in cell death was found for both types of epithelial cells in males from the contaminated area, indicating that dioxins had a more pronounced effect and that years of residence and aging affected the results inversely. Thus, the increase in apoptosis could suggest a dioxin effect and serve as a marker for cellular damage generally. However, the small sample size and the lack of a dioxin association with oral or nasopharyngeal cancers limits the confidence with which conclusions can be drawn from this study.

There is increasing investigation into the mechanics of the interactions between TCDD (and dioxin-like chemicals) and the normal function and development of the blood–brain barrier. Filbrandt et al. (2004) first demonstrated that TCDD was active in regulating gene expression in isolated murine cerebral vascular endothelial cells and astrocytes. X. Wang et al. (2011) demonstrated that TCDD exposure results in an increased expression and activity of several xenobiotic efflux transporters at the blood–brain barrier in rats and isolated capillaries in vitro. This has the potential to alter the delivery of drugs or endogenous chemicals to the brain. Miyazaki et al. (2016) reported that exposure to TCDD during blood–brain barrier formation disrupts and impairs blood–brain barrier function in part by the suppression of glial cell derived neurotrophic factor action, which may contribute to the adverse effects of TCDD on the fetal central nervous system. In a human cerebral microvascular endothelial cell line, Jacob et al. (2015)

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did not observe a change in the expression or activity of two of the major efflux transporters in the human cerebral endothelium (ABCB1 and ABCG2) after exposure to TCDD. In a second study using the same cell line, Jacob et al. (2017) demonstrated that TCDD exposure increased the levels of the pro-inflammatory cytokines IL-1β and IL-6, which could induce blood–brain barrier breakdown and contribute to the pathogenesis of a variety of neurologic disorders.

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5

Background on Selected Epidemiologic Studies and Populations

The process of updating the evidence base of possible health effects of the chemicals of interest (COIs)—2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); 4-amino-3,5,6-trichloropicolinic acid (picloram); and dimethyl arsenic acid (DMA or cacodylic acid)—has involved the evaluation and integration of thousands of epidemiologic publications over successive reports. The search strategy used to identify these publications, along with refinements that have been made since the initial volume of the series was prepared, are described in Chapter 3.

In addition to reviewing studies involving exposures to the specific COIs, the current and previous Veterans and Agent Orange (VAO) committees have considered studies that examined compounds chemically related to the herbicides used in Vietnam, such as 2-(2-methyl-4-chlorophenoxy) propionic acid, hexachlorophene, and chlorophenols, particularly 2,4,5-trichlorophenol. If a publication did not specify the herbicides or polychlorinated biphenyls (PCBs) with dioxin-like actions to which study participants were exposed or the magnitude of exposure, those limitations were considered when weighing the evidence of each publication. The committee considers studies of exposure to PCBs and other dioxin-like chemicals informative if their results were reported in terms of TCDD toxic equivalents (TEQs) or concentrations of specific congeners of dioxin-like chemicals. The details of the exposure assessments conducted within individual studies are presented in this chapter, whereas generic issues of exposure assessment are discussed in Chapter 3 along with the special challenges involved in characterizing and reconstructing the herbicide exposures of Vietnam veterans.

This chapter presents study design and other important methodologic information on populations of Vietnam veterans, occupational cohorts, and

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environmentally exposed groups that have been reported on repeatedly, often for many health outcomes, as well as on case-control studies that have generated multiple epidemiologic publications relevant to the VAO series. This integrative approach has been taken to avoid repeating design information in multiple health-outcomes chapters and to make evident to the reader the extensive degree of interrelationship among many of the published analyses that have been reviewed in the course of the VAO series. If new results are based on updating information from or adding subjects to previously studied populations or use a subset of the original study population, then this synthesis considers the redundancy among studies while recognizing that separately reported information can impart new relevance to other data on a study population. Such clusters of studies are useful in describing the course of a population's response to an exposure, and joint consideration of an entire body of research on a population may yield insights into relationships with potential confounding factors. The various study designs have strengths and weaknesses that influence the evidentiary weight that they contribute, and these factors are addressed in the health-outcomes chapters. One-time reports on a study population that addressed only a single health outcome are not described in this chapter, but instead are described and critiqued in the sections of the report that discuss the results related to that particular health outcome.

Many of the cohorts that have contributed to the cumulative findings of the VAO committees, occupational cohorts in particular, are no longer being followed. For completeness, these cohorts are mentioned briefly in this chapter and, where relevant, in the body of this report. Additional detailed background information on them is available in the earlier volumes of the series. This chapter is intended to give a brief overview of those major cohorts and studies that have contributed to the evidence base of potential health outcomes that stem from exposure to the COIs and for which new information has become available on the incidence or prevalence of disease and other health outcomes since *Update 2014*. It is not intended to be a compendium of every study or population ever reviewed in the VAO series.

Many groups potentially exposed to the COIs have been monitored periodically, including the cohorts of the International Agency for Research on Cancer (IARC) and the National Institute for Occupational Safety and Health (NIOSH); residents of Seveso, Italy; and Ranch Hand and Army Chemical Corps (ACC) personnel who served in Vietnam. Discussions of the specific cohorts in this chapter include references both to publications discussed in previous VAO reports and to new publications. In drawing its conclusions, the committee combined the evidence in new publications and the evidence synthesized from *Update 2014*, taking into account the interdependence of related publications.

Individual researchers who belong to the research consortia that are evaluating cohorts in large multicenter studies (such as the IARC and NIOSH cohort studies) sometimes publish reports based on subsets of study participants who they themselves are monitoring. VAO committees consider all published reports, including those based on entire cohorts and those based on subcohorts.

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In drawing its conclusions, the committee factored in both types of studies, taking into consideration the interdependence among related studies. In particular, some subcohort studies have access to information not available for the entire cohort, such as data on individual serum TCDD concentrations and personal information that can be used to adjust for additional confounders. Furthermore, in the case of analyses based on an entire cohort that include data from a subcohort as a subset, using the reports on the subcohort as part of the evaluation might provide additional information on the consistency of the relationships among subcohorts, such as whether there are important subcohort-by-exposure interaction effects that were not considered in the full-cohort analysis. As long as the design and analysis methods of the study populations are recognized, VAO committees have been less concerned about over-weighting unstable positive findings based on small subgroups or giving "repeated consideration" to duplicative results than they would have been if a quantitative meta-analysis were being undertaken.

The chapter is organized to present the study populations in the order that roughly reflects the importance attributed to the data generated (Vietnam veterans, occupationally exposed workers, and people who have been environmentally exposed). It begins with Vietnam veterans and first covers studies conducted in the United States by the Air Force, the Centers for Disease Control and Prevention (CDC), the Department of Veterans Affairs (VA), and other groups, before describing studies of veterans from other countries (particularly Australia, New Zealand, and South Korea) who served in Vietnam. The section "Occupational Studies" covers studies of workers who, through employment or other work (other than military service), were exposed to the COIs and dioxin-like chemicals, including production workers, agriculture and forestry workers (including herbicide and pesticide applicators), and other groups (e.g., sawmill and paper and pulp workers). Again, studies of U.S. workers are presented before those of international cohorts. The section "Environmental Studies" covers studies of populations exposed to the COIs and dioxin-like chemicals from non-occupational sources and includes assessments of the general population, such as the National Health and Nutrition Examination Survey (NHANES), and also assessments of people who had unusually high exposures because of war-related or industrial sources in their residential neighborhoods (such as the residents of southern Vietnam; suburban Taichung, Taiwan; and Chapaevsk, Russia) or accidents (such as Seveso, Italy, and the Yusho poisoning in Western Japan). The chapter ends with a section that addresses the publications that are based on repeatedly mentioned case-control study populations; the case-control studies that assessed Vietnam-veteran status, however, are included in the section on veteran studies, and nested case-control studies are presented along with the cohorts from which they were derived.

Because of the breadth of literature reviewed in this chapter, Figure 5-1 provides the reader with a comprehensive overview of the individual study populations that have been reviewed in the course of the VAO series, although not all of these populations are discussed in this chapter or even in this volume.

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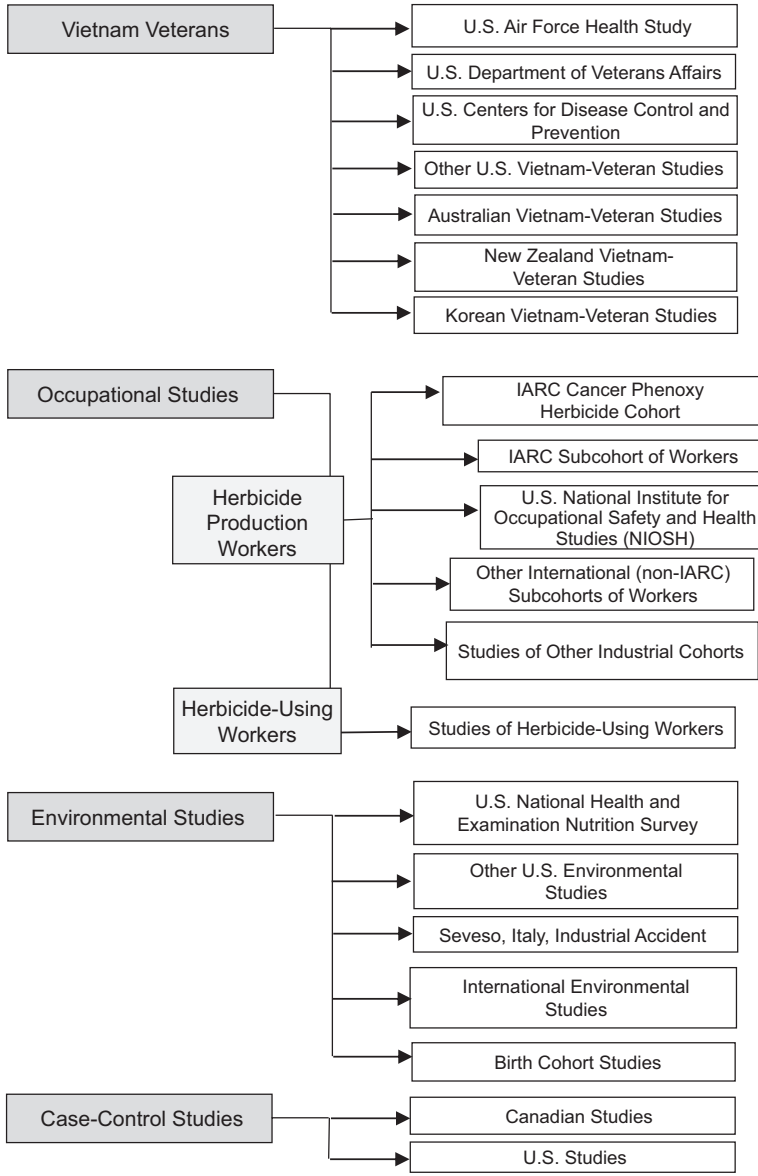

FIGURE 5-1 Overview of the individual study populations reviewed in the VAO series.

continued

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*Army Chemical Corps *Female Vietnam Veterans *Proportionate Mortality *VE-HEROeS *Other VA Studies
*National Vietnam Veterans Readjustment Study

*Birth Defects Study *Agent Orange Validation Study *Vietnam Experience Study *Selected Cancers Study

*American Legion Study *State-specific studies

*Australian Vietnam Veterans *Australian Vietnam Veterans Family Study *Sample of 1,000 Veterans
*Australian Conscripted Army National Service *Birth Defects in Australian Infants

*TCDD Concentrations in Korean Vietnam Veterans *Korean Vietnam Veterans Health Study
*Other Studies of Korean Vietnam Veterans

*Austria *Denmark *Germany *Great Britain *Netherlands *New Zealand

*NIOSH PCP Cohort *NIOSH Cross-Sectional Medical Study
*NIOSH TCDD Mortality Cohort: *Monsanto Mortality Study *Dow Herbicide Production Workers

*BASF Ludwigshafen Plant (Germany) *Czechoslovakia

*Waste Incineration (Japan, Korea) *Paper and Pulp Cohorts (United States, Finland, Denmark, IARC)
*Sawmill Workers (Canada, New Zealand) *Automobile Foundry Workers (China)
*Other Chemical Plants (Croatia, Germany, Italy, Russia, United Kingdom, United States)

*US: Agricultural Health Study, California United Farm Workers of America Study
*International Herbicide Users: *Argentina *Australia *Canada *Denmark *Finland *Germany *Iceland
*India *Italy *Mexico *Netherlands *New Zealand *Norway *Sweden

*Anniston, AL Community Health Survey *Times Beach and Quail Run Cohort *Iowa Women's Health Study
*Salinas Mothers and Children Cohort *Longitudinal Investigation of Fertility and the Environment Study

*Seveso Full Cohort *Seveso Women's Health Study

Country-specific Environmental Studies: *Belgium *Brazil *Canada *China *Denmark *Finland *France
*Germany *Greece *Hong Kong *Italy (other than Seveso) *Japan *Korea *Netherlands *Nicaragua
*Norway *Russia *Spain *Sweden *Taiwan *Vietnam

*Danish Fetal Origins 1988–1989 Cohort *Norwegian Mother and Child Cohort Study
*Duisburg (Germany) Birth Cohort *Hokkaido (Japan) Study on Environment and Children's Health
*Other birth cohorts and mother-child pair studies in Belgium, Denmark, Netherlands, Taiwan, and Vietnam

*Cross-Canada Study of Pesticides and Health

*National Birth Defects Prevention Study *Upper Midwest Health Study

FIGURE 5-1 Continued

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VIETNAM-VETERAN STUDIES

Studies of Vietnam veterans who might have been exposed to herbicides, including Agent Orange, have been conducted in the United States at the national and state levels and in Australia, South Korea, and New Zealand. Exposures have been defined in various ways, and health outcomes have been evaluated with reference to various comparison or control groups. This section is organized primarily by research organization or sponsor because it is more conducive to a methodical presentation of the studies. The means by which herbicide or dioxin exposures were characterized varies from the individually specified exposures of Ranch Hand and ACC personnel, as reflected in serum TCDD measurements, to the use of service in Vietnam as a surrogate for TCDD exposure in some studies.

Several comparison groups have been used for veteran cohort studies: Vietnam veterans who were stationed in areas where herbicide-spraying missions were unlikely to have taken place; Vietnam-era veterans who were in the military at the time of the conflict but did not serve in Vietnam; veterans who served in other wars or conflicts, such as the Korean War and World War II; and various state and national populations. In all of the studies of Vietnam veterans, whether or not the study participants were American, the study participants were the target population of the committee's charge, and they were assumed to have had a higher probability of exposure to the COIs than people who did not serve in Vietnam, regardless of whether there was any information on individual exposures beyond the mere fact that they were deployed to Vietnam.

The studies in the publication period considered in the present update examined a range of health outcomes among Vietnam veterans with service history from the United States as well as those from New Zealand. These included new analyses of the Air Force Health Study cohort (AFHS) (Landgren et al., 2015; Mazur et al., 2013, 2014), ACC personnel (Cypel et al., 2016), several publications from VA Medical Centers (Krishnamurthy et al., 2016; Nosrati et al., 2014; Ovadia et al., 2015), and a 20-year hospitalization study of New Zealand Vietnam veterans (Cox et al., 2015).

Air Force Health Study

Reports and findings from the AFHS have provided important information that has been incorporated throughout the VAO series, including the current volume, and it continues to be considered an important cohort in the committee's assessment of the overall evidence of exposure to the COIs and health outcomes. Active data collection from this cohort was completed in 2002, but VAO committees have remained interested in reviewing additional publications that provide longitudinal analysis of the vast amount of information assembled or that make use of the collection of preserved biologic samples. As yet, the few new published findings have been disease specific and not always focused on the effects of

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exposure to the herbicides, but have grouped all participants to examine outcomes related to aging (IOM, 2015).

Major defoliation activities in Vietnam were conducted by Air Force personnel as part of Operation Ranch Hand. Veterans who took part in the defoliation activities became the first subpopulation of Vietnam veterans to receive special attention with regard to herbicide exposure and have become known as the Ranch Hand cohort within the AFHS. To determine whether exposure to herbicides, including Agent Orange, had adverse health effects on these veterans, the Air Force made a commitment to Congress and the White House in 1979 to conduct an epidemiologic study of Ranch Hand personnel (AFHS, 1982). The study protocol had three components: a retrospective mortality study, a retrospective morbidity study, and a 20-year prospective follow-up study with longitudinal data and biospecimens collection. Details on the mortality and retrospective morbidity arms can be found in prior reports (IOM, 2006b, 2015). The prospective study arm has been the focus of multiple reports on a variety of health outcomes in the cohort as well as new research using these assets.

Prospective Study Design and Data Collection

Records from the National Personnel Records Center and the Air Force Human Resources Laboratory were searched and cross-referenced to identify all Ranch Hand personnel (AFHS, 1982; Michalek et al., 1990). The exact number of Ranch Hands varies among published reports, depending on the time frame of identification, but the most widely used estimate is 1,242, which reflects the number who served in Vietnam and who were not killed in action. Ultimately, however, not all of those who have been identified actually participated in the AFHS (some were deceased before the study began, others were unlocatable, and a small number refused to participate) (IOM, 2015). A comparison population of 24,971 C-130 crew members and support personnel who served in the U.S. Air Force between 1962 and 1971 and were assigned to duty in Southeast Asia but who were not occupationally exposed to tactical herbicides (AFHS, 1983, 1984a) was selected from the same data sources. Each Ranch Hand was matched to a pool of 8 to 10 comparisons, who were selected based on the first living and compliant person randomly selected from the individual-level pool. Individual comparison participants remained associated with their matched Ranch Hand for the duration of the study, but those who died, dropped out, or were lost to follow-up were replaced with the next best comparable control who was living and agreed to participate (AFHS, 1982). Comparison participants were individually matched for age, type of job (differentiated into five categories: officer/pilot, officer/navigator, officer/other, enlisted/flight engineer, and enlisted/other), and race (white or not white) to control for possible differences in the development of chronic disease that may relate to age, race, or educational and socioeconomic status (AFHS, 1984a). To control for the many potential confounders related to the physical and

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psycho-physiologic effects of combat stress and the Southeast Asia environment, Ranch Hands were matched to control participants who performed similar combat or combat-related jobs (AFHS, 1982). Comparisons were assumed to be similar to the Ranch Hands regarding lifestyle, training profiles, and socioeconomic factors. Although not representative of the U.S. population, the sample was diverse in terms of socioeconomic status and educational background (IOM, 2015).

The prospective follow-up consisted of six comprehensive exams that began with the baseline exam in 1982 and occurred thereafter in years 3, 5, 10, 15, and 20 of the study (the final physical exam was conducted in 2002 and final analysis for the formal AFHS study was completed in 2006). Morbidity was ascertained through comprehensive questionnaires and accompanying physical examination, which included more than 200 laboratory and clinical tests (although the number and type of laboratory tests performed at each physical examination changed over time, reflecting changes in science and technology). Questionnaire data included information relating to demographics; employment; child and family health; health habits; recreation, leisure, and physical activities; toxic exposures; military experience; and wartime herbicide exposure. Data collected during the physical examinations included indices of health status that encompassed general health and endpoints by major organ system. Additional sources of data collected in the course of the AFHS included medical records from the participants' physicians, dentists, and other health providers; vital status records, such as birth and death certificates; information on the participants' families, including spouses and children who were under 18 years old at the time of the exam; and military administrative records that contained duty station orders, flight records, performance reports, awards and decorations, and discharge documents (IOM, 2015). The number of Ranch Hand and comparison participants who completed the questionnaire and physical exam differed at each follow-up. In all, 2,758 individuals participated in at least one exam cycle.

Over the course of the AFHS, more than 91,000 unaliquoted biospecimens were collected as part of the physical exam component. The number of samples varied by type of specimen, participant, and cycle. Although some samples were collected as part of the laboratory testing and work-ups, additional biospecimens samples were collected from study participants at each exam cycle and preserved to be used for future analyses. Serum and urine were collected longitudinally across multiple cycles, while semen and whole blood were collected at a single exam cycle. Adipose tissue was collected from a subset of individuals at one exam cycle. To obtain samples for a TCDD assay carried out by the CDC on 777 Ranch Hand and 1,174 matched comparisons, a separate blood draw was performed during cycles 3–6 (see discussion on estimating exposure in the cohort, below). Multiple blood samples were drawn for a subset of the population for use in dioxin biological half-life and other studies.

Results have been published for baseline morbidity (AFHS, 1984a) and baseline mortality (AFHS, 1983) and for reproductive outcomes (AFHS, 1992;

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Michalek et al., 1998a,c; Wolfe et al., 1995). Mortality updates have been published for 1984–1986, 1989, and 1991 (AFHS, 1984b, 1985, 1986, 1989, 1991b). An interim technical report updated cause-specific mortality in Ranch Hands through 1993 (AFHS, 1996). Michalek et al. (1998b) and Ketchum and Michalek (2005), respectively, reported on 15-year and 20-year follow-ups of post-service mortality in the Ranch Hand veterans, updating an earlier cause-specific mortality study by Michalek et al. (1990). Many analyses presented in the voluminous reports on the follow-up examinations of 1984, 1987, 1992, 1997, and 2002, which are cited as AFHS (1987, 1990, 1995, 2000, 2005), have been deemed not useful for the purposes of the VAO reviews because they were limited to comparisons of data on those in the cohort who were still alive and participated in a particular examination.

Exposure Estimation

As described in Chapter 2, the results of biologic-marker studies of Ranch Hand personnel have been consistent with their being exposed, as a group, to TCDD. When the Ranch Hand cohort was classified by military occupation, a higher level of serum TCDD was detected in people whose jobs involved more frequent handling of herbicides (AFHS, 1991a). The exposure index initially proposed in the AFHS relied on military records that documented spraying missions of TCDD-containing herbicides (Agent Orange, Agent Purple, Agent Pink, and Agent Green) as reported in the Herbicide Reporting System tapes for the period starting in July 1965. For exposure before July 1965, exposure information would be based on military procurement records and dissemination information. In 1991 the record-based exposure index was compared with the results of the Ranch Hand serum-TCDD sampling conducted on personnel at least 10 years after their service in Vietnam. The exposure index and the TCDD serum levels, which the authors referred to as body burden, correlated weakly.

Blood samples for use in determining serum TCDD concentrations were drawn at the periodic examinations conducted in 1982 (cycle 1) from 36 Ranch Hands (Pirkle et al., 1989), in 1987 (cycle 3) from 866 Ranch Hands (AFHS, 1991a), in 1992 (cycle 4) from 455 Ranch Hands (AFHS, 1995), and in 1997 (cycle 5) from 443 Ranch Hands (AFHS, 2000). For veterans whose TCDD was not measured in 1987 but was measured later, the later measurement was extrapolated to 1987 by using a first-order kinetics model with a constant half-life of 7.6 years.

Michalek et al. (1995) developed several indexes of herbicide exposure of members of the Ranch Hand cohort and tried to relate them to the measurements of serum TCDD from 1987 to 1992. Self-administered questionnaires completed by the Ranch Hand veterans were used to develop several indexes of herbicide or TCDD exposure: the number of days of skin exposure, the percentage of skin area exposed, and the product of the number of days of skin exposure, the percentage

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of skin exposed, and a factor for the concentration of TCDD in the herbicide. A fourth index, which used no information gathered from individual study participants, was calculated by multiplying the volume of herbicide sprayed during a person's tour of duty by the concentration of TCDD in herbicides sprayed in that period and then dividing the product by the number of crew members in each job specialty at the time.

Each of the four indexes tested was significantly related to serum TCDD concentrations, although the models explained only 19% to 27% of the variability in serum TCDD concentrations. Days of skin exposure had the highest correlation. Military job classification (for example, Ranch Hand combat troops, Ranch Hand administrators, Ranch Hand flight engineers, and Ranch Hand ground crew), which is not included in any of the four indexes, explained 60% of the variability in serum TCDD. When the questionnaire-derived indexes were applied within each job classification, days of skin exposure added statistical significance, but not substantially, to the variability explained by job alone.

Ranch Hands were divided into three categories on the basis of their potential exposure:

- *Low potential*. Pilots, copilots, and navigators. Exposure was primarily through preflight checks and spraying missions.
- *Moderate potential*. Crew chiefs, aircraft mechanics, and support personnel. Exposure could occur by contact during de-drumming and aircraft loading operations, onsite repair of aircraft, and repair of spray equipment.
- *High potential*. Spray-console operators and flight engineers. Exposure could occur during operation of spray equipment and through contact with herbicides in the aircraft.

At times, other metrics of exposure were also applied. For example, rank was used as a surrogate of exposure because officers (pilots, copilots, and navigators) were unlikely to handle the herbicides.

Pavuk et al. (2014) analyzed serum concentrations of TCDD and dioxin-like chemicals (i.e., polychlorinated dibenzo-*p*-dioxins [PCDDs], polychlorinated dibenzofurans [PCDFs], and PCBs) from samples collected in 2002 from 777 Ranch Hand and 1,173 comparison subjects. In addition, the results were compared with serum samples from 436 age- and gender-matched adults from the NHANES research initiative. The main findings showed that median serum TCDD levels were more than two times higher in the Ranch Hand veterans than in either the AFHS comparison veterans or the NHANES comparison group. However, the absolute values of serum TCDD levels, as well as the group differences in median serum TCDD levels, were substantially lower than results from prior serum samples collected in 1987. For the other dioxin-like chemicals, the concentrations in 2002 were similar in all three groups. These data demonstrate the unique TCDD signature experienced from herbicide exposure in Vietnam and

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indicate that, over time, the elimination rate is higher than the ongoing intake rate from background exposure to TCDD in both groups of AFHS veterans.

Analyses of the serum TCDD readings were included in the 1987 examination report (AFHS, 1991a). Other Ranch Hand publications have addressed the relationship between serum TCDD and reproductive hormones (Henriksen et al., 1996); diabetes mellitus, glucose, and insulin (Henriksen et al., 1997); skin disorders (Burton et al., 1998); preterm birth and infant death (Michalek et al., 1998a); sex ratios (Michalek et al., 1998c); skin cancers (Ketchum et al., 1999); insulin, fasting glucose, and sex-hormone-binding globulin (Michalek et al., 1999a); immunologic responses (Michalek et al., 1999b); diabetes mellitus (Longnecker and Michalek, 2000; Steenland et al., 2001); cognitive function (Barrett et al., 2001); hepatic abnormalities (Michalek et al., 2001b); peripheral neuropathy (Michalek et al., 2001c); hematologic results (Michalek et al., 2001a); psychologic functioning (Barrett et al., 2003); correlations between diabetes and TCDD elimination (Michalek et al., 2003); thyroid function (Pavuk et al., 2003); cancer incidence (Akhtar et al., 2004; Pavuk et al., 2005); insulin sensitivity (Kern et al., 2004); prostate cancer (Pavuk et al., 2006); serum testosterone and the risk of benign prostate hyperplasia (Gupta et al., 2006); and diabetes and cancer incidence (Michalek and Pavuk, 2008).

The tendency of the AFHS researchers to use different cutpoints and population definitions for analogous analyses suggests that they used an a posteriori selection that may have influenced the results. For example, Michalek and Pavuk (2008) allude to the commonly held assumption that Agent Orange was more heavily contaminated earlier in the war as the motivation for making various temporal partitions in their analyses, but the choices were not consistent among studies. With respect to the development of cancer, service in 1968 or earlier was considered to have been in the critical exposure period, whereas for diabetes, the critical exposure period was considered to be 1969 or earlier. Additionally, the construction of low- and high-exposure variables based on "days of spraying" was done differently for cancer than it was diabetes. Days of spraying were grouped into 30-day blocks for cancer, and into blocks of 90 or more days for diabetes.

Impact of the AFHS on VAO Reviews

Ostensibly, the AFHS was designed to answer exactly the question that the VAO series is asking: Is exposure to the herbicides used during the Vietnam War associated with long-term health outcomes or outcomes in the offspring of exposed veterans? The AFHS is perceived by many to be the central piece of research for decision making by the VAO committees because it used longitudinally collected data and objective, quantifiable measures of TCDD exposure through serum samples, on a population that was directly exposed to the COIs in the Vietnam theater. Unlike many other studies of Vietnam veterans, data on alcohol

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use and smoking status were collected and included in the analysis when they were known risk factors for the outcome of interest. However, the AFHS also has important limitations that all VAO committees have had to consider.

Although the study was carefully designed to match Ranch Hand and comparison subjects to minimize bias to the extent possible, the AFHS population is likely not representative of the entire population of Vietnam veterans, so its findings might not be generalizable to all Vietnam veterans. The 1987 TCDD serum assay found the comparison subjects to also have elevated levels of TCDD, although the exposure was significantly higher in the Ranch Hand group than in the comparison group. Therefore, the comparison is not an ideal exposed-versus-unexposed comparison but rather a high-exposure-versus-low-exposure comparison (IOM, 2006b, 2015). The exposure in the comparison group might also make the study findings vulnerable to bias toward the null if the difference in exposures between the AFHS group and the comparison group was not large enough to allow an association between exposure and outcome to be detected. That problem does not affect the validity of positive findings, however. Similarly, for AFHS analyses that used non-AFHS Vietnam veterans as the comparison group, those individuals might also have been exposed to the COIs, which would likewise influence results toward the null.

The AFHS might be underpowered for detecting small effects, especially rare outcomes, because of its relatively small sample size. Therefore, its findings are vulnerable to false negatives (failure to detect an important association). This also raises questions about the stability of positive findings; this is somewhat less of a problem if the findings are repeated over examination cycles, although the results of the examination cycles themselves are not fully independent repetitions.

Three new studies have been published using AFHS data since *Update 2014*. In the first study, Landgren et al. (2015) used data and serum samples from 479 Ranch Hands and 479 comparison veterans to examine the association between serum TCDD levels and the presence of monoclonal gammopathy of undetermined significance (MGUS). After the model had been adjusted for several demographic and clinical factors, Ranch Hands were found to have a 2.4-fold increased risk for MGUS. This study is the first to correlate an objective measurement of levels of TCDD exposure with MGUS and additional detail of it is found in Chapter 7. The second study used AFHS data from 991 AFHS participants to examine the relationship between testosterone levels and the levels of fasting glucose and, therefore, a diagnosis of type 2 diabetes (Mazur et al., 2014). The authors found that low testosterone levels in men were an independent risk factor (comparable to aging and obesity) for high fasting glucose and, therefore, that testosterone was a weak predictor of a diagnosis of type 2 diabetes. In a second publication based on AFHS data, Mazur et al. (2013) used data from the same 991 AFHS subjects to examine the relationship of obesity to individual and population-level declines in testosterone. Over 20 years of follow-up, mean testosterone levels declined at least twice as much as would have been

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expected from cross-sectional estimates of the decline usually associated with aging. However, because neither of the Mazur studies considered exposure status or TCDD levels in the analysis, these studies were not considered to be relevant to the committee's charge even though they were conducted using AFHS data.

Department of Veterans Affairs

Army Chemical Corps Cohort

Analyses of members of the ACC were conducted by VA, whose other research efforts on Vietnam veterans are discussed together below. Like the Ranch Hand personnel, ACC personnel performed chemical operations involving the direct handling and distribution of herbicides, but instead of using planes, they performed these tasks on the ground and by helicopter in Vietnam. Nearly 1,000 men serving in ACC units were deployed to Vietnam between 1966 and 1971. ACC members were responsible for the storage, preparation (handling and mixing), and application of herbicides, tear gas, and napalm among other chemicals. ACC units were also tasked with cleaning and maintenance of the equipment used to prepare and apply the chemicals (Thomas and Kang, 1990). Because the ACC personnel were expected to have been highly exposed to herbicides, VAO committees recommended studying this important group of Vietnam veterans (IOM, 1994) and later encouraged the publication of the study's findings (IOM, 2005). The availability of serum TCDD concentrations in a subset of this cohort of Vietnam veterans has made its findings particularly useful in appraising possible associations with various health outcomes. The primary strengths and limitations of the ACC studies are similar to those of the AFHS.

ACC service members were belatedly identified using morning reports for all ACC units known to have been assigned to Vietnam between 1966 and 1971 for a study of health effects and mortality related to herbicide exposure (Thomas and Kang, 1990). However, the findings of increased risk of certain outcomes, such as digestive diseases, were based on small numbers of cases and cannot be associated with particular exposures since serum samples or other objective measures of exposure were not collected. In an extension of that study, Dalager and Kang (1997) compared the mortality of ACC veterans who deployed to Vietnam with the mortality of those who did not. For that study, ACC veterans were identified using a combination of the same morning reports for ACC units stationed in Vietnam as used by Thomas and Kang (1990), Defense Manpower Data Center tapes of Vietnam-era Army personnel with a military occupational specialty code indicating a chemical operations position between 1971 and 1974, and class rosters for chemical courses conducted at the Army Chemical School at Fort McClellan, Alabama, from 1965 to 1972. Analyses compared cause-specific mortality among Vietnam-deployed ACC men (n = 2,872) and those who never deployed to Southeast Asia (n = 2,737), and also compared each group to the

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standardized U.S. population. Because all men who served in ACC units were stationed at Fort McClellan for at least some time, and Fort McClellan is in close proximity to Anniston, Alabama, where Monsanto operated a plant that produced PCBs, all ACC veterans were likely exposed to at least low levels of these and other chemicals. Therefore, comparisons using deployed and non-deployed ACC men are likely to be biased toward the null due to this baseline of increased exposure, but this bias is not the case for analyses that used the standardized U.S. population as the comparison group.

The results of an initial feasibility study that measured serum dioxin levels in a subset of Vietnam-deployed and nondeployed ACC veterans were reported by Kang et al. (2001). The researchers recruited 565 veterans: 284 Vietnam veterans and 281 non-Vietnam veterans as controls. Blood samples were collected in 1996 from 50 Vietnam veterans and 50 control veterans, and 95 of the samples met CDC standards of quality assurance. A comparison of both groups showed that the geometric mean TCDD concentrations did not differ between groups ($p = 0.6$). The 50 Vietnam-deployed veterans were then stratified into those who sprayed herbicides and those who did not, based on self-reported information. The sprayers had higher TCDD concentrations than those who reported no spraying activities. The authors concluded that Agent Orange exposure was a likely contributor to TCDD concentrations in Vietnam veterans who had a history of spraying herbicides.

Following the 2001 feasibility study Kang et al. (2006) reported the findings from the larger study of this population of ACC veterans. A health survey was administered by telephone to 1,499 Vietnam-deployed veterans and 1,428 non-Vietnam-deployed veterans. Exposure to herbicides was assessed by analyzing serum specimens from a sample of 897 veterans for dioxin. Consistent with the findings from the feasibility study, veterans who reported spraying herbicides had significantly higher TCDD serum concentrations than veterans who did not report herbicide spraying.

Having determined the vital status—that is, whether an individual was alive or had died—of the ACC personnel through 2005, Cypel and Kang (2010) presented results on mortality from the following causes: cancers (oral and pharyngeal, digestive, respiratory, prostate, testicular, skin, brain, and lymphopoietic [leukemia]), diabetes, circulatory conditions (hypertension and cerebrovascular), respiratory conditions (pneumonia, influenza, and chronic obstructive pulmonary disease), and cirrhosis of the liver. The study compared 2,872 ACC personnel who served in Vietnam with 2,737 ACC personnel who did not serve in Vietnam, using survival analysis that controlled for race, age at entry into follow-up, rank, and duration of military service. It also compared 662 ACC personnel who served in Vietnam and reported spraying herbicides with 811 who did not serve in Vietnam and did not report spraying herbicides, controlling for additional covariates of body mass index (BMI) and smoking status obtained in the telephone survey. Mortality in both cohorts was also compared with the expected mortality

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in U.S. males. Concerns were raised over the lack of adjustment for smoking status in the analysis of respiratory diseases in Vietnam-deployed veterans and non-Vietnam-deployed veterans. (The subcohort analyses that compared sprayers with nonsprayers were adjusted for smoking status.)

In a new study of ACC veterans, Cypel et al. (2016) analyzed the results of a 2013 survey of 3,086 ACC veterans that compared prevalence of self-reported, physician-diagnosed hypertension in both Vietnam-deployed and non-Vietnam-deployed herbicide sprayers and non-sprayers. This study, which is reviewed in Chapter 10, reports that the mean serum TCDD level in ACC Vietnam-service sprayers was 4.3 parts per trillion (ppt) (lipid based) as compared with a mean level of 9.5 ppt in AFHS participants.

U.S. Female Vietnam Veterans Cohort

Although estimates have varied, the most recent estimates from VA are that 7,500 U.S. women served in Vietnam between August 1964 and May 1975 (VA, 2017a). The vast majority of them served as combat nurses—mostly in the Army Nurse Corps—but some also served in the Women's Army Corps and the Air Force, Navy, and Marine Corps (Spoonster-Schwartz, 1987; Thomas et al., 1991; VA, 2017a).

In 1986, Public Law (PL) 99-972 was enacted. It required that an epidemiologic study be conducted to examine the long-term adverse health effects on female Vietnam veterans who had exposure to traumatic events, exposure to herbicides such as Agent Orange or other chemicals or medications, or any other related experience or exposure during such service. The first study that VA conducted to assess mortality in female Vietnam veterans was by Thomas et al. (1991). No comprehensive record of female personnel who served in Vietnam in 1964–1972 existed, so the researchers gathered military service data from each branch of the armed forces through December 31, 1987. Female Army and Navy personnel were identified from morning reports and muster rolls of hospitals and administrative support units where women were likely to have served. Military personnel were identified as female by their names, leaving open the possibility that some women may have been inadvertently excluded from the analysis. Women who served in the Air Force and Marine Corps were identified through military records. The combined roster of all female personnel from the military branches was considered by the researchers to be generally complete. A comparison group of female veterans was identified through the same process as the women who served in Vietnam but the comparison group had not served in Vietnam during their military service. Demographic information and information on overseas tours of duty, unit assignments, jobs, and principal duties were abstracted from military records. Mortality information was obtained from VA's Beneficiary Identification Records Locator Subsystem, the Social Security Administration, the Internal Revenue Service, the National Death Index (NDI),

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and military personnel records. Women whose service in the military fell outside the period of interest, whose records were missing data, or who served in Southeast Asia but not in Vietnam were excluded. The analysis included 132 deaths among 4,582 female Vietnam veterans and 232 deaths among 5,324 comparison veterans who served in the military from July 4, 1965, to March 28, 1973, which was when combat operations occurred. Cause-specific mortality was derived for both groups of veterans and compared with mortality in U.S. women with adjustments for race, age, and calendar period. Dalager et al. (1995a) updated mortality in the original cohort through December 31, 1991, using the same study protocol as Thomas et al. (1991). After updating the mortality figures and adjusting the existing cohort on the basis of new information about the study groups based on the inclusion criteria, an additional 4 Vietnam-deployed veterans and 1 comparison veteran were included in the final study population (Dalager et al., 1995a).

Updates of mortality among women Vietnam veterans have been published periodically. Cypel and Kang (2008) conducted a mortality study of female veterans who deployed to Vietnam, comparing them with a control group of women veterans matched on rank and military occupation who were in the military at the same time period but who were not deployed to Vietnam. Kang and colleagues (2014a) updated total and cause-specific mortality analyses of female U.S. Vietnam-era veterans through December 31, 2010, using the same sources to determine vital status as were used by Thomas et al. (1991) and Dalager et al. (1995a). For deaths that occurred before 1992, the cause of death was ascertained from official death certificates. For deaths occurring on or after January 1, 1992, cause-of-death information was obtained from NDI Plus, which codes the cause of death by the *International Classification of Diseases* (ICD) system. The underlying causes of death were formally assigned by a qualified nosologist. This mortality update was structured as a retrospective cohort study consisting of three study groups of female veterans who served during the Vietnam era using the same dates as Thomas et al. (1991) and Dalager et al. (1995a). The first group included 4,734 female veterans who served in Vietnam, the second group consisted of 2,062 female veterans who served near Vietnam, and the third group included 5,313 female veterans who did not deploy outside of the United States. Mortality comparisons were made using either the non-deployed U.S. cohort or women of the U.S. general population, adjusted for age, race, and calendar year, as the reference group. Of the total sample of 12,109 female veterans, 2,743 (23%) were deceased by the study end date of December 31, 2010, and the cause of death was available for 96.2% of the deaths. The adjusted total mortality and heart-disease-specific rates were lower in the female Vietnam veterans than in the U.S. Vietnam-era female veterans or in the U.S. general population. The cancer mortality rate was approximately equal between the female Vietnam veterans group and both the U.S. cohort of female veterans and the U.S. general population. When the analysis was constrained to nurses only (approximately two-thirds of the study cohort), higher adjusted mortality rates for pancreatic, brain, and

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other nervous system cancers were reported for the female Vietnam veterans. Whereas all reports from the female U.S. Vietnam-veteran cohort provide direct information on the health and mortality status of female military personnel who served in Vietnam, the limitations of the results must be kept in mind. Specifically, female veterans likely experienced low herbicide exposure because they were not involved in applying herbicides or engaged in direct combat, and their in-country tours of duty were generally limited to 1 year and at fixed locations that were not in proximity to known defoliated areas. In summary, this analysis does not provide evidence of a higher risk of total or cause-specific mortality in female Vietnam-deployed veterans compared with non-deployed female Vietnam veterans and the U.S. general population. The suggestion of higher rates of mortality from pancreatic, brain, and other nervous system cancers among Vietnam nurse veterans should be cautiously interpreted, given the study's limitations and the large number of causes of mortality examined.

VA also published studies of pregnancy outcomes and gynecologic cancers—namely, neoplasms of the cervix, uterus, and ovary—in U.S. female Vietnam veterans (Kang et al., 2000a,b). Army veterans were identified from a list obtained by the Army and Joint Services Environmental Support Group; computerized lists were also provided by the Air Force, Navy, and Marine Corps. Military-service data were abstracted from personnel records. Of 5,230 eligible veterans, 4,390 with a documented tour of duty in Vietnam were alive on January 1, 1992. From a pool of 6,657 women whose military units did not serve in Vietnam, 4,390 veterans who were alive on January 1, 1992, were randomly selected as controls. After the research group excluded 250 veterans and 250 nonveterans who participated in a pilot study as well as those who could not be located (n = 370), who were deceased (n = 339), or who declined to participate (n = 775, 13% of Vietnam veterans and 17% of non-Vietnam veterans), 6,430 women completed a full telephone interview, and another 366 women completed only a short, written questionnaire. The information collected included demographic background, general health, lifestyle, menstrual history, pregnancy history, pregnancy outcomes, and military experience, including nursing occupation and combat exposure. Information on pregnancy risks and complications—including smoking, infections, medications, exposure to X-rays, occupational history, and exposure to anesthetic gases, ethylene oxide, herbicides, and pesticides—was collected for each pregnancy. In Kang et al. (2000a), for each woman veteran, the first pregnancy following the beginning of Vietnam service was designated as the index pregnancy. For the comparison group, the first pregnancy after July 4, 1965, was designated as the index pregnancy. The study analyzed data on 3,392 Vietnam and 3,038 non-Vietnam veterans and on 1,665 Vietnam and 1,912 non-Vietnam veteran index pregnancies. In Kang et al. (2000b), a self-reported history of gynecologic cancers (defined by the authors as cancers of the breast, ovary, uterus, and cervix) was collected. The authors attempted to "retrieve hospital records on all reported cancers as far back as 30 years." Of records successfully

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found, 99% of the breast cancer and 90% of all cancer diagnoses were confirmed. The authors did not provide specific data on diagnosis confirmation for the three sites other than the breast, but they stated that Vietnam status was not associated with a greater likelihood of finding confirmatory medical records.

After the publications by Kang et al. (2000a,b), Congress passed PL 106-419, which provides compensation for the children of female Vietnam veterans who are born with birth defects unrelated to an existing familial disorder, to a birth-related injury, or to a fetal or neonatal infirmity with a well-established cause. The legislation covers 18 birth defects, including cleft lip or palate, congenital heart disease, hypospadias, neural-tube defects, and Williams syndrome. A complete list of covered birth defects can be found in 38 CFR 3.815.

Proportionate-Mortality Cohort

Among the earliest reports on health outcomes in Vietnam veterans was a proportionate-mortality study by Breslin et al. (1988). The participants were Army and Marine Corps ground troops (all men) who served at any time from July 4, 1965, through March 1, 1973. A list of 186,000 Vietnam-era Army and Marine Corps veterans who were reported deceased as of July 1, 1982, was assembled from VA's Beneficiary Identification Records Locator Subsystem. From this list, 75,617 individuals were randomly selected for inclusion in the study. The information extracted from the selected military records included duty stations, dates of tours, branch of military service, date of birth, sex, race, military occupation specialty codes, education level, type of discharge, and confirmation of service in Vietnam. Additional information was extracted on veterans who served in Southeast Asia, including the first and last dates of service in Southeast Asia, the military unit, and the country where the veteran served. For the final sample of Army and Marine Corps veterans, the cause of death was ascertained from death certificates or Department of Defense (DoD) Report of Casualty forms for 24,235 men who served in Vietnam and 26,685 men who did not serve in Southeast Asia. Each veteran's cause of death was coded by a nosologist who used ICD-8. Exposue to herbicides or other environmental factors was not considered in the analysis. Deaths from external causes (accidents, poisonings, and violence) were slightly eleveated among Vietnam veterans who served in the Army but not among marines who served in Vietnam. Death from any cancer was elevated among marines who served in Vietnam but not Army veterans. When examined by type of cancer, proportionate mortality ratios were not elevated for Army veterans, but proportionate mortality ratios for lung and non-Hodgkin lymphoma (NHL) were elevated among the Marine Corps veterans.

Using the proportionate-mortality cohort assembled by Breslin et al. (1988), Burt et al. (1987) conducted a nested case-control study of NHL with controls selected from among the cardiovascular-disease deaths. Although unrecognized at the time of that publication, using cardiovascular deaths as the control group

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biased estimates of NHL toward the null because some cardiovascular diseases, such as hypertension, are associated with exposure to herbicides, see Chapter 10. When all Army and Marine Corps veterans who were deployed to Vietnam were compared with veterans who did not serve in Vietnam, no excess of mortality from NHL was found. When stratified by service branch and age, only marines with combat roles or who served in Vietnam from 1967 to 1969 (when herbicide spraying was greatest) had statistically significant increased odds of death from NHL. In a follow-up of the Breslin et al. (1988) study, Bullman et al. (1990) compared cause-specific proportionate mortality of 6,668 Army I Corps Vietnam veterans, who served in the northernmost part of South Vietnam in a combat zone designated as Military Region I by the U.S. military, with 27,917 Army Vietnam-era veterans who had not served in Vietnam. The subjects studied by Bullman et al. included the study population identified by Breslin et al. and an additional 9,555 Army Vietnam-era veterans whose deaths were identified after VA's Beneficiary Identification Records Locator Subsystem database was searched for mortality data through December 31, 1984. Deaths from external causes (accidents, poisonings, and violence) were found to be slightly eleveated among Army I Corps Vietnam veterans, particularly deaths attributed to motor vehicle accidents and accidental poisonings. Similarly, Watanabe et al. (1991) updated the Vietnam-veteran mortality experience reported by Breslin et al. (1988) by extending the follow-up from January 1, 1982, to December 31, 1984. An additional 11,325 deceased Army and Marine Corps Vietnam-era veterans were identified from the period and included in the study. The study population for Watanabe et al. consisted of 62,068 military veterans, of whom 29,646 served in Vietnam and 32,422 never served in Southeast Asia. Proportionate-mortality ratios were calculated for three referent groups: branch-specific (Army and Marine Corps) non-Vietnam veterans, all non-Vietnam veterans combined, and the U.S. male population. Deaths from external causes were again statistically significantly elevated among Vietnam-deployed marines compared with non-Vietnam veterans and Army veterans who served in Vietnam compared with Army veterans who did not serve in Vietnam and all non-Vietnam veterans. Cancer of the larynx was statistically significantly higher among Vietnam-deployed Army veterans than either non-Vietnam Army veterans or all non-Vietnam veterans but lung cancer was only significantly different for Army Vietnam veterans compared with all non-Vietanm veterans. Deaths from lung cancer, NHL, and Hodgkin disease were all statistically significantly elevated for Marine Corps veterans who had served in Vietnam compared with marines who had not served in Vietnam only. A third follow-up proportionate-mortality study (Watanabe and Kang, 1996) used the veterans from Breslin et al. (1988) and Watanabe et al. (1991) and included an additional 9,040 randomly selected Vietnam-era veterans who died from July 1, 1984, through June 30, 1988. The final study included 70,630 veterans—33,833 who had served in Vietnam and 36,797 who had never served in Southeast Asia. The analyses were performed using the same referent groups described in Watanabe

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et al. (1991). Just as in the previous analyses of mortality, Army and Marine Corps Vietnam veterans had statistically significant excesses of deaths from external causes. Army Vietnam veterans had statistically significant excesses of deaths for laryngeal cancer and lung cancer when compared to both Army non-Vietnam veterans and all non-Vietnam veterans. Results showing statistical significance for Marine Corps Vietnam veterans varied according to the referent population used (non-Vietnam marine veterans or all non-Vietnam veterans). When compared with non-Vietnam marine veterans, marine Vietnam veterans had significantly elevated proportionate mortality ratios for deaths from NHL, Hodgkin disease, and cancers of the pancreas, lung, and skin. Deaths from circulatory diseases were statistically significantly lower among Marine Corps Vietnam veterans than marines who did not serve in Vietnam and all non-Vietnam veterans. Marine Corps Vietnam veterans also had significant excesses for lung cancer and skin cancer compared with all non-Vietnam veterans. Proportionate mortality ratios for deaths due to respiratory and digestive diseases were statistically significantly lower among marine Vietnam veterans than all non-Vietnam veterans. Compared with the standardized U.S. population, deaths from many of the major categories of organ systems were statistically significantly lower among both the Vietnam-deployed and non-deployed veterans. However, cancers overall were higher among the Vietnam-deployed and non-deployed Army veteran groups and the Marine Corps non-Vietnam veteran group. Lung cancer deaths were significantly higher among both Army veteran groups and the Marine Corps Vietnam-deployed group compared with the U.S. population. Using the U.S. population for comparison, proportionate mortality ratios were statistically significantly higher for prostate cancer in Army non-deployed veterans and skin cancer for Marine Corps veterans that served in Vietnam.

National Vietnam Veterans Readjustment and Longitudinal Studies

In response to concerns about the health and well-being of Vietnam veterans, in 1983 Congress passed PL 98-60, which directed VA to contract for an independent national study looking at the frequency and effects of posttraumatic stress disorder (PTSD) and related postwar psychological issues. In 1984 the contract for the National Vietnam Veterans Readjustment Study was awarded to the Research Triangle Institute (RTI). Several publications resulted from that work (Currier and Holland, 2012; Schlenger et al., 2015a; Yager et al., 2016) but given the focus on mental health, most are not relevant to the VAO series.

In 2000 Congress passed PL 106-419, which directed VA to contract for a follow-up study, the National Vietnam Veterans Longitudinal Study, to determine the effects of PTSD over a 25-year period and postservice adjustment. The study was awarded to Abt Associates (VA, 2018b). A total of 1,450 veterans participated in at least one of the study phases of the National Vietnam Veterans Longitudinal Study, which included a self-administered paper health questionnaire,

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a computer-assisted telephone health interview, and a telephone mental health interview conducted by a professional clinical interviewer (Marmar et al., 2015). Results from this second effort have been recently published (Marmar et al., 2015; Schlenger et al., 2015b, 2016; Steenkamp et al., 2017). But because the focus of this research is mental health outcomes, the results are not considered by VAO update committees. The data collected from veterans who participated in both the National Vietnam Veterans Readjustment Study and the National Vietnam Veterans Longitudinal Study are now part of the National Vietnam Veterans Longitudinal Study Registry, which was acquired in 2015 and is housed at VA's Seattle Epidemiologic Research Information Center in Seattle, Washington (VA, 2018a). However, as noted previously, VAO committees do not consider mental health conditions in their review of health outcomes of Vietnam veterans.

Most recently, VA has undertaken a new research initiative to assess the current health of Vietnam veterans who served on the ground, Blue Water Navy veterans, and veterans who served elsewhere during the Vietnam Era (1961–1975). The Vietnam Era Health Retrospective Observational Study (VE-HEROeS) is designed to compare the overall health, lifestyle characteristics, and aging-related conditions of these veterans with similarly aged U.S. residents who never served in the military. The committee invited the lead investigator, Dr. Victoria Davey, to discuss the project. The study was designed to compare a retrospective cohort of Vietnam veterans, with all service branches represented, with Vietnam-era veterans who were deployed to countries other than Vietnam, Cambodia, or Laos and with members of the U.S. general population who never served in the military. Recruitment was based on a sampling frame that was constructed to be a randomly selected sample of persons from VA's database of all veterans (both users of VA health care or benefits and those not using VA services). The questionnaire collected information on the following topics: military service (combat experience, chemical and other exposures, re-entry into civilian life, or no military service), general health (neurologic conditions, infections, presumptive conditions, cancer, hypertension, and mental health conditions), experience with aging, lifestyle factors (tobacco use, health care use, living arrangements), and health experiences of descendants (nine questions on birth defects and other conditions of children and grandchildren). A medical records review is being conducted of a small subset of participants (n = 4,000) to validate the questionnaire information (Davey, 2017). The data are being analyzed, and although some abstracts and posters containing a few findings have been submitted to VA research and outside conferences, no results had been published in the peer-reviewed literature as of January 31, 2018.

Other VA Studies

VA also conducted studies that focused on specific health outcomes, using data from VA's Agent Orange Registry, a computer database containing health information on Vietnam veterans who voluntarily undergo examinations in a

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VA hospital. This registry was established in 1978 to monitor health complaints or problems of Vietnam veterans that potentially could be related to herbicide exposure during their military service in Vietnam, but it was not intended to be a research program (Dick, 2015). The registry was established also as a means to facilitate increased access of Vietnam veterans to the VA health care system. Veterans are eligible to participate if they had any active military service in the Republic of Vietnam between 1962 and 1975 and express a health concern related to herbicide exposure. Beginning in 2011, eligibility has been expanded to include veterans who served along the Korean Demilitarized Zone between 1968 and 1971, veterans who served in certain units in Thailand, and veterans who were involved in the testing, transporting, or spraying of herbicides for military purposes (Dick, 2015). The examinations that these veterans undergo consist of an exposure history (based on self-reports that are not verified by DoD records), a medical history, laboratory tests if indicated, and an examination of the organ systems most commonly affected by toxic chemicals. The quality, consistency, and usability of data from this registry—and indeed from all registries with voluntary participation that rely on self-reported information—are limited. As of July 6, 2017, this registry contained information on 676,774 veterans who had undergone an initial examination and there had been 79,846 follow-up evaluations, for a total of 756,620 examinations (VA, 2017b). Some VA investigators have used participation in the registry as a surrogate of herbicide exposure (Clemens et al., 2014; Nosrati et al., 2014; Ovadia et al., 2015).

Other VA analyses have relied on self-reported exposure to herbicides (Beard et al., 2016, 2017; Krishnamurthy et al., 2016; Le et al., 2016; Q. Li et al., 2013). Two other VA studies (Baumann Kreuziger et al., 2014; Mescher et al., 2018) determined exposure to Agent Orange (or presumably other herbicides) by reviewing claims of exposure submitted by veterans that were reviewed by VA benefits and compensation officers who used service records to confirm that locations and timeframes of deployment corresponded to sprayed areas.

VA has evaluated specific health outcomes, including carrying out case-control studies of soft-tissue sarcoma (STS) (Kang et al., 1986, 1987), NHL (Dalager et al., 1991), Hodgkin lymphoma (HL) (Dalager et al., 1995b), testicular cancer (Bullman et al., 1994), and lung cancer (Mahan et al., 1997). Each study created and applied a different measure of surrogate herbicide exposure because objective measures, such as serum TCDD analyses, were not available. Other VA studies included a post-service mortality analysis (separate from the proportionate mortality cohort) of marines that compared those who served in Vietnam (n = 10,716) with Vietnam-era marines (n = 9,346) (Watanabe and Kang, 1995), and a small case-control study that compared dioxin and dibenzofuran concentrations in the adipose tissue of 36 Vietnam veterans and 79 non-Vietnam veterans and a sample of U.S. men born in 1936–1954 (Kang et al., 1991). All tissue samples were archived specimens from the EPA's National Human Adipose Tissue Survey and

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had been collected by hospitals and medical examiners from men who died from external (non-combat) causes or surgical procedures.

VA has examined other outcomes in Vietnam veterans: PTSD (Bullman et al., 1991; Goldberg et al., 1990; True et al., 1988), suicide and motor-vehicle crashes (Bullman and Kang, 1996; Farberow et al., 1990), tobacco use (McKinney et al., 1997), and self-reported physical health (Eisen et al., 1991). The studies have been included for completeness, but the outcomes that they address are outside the purview of this committee. *VAO* and *Update 1998* discussed them in detail; most did not deal with exposure to herbicides specifically, and the exposure to "combat" was evaluated as the risk factor of interest. As noted VA has also initiated a study to update the mortality experience of Vietnam veterans. This update is expected to update the rates, causes, and patterns of overall and cause-specific mortality from 1979 through 2014 of all Vietnam veterans compared with all Vietnam-era veterans and the general U.S. population (Davey, 2017).

Centers for Disease Control and Prevention Studies

Surveys of U.S. Vietnam veterans who were not part of the Ranch Hand or ACC groups indicated that 25% to 55% believed they were exposed to herbicides (CDC, 1989b; Erickson et al., 1984a,b; S. D. Stellman and J. M. Stellman, 1986). Several attempts have been made to estimate the exposures of Vietnam veterans who were not part of the Ranch Hand or ACC groups. CDC has undertaken a series of studies to examine various health outcomes in Vietnam veterans as directed by Congress in the Veterans Health Programs Extension and Improvement Act of 1979 (PL 96-151) and the Veterans' Health Care, Training, and Small Business Loan Act of 1981 (PL 97-72). The first of these CDC studies was a case-control interview study of birth defects in the offspring of men who served in Vietnam, which included developing an exposure opportunity index to score herbicide exposure (Erickson et al., 1984a,b).

Agent Orange Validation Study

The CDC Agent Orange study (CDC, 1985) was initiated in response to a 1983 request by the U.S. government to conduct a study of possible long-term health effects in Vietnam veterans exposed to herbicides. The study attempted to classify veterans' service-related exposures to herbicides by determining the proximity of troops to herbicide spraying through the use of military records to track troop movement and the use of the HERBS tapes[1] to locate herbicide-spraying patterns.

[1]The HERBS tapes were a digital record of herbicide spray missions compiled by the Department of Defense in the early 1970s. Information on them is presented in the original *VAO* report (IOM, 1994).

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In 1987, CDC conducted the CDC Agent Orange Validation Study to test the validity of the various indirect methods used to estimate the exposure of ground troops to herbicides in Vietnam. The study measured serum TCDD in a non-random sample of Vietnam veterans and in Vietnam-era veterans who did not serve in Vietnam (CDC, 1988b). Vietnam veterans were selected for the study on the basis of the number of herbicide exposure events that they were thought to have experienced, based on the number of days their unit was within 2 kilometers and 6 days of a recorded herbicide-spraying event. Blood samples were obtained from 66% of 646 Vietnam veterans and from 49% of the eligible comparison group of 97 veterans. More than 94% of those whose serum was obtained had served in one of five battalions.

The median serum TCDD concentration in Vietnam veterans in 1987 was 4 ppt (range: < 1 to 45 ppt, but only two veterans had concentrations above 20 ppt). The "low" exposure group consisted of 298 Vietnam veterans, the "medium" exposure group 157 veterans, and the "high" exposure group 191 veterans. The distribution of TCDD measurements was nearly identical to that in the control group of 97 non-Vietnam veterans. The CDC validation study concluded that study participants could not be distinguished from controls on the basis of serum TCDD. In addition, neither record-derived estimates of exposure nor self-reported exposure to herbicides could predict which Vietnam veterans would have high serum TCDD at the time of the study (CDC, 1988b, 1989a). The report concluded that it was unlikely that military records alone could be used to identify a large number of veterans who might have been heavily exposed to TCDD in Vietnam. The serum TCDD measurements in Vietnam veterans also suggested that the exposure to TCDD in Vietnam was substantially lower, on average, than that of persons exposed as a result of the industrial explosion in Seveso, Italy, or that of the heavily exposed occupational workers who have been the focus of many of the studies evaluated by VAO committees. The assessment of average exposure does not eliminate the possibility that some Vietnam veterans had heavy exposures.

Vietnam Experience Study

Using exposure estimates from the Agent Orange Validation Study, CDC conducted the CDC Vietnam Experience Study, a historical cohort study of the health experience of Vietnam veterans (CDC, 1989b). The study was divided into three parts: physical health, reproductive outcomes and child health, and psychosocial characteristics (CDC, 1988a,c,d). CDC examined post-service mortality (through 1983) in a cohort of 9,324 U.S. Army veterans who served in Vietnam and in 8,989 Vietnam-era Army veterans who served in Germany, Korea, or the United States (Boyle et al., 1987; CDC, 1987). In other studies using the data collected from the Agent Orange Validation Study, O'Brien et al. (1991) combined the mortality and interview data to identify veterans who developed

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NHL. Decoufle et al. (1992) evaluated the influence of self-reported exposure to herbicides on self-reported adverse health outcomes. Finally, in a follow-up of CDC's Vietnam Experience Study cohort, Boehmer et al. (2004) reported findings on mortality from 1965 through 2000.

Selected Cancers Study

The CDC Selected Cancers Study (CDC, 1990a) was designed to investigate the effects of military service in Vietnam and of exposure to herbicides on the health of American veterans, with a specific focus on the risk of developing NHL (CDC, 1990b), STS and other sarcomas (CDC, 1990c), HL (CDC, 1990d), and nasal, nasopharyngeal, and primary liver cancers (CDC, 1990d).

Other U.S. Vietnam-Veteran Studies

American Legion Study

The American Legion, a voluntary service organization for veterans, conducted a cohort study of the health and well-being of Vietnam veterans who were members. The studies examined physical health and reproductive outcomes, social-behavioral consequences, and PTSD in veterans who had served in Southeast Asia and elsewhere (Snow et al., 1988; J. M. Stellman et al., 1988; S. D. Stellman et al., 1988a,b). No additional studies have been published on the cohort.

State Studies

Several states have conducted studies of Vietnam veterans, most of which have not been published in the scientific literature. The VAO and Update 1996 committees reviewed studies of veterans of Hawaii (Rellahan, 1985), Iowa (Wendt, 1985), Maine (Deprez et al., 1991), Massachusetts (Clapp, 1997; Clapp et al., 1991; Kogan and Clapp, 1985, 1988; Levy, 1988), Michigan (Visintainer et al., 1995), New Jersey (Fiedler and Gochfeld, 1992; Kahn et al., 1988, 1992a,b,c), New Mexico (Pollei et al., 1986), New York (Greenwald et al., 1984; Lawrence et al., 1985), Pennsylvania (Goun and Kuller, 1986), Texas (Newell, 1984), West Virginia (Holmes et al., 1986), and Wisconsin (Anderson et al., 1986a,b). Chamie et al. (2008) examined the association between herbicide exposure and prostate cancer in Vietnam-era veterans using the VA health system in northern California; the reliability of this study of about 13,000 men is limited by its reliance on self-reported exposure status and by the exclusion of prostate cases diagnosed before 1998, before computerized records became available. No additional single-state studies have been identified.

Grufferman et al. (2014) evaluated the role of parental military service in Vietnam and service-related exposures and the risk of rhabdomyosarcoma in

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offspring using data from the Intergroup Rhabdomyosarcoma Study Group clinical trial, which included hospitals in 46 U.S. states and the District of Columbia from 1982 to 1988.

Australian Vietnam-Veteran Studies

The Australian government has commissioned a number of studies to follow the health outcomes of Australian veterans who served in Vietnam. Although the Australians did not participate in herbicide spraying, there is a possibility that they may have been exposed to the herbicides if stationed or passing through areas that were sprayed.

Australian Vietnam Veterans

The Australian Vietnam veterans study population corresponds to the cohort defined by the Nominal Roll of Vietnam Veterans, which lists Australians who served on land or in Vietnamese waters from May 23, 1962, to July 1, 1973, including military and some non-military personnel of both sexes. People who served in any branch of service in the defense forces and citizen military forces (such as diplomatic, medical, and entertainment personnel) were considered. The comprehensive studies, however, are limited to male members of the military, and most of the analyses focus on men in the defense forces—the Army (41,084), the Navy (13,538), and the Air Force (4,570). One investigation examined the possibility of an association between Vietnam service and cancer incidence by comparing diagnoses from 1982 to 2000 among male Vietnam veterans with those in the general population of Australia (ADVA, 2005a). The results in that report supersede those reported by the Australian Department of Veterans' Affairs (CDVA 1998a). Morbidity in female Vietnam veterans had also been studied (CDVA, 1998b). Additional case-control studies of the incidence of adrenal gland cancers, leukemias, and NHL were conducted in this population (AIHW, 1999, 2000, 2001).

There have been several studies of mortality among Australian Vietnam veterans (CDVA, 1997; CIH, 1984a,b,c; Crane et al., 1997a,b; Evatt, 1985; Fett et al., 1987a,b; Forcier et al., 1987). The latest one (ADVA, 2005b), which considered the causes of death of men in all branches of service through 2001 compared with that of the general Australian population and reported by branch of service, supersedes the others.

Australian Vietnam Veterans Family Study

The Australian Department of Veterans' Affairs has published four large volumes that summarize the results of studies conducted among family members of Vietnam-era veterans (ADVA, 2014a,b,c,d). The purpose of this study was to

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better understand the long-term impacts of service on the health and welfare of the families of Australian Vietnam veterans. The first volume (2014a) provided an overview of the entire effort. The second (2014b) assessed the health of the family members with more emphasis placed on the details of psychological and social well-being, rather than adverse impacts on physical health. The third (2014c) investigated mortality among members of the veterans' families, while the final volume (2014d) discussed qualitative information gathered in the course of the entire study. Although responses were collected on spouses and partners of the veterans, the analyses focused on outcomes reported by the children of the veterans. The wide range of outcomes examined for the family members themselves included mental health outcomes, pregnancy and birth defect outcomes, physical health, social functioning, and mortality. Because many of the health outcomes reported for these family members are not central to the charge of the committee (e.g., mental health and social functioning), minimal consideration was given to these publications.

From the roster of Australian Vietnam veterans, more than 10,000 Australians who had served in the Vietnam War were randomly selected and contacted, along with their family members, for potential participation in the study. The Vietnam veterans who were identified and ultimately selected included 3,940 who were randomly selected and 2,569 who self-selected into the study based on media publications announcing that the study would be conducted.

The primary comparison group consisted of family members of non-deployed Vietnam-era personnel. These personnel comprised 3,967 randomly selected non-deployed era veterans and 418 who self-selected into the study. Thus, there were far more Australian Vietnam veterans who self-selected into the study than non-deployed Australian Vietnam-era veterans who self-selected, and the percentage of the Vietnam veterans who self-selected was much higher than the percentage of non-deployed Vietnam-era veterans who self-selected. In total, the family members of Vietnam veterans included 2,199 sons and daughters, of whom 1,385 were examined for pregnancy and birth defect–related outcomes.

When there is no specific exposure information provided, the VAO series has considered these comparisons of deployed versus non-deployed groups to cover potential exposure to all the COIs and thus the most relevant measures for their task. Such contrasts, however, also cover all aspects of the deployment experience, and in this set of Australian studies there was considerably more concern about the psychological effects on the veterans (especially PTSD) and their secondary impact on the veterans' family members, which would not be expected to be an effect of herbicide exposure. Some analyses have been conducted among all study participants, and some analyses were stratified by the type of enrollment (random versus self-selected). The committee fully recognized the potential reporting biases that may have emanated from the self-selected cohort, and thus it placed considerably more weight on the results derived for the randomly selected cohort, as did the researchers themselves.

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Australian Conscripted Army National Service

The Australian Conscripted Army National Service study population is a subset of the veterans considered in the overall Australian Vietnam Veterans study group. The 19,240 conscripted male Army veterans deployed to Vietnam ("National Service" veterans) were compared with 24,729 non-deployed counterparts ("National Service non-veterans"). The results on death and cancers in the Australian conscripted Army National Service veterans (ADVA, 2005c) supersede those of earlier internal comparisons of deployed and non-deployed Vietnam War–era National Service veterans (CIH, 1984a,b,c; Crane et al., 1997b; Fett et al., 1987a,b). Those government-sponsored studies of Australian Vietnam veterans did not characterize the veterans' exposure to the herbicides sprayed in Vietnam beyond the fact that they had served on land or in Vietnamese waters from May 23, 1962, through July 1, 1973. It is the convention of VAO committees to regard Vietnam veterans in general as being more likely to have received higher exposures to the COIs than the general public, but ideally that assumption should have been validated by more objective measurements of exposure, such as serum measurements, in a sample of Australian Vietnam veterans.

Sample of 1,000 Australian Vietnam Veterans

O'Toole et al. (1996a,b,c) studied a broad spectrum of health issues in a random sample of 1,000 Australian Vietnam veterans (both regular enlisted and conscripted Army National Service members) selected from Australia's comprehensive roster of 57,643 service members who were deployed to Vietnam. In wave 1, conducted in 1990–1993, 641 members of the sample were located and interviewed. In wave 2, conducted in 2005–2006, O'Toole et al. (2009) obtained responses from 450 (51.4% of those not known to have died). A total of 391 veterans responded to both waves. The Australian Bureau of Statistics' National Health Survey was administered in both waves, and additional data were collected on combat experience, PTSD, and general psychiatric status. The veterans' self-reported health status was compared with that of the general male Australian population gathered during the government's administration of the same survey in 1989–1990 and 2004–2005; it is not clear that this instrument was administered to the two groups under comparable conditions. The low response rates make the findings vulnerable to nonresponse bias, and the self-report measures of health conditions might be of low validity and subject to recall bias. The committee for *Update 2010* was skeptical about the reliability of the nearly uniform findings of statistically increased prevalence of nearly 50 health conditions.

O'Toole et al. (2010) reported on mortality in the sample through 2004 as related to previously gathered information on psychosocial factors that are not within the scope of VAO reviews. It is of interest, however, that they found that 11.7% of the veterans in the sample had died by the end of 2004. Additional

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publications using the survey results from waves 1 and 2 have been published on the course of combat-related PTSD (O'Toole and Catts, 2017) and the intergenerational transmission of PTSD in the offspring of these veterans (O'Toole et al., 2017). However, as mental health outcomes related to combat cannot easily be teased apart from any potential effects of herbicide exposure, such publications are not reviewed in depth.

Birth Defects in Australian Infants

The Australian government sponsored a case-control study of 8,517 infants with congenital anomalies born in 1966–1979 at 34 hospitals in New South Wales, Victoria, and in the Australian Capital Territory; the infants were matched by period of birth, mother's age, hospital, and means of hospital payment to live-born infants without diagnosed birth defects (Donovan et al., 1983, 1984; Evatt, 1985). The fathers of infants in both groups were identified and their names compared with those on the roster of men who had served in the Australian Army in 1962–1972; additional means of verification were used to determine whether a child's father was in the Army during this interval (329 cases and 338 controls) and also whether the father was deployed to Vietnam (127 cases and 123 controls). After adjustments were made for maternal age, infant sex, multiple births, and father's place of birth, conditional logistic regression was used to compare the Vietnam veterans (National Service or regular Army) to other era veterans and to all other fathers for all birth anomalies and for seven diagnostic groups.

Korean Vietnam-Veteran Studies

Military personnel of the Republic of Korea served in Vietnam from 1964 through 1973. Studies of the health of these personnel have been pursued by several researchers.

Study of TCDD Concentrations in Korean Vietnam Veterans

J. S. Kim et al. (2001) attempted to use serum dioxin concentrations to validate an index for estimating group exposure. The study involved 720 veterans who served in Vietnam and 25 veterans who did not. The exposure index was based on herbicide-spraying patterns in military regions where Korean personnel served, time and location data on the military units stationed in Vietnam, and an exposure score derived from self-reported activities during service. A total of 13 pooled samples were submitted to CDC for serum dioxin analyses. One analytic sample was prepared from the pooled blood of the 25 veterans who did not serve in Vietnam. The remaining 12 samples were intended to correspond to 12 exposure categories; each was created by pooling blood samples from 60 veterans. The 12 exposure categories ultimately were reduced to four exposure groups, each

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representing a quartile of 180 Vietnam veterans but characterized by only three serum TCDD measurements.

The paper by J. S. Kim et al. (2001) reported highly significant Pearson correlation coefficients and results of multiple logistic-regression analysis. The statistical analyses apparently were based on the assignment of the pooled serum dioxin value to each individual in the exposure group. The multiple regression analysis evaluated such variables as age, BMI, and consumption of tobacco or alcohol. In a later report on the same exposure groups and serum dioxin data, the authors corrected their analysis (J. S. Kim et al., 2003). A correlation was observed between serum dioxin concentrations and ordinal exposure categories, but the correlation was not statistically significant. The authors attributed the lack of statistical significance to the small sample size, and they noted that the data exhibited a distinct monotonic upward trend; the average serum dioxin concentrations were 0.3, 0.6, 0.62, 0.78, and 0.87 pg/g (lipid-adjusted) for, respectively, exposure categories 0 through 4. The decision to pool blood samples from a large number of persons in each exposure set (J. S. Kim et al., 2001) greatly reduced the power of the validation study. Instead of 180 samples in each of the final exposure categories, the pooled analysis produced only three samples in each category. The lipid-adjusted serum TCDD concentrations in the 12 pooled samples from Vietnam veterans ranged from 0.25–1.2 pg/g, whereas the single sample from the non-Vietnam veterans contained 0.3 pg/g. The narrow range of results makes the biologic relevance of any differences questionable.

Thus, it appears that there was not a clear separation in terms of TCDD levels between Korean Vietnam veterans and non-Vietnam veterans. Furthermore, the range of mean values in the four Vietnam veteran exposure categories was narrow, and all concentrations were relatively low (less than 1 pg/g). The relatively low serum dioxin concentrations observed in the 1990s in those people are the residuals of substantially higher initial concentrations, as has been seen in other Vietnam veteran groups. However, the concentrations reported in the Korean veterans study are significantly lower than those reported in American Vietnam veterans in the 1988 CDC Agent Orange Validation Study, which was nonetheless unable to distinguish Vietnam veterans from non-Vietnam veterans on the basis of serum dioxin (CDC, 1988b). The Korean authors were able to construct plausible exposure categories based on military records and self-reporting, but they were unable to validate the categories with serum dioxin measurements.

Korean (Vietnam) Veterans Health Study

Six publications have been reviewed from an exceptionally large epidemiological study of more than 180,000 Korean Vietnam veterans, denoted herein as the "Korean study." It is much larger in scope than all of the other published epidemiological studies conducted among Vietnam veterans. The Korean study provides results for a very large set of health outcomes, including rare conditions,

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as well as non-fatal outcomes and cause-specific mortality. The research methodology used in the Korean study was very carefully evaluated by the Update 2014 committee (the first committee to examine these publications) because the study used multiple methods of exposure ascertainment and health outcome ascertainment. The careful evaluation was done so that across all health outcomes, committee members would weigh the results from the Korean study in a consistent manner and take into account the strengths and limitations from this large body of data.

For the Assessment of the Potential Exposure to Herbicides Publications on the Korean study have relied on multiple methods for the exposure assessment (referred to imprecisely in the Yi articles as Agent Orange). First, a self-report perceived exposure index was used to query Korean veterans as to how they might have been exposed to herbicides in Vietnam (Yi et al., 2013a,b). Responses to six questions on a postal survey were used to derive a four-tiered categorization of self-perceived herbicide exposure (Yi et al., 2013a); see Table 5-1. The initial four-tier scale (high, moderate, low, and none) was further compressed into simply "high" or "low" for many analyses. The perceived herbicide exposure estimates were highly correlated with the health outcomes, indicating the possibility of recall bias.

In the second method, an objective exposure opportunity index (EOI) score of exposure potential was calculated for each veteran based on the proximity of the veteran's military unit to herbicide-sprayed areas (Yi et al., 2014a,b). The Korean investigators obtained locations and calendar date histories for the military units represented in their cohort and used this information as input to obtain EOI scores from its model (J. M. Stellman et al., 2003b), which consolidates all the temporal and spatial information gathered from the original military records on the herbicide spray missions conducted in Vietnam. The investigators classified the resulting EOI scores using two- and four-group categorizations

TABLE 5-1 Distribution of Perceived Herbicide Exposure Among 114,562 Korean Vietnam Veterans[a]

2 Groups	Exposure Questions	4 Groups	Prevalence
High	1. Sprayed herbicides	High	16.1%
	2. Handled herbicide spray equipment		
	3. Present during herbicide spraying	Moderate	35.7%
	4. Got herbicide on skin or clothing		
Low	5. Walked through sprayed area	Low	13.2%
	6. Exposed in other ways (not listed above)		
	Answered "no" to all six questions	None	34.9%

[a]Exposure assigned based on their self-reported responses to a postal survey.
SOURCE: Adapted from Yi et al., 2013a.

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and multiple aggregations of military units. The initial four-tier scale (high, moderate, low, and none) was further compressed into simply "high" or "low" for many analyses, the details of which are presented in Tables 5-2 and 5-3. The self-reported perceived exposures are not directly comparable to the objective EOI scores, which were designed to assess the exposure opportunity that would result from unintended proximity to herbicide spraying and not the direct result of duties that required handling or applying herbicides (IOM, 2008).

TABLE 5-2 Distribution of EOI Scores on Two-Level Scale in Epidemiology Studies Among Korean Vietnam Veterans[a]

Exposure Category (log_{10} EOI Score)	Yi et al. (2014a)[b] Division/Brigade or Battalion/Company (n = 111,726)[d]	Yi et al. (2014b)[c] Division/Brigade (n = 180,251)[e]
Low (< 4.0)	62.0%	52.4%
High (≥ 4.0)	38.0%	47.6%

[a]Details of the two-level exposure classification is described in the Ohrr et al. (2006, publication in Korean).

[b]Battalion/company level EOI score assigned for combat units only.

[c]The overall range of log EOI scores in Yi et al. (2014b) is 0.0–5.8.

[d]Yi et al. (2014a), 111,726 veterans analyzed for disease prevalence.

[e]Yi et al. (2014b), 180,251 veterans analyzed for cancer outcomes.

SOURCES: Adapted from VAO Update 2014 (NASEM, 2016a).

TABLE 5-3 Distribution of EOI Scores on Four-Level Scale[a]

Source	Yi et al. (2013a)		Yi et al. (2014a)	
Exposure Category (log_{10} EOI score)	Division/ Brigade (n = 96,126)[b]	Battalion/ Company (n = 96,126)	Division/Brigade or Battalion/ Company[c] (n = 111,726)[d]	Division/ Brigade (n = 180,251)[e]
None (< 0.1)	20.1%	26.1%	30.9%	25.2%
Low (0.1 ≤ EOI < 4.0)	28.2%	33.1%	31.2%	27.2%
Medium (4.0 ≤ EOI < 5.0)	31.1%	21.5%	20.1%	28.3%
High (≥ 5.0)	20.6%	19.3%	17.9%	19.3%

[a]Details of the four-level exposure classification is described in Ohrr et al. (2006, publication in Korean).

[b]Yi et al. (2013a), 96,126 veterans analyzed for self-reported disease prevalence. Log (EOI score) mean and range not reported.

[c]Battalion/company level EOI score assigned for combat units only.

[d]Yi et al. (2014a), 111,726 veterans analyzed for disease prevalence. Log (EOI score) mean 2.6 ± 2.2 (range 0.0–6.2).

[e]Yi et al. (2014b), 180,251 veterans analyzed for cancer outcomes. Log (EOI score) range 0.0–5.8.

SOURCE: Adapted from VAO Update 2014 (NASEM, 2016a).

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In developing the EOI scores, military units were aggregated at two levels: the larger brigade/division level and the smaller battalion/company level. The distributions of EOI scores are similar across the Korean study publications, regardless of military unit aggregation. The Update 2014 committee noted that the proportion of veterans in the "high" exposure category may be too large and the individuals too similar to the lower categories to detect the true strength of associations between exposure and adverse health conditions. The committee proposed that an exposure classification that put only the top 10% or 15% of individuals in the "high" category would have been better for the purpose of identifying adverse health effects due to exposure.

The Korean study overcame significant logistical challenges in applying the EOI model to a large-scale epidemiologic study of a broad spectrum of health effects. The Update 2014 committee found that although there were likely sources of error in the EOI method for modeling herbicide exposures of Vietnam veterans, there was no indication of systematic bias in the rank ordering of exposure scores developed by this method. Furthermore, since nondifferential misclassification of exposure would bias measures of association toward the null, the observed statistically significant relationships found between EOI scores and health effects are likely to be real.

For the Assessment of the Health Outcomes of Interest As with exposure assessment, multiple methods were used to ascertain health outcomes in the Korean study. First, veterans self-reported all current and physician-diagnosed diseases. The diseases were classified into seven groups of diseases: cancers, circulatory diseases, respiratory diseases, digestive diseases, neuromuscular diseases, endocrine diseases, and other diseases. Within the major disease groups, self-reporting was further provided for 17 cancers (including stomach cancer, liver cancer, and lung cancer), 13 circulatory diseases (including hypertension, myocardial infarction, and angina), 5 respiratory diseases (including chronic bronchitis and emphysema), 6 digestive diseases (including gastritis and peptic ulcer), 4 neuromuscular diseases (including central nervous system disorders and peripheral neuropathy), 2 endocrine diseases (diabetes and hypothyroidism), and 4 other diseases (including renal failure and skin disease).

Second, incidence data for the specific types of cancer reported in the cohort were obtained from the Korean Cancer Incidence Database (1992–2003) and classified according to ICD-10.

Third, prevalent cases of individual disease conditions were identified by extracting claims data from the Korea National Health Insurance service during the period January 1, 2000, to September 30, 2005. Data on health outcomes were also obtained through a review of medical care covered directly by the Korean government through the Veterans Health Service during the same period. The health outcomes that were examined included the prevalence of endocrine

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diseases (ICD-10 E00–E90), neurologic diseases (G00–G99), circulatory diseases (I00–I99), respiratory diseases (J00–J99), and digestive diseases (K00–K93).

Fourth, vital status of Korean Vietnam veterans and the underlying causes of deaths were ascertained by use of the 1992–2005 death records of the National Statistical Office. Categories included all causes of death, 23 specific cancers, and 36 specific causes other than cancer.

Using these multiple methods for exposure classification and health outcome ascertainment, associations between metrics of herbicide exposure potential and health outcomes were derived. First, in some analyses, the health experiences of Korean Vietnam veterans, as a function of their exposure status, was compared to the health status of age-matched adults in the Korean general population. This method is known as an "external" control group. Second, some analyses were performed among Korean Vietnam veterans with the lowest herbicide exposure classification serving as the comparison group. This method is known as an "internal" control group.

The above variations in exposure assessment, health outcome ascertainment, and the use of internal and external comparison groups have significant implications for the appropriate interpretation of results from the Korean study. In considering these variations, the committee kept in mind the following methodological principles and empirical observations:

1. Whereas self-reported exposure may be reliable and valid in some research circumstances, it is generally considered less reliable and valid than objectively obtained estimates of exposure (Zajacova and Dowd, 2011). The potential for recall bias is of particular concern, and the likelihood of this bias occurring increases with the length of time from the potential exposure to the incidence of disease.
2. In acquiring health outcome data, objective sources (e.g., a cancer registry or health claims system) are generally preferred over self-report outcome data, assuming that the objective source of outcome data is largely comprehensive.
3. For morbidity and mortality analyses, the estimation and validity of relative risk may be more prone to bias when an external control group is used (e.g., the general population) than when an internal control group is used (Monson, 1990). This may be due to the "healthy warrior" effect. That is, in order to be accepted to military service and deploy, members must meet a high standard of general and physical fitness, whereas the general population includes some individuals of poor health. Using an internal control group, so long as the veteran groups are similar or adjusted for potential confounding variables, alleviates concerns of bias due to the healthy warrior effect.
4. Relative risk estimates that are only slightly above (e.g., 1.1) or below (e.g., 0.9) the null value of 1.0 may achieve statistical significance because of the large number of subjects in the study population, but may

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still reflect bias (e.g., selection or confounding bias) and be of less clinical significance than relative risk estimates of larger magnitude.

Therefore, when reviewing results within and across publications from the Korean study, the Update 2014 committee gave very limited overall weight to self-reported exposure data and self-reported health-outcomes data compared to objective measurements of the chemicals and health outcomes of interest. Also, more weight was given to the relative risk estimates of mortality and cancers derived from the use of an internal control group than from the use of the general population in order to minimize concern about a healthy warrior effect. Finally, less weight was afforded to statistically significant associations close to the null value (e.g., ranging from 0.9 to 1.1) than to those further from the null in order to account for differences of questionable clinical significance arising from this large study's statistical power and to account for modest selection bias and confounding.

Brief Reviews of Individual Publications on the Korean Veterans Health Study

No new publications on the Korean study were identified for the current update. Relevant results from these publications are presented in each of the applicable health outcome sections under the heading of *Conclusions from VAO and Previous Updates*. However, to avoid redundancy, each of the publications is reviewed here, with a focus on the methods used.

In Yi (2013), a total of 185,265 Korean men, who had served in Vietnam from 1964 to 1973 and who were alive in 1992 were followed for cancer incidence from 1992 to 2003. Cancer diagnoses were ascertained via linkage with the Korean National Cancer Incidence Database, whereas cancer deaths were identified using National Statistical Office records. Cancer incidence and cancer mortality were not examined in terms of the veterans' herbicide exposure during military service in Vietnam. Age-adjusted incidence and standardized incidence ratios were calculated using the Korean male population during 1992 to 2003 as the reference population (Yi, 2013). The overall cancer incidence among Vietnam veterans was not higher than in the general male population. However, when the incidence was analyzed by cancer type, Vietnam veterans and subgroups of the study population classified by military rank (enlisted; non-commissioned officer; officer) experienced a higher incidence of several cancers, including prostate cancer, T-cell lymphoma, lung cancer, bladder cancer, kidney cancer, and colon cancer, than the general Korean population. In Yi et al. (2013a), exposure of 114,562 Korean Vietnam veterans was assessed using two methods, as previously described in the section For the Assessment of the Potential Exposure to Herbicides. The first method was perceived self-report herbicide exposure in which veterans were categorized as having either "low" or "high" perceived exposure.

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The second method used to estimate herbicide exposure was more objective and based on the proximity of each veterans' military unit to herbicide-sprayed areas using the EOI model developed by J. M. Stellman et al. (2003b) and veterans were again classified using "high" and "low" group exposure categorizations. Associations were reported between self-reported diseases and high versus low exposures. All disease outcomes were based on self-report and classified into seven groups of diseases: cancers, circulatory diseases, respiratory diseases, digestive diseases, neuromuscular diseases, endocrine diseases, and other diseases. Subtypes of disease were reported for each disease condition. The Update 2014 committee was concerned about the reliability of self-reported exposure and health data because the use of such data in Yi et al. (2013a) uniformly yielded highly significant statistical associations across an exhaustive spectrum of disease conditions, while the use of the objective EOI method of exposure classification and documented reports of adverse health outcomes in the later publication on this population produced more variable results. The observation of inconsistencies when theoretically more reliable measures of health and exposure were analyzed reinforced the concern about the findings based on self-report in the Korean study.

Yi et al. (2013b) examined the serum levels of TCDD in 102 of these Korean Vietnam veterans for three purposes:

1. to assess their use as a potential objective tool for herbicide exposure;
2. to determine their correlation to self-reported exposure (six-item questionnaire); and
3. to evaluate how they related to age, BMI, and smoking.

In 2002 serum samples were collected, and a health examination was performed. For an objective assessment of herbicide exposure, EOI scores were again derived using the model by J. M. Stellman et al. (2003b), and veterans were classified as low versus high exposure or in four categories consisting of none, low, moderate, or high exposure. The serum TCDD concentrations among the Korean Vietnam veterans were lower than those reported in other studies of Korean and U.S. Vietnam veterans, and concentrations were not associated with herbicide exposure indices or with age, BMI, or smoking. The net value of this study is the observation that the assessment of serum levels of TCDD among veterans long after service in Vietnam (e.g., 40 years or more) may be of very limited value as a metric for herbicide exposure unless individuals were exposed to very high levels during military service.

Yi and Ohrr (2014) examined the incidence of cancer among 180,251 Korean Vietnam veterans from 1992 through 2003 using EOI scores for exposure assessment. The incidence of cancer was determined through a review of records from the Korea National Cancer Incidence Database. Overall, the veterans classified with high exposure had a small yet statistically significant higher risk of cancer

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than the veterans classified with low exposure. Compared with low exposure, high herbicide exposure appeared to be most related to an elevated risk of cancers of the mouth, salivary glands, stomach, and small intestine. The objective classification of both herbicide exposure and cancer incidence is considered a strength of this study over other publications from this cohort that used self-report data for analyses.

Again, using EOI scores to estimate herbicide exposure, Yi et al. (2014a) examined the prevalences of a wide range of disease conditions, specifically, those pertaining to the endocrine, nervous, circulatory, respiratory, and digestive systems. Health information was derived through a review of claims data from the Health Insurance Review and Assessment Service of Korea from January 1, 2000, to September 30, 2005. Overall, and compared with low exposure, high herbicide exposure was associated with a statistically significantly higher prevalence of hypothyroidism, autoimmune thyroiditis, other endocrine gland disorders including pituitary gland disorders, as well as amyloidosis and Alzheimer disease. As with Yi and Ohrr (2014), the objective classification of both herbicide exposure and disease prevalence is considered a strength of this study.

Finally, Yi et al. (2014b) used objective classifications of both herbicide exposure and cause-specific mortality to analyze cause-specific mortality in 180,639 Korean Vietnam veterans. The EOIs were used as the basis for two characterizations of herbicide exposure: as "low" versus "high" and as per unit increase based on a log-transformed scale. The incidence of mortality and cause of death were ascertained by the use of death records from the National Statistical Office for the period 1992–2005. Veterans with high herbicide exposure were found to have 10% increased long-term risk of mortality and 13% increased cancer mortality. The observed cause-specific cancer mortality estimates were very imprecise, but were highest for thyroid cancer, chronic myeloid leukemia, small intestine cancer, and bladder cancer.

Other Studies of Korean Vietnam Veterans

Epidemiologic studies have also looked at immunotoxicologic outcomes (H. A. Kim et al., 2003) and skin and general disease patterns (Mo et al., 2002) in Korean Vietnam veterans who were exposed to herbicides during the Vietnam War. One case-control study of the association between exposure to TCDD and recovery outcomes (hypertension, hyperlipidemia, and the rate and severity of major adverse coronary events) in male veterans who presented with acute coronary syndrome was also examined, but the study findings are not informative about associations between TCDD and acute coronary syndrome itself. Two new studies of Korean veterans who served in Vietnam were identified for the current update. Y. S. Yang et al. (2016) used a case-control design to examine the association between exposure to Agent Orange and Parkinson disease. Han et al. (2016) conducted a small hospital-based case-control study of acute ischemic

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stroke to compare vascular features among those presumed to have been exposed to Agent Orange (based on deployment in Vietnam) and those veterans who did not serve in Vietnam.

New Zealand Vietnam-Veteran Studies

McBride et al. (2013) followed 2,783 male veterans from New Zealand who served in Vietnam between 1964 and 1972. Their status with respect to cancer incidence and mortality was determined from 1988 through 2008. This cohort included 84% of all 3,322 Vietnam veterans from New Zealand who had survived service in Vietnam. Standardized incidence and mortality ratios were generated by comparing the observed incident cases and deaths in this cohort with the corresponding expected numbers of new cases and deaths rates from the general male population of New Zealand. For all-cause mortality, the Vietnam veterans had significantly lower rates than the New Zealand general population. Cancer mortality and overall incidence were similar between Vietnam veterans and the New Zealand general population, as was heart disease mortality. In contrast, New Zealand Vietnam veterans appeared to be at higher risk of cancers of the head and neck and oral cavity, pharynx, and larynx as well as of incident chronic lymphoid leukemia (also known as chronic lymphocytic leukemia) than was the New Zealand general population. Although the follow-up of this cohort was long (20 years), the study did not have information on cancer incidence and mortality in the time period immediately after the service (i.e., between 1972 and 1988). It also lacked an internal comparison group, and information on potential confounding factors including smoking, drinking habits, and human papilloma virus status was not available, which limits the interpretation of the data, particularly regarding incident cancers. Moreover, it was assumed that any veteran who had been deployed had been exposed to the herbicides, and the presumed exposure was not validated through more objective measures such as serum concentrations or even more targeted self-reported questions of exposure.

For the current report, one new follow-up publication of the New Zealand Vietnam veteran cohort was identified. Cox et al. (2015) used hospital discharge records from 1988 to 2009 to report the prevalent health conditions among the same cohort of 2,783 New Zealand Vietnam veterans used by McBride et al. (2013). For participants 30 years of age or older, person-years of follow-up were calculated by 5-year age categories. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. The comparison group was the standardized general population of New Zealand. Standardized hospitalization ratios and 99% confidence limits were calculated and reported as a means to control for the multiple tests performed for various outcomes. Overall, this study found that hospital admissions due to all causes combined was slightly higher for Vietnam veterans than for the standardized population of New Zealand. Multiple

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outcomes by system and specific conditions were reported; however, several of the outcomes are not generally conditions for which an individual would be hospitalized (e.g., diabetes, cataract, anxiety disorder), thereby limiting the utility of some of the findings reported. This study is also subject to the same limitations discussed for McBride et al. (2013).

OCCUPATIONAL STUDIES

Several occupational groups in the United States and elsewhere have been exposed to the COIs. Exposure characterization in studies of these groups varies widely in the metric used, the extent of detail, confounding exposures, and whether individual, surrogate, or group (ecologic) measures are used. Some studies reviewed in earlier VAO reports used job titles as broad surrogates of exposure; others rely on disease-registry data.

The VAO committees have reviewed many epidemiologic studies of occupationally exposed groups for evidence of an association between exposure to TCDD or to the herbicides used in Vietnam and health risks. In reviewing the studies, the committee considered two types of exposure separately: exposure to 2,4-D or 2,4,5-T and exposure to TCDD from 2,4,5-T or other sources. That separation is necessary because some health effects could be associated with an exposure to 2,4-D or 2,4,5-T in the absence of substantial TCDD exposure. After the dioxin contamination of phenoxy herbicides was recognized, production conditions were modified to minimize that contamination, and the use of the products most likely to contain TCDD (2,4,5-T and Silvex) was banned. As a result, workers who were exposed to phenoxy herbicides only after the late 1970s are not assumed to have been at risk for exposure to TCDD. The distinction is particularly important for workers in agriculture and forestry, including farmers and herbicide appliers, whose exposure is primarily the result of mixing, loading, and applying herbicides.

The committee also considered studies of occupational exposure to dioxins, focusing on workers in chemical plants that produced phenoxy herbicides or chlorophenols, which tend to be contaminated with PCDDs. Waste-incineration workers were also included in the occupation category because they can come into contact with dioxin-like chemicals while handling the byproducts of incineration. Other occupationally exposed groups included were pulp-and-paper workers exposed to dioxins through bleaching processes that use chlorinated compounds and sawmill workers exposed to chlorinated dioxins, which can be contaminants of the chlorophenates used as wood preservatives.

Because no new information has been published on several of these cohorts for years or decades and detailed information on them is available in previous volumes of the series, this section offers only a brief overview of those major occupational cohorts and studies that have contributed to the evidence base on potential health outcomes stemming from exposure to the COIs but for which no

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new information for the current volume is available. Instead, the focus here is on the design and methodology of studies for those occupational cohorts for which new information is available in this report.

Studies of Herbicide Production Workers

International Agency for Research on Cancer (IARC)

A multisite study by IARC involved 18,390 production workers and phenoxy herbicide sprayers working in 10 countries (Saracci et al., 1991). The full cohort was established by using the International Register of Workers Exposed to Phenoxy Herbicides and Their Contaminants. Twenty cohorts were combined for the analysis: one each in Australia, Austria, Canada, Finland, and Sweden; two each in Denmark, Italy, the Netherlands, and New Zealand; and seven in the United Kingdom. There were 12,492 production workers and 5,898 sprayers in the full cohort. Several studies published using data from this cohort were reviewed by the first few VAO committees and contributed to conclusions on some cancers (Kogevinas et al., 1992, 1993, 1995, 1997), but no new studies of the IARC cohort have been published since *Update 1998*.

Several of the subcohorts that make up the IARC cohort have generated independent reports that have been evaluated separately by VAO committees to garner additional insights, such as the results associated with TCDD concentrations measured in various subjects: Austrian production workers (Jäger et al., 1998; Neuberger et al., 1998, 1999), British production workers (Coggon et al., 1986, 1991, 2015), Danish production workers (Lynge, 1985, 1993), Dutch production workers (Boers et al., 2010, 2012; Bueno de Mesquita et al., 1993; Hooiveld et al., 1998), German production workers (Becher et al., 1992, 1996; Flesch-Janys, 1997; Flesch-Janys et al., 1995, 1996; Manz et al., 1991), and New Zealand production workers (Burns et al., 2010; Collins et al., 2009a; McBride et al. 2009a,b; 't Mannetje et al., 2005, 2017, 2018). Several of the component cohorts have not been the subject of any separate publications: Australian herbicide sprayers, Canadian herbicide sprayers, Finnish production workers, two cohorts of Italian production workers, and Swedish production workers. The U.S. NIOSH cohort was added to the IARC cohort as of the 1997 publication by Kogevinas et al. and is discussed after the international production-worker cohorts.

Previous committees have examined studies of several international production-worker or herbicide-using worker cohorts, including many that were subcohorts of IARC. No new studies were identified by the committee for inclusion in the current report on many of these groups, including

- BASF Ludwigshafen Plant workers, Germany, who were involved in an accident in 1953 when some of the workers experienced extremely high

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exposures to TCDD during the manufacture of trichlorophenol (TCP), or as a result of the accident, or following the accident during reactor cleanup, maintenance, or demolition (Ott and Zober, 1996; Thiess et al., 1982; Zober et al., 1990, 1994, 1997). (This group was not in the IARC cohort.)

- Two Dutch subcohorts of the IARC cohort consisting of 2,106 male workers employed in two manufacturing factories producing and formulating chlorophenoxy herbicides: 2,4,5-T in factory A from 1955 through 1985, and 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2-(2-methyl-4-chlorophenoxy) propionic acid (Mecoprop, MCPP), and 2,4-D in factory B from 1965 through 1986. Accordingly, members of both subcohorts had potential exposure to phenoxy herbicides, but only those in factory A would have been exposed to TCDD. Several analyses have been made using the entire cohort population or subsets of workers from chemical production factory A and factory B—these have examined different endpoints and applied different methods and measures to estimate exposure to TCDD (Boers et al., 2010, 2012; Bueno de Mesquita et al., 1993; Hooiveld et al., 1998; Saberi Hosnijeh et al., 2011, 2012a,b,c, 2013a,b).
- German production workers consisting of four German cohorts added to the IARC cohort as of 1997: the Boehringer–Ingelheim cohort, which had a high potential for TCDD exposure from the production of TCP and 2,4,5-T from 1951 to 1954 and from 1957 to 1984 (Flesch-Janys, 1997; Flesch-Janys et al., 1995; Manuwald et al., 2012; Manz et al., 1991), a cohort in the BASF Ludwigshafen plant that did not include those involved in the 1953 accident, and cohorts in two Bayer plants (Becher et al., 1992, 1996). All the plants were involved in the production of phenoxy herbicides or chlorophenols.
- Workers in Czechoslovakia (n > 80) who were exposed to TCDD during the production of 2,4,5-T (Pazderova-Vejlupková et al., 1981; Pelclová et al., 2001, 2002, 2007, 2009, 2011; Urban et al., 2007).

New Zealand Production Workers Two new studies on this cohort were identified ('t Mannetje et al., 2017, 2018). The Dow AgroSciences plant in New Plymouth, New Zealand, produced phenoxy herbicides from the late 1950s through the mid-1980s. This plant also produced picloram, one of the COIs about which very little information is available. The New Zealand cohort was incorporated into the original IARC cohort.

Complete employment records for 1969–1984 (referred to as the 1984 cohort) were available and 't Mannetje et al. (2005) used them to report on the mortality experience through 2000 of the 713 men and 100 women who had worked at least 1 month in that period. McBride et al. (2009a) conducted expanded analyses and updated previous analyses of cause-specific mortality from both cancers and other conditions. The cohort was increased to 1,599 participants (the 1988 cohort),

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including a substantial number of people who had minimal opportunity for exposure, by extending the employment period for eligibility to November 1, 1988, and removing the requirement that employment lasted at least 1 month. A subsample of the 1988 cohort participated in a serum-dioxin analysis that found that 70% had been exposed. McBride et al. (2009b) further expanded the cohort to 1,754 participants (the 2003 cohort) by further extending eligibility to anyone who had worked at the site at any time until October 1, 2003. Both enlarged cohorts were followed through 2004. The New Zealand Health Information Service Mortality Collection was used to identify deaths, and exposure status was classified according to work experience. Collins et al. (2009a) described the group's serum TCDD concentrations overall, and Burns et al. (2010) performed analyses to determine what factors might predict serum TCDD: age, BMI, and employment history were found to be significant determinants. Both Collins et al. (2009a) and C. J. Burns et al. (2010) reported standardized mortality ratios adjusted for age, sex, and calendar age and used the New Zealand general population as the reference population. For the 1988 cohort, effect estimates were stratified by exposure status (ever exposed and never exposed) and by predicted cumulative exposure categories. For the 2003 cohort, standardized mortality ratios were reported for the entire cohort and stratified by employment duration (less than 3 months and at least 3 months) and by latency (15 years of latency and less than 15 years of latency). For the 1988 cohort, proportional-hazards survival analysis was also used to test the association between mortality and predicted cumulative exposure categories.

The New Zealand studies have several important limitations. The sample loss was substantial: 13% were lost to follow-up in both cohorts, and 8% of the 1988 cohort and 9% of the 2003 cohort emigrated. If subject loss was nonrandom, then the study findings might be vulnerable to sample selection bias. In addition, the inclusion in the 2003 cohort of the employees hired as recently as 2003 is questionable. It appears that no deaths were observed in the increment between the 1988 cohort and the 2003 cohort (those hired since 1988), presumably because these participants were relatively young. The inclusion of the incremental participants might dilute the power of the study to detect the effects of TCDD exposure on health outcomes that require a long latent period; participants who have not yet "matured" through the latent period might be contributing noise rather than signal to the analyses. The Update 2010 committee, therefore, did not give substantial weight to the dose–response findings of McBride et al. (2009b). The serum concentrations of dioxins and furans observed in a subset of the workers in the Dow phenoxy-herbicide plant in New Zealand have been used in estimating individual exposure (Aylward et al., 2010; Collins et al., 2009a).

National Institute for Occupational Safety and Health (NIOSH) Studies

NIOSH has collected data on and followed several groups of U.S. workers exposed to the committee's COIs, including the dioxin registry; the

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pentachlorophenol (PCP) cohort (Ruder and Yiin, 2011); the cross-sectional medical study of workers in plants producing 2,4,5-TCP and 2,4,5-T or 2,4,5-TCP, 2,4,5-T, and hexachlorophene (Alderfer et al., 1992; Calvert et al., 1991, 1992, 1994, 1998, 1999; Egeland et al., 1994; Halperin et al., 1995, 1998; Kayajanian, 2002; Lawson et al., 2004; Sweeney et al., 1993, 1996, 1997–1998); and the TCDD mortality cohort. The NIOSH cohort was added to the IARC cohort as of the 1997 publication by Kogevinas et al. No new studies on the entire NIOSH cohort have been published since those reviewed in *Update 2006*.

NIOSH TCDD Mortality Cohort Since 1978, NIOSH has compiled an extensive set of data on chemical production workers potentially contaminated with TCDD in 1942–1984. More than 5,000 workers who were involved in production or maintenance in any of 12 companies were identified from personnel and payroll records; 172 additional workers identified previously by their employers as being exposed to TCDD were also included in the study cohort (Suskind and Hertzberg, 1984). The employees' possible exposure resulted from working with various substances of which TCDD was a contaminant: 2,4,5-TCP, 2-(2,4,5-trichlorophenoxy) propionic acid (Silvex, 2,4,5-TP), 2-(2,4,5-trichlorophenoxy) ethyl 2,2-dichloropropionate (Erbon), O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate (Ronnel®), and hexachlorophene. The 12 plants involved were large manufacturing sites of major chemical companies, so many of the participants were potentially exposed to many other compounds, some of which could be toxic and carcinogenic.

Exposure status was determined initially through a review of process operating conditions, employee duties, and analytic records of TCDD in industrial hygiene samples, process streams, products, and waste (Fingerhut et al., 1991). Occupational exposure to TCDD-contaminated processes was confirmed by measuring serum TCDD in 253 cohort members. The duration of exposure, defined as the number of years an employee had worked in processes contaminated with TCDD, was used as the primary exposure metric in the study. The use of duration of exposure as a surrogate for cumulative exposure was based on a correlation (Pearson correlation efficient, 0.72) between log-transformed serum TCDD levels and the number of years worked in TCDD-contaminated processes. The duration of exposure of individual workers was calculated from work records, and exposure-duration categories were created: < 1 year, 1–<5 years, 5–<15 years, and ≥15 years. In some cases, information on the duration of exposure was not available, so a separate metric, duration of employment, was defined as the total time that each worker was employed at the study plant. Fingerhut et al. (1991) used the exposure measures in assessing mortality through 1987.

A follow-up study (Steenland et al., 1999) examined the association between TCDD exposure and cause of death through 1993; it examined specific health outcomes, including cancers (all and site-specific), respiratory disease, cardiovascular disease, and diabetes. The researchers used a more refined exposure

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assessment than had previous analyses. It excluded workers whose records were inadequate to determine the duration of exposure, and this reduced the number of study participants to a subcohort of 3,538 workers (69% of the overall cohort). The exposure assessment for that subcohort was based on a job–exposure matrix that assigned each remaining worker a quantitative exposure score for each year of work (Piacitelli and Marlow, 1997).

Subcohorts of the NIOSH TCDD Mortality Cohort

Monsanto The NIOSH study cohort (Fingerhut et al., 1991) included employees of the Monsanto facility in Nitro, West Virginia, which produced 2,4,5-T in 1948–1969. Zack and Suskind (1980) examined the mortality experience of the 121 men who had chloracne associated with an unintentional release that occurred on March 8, 1949. Other studies considered mortality and other health outcomes in additional workers involved in numerous aspects of 2,4,5-T production at the Monsanto plant (Collins et al., 1993; Moses et al., 1984; Suskind and Hertzberg, 1984; Zack and Gaffey, 1983). No additional studies on those participants alone have been published; they have since been followed as part of the NIOSH and IARC cohorts.

Dow Production Workers At the Dow plant in Midland, Michigan, 2,4-D, TCP, and PCP were manufactured over different, overlapping time periods and exposed different populations of workers. The cohort of TCP workers who were potentially exposed specifically to TCDD is one of the eight cohorts in the NIOSH cohort of dioxin-exposed U.S. workers that were entered into the IARC phenoxy herbicides cohort. Dow PCP workers who were not exposed to TCDD are not in the IARC or NIOSH cohorts for TCDD, but this group is one of four cohorts included in NIOSH's PCP cohort (Fingerhut et al., 1984; Ruder and Yiin, 2011). Dow assembled a large cohort at the Midland, Michigan, plant (Bond et al., 1989a; Cook et al., 1986, 1987). TCDD exposure in the cohort was defined by chloracne diagnosis (Bond et al., 1989b). Within the cohort, a subcohort study of women (Ott et al., 1987) and a case-control study of STS (Sobel et al., 1987) were conducted. The Dow cohorts have been followed as part of the NIOSH and IARC cohorts since 1991 and 1997, respectively.

C. J. Burns et al. (2011) reported on cancer incidence in 2,4-D production workers in the Dow Midland plant. The exposed cohort consisted of 1,316 men who worked in 2,4-D operations from 1945 through 1994 and who were alive on January 1, 1985, when the Michigan statewide cancer registry was initiated. Exposure was considered both as a discrete category (exposed cohort members versus a non-exposed reference population) and as a cumulative variable estimated as (job-specific exposure estimate) × (duration on the job) summed over all jobs held since 1945. Workers were stratified into three categories according to their estimated cumulative exposure. The cohort was followed from 1985

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through 2007. Cancer incidence was ascertained from the Michigan statewide cancer registry, and data were linked to two other states where cohort members might reside. Three nested cohorts were used for statistical analyses in order to address potential problems with missing data due to migration outside the three states, with data linkage. Cohort 1 consisted of the entire exposed cohort regardless of residency (1,316 people who had 25,267 person-years of follow-up). Cohort 2 required Michigan residency: follow-up was terminated when a person was no longer a Michigan resident because company records showed a permanent non-Michigan address (those who died were classified as residents if Michigan was listed as the state of residency on their death certificates) (1,256 who had 23,354 person-years). Cohort 3 was the most restrictive and required Michigan residency; follow-up was terminated as soon as a person was no longer known to be a Michigan resident (1,108 who had 18,897 person-years). People of unknown residency status were assumed to remain Michigan residents and were included in the follow-up for Cohort 2 but were excluded from Cohort 3. Standardized incidence ratios were derived for all three cohorts, with Michigan white males as the reference population; Fisher's exact confidence intervals were used to characterize the uncertainty. For Cohort 2, additional analyses were conducted by using the National Cancer Institute's Surveillance, Epidemiology, and End Results registry population and a regional population as the reference populations. The cohort was stratified according to cumulative duration and cumulative exposure categories. There were several concerns that the study findings might be biased. First, the study cohort might be healthier than the general population being used as the reference population. Second, the lack of a latency period in the study design might lead to an attenuation effect on the risk estimates. Third, Cohort 2, which was the researchers' focus in the study, might be vulnerable to an attenuation effect because of the uncertainty in residency status. The results on Cohort 3 are considered the least subject to bias and therefore the most reliable. Although Cohort 3 was the smallest group and as such is also subject to the most variability, consistency in results among the three cohorts was considered confirmatory.

All Dow TCP-Exposed Workers TCP was produced in Dow's Midland facility from 1942 to 1979, and 2,4,5-T was produced there from 1948 to 1982. Collins et al. (2009b) reported on the vital status through 2003 of 1,615 people who worked with TCP or 2,4,5-T from 1942 through 1982; 58,743 person-years were accumulated, and 662 deaths were observed. Standardized mortality ratios for cause-specific mortality in the cohort—with and without the overlap of 196 people who were also part of the PCP cohort (reviewed in Collins et al., 2009c)—were calculated by using the U.S. population as the reference population and using the Occupational Mortality Analysis Program. Work history records were used to determine length of exposure. Serum samples used for measuring the levels of six types of dioxins were collected for 431 TCP and PCP workers. Historic concentrations for each dioxin congener were calculated

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from the median concentrations from the serum samples and the known half-lives associated with each congener. A job exposure matrix was created for both the TCP and PCP production facilities based on measured concentrations for workers in different jobs. A pharmacokinetic model was applied to job-specific concentrations to the work history of each member of the study group to estimate their time-dependent serum concentration profiles for each dioxin congener (i.e., TCDD, as well as Hexa-CDD, Hepta-CDD, and Octa-CDD). Collins et al. (2016) reported the results of an additional follow-up of vital status to TCP and PCP production workers. Vital status was determined for the cohort through December 2011; a total of 1,198 deaths since 1979 were found.

Dow PCP Production Workers These workers were engaged in the manufacture of PCP from 1937 to 1980 in the same plant where the TCP cohort worked. Unlike TCP, PCP did not contain TCDD, but it did contain other highly chlorinated dioxin congeners, and 20% of the PCP workers had suffered from chloracne. Dow performed an exposure risk assessment on the subset of its manufacturing workers who were exposed to PCP (Ramlow et al., 1996). The exposure assessment evaluated the available industrial-hygiene and process data, including recollections from employees about their job, processes, and changes in processes as well as data from engineering controls, measurements from surface wipes, and exposure-monitoring data from area sampling and personal breathing zones. Jobs in the "flaking/prilling/packaging area" were determined to have a higher potential exposure because of dermal exposure to airborne PCP; the industrial-hygiene data suggested a difference of about a factor of 3 between the areas of highest and lowest potential exposure. An estimated exposure-intensity score of between 1 and 3 (from lowest to highest potential exposure intensity) was assigned to each job. Information concerning the use of personal protective equipment was deemed to be unreliable. For each participant, cumulative PCP and TCDD exposure indexes were calculated by multiplying the duration of each exposed job by its estimated exposure intensity and then summing across all exposed jobs.

Collins et al. (2009c) conducted a mortality study of the Dow PCP production workers with the accrual of years at risk starting at the beginning of 1940 and followed through 2003; the TCP workers were followed over the same years (Collins et al., 2009b). The cohort consisted of 773 PCP workers; 27,035 person-years were accumulated, and 370 deaths were observed. Standardized mortality ratios for the PCP cohort (with and without the overlap of 196 people in the TCP cohort) were reported for cause-specific mortality, with the U.S. population as the referent population. Proportional-hazards survival analysis was also used to assess the association between mortality and predicted cumulative exposure as a function of the total TCDD TEQ. An updated mortality analysis that follows the cohort of PCP and TCP workers though 2011 and reports cause-specific mortality on several cancers and other diseases (Collins et al., 2016) is reviewed in the current update.

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Dow TCDD-Exposed Production Workers Dow conducted a study of 204 workers engaged in the production of 2,4,5-T (Ott et al., 1980) and another study of 61 TCP manufacturing workers who had chloracne (Cook et al., 1980). Industrial hygienists developed a job-exposure matrix that ranked employee exposures as low, moderate, or high on the basis of available air-monitoring data and professional judgment. The matrix was merged with employee work histories to assign an estimate of exposure to each job. A cumulative dose was then developed for each of the 878 employees by multiplying the representative 8-hour time-weighted average exposure value for each job by the number of years in the job and then adding the products for all jobs. A 2,4-D time-weighted average of 0.05 mg/m^3 was used for low, 0.5 mg/m^3 for moderate, and 5 mg/m^3 for high exposure. The exposure estimates do not appear to have taken into account the role of dermal exposure in the facilities. It is not clear to what extent the use of air measurements alone can provide accurate classification of workers into low-, moderate-, and high-exposure groups. The study apparently did not include biologic monitoring of 2,4-D.

Bond et al. (1983) investigated potential exposure to TCDD and morbidity in the sets of workers reported on by Cook et al. (1980) and Ott et al. (1980). Potential TCDD exposure and reproductive outcomes were studied in the offspring of 930 men who worked with chlorophenol from 1939 through 1975 (Townsend et al., 1982). Dow employees who had a diagnosis of chloracne or who were classified as having chloracne on the basis of a clinical description were followed prospectively for mortality (Bond et al., 1987). There was a succession of mortality studies of workers involved in 2,4-D production in several of the plants (Bloemen et al., 1993; Bond et al., 1988; C. J. Burns et al., 2001). These studies also used the same exposure-assessment procedures.

Bodner et al. (2003) published a 10-year follow-up of the work of Cook et al. (1986), comparing the mortality experience of 2,187 male workers at Dow who were potentially heavily exposed to dioxin before 1983 with that of the NIOSH and IARC cohorts. Dow researchers have published a study of serum dioxin concentrations measured in 2002 in former chlorophenol workers (Collins et al., 2006). Most of the workers in the study were included in the NIOSH and IARC cohorts. The authors used their data to estimate worker exposure at the time of exposure termination by using several pharmacokinetic models. They concluded that their findings were consistent with those of other studies that reported high serum dioxin concentrations in chlorophenol workers after occupational exposure.

Aylward et al. (2013) examined the elimination rates of dioxin congeners in former chlorophenol workers from the Midland plant. Blood samples from 56 former chlorophenol workers were taken and examined in 2004–2005 and then resampled in 2010. The purpose of this analysis was to estimate half-life reductions for TCDD TEQs, which, in aggregate, were 9.3 years for the dioxin congeners analyzed. This analysis is informative with respect to estimating elimination rates over time for the COIs as the Vietnam-veteran cohort continues to age.

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Studies of Other Industrial Cohorts

VAO committees have reviewed several other occupational cohorts and studies of industrial workers over the series. The countries and industries have included

- Chinese automobile foundry workers (L. Wang et al., 2013);
- pulp and paper workers exposed to TCDD and other dioxins that can be generated by the bleaching process during the production and treatment of paper and paper products in the United States (Henneberger et al., 1989; Robinson et al., 1986; Solet et al., 1989), Finland (Jäppinen and Pukkala, 1991), Denmark (Rix et al., 1998), and an IARC cohort of paper and pulp workers in 11 countries (McLean et al., 2006);
- sawmill workers with dermal and inhalation exposure through the use of pentachlorophenates (which are contaminated with higher-chlorinated PCDDs [Cl6–Cl8]) in British Columbia, Canada (Dimich-Ward et al., 1996; Heacock et al., 1998; Hertzman et al., 1997), and New Zealand (McLean et al., 2009), or to tetrachlorophenates (which are less contaminated with higher-chlorinated PCDDs) (McLean et al., 2009) in New Zealand; and
- waste incineration workers in Japan (Yamamoto et al., 2015; Yoshida et al., 2006) and Korea (Oh et al., 2005).

Studies of U.S. workers in other chemical plants have also included studies of 2,4-D and 2,4,5-T production workers (Poland et al., 1971), white men employed at a chemical plant that manufactured flavors and fragrances (Thomas, 1987), and workers producing PCP, lower-chlorinated phenols, and esters of chlorophenoxy acids (Hryhorczuk et al., 1998).

International cohorts of chemical workers exposed to the COIs have included UK chemical workers exposed to TCDD as a result of an industrial accident in 1968 (Jennings et al., 1988; May, 1973, 1982, 1983), 2,4-D production workers in the former Soviet Union (Bashirov, 1969), and Croatian workers exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-D, and malathion) during the production process (Garaj-Vrhovac and Zeljezić 2002). Some of these worker populations, such as the Chinese automobile foundry workers, were reported on only once, and others were prospectively or retrospectively followed for years, such as waste incineration workers in Japan (Yamamoto et al., 2015; Yoshida et al., 2006). Summaries of these studies are included in the update in which they were first identified. For the current volume, morbidity and mortality outcomes of worker cohorts at an electric arc furnace in Italy (Cappelletti et al., 2016) and at a transformer and capacitor recycling plant in Germany (Fimm et al., 2017; Haase et al., 2016; Putschogl et al., 2015) are reviewed.

Inadequate occupational hygiene at a recycling plant for capacitors and transformers in Dortmund, Germany, led to a significant body burden of PCBs

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in workers, their family members, and people working or living in the immediate vicinity of the plant. A medical surveillance program, Health Effects in High-Level Exposure to PCB, was initiated to provide biomonitoring to identify potential health risks related to PCB exposure for the affected population (Kraus et al., 2012). Workers of and residents around this plant were exposed to both dioxin-like (PCBs 105, 114, 123, 156, 157, 167, and 189) and non-dioxin-like PCBs as a result of contamination of the area. Participation in the surveillance program was voluntary, and data and blood samples were collected at three time points: immediately (beginning in 2010), 1 year, and 2 years after exposure. PCB congeners were measured in serum using blood samples collected from the participants, and urine samples were also collected at the three time points. Of the eligible 294 adults, 148 participated at all three time points. Three studies using these data were published and are reviewed in this update. Putschogl et al. (2015) studied associations with neurotransmitter metabolites of dopamine and norepinephrine in urine as markers of targeted effects on these specific neurotransmission pathways, and Haase et al. (2016) confirmed that PCB exposure can modify lymphocyte profiles. However, neither of these analyses of metabolites or immune cells is diagnostic of a specific health outcome, and they are therefore only of tangential relevance to the committee's charge. Fimm et al. (2017) examined a broad range of cognitive functions covering attention, executive processing, reasoning, memory, and motor performance in this cohort.

Studies of Herbicide-Using Workers

The original VAO committee and committees up through *Update 2006* were more inclusive of identified studies from the literature searches. Several of the early studies reviewed and included in the updates had nonspecific, inferred exposure characterizations based on "usual occupation" from death certificates, self-reported "current occupation," or census tract or area of residence (e.g., rural or farming areas). The studies focused primarily on farmers and people employed in the agricultural industry, but workers in forestry and other outdoor occupations, such as highway-maintenance workers, are also likely to have been exposed to herbicides and other chemicals. The problem with using such nonspecific surrogates of exposure is that it will influence and change the effect estimates (either toward or away from the null depending on the types of bias present) making it impossible to know the true effect of the COIs. Occupation or job titles do not provide information on the duration or the intensity of the exposure, and they cannot be used to determine whether a worker was exposed to a specific agent. Even those studies that collected more details on the number of years of employment in the agriculture industry, broad categories of the chemicals used (e.g., herbicides, insecticides, fungicides, etc.), specific classes of chemicals or names, the frequency of application, the use of personal protective equipment, and the method of application may be limited by recall or information bias (depending on

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whether other records are available) and do not provide much evidentiary weight if the specific agents and chemicals used are not collected.

Since the first VAO report, exposure assessments in epidemiology studies have been increasingly exact in both specificity and amount, enabling the committees of the more recent updates to establish stricter criteria for accepting exposure as sufficiently specific for results to be added to the evidentiary database. The current committee now seeks results expressed in terms of the five COIs for this project or their analogues, and it regards classification based only on job title as inadequate. Data derived from studies in which exposure is described nonspecifically as "herbicide" can at most be used as supportive evidence. According to the policy established by the Agent Orange Act of 1991, studies of Vietnam veterans are presumed to involve relevant exposure, as are studies of workers at a particular plant during a period when it is known to have been producing phenoxy herbicides or other chemicals recognized as having been contaminated with TCDD.

The most reliable information on health effects among American herbicide applicators and users exposed to the COIs has come from prospective investigations of the Agricultural Health Study (AHS). More than 200 publications have reported on outcomes from this cohort, although not all have focused on the use of 2,4-D or other COIs. Several new publications from the AHS were reviewed in the current update. VAO and update committees have also reviewed studies of other populations of farmers and agricultural workers conducted among state populations and as part of larger cohorts such as the CropLife America Farm Family Exposure Study (Mandel et al., 2005) and the California United Farm Workers of America Study (Mills and Yang, 2005, 2007; Mills et al. 2005). In addition to studies of herbicide users in the United States, several international publications have been reviewed by VAO committees.

Agricultural Health Study

The AHS is a prospective investigation of cohorts of private pesticide applicators (farmers), their spouses, and commercial pesticide applicators in Iowa and North Carolina, with a total of 89,658 participants, including 57,311 applicators (82% of those seeking licensing) and 32,347 spouses (75% of all spouses). The applicators are predominantly male, and the spouses are predominantly female. The AHS is sponsored by the National Cancer Institute, the Environmental Protection Agency, and the National Institute of Environmental Health Sciences. Enrollment in the study was offered to applicants for applicator certification in Iowa and North Carolina. The project's website (www.aghealth.nih.gov) provides many details about the study, including a specification of which pesticides were the subject of information gathered from the enrollment forms and mailed questionnaires (Alavanja et al., 1994).

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In phase I (1993–1997), the enrollment form for both commercial (8.6%) and private (largely farmers) applicators asked for details on the use of 22 pesticides (10 herbicides, including 2,4-D; 9 insecticides; 2 fungicides; and 1 fumigant) and yes–no responses as to whether 28 other pesticides (8 herbicides, including 2,4,5-T and Silvex; 13 insecticides; 4 fungicides; and 3 fumigants) had ever been used. A subset of 24,034 applicators also completed and mailed back a take-home questionnaire (response rate 42%). The questionnaire asked for details about use of the 28 pesticides with yes–no information on the enrollment form and for yes–no responses as to whether 108 other pesticides (34 herbicides, including organic arsenic, which would cover cacodylic acid; 36 insecticides; 29 fungicides; and 9 fumigants) had ever been "frequently" used. Although no pronounced differences in demographics, medical histories, or farming practices were found between those who completed the questionnaire and those who did not (Tarone et al., 1997), it is still possible that selection bias might have compromised the validity of any studies based on the questionnaire because of differences that might not have been captured in the enrollment form. Dosemeci et al. (2002) published an algorithm designed to characterize the personal exposures of that population. Weighting factors for the key exposure variables were developed from the literature on pesticide exposure. This quantitative approach has the potential to improve the accuracy of exposure classification for the cohort, but the published epidemiologic studies reviewed as part of the VAO series do not appear to have used that method.

Phase II was a 5-year follow-up conducted in 1999–2003. Computer-assisted telephone interviews were completed by 60,138 participants. The interviews specified "pesticides" in general to include herbicides. They asked about specific pesticides on individual crops; for several crops, only if atrazine or 2,4-D was specified was a participant asked whether it had been used alone or as part of the manufacturer's mixture. A full pesticide list was not posted on the website with the follow-up questionnaire. In addition, dietary histories were completed by 35,164 respondents, and buccal-cell samples were gathered from 34,810 participants. The rate of response to the phase II survey—67% overall and 63% of the original cohort of 55,748 male applicators—was modest and leaves some room for selection bias to compromise the validity of studies based on the survey. In phase III (2005–2010), responses to an updated computer-assisted telephone interview were provided by 43,426 participants.

Numerous reports on the AHS cohort have been considered in the VAO series, and several new published studies have been reviewed in the current update (Bonner et al., 2017; Christensen et al., 2016; Henneberger et al., 2014; Koutros et al., 2016; LaVerda et al., 2015; Lebov et al., 2015, 2016; Parks et al., 2016). All have developed pesticide-exposure estimates or exposure categories from self-administered questionnaires, but only those studies that have considered exposure to the COIs are considered most relevant to VAO committees. The results on the relative rates of individual conditions seem comparable in exposure specificity

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with findings in production cohorts in which not all of the workers included were necessarily exposed to the COIs and may have had additional toxic exposures. The AHS questionnaire collected detailed information regarding herbicide use; 2,4-D was the most commonly reported herbicide. Because all relevant studies have been reviewed in detail in the VAO report that corresponded to their publication date, only an overview is presented here. Details of the studies reviewed for the first time in the current volume are presented in the section corresponding to the health outcome of interest. Using various subsets of the study population, they have addressed a variety of health outcomes associated with one or more of the COIs, including

- all-cause and cause-specific mortality (Blair et al., 2005a; Waggoner et al., 2011) and morbidity (Alavanja et al., 2005; Blair et al., 2005b);
- incidence of prostate cancer (Alavanja et al., 2003, 2005), lung cancer (Alavanja et al., 2004), breast cancer (Engel et al., 2005), colorectal cancer (W. J. Lee et al., 2007), cutaneous melanoma (Dennis et al., 2010), all cancers (Alavanja et al., 2005; Koutros et al., 2010a, Samanic et al., 2006), and risk factors for pancreatic cancer (Andreotti et al., 2009);
- neurotoxicity of chronic exposure to modest amounts of pesticides (Kamel et al., 2005), neurologic symptoms including memory and concentration problems (Kamel et al., 2007a), Parkinson disease (Kamel et al., 2007b, 2014; Tanner et al., 2011), and amyotrophic lateral sclerosis (Kamel et al., 2012);
- reproductive effects (Farr et al., 2004, 2006) and cancer risk in the 21,375 children of pesticide appliers born in 1975 or later (Flower et al., 2004);
- diabetes in applicators and their wives (Montgomery et al., 2008; Starling et al., 2014) and history of gestational diabetes (Saldana et al., 2007);
- incidence of and mortality from myocardial infarction (Mills et al., 2009) and mortality from stroke (Rinsky et al., 2013);
- respiratory symptoms and disorders: chemical predictors of wheeze (Hoppin et al., 2002, 2006b), hypersensitivity pneumonitis (Hoppin et al., 2007b), chronic bronchitis (Hoppin et al., 2007a; Valcin et al., 2007), atopic and nonatopic asthma in women (Hoppin et al., 2008), and allergic and nonallergic adult-onset asthma (Hoppin et al., 2009);
- thyroid disease among AHS female spouses (Goldner et al., 2010) and male pesticide applicators (Goldner et al., 2013); and
- genetic markers (single nucleotide polymorphisms and candidate genes) and outcomes of prostate cancer risk (Andreotti et al., 2012; Barry et al., 2011, 2012; Karami et al., 2013; Koutros et al., 2010b, 2011), and telomere length (Hou et al., 2013).

Health outcomes examined by a single study that have accounted for and presented results on relevant COI exposures include doctor visits resulting from

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pesticide exposure (Alavanja et al., 1998), rheumatoid arthritis (De Roos et al., 2005b), hearing loss (Crawford et al., 2008), and rhinitis in the past 12 months (Slager et al., 2009).

Several additional publications have discussed pesticide-use patterns in the population (Hoppin, 2005; Hoppin et al., 2006a; Kirrane et al., 2004; Samanic et al., 2005), estimated amounts of absorption of 2,4-D and chlorpyrifos exposures (Thomas et al., 2010), and have developed (Dosemeci et al., 2002) or refined (Coble et al., 2011) an algorithm for estimating pesticide exposure intensity. Other studies have focused on examining a variety of exposure issues, including the effect of exposure misclassification in the AHS (Blair et al., 2011) and using multiple imputation methods to assign pesticide use values for nonresponders and other missing data (Heltshe et al., 2012).

International Studies of Herbicide Users

Several studies have been reviewed in the VAO series that have published results of health effects on herbicide users in many different countries. In some countries, large studies were designed and large cohorts were followed, producing several publications that have been reviewed in the VAO series; those cohorts are described in brief by country of origin.

The Canadian Ontario Farm Family Health Study examined exposure to phenoxyacetic acid herbicides, including 2,4-D, and several fertility, reproductive, and pregnancy outcomes (Arbuckle et al., 1999a,b, 2001; Curtis et al., 1999; Savitz et al., 1997) and birth defects (Weselak et al., 2008). Biomonitoring was conducted in a subset of participants to evaluate the validity of the self-reported predictors of exposure (Arbuckle and Ritter, 2005; Arbuckle et al., 2002, 2005) and to examine other potential indirect sources of exposure to herbicides (Arbuckle et al., 2006). A second large cohort of 156,242 Canadian male farmers from three provinces, known as the Canadian Farm Operator Study, was assembled, and information on herbicide use in the cohort was used to determine the risk of specific causes of death in that population: NHL (Morrison et al., 1994; Wigle et al., 1990), prostate cancer (Morrison et al., 1993), brain cancer (Morrison et al., 1992), multiple myeloma (Semenciw et al., 1993), and leukemia (Semenciw et al., 1994). Other Canadian studies of agricultural workers have evaluated immune, neurobehavioral, and lung function of farmers who mixed and applied commercial formulations that contained chlorophenoxy herbicides and of residents in an agricultural area of Saskatchewan, Canada (Faustini et al., 1996), as well as asthma and pesticide use among male farmers in Saskatchewan (Senthilselvan et al., 1992). Mortality in men employed by a Canadian public utility, who were likely exposed to herbicides similar to those used in Vietnam, has also been reviewed (Green, 1987, 1991).

In Denmark, three studies reported on a cohort of Danish gardeners who had been exposed to herbicides (E. S. Hansen et al., 1992, 2007; Kenborg et

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al., 2012), and one study examined cancer incidence among Danish farmers (Ronco et al., 1992). Danish gardeners were identified from union worker records on May 1, 1975. Most of the women (n = 859) worked in greenhouses, where herbicides are not routinely used; the men (n = 3,156), however, were known to be highly exposed to pesticides and herbicides, which included the phenoxy herbicides 2,4-D, 2,4,5-T, and MCPA. Vital status of the entire cohort through 1984 was achieved. Person-years at risk were calculated and reported for a latent period of 10–15 years. E. S. Hansen et al. (1992) determined the incidence of cancer in this cohort from 1975 to 1984 and compared those numbers with expected numbers calculated from incidence rates in the general Danish population. E. S. Hansen et al. (2007) used analogous methods to extend the follow-up period for the men through 2001 but used year of birth as a surrogate for intensity of exposure, with high exposures assumed for those who had been born before 1915, low exposures for those born in 1934 or later, and intermediate exposure for those born in between. Using this same cohort of Danish gardeners but restricted to men alive and living in Denmark at the beginning of 1977 (n = 3,124), Kenborg et al. (2012) reported on the incidence of Parkinson disease as a primary diagnosis from 1977 through 2008 and compared the results with the observed incidence of Parkinson disease in all Danish men by calendar period and age group. Kenborg et al. (2012) also reported on the incidence of lung, larynx, and bladder cancers in the cohort compared with the incidence in the general male Danish population. For the current update, a study of parental employment in farming and agriculture and cryptorchidism in offspring using the Danish National Patient Registry (Jorgensen et al., 2014) was identified and reviewed.

Three studies reporting on or updating mortality or cancer morbidity in Finnish men who had applied 2,4-D and 2,4,5-T for at least 2 weeks in 1955–1971 through work removing brush have been reviewed in the VAO series (Asp et al., 1994; Riihimaki et al., 1982, 1983).

Studies of Italian herbicide users have primarily been conducted among farmers in particular regions. Gambini et al. (1997) examined cancer mortality in a cohort of rice growers in northern Italy. Two studies reported on a cohort of male farmers in Italy's southern Piedmont region who were licensed to use agricultural pesticides in 1970–1974, and the use of phenoxy herbicides in the area was reported to be twice the national average. Corrao et al. (1989) evaluated cancer incidence in the 25,945 men in the cohort on the basis of new diagnoses from hospital admissions in 1976–1983. In a continuation of that study, Torchio et al. (1994) reported on mortality through 1986 in the 23,401 men who were residents of the Piedmont area at the time of registration, stratified by the location of their residences (lived near arable land, near woodlands, and near mixed-use land).

The original VAO committee reviewed several studies that used data from the Swedish Cancer-Environment Register, which contained data from the Swedish Cancer Registry and individual responses from the 1960 and 1970 national censuses, including data on current occupation. Publications that used the Swedish

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Cancer-Environment Register included a study of cancer incidence and farm work (Wiklund, 1983); studies of STS and malignant lymphoma in agricultural and forestry workers (Wiklund and Holm, 1986; Wiklund et al., 1988a); and a study of the risk of multiple myeloma in relation to various occupational activities (Eriksson and Karlsson, 1992). Two studies of cancer mortality in Swedish railroad workers who were exposed to 2,4-D, 2,4,5-T, and other herbicides were also examined (Axelson and Sundell, 1974; Axelson et al., 1980). Other studies reviewed by the committee addressed mortality and cancer incidence in a cohort of Swedish lumberjacks (Thörn et al., 2000) and cancers in Swedish pesticide and herbicide applicators (Dich and Wiklund, 1998; Wiklund et al., 1987, 1988b, 1989a,b).

For some countries, only one or two studies have been published on exposure to the COIs and health outcomes (mortality, prevalence, or incidence) among herbicide handlers. Many of these studies were small and, especially the earlier studies, lacked adequate or specific exposure information. These have included: Argentinian farmers (Butinof et al., 2015; Lerda and Rizzi, 1991); Australians exposed to phenoxy herbicides (Fritschi et al., 2005); Dutch forestry workers exposed to 2,4,5-T (van Houdt et al., 1983) and Dutch male herbicide applicators (Swaen et al., 1992, 2004); German male agricultural plant-protection workers in the former German Democratic Republic who spent at least 5 years during 1948–1972 applying pesticides, some of which were phenoxy herbicides (Barthel, 1981); Icelanders whose occupation may have exposed them to 2,4-D (Zhong and Rafnsson, 1996); Indian herbicide sprayers and warehouse employees (Linga Reddy et al., 2015); Mexican agricultural workers (Carbajal-López et al., 2016); New Zealand forestry workers (Reif et al., 1989); and offspring of Norwegian farmers (Kristensen et al., 1997).

ENVIRONMENTAL STUDIES

Industrial accidents have led to the evaluation of long-term health effects in non-worker populations that live near areas with fairly high environmental concentrations of the COIs. Effects on residents around normally operating industrial operations, such as waste incinerators, and even on people exposed only to "background" concentrations have also been studied. The systematic follow-up studies that have been conducted on the Seveso population and the numerous analyses of the population-level data collection effort of NHANES have contributed prominently to the evidence base considered by VAO committees.

Environmental exposures to the COIs almost never occur in isolation. Exposures to dioxin-like chemicals generally occur as part of mixtures that also include non–dioxin-like chemicals that tend to correlate with the dioxin-like chemicals, so it is not surprising that specific chemicals measured in a person's serum also tend to correlate with one another; this collinearity means that it is difficult for epidemiologic studies to attribute any observed association to a particular chemical configuration (Longnecker and Michalek, 2000).

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Environmental studies are presented below, beginning with the United States, then summarizing major international cohorts.

U.S. Environmental Studies

Several populations with environmental exposures to the COIs have been reviewed in the course of the VAO series. Descriptions of those populations and cohorts for which no new information has been identified since the last VAO update report (e.g., Anniston, Alabama, Community Health Survey; the Great Lakes Fish Consumption Study; the Iowa Women's Health Study; and Times Beach and Quail Run, Missouri) can be found in Chapter 6 of *Update 2014* (NASEM, 2016a) and the volume that first reviewed the study following its publication. Studies of U.S. populations that the committee identified and that have not been the focus of multiple ongoing follow-up or publications are summarized in the chapters related to the health outcome of interest.

National Health and Nutrition Examination Survey

In the early 1960s the CDC National Center for Health Statistics began the NHANES program as a means of monitoring and assessing the health and nutritional status of people of all ages living in the United States. In 1999 the survey became a continuous program that has a changing focus on a variety of health and nutrition measurements in order to meet emerging needs. A rich variety of data—demographic and socioeconomic data; dietary information; medical, dental, and physiologic assessments; and the serum concentrations of persistent organic pollutants (POPs), including specific congeners of dioxins, furans, and PCBs—are collected through in-person interviews, health examinations, and blood samples obtained from a nationally representative sample of adults and children in the noninstitutionalized U.S. population. Information obtained from NHANES data is used to determine the prevalences of diseases, to assess nutritional status, and to establish national standards of height, weight, and blood pressure. Researchers also conduct analyses of the NHANES data for epidemiologic studies and medical research on various health outcomes using serum concentrations of various compounds to determine associations.

Starting with the preparation of *Update 2008*, VAO committees began seeing a stream of publications addressing the possible association of some pesticides and various individual and grouped dioxin-like chemicals with the occurrence of a variety of health outcomes as assessed by the surveys for particular temporal spans. NHANES data from 1999 to 2002 were used to evaluate the relationships of the COIs with cardiovascular disease (Ha et al., 2007); diabetes, metabolic syndrome, insulin resistance, and arthritis (D. H. Lee et al., 2006, 2007a,b,c); peripheral neuropathy and poor glycemic control (A1C $\geq$ 7.0%) (D. H. Lee et al., 2008); obesity via BMI and waist circumference (Elobeid et al., 2010); and

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thyroid-hormone concentrations (Turyk et al., 2007). Hypertension over this time period was examined by Ha et al. (2009) and Everett et al. (2008a), but Everett et al. also provided additional information for the years 2003–2004 in a subsequent commentary (Everett et al., 2008b). Peters et al. (2014) added NHANES data for 2005–2008 to the sets for 1999–2002 and 2003–2004 previously analyzed by Everett et al. (2008a,b) for the association of blood pressure with blood concentrations of dioxin-like PCBs 126 and 169 and mono-ortho PCBs 118 and 156. Using this expanded data set, they developed a model to predict blood PCB concentrations using generally available variables (age, sex, ethnicity, and blood lipid levels). However, because only a single mono-ortho dioxin-like PCB was used in combination with PBCs having no dioxin-like activity, this work does not augment the results previously published by Everett et al. (2008a,b) for VAO purposes.

As additional cycles of NHANES were conducted and became available for research, new analyses used data collected over multiple cycles. For example, serum samples collected from NHANES participants between 1999–2004 were analyzed for organochlorine pesticides, POP residues, and dioxin-like chemicals, including PCDDs, PCDFs, and PCBs. M. R. Cho et al. (2011) reported on the associations between bone mineral density and exposures to POPs and organochlorine pesticides. Y. S. Lin et al. (2012) examined samples for levels of dioxin-like chemicals (PCDDs, PCDFs, and PCBs based on TEQs) and their associations with total and cause-specific (cardiovascular and cancer) mortality, through 2006, based on ICD-10 codes. Everett and Thompson (2014) examined the relationship between dioxins (including TCDD) and dioxin-like PCBs (TEQs were calculated for six different dioxins and eight dioxin-like PCBs) and the prevalence of diabetic nephropathy. Using 2003–2004 NHANES data, Y. M. Lee et al. (2013) evaluated the associations between toxic equivalency factors (TEFs) for organochlorine pesticides as well as PCDDs, PCDFs, and PCBs and the risk of hyperuricemia in subjects 20 years of age and older. Jones et al. (2011) examined the association between urinary arsenic and hypertension and blood pressure in NHANES 2003–2008 participants. Each of the relevant NHANES studies and their results have been summarized in detail previously. For the current update, 1999–2004 NHANES data were used to examine the relationship of serum concentrations of dioxin-like PCBs and other relevant chemicals with cancer (S. A. Kim et al., 2015; Morgan et al., 2017), diabetes and nephropathy (Everett and Thompson, 2016), cardiovascular outcomes (S. A. Kim et al., 2015), and possible indicators of immune dysfunction (Serdar et al., 2014). NHANES data from cycles 1999–2000 and 2001–2002 were used to examine the correlation between serum concentrations of dioxin-like PCBs and cognitive impairment in adults (Przybyla et al., 2017), and NHANES data from the 2011–2012 cycle were used to study whether different sets of urinary environmental chemical concentrations are risk factors of high blood pressure (Shiue et al., 2014).

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Priority Toxicant Reference Range Study The study population for the Priority Toxicant Reference Range Study was a subgroup of participants aged 20–59 years in NHANES III (1988–1994), which was established to characterize the levels of 44 environmental toxicants (including 2,4-D and its metabolite 2,4-dichlorophenol) in urine and blood (regarded as indicators of internal dose). Unlike overall NHANES samples, which were established by rigorous statistical sampling procedures to be representative samples of the U.S. population, this study sample is regarded as a convenience sample because its 1,338 members had voluntarily provided an additional 20 ml of blood and had responded to an extra questionnaire during their regular NHANES medical examination (Needham et al., 1995).

Schreinemachers (2010) examined the association in 727 healthy adults between exposure to 2,4-D, as indicated by its presence in urine, and biomarkers that are linked to the pathogenesis of acute myocardial infarction and type 2 diabetes, namely, serum high density lipoprotein, triglycerides, total cholesterol minus high density lipoprotein, insulin, C-peptide, plasma glucose, and thyroid-stimulating hormone. Urinary 2,4-D was detectable in 102 (14%) samples, with concentrations of 1–28 mg/dL. The outcome variables were compared between participants with and without detectable urinary 2,4-D by using Wilcoxon's rank-sum test.

Krieg (2013) performed a limited assessment of cognition in 700 adults. Twelve pesticide metabolites were measured in the urine, including two chemicals found in the urine after 2,4-D exposure: unmetabolized 2,4-D and 2,4-dichlorophenol. The analysis investigated the association of urine pesticide metabolite concentrations with the results of three neurobehavioral tests (simple reaction time, symbol-digit substitution, and serial digit learning).

Longitudinal Investigation of Fertility and the Environment Study

The Longitudinal Investigation of Fertility and the Environment (LIFE) Study examined environmental influences on human fecundity and fertility. Participants were 501 male partners of couples discontinuing contraception for the purposes of becoming pregnant who were recruited in Michigan and Texas during 2005–2009. Upon enrollment, in-person interviews were conducted with each male partner to ascertain health, demographic, and reproductive histories. All data and biospecimens were collected in the home, and baseline interviews were followed by a standardized anthropometric assessment for the determination of BMI conducted by research nurses who also obtained non-fasting blood (10 mL) for quantification of serum chemicals and lipids. The quantification of POPs in serum included polybrominated biphenyl 153, 9 organochlorine pesticides, and 10 polybrominated diphenyl ethers. PCBs with TEFs included 105, 114, 118, 156, 157, 167, and 189. A baseline semen sample was obtained followed by a second sample approximately 1 month later irrespective of couples' pregnancy status. A total of 35 semen parameters were measured including 5 that reflected general characteristics

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(volume, straw distance, sperm concentration, total sperm count, hypo-osmotic swollen), 8 motility measures, 12 morphometry measures, 8 morphology measures, and 2 sperm chromatin stability assay measures. Two publications from this study were identified and are reviewed in detail in Chapter 8: Mumford et al. (2015) examined the relationship between exposure to a number of persistent organic pollutants and semen quality, and Robledo et al. (2015) used blood samples from adult couples to characterize the relationship between the concentrations of a number of persistent organic pollutants and birth outcomes.

International Environmental Studies

Several studies of international populations that were environmentally exposed to the COIs have been reviewed by VAO committees. As this chapter is not intended to be a compendium of every study ever reviewed in the VAO series, only those cohorts and groups with environmental exposures that have contributed more than one study for review by VAO committees and for which new information is available are considered here. A number of reviewed studies have used data analyzed from birth cohorts. For example, results from the Norwegian Mother and Child Cohort Study on prenatal exposure to dioxin-like PCBs and health outcomes have been previously reviewed (Papadopoulou et al., 2013; Stølevik et al., 2011). The Norwegian Mother and Child Cohort Study is a prospective, population-based pregnancy cohort that recruited more than 100,000 pregnant women (resulting in about 114,000 children) and more than 75,000 fathers from 1998 to 2008 to study the causes, variability, and trajectories of diseases over the life course. The participation rate was relatively low (41%), perhaps due, in part, to the requirement to be able to fluently read Norwegian. Questionnaires were administered to collect health data, demographic factors, lifestyle exposures, and developmental progress (Magnus et al., 2016). Blood samples were collected twice from the mothers (first at the ultrasound appointment at 17–18 weeks of gestation and later after delivery) and were processed and stored. A single blood sample was collected from fathers at the ultrasound appointment. A sample from the cord blood was drawn at delivery (Paltiel et al., 2014). New results from Caspersen et al. (2016) are presented in Chapter 8.

A second birth cohort for which several published studies have been reviewed in the VAO series is the Duisburg Birth Cohort Study (Nowack et al., 2015; Rennert et al., 2012; Winneke et al., 2014). This cohort consists of 232 healthy mother–infant pairs, in which the mothers were recruited between 2000 and 2002 in Duisburg, Germany; had no serious complications or illnesses during pregnancy or parturition; and gave birth to children who were born at term (weeks 38–42 of pregnancy). Maternal blood samples were taken during weeks 28–43 of gestation, and samples of maternal milk were collected from nursing mothers during the first 3 weeks after parturition. Both samples were tested for dioxins, dioxin-like PCBs, and six indicator PCBs (Wilhelm et al., 2008).

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Other prenatal exposures studies have examined exposure to endocrine-disrupting chemicals (including dioxins) in Belgian children (Delvaux et al., 2014) and birth weight and development (Halldorsson et al., 2009; Olsen et al., 2001) and childhood growth (Wohlfahrt-Veje et al., 2014) in Danish children who were exposed prenatally to dioxins and dioxin-like chemicals. Two publications that used data from the Danish Fetal Origins 1988–1989 Cohort, in which offspring were followed for 20 years, were identified for the current update. The first study examined prenatal levels of maternal POPs in serum and the risk of asthma in offspring (S. Hansen et al., 2014), and the second examined allergic sensitization and lung function in offspring (S. Hansen et al., 2016). The effects of prenatal and lactational dioxin and PCB exposure on several neurodevelopment and functioning parameters have been examined in a longitudinal assessment of Dutch children (Berghuis et al., 2014; ten Tusscher et al., 2014). However, these Belgian, Danish, and Dutch studies have potential relevance only to female Vietnam veterans with pregnancy subsequent to military service.

In addition to the many studies that used birth cohorts to examine point-in-time or longitudinal health effects of prenatal exposure to dioxins and dioxin-like chemicals, several cohorts of people who were exposed as adults to the COIs from their residential environments have been established and followed. These have included studies from the Dutch LifeLines cohort study, a multidisciplinary prospective population-based cohort study examining health and health-related behaviors of persons living in the northern region of the Netherlands (de Jong et al., 2014); Finnish fishermen and their wives who were exposed to higher amounts of dioxins and PCBs through consumption of contaminated fish (Turunen et al., 2008, 2012); and French residents in the vicinity of municipal solid-waste incinerators that had high levels of TCDD emissions, who were examined for cancer (Floret et al., 2003; Viel et al., 2000, 2008a,b, 2011) or birth defects (Cordier et al., 2004, 2010). For the current update, the subjects of studies of the effects of environmental exposures have included populations in Belgium (Den Hond et al., 2015; Van Larebeke et al., 2015), Brazil (Cremonese et al., 2017; Ueker et al., 2016), Canada (Singh and Chan, 2017; Thomas et al., 2015), China (J. Z. Yang et al. 2015; X. L. Yang et al., 2015; J. Zhang et al., 2014), France (Danjou et al., 2015; Kalfa et al., 2015; Mayhoub et al., 2014; Nicolle-Mir, 2014; Ploteau et al., 2017), Germany (Fimm et al., 2017; Haase et al., 2016; Putschogl et al., 2015), Greece (Vafeiadi et al., 2017), Hong Kong (Hui et al., 2016), Italy (Cappelletti et al., 2016), Korea (Lim et al., 2017), Nicaragua (Raines et al., 2014), Norway (Koutros et al., 2015), Russia (Galimova et al., 2015), and Spain (Martínez-Zamora et al., 2015; Paul et al., 2017).

Some environmental exposures, such as the large accidental release of TCDD in Seveso, Italy, have been the subject of multiple studies and long-term follow-up. For many of these populations new studies continue to be published, and those that have adequate exposure specificity and assessment are reviewed by

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VAO committees. Additional background on those groups is summarized in more detail below.

Seveso, Italy

On July 10, 1976, a large industrial accident caused by an uncontrolled reaction during TCP production in Seveso, Italy, resulted in chemical release to the surrounding area that created an environmental exposure to TCDD. The degree of TCDD contamination in the soil has been used extensively as a means of imputing exposures of members of the population. Three areas were defined on the basis of soil sampling: Zone A (556 people), the most heavily contaminated, from which all residents were permanently evacuated within 20 days; Zone B (3,920), an area of lower contamination that all children and women in the first trimester of pregnancy were urged to avoid during daytime; and Zone R (26,227), a region with some contamination in which the consumption of local crops was prohibited (Bertazzi et al., 1989a,b). The sample sizes differ among follow-up studies, presumably because of migration; the sample sizes given above were reported in Bertazzi et al. (1989b).

Cohort of Entire Exposed Population

Data on serum TCDD concentrations in Zone A residents have been presented by Mocarelli et al. (1990, 1991) and by CDC (1988e). In the 10 residents who had severe chloracne, TCDD concentrations were 828–56,000 ppt of lipid weight (median 16,600 ppt). In 9 residents without chloracne (one sample was lost), TCDD concentrations were 1,770–10,400 ppt (median 4,540 ppt). TCDD was undetectable in all control participants but one. The highest of the concentrations exceeded any that had been estimated at the time for TCDD-exposed workers on the basis of backward extrapolation and a half-life of 7 years. Data on nearby soil concentrations, the number of days that a person stayed in Zone A, and whether local food was consumed were considered in evaluating TCDD, but none of those data correlated with serum TCDD, which suggested strongly that the important exposure was from fallout on the day of the accident. The presence and degree of chloracne correlated with TCDD. Adults seemed much less likely than children to develop chloracne after acute exposure, but surveillance bias could have affected that finding. More recent updates (Bertazzi et al., 1998, 2001) have not changed the exposure-assessment approach.

A number of studies of the Seveso population have used lipid-adjusted serum TCDD concentrations as the primary exposure metric (Baccarelli et al., 2002; Eskenazi et al., 2002a,b, 2003, 2004, 2005, 2007, 2010, 2014; Landi et al., 2003). Fattore et al. (2003) measured the current air concentrations of PCDDs in Zones A and B and compared them with measurements in a control area near Milan.

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The authors concluded that a release from PCDD-contaminated soil did not add appreciably to air concentrations in the Seveso study area. Finally, Weiss et al. (2003) collected breast milk from 12 mothers in Seveso and compared TCDD concentrations in the milk with concentrations in a control population near Milan, and they found that the TCDD concentrations in milk from mothers in Seveso were twice as high as in controls. The authors concluded that breastfed children in the Seveso area were likely to have higher body burdens of TCDD than children in other areas.

Several cohort studies have been conducted using the Zone A, Zone B, and Zone R exposure categories. There have been multiple long-term follow-up investigations of the health outcomes, especially cancers, of Seveso residents. Bertazzi and colleagues, for example, conducted 10-year mortality follow-up studies of adults (Bertazzi et al., 1989a) and children who were 1–19 years old at the time of the accident (Bertazzi et al., 1989b, 1992), 15-year follow-up studies (Bertazzi et al., 1997, 1998), and a 20-year follow-up study (Bertazzi et al., 2001). Pesatori et al. also conducted a 15-year follow-up study to update non-cancer mortality (1998), and a 20-year follow-up of incident cancers (2009). Consonni et al. (2008) reported on the 25-year follow-up (through 2001) vital status of residents ("present") in the Seveso area and reference territory at the time of the Seveso accident and of immigrants and newborns ("non-present") in the 10 years thereafter. Cause-specific mortality was determined for each zone, compared with that in the comparison cohort, and adjusted for presence at the accident, sex, age, and time since the Seveso accident. Most recently, Eskenazi et al. (2018) published a review summarizing the results of research studies conducted in this population over the 40 years since the explosion as well as areas of continuing investigation, including effects in children and grandchildren of both men and women exposed to dioxin in the three zones of exposure.

In addition to a 2-year prospective controlled study of workers potentially exposed to TCDD during the cleanup of the most highly contaminated areas after the accident (Assennato et al., 1989a), other studies have examined specific health effects associated with TCDD exposure in Seveso residents—chloracne, birth defects, and spontaneous abortion—as well as crude birth and death rates (Bisanti et al., 1980); the distribution of chloracne in Seveso children (Caramaschi et al., 1981); chemicals in the blood and urine of children who had chloracne (Mocarelli et al., 1986); chloracne and peripheral nervous system conditions (Barbieri et al., 1988); dermatologic and laboratory tests in a group of the children who had chloracne and in a group of controls (Assennato et al., 1989b); health status and TCDD concentrations in chloracne cases and non-cases (Baccarelli et al., 2005a) that had been recruited previously by Landi et al. (1997, 1998); hepatic enzyme–associated conditions (Ideo et al., 1982, 1985); abnormal pregnancy outcomes (Mastroiacovo et al., 1988); cytogenetic abnormalities in maternal and fetal tissues (Tenchini et al., 1983); neurologic disorders (Boeri et al., 1978; Filippini et al., 1981); cancers (Bertazzi et al., 1993; Pesatori et al., 1992,

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1993, 2008, 2009); the sex ratio of offspring who were born in Zone A (Mocarelli et al., 1996); birth weight and neonatal thyroid function (Baccarelli et al., 2008); immunologic effects (Baccarelli et al., 2002); effects on reproductive hormones and sperm quality (Mocarelli et al., 2008, 2011); aryl hydrocarbon receptor (AHR)-dependent pathway and toxic effects of TCDD in humans (Baccarelli et al., 2004); effects of TCDD-mediated alterations in the AHR-dependent pathway in people who lived in Zones A and B (Landi et al., 2003); and NHL-related (14;18) translocation prevalence and frequency in dioxin-exposed healthy people in Seveso (Baccarelli et al., 2006). Baccarelli et al. (2005b) reviewed statistical strategies for handling non-detectable readings or readings near the detection limit in dioxin-measurement datasets. They recommended that a distribution-based multiple-imputation method be used to analyze environmental data when substantial proportions of observations have non-detectable readings.

Seveso Women's Health Study

The Seveso Women's Health Study (SWHS) was undertaken to evaluate the association between individual serum TCDD concentrations and reproductive effects in women who resided in Seveso at the time of the 1976 accident. From a pool of 1,271 eligible women who were between infancy and 40 years old at the time of the accident, who had resided in Zone A or B at that time, and for whom adequate serum remained from the samples collected shortly after the explosion, 981 were enrolled in the study group in 1996–1998 (80% participation rate). All the women were interviewed by a nurse blinded to their exposure status, and each subset received gynecologic examinations. Medical records of those who reported ever having received a diagnosis of cancer were obtained and subjected to blind review by a pathologist. The stored samples were used for new TCDD analyses with improved analytic techniques that had become available.

As an initial step in the SWHS, Eskenazi et al. (2001) tested the validity of exposure classification by zone. Investigators measured serum TCDD in samples collected in 1976–1980 from 601 residents (97 in Zone A and 504 in Zone B). A questionnaire that the women completed in 1996–1998 included age, chloracne history, animal mortality in the vicinity, consumption of homegrown food, and the woman's location at the time of the explosion. Participants did not know their TCDD concentrations at the time of the interview, but most knew their zones of residence. Interviewers and TCDD analysts were blinded to the participants' zones of residence. The zone of residence explained 24% of the variability in serum TCDD. Adding the questionnaire data improved the regression model to the point that it explained 42% of the variability. Those findings demonstrate a significant association between zone of residence and serum TCDD, but much of the variability in TCDD concentration is still unexplained by the models. Warner et al. (2005) used a chemical-activated luciferase-gene expression bioassay and a high-resolution isotope-dilution gas-chromatography mass-spectrometry assay

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to measure PCDDs, PCDFs, and PCBs in the serum of 78 women who resided near Seveso in order to determine average total dioxin-like chemical TEQs; the two methods produced similar results. Warner et al. (2014) compared the concentrations of TCDD in serum samples taken in 1976 and in 1996 to examine characteristics that could be used to predict 1996 TCDD concentrations. They also presented an updated estimate of the TCDD elimination half-life over the 20-year period since the uncontrolled release took place.

In one study, the women enrolled in the SWHS were assessed for cancer incidence in the 20 years following the accident (Warner et al., 2002). Warner et al. (2011) later added more than 10 years of observations on cancer incidence in the women in the SWHS, covering the period from the 1976 explosion through 2009. Of the 981 women who had participated in the first study, 833 were located, alive, and willing to participate in the second. Each was re-interviewed, provided clinical measurements, and consented to a medical record review to confirm her cancer diagnosis. A subset underwent bone-density testing. The average age was 50.8 years. An additional 45 cancers had been diagnosed, for a total of 66 cases, of which 33 were breast cancers. Thyroid cancer was the next most prevalent, with 7 cases, and the 15 other types of cancer observed had at most 3 cases. After adjusting for the women's age at the time of the accident and for marital status, the researchers found that the association between the risk of any cancers and the lipid-adjusted, log-transformed serum TCDD concentrations at the time of the accident was significantly higher in the subjects than in controls. The availability of serum TCDD concentrations measured from blood samples gathered fairly soon after the single-substance accident (which minimizes uncertainty about what exposure had been experienced and reduces the need for back-extrapolation) contributes substantially to the value of the results.

A series of studies have examined the associations between serum TCDD and a variety of endpoints related to female reproductive functioning: menstrual cycle (Eskenazi et al., 2002a), endometriosis (Eskenazi et al., 2002b), pregnancy outcome (Eskenazi et al., 2003a), age at menarche and age at menopause (Eskenazi et al., 2005), and age at menarche in women who were premenarcheal at the time of the explosion (Warner et al., 2004). Eskenazi et al. (2007) and Warner et al. (2007) examined the incidence of fibroids and ovarian function, respectively, in SWHS participants.

Eskenazi et al. (2010) examined the relationship between serum TCDD around the time of the accident and time to pregnancy in 463 SWHS participants who had attempted pregnancy since the accident and who were no more than 40 years old at the time of the accident. The main analysis was restricted to the 278 women who had delivered live births that were not the results of contraceptive failure. Effect estimates for the associations between TCDD and fecundity and between TCDD and infertility were adjusted for several factors including maternal age, maternal smoking in the year before conception, parity, menstrual-cycle irregularity, oral-contraceptive use in the year before attempt, paternal age near

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the time of conception, and the history of reproductive and endocrine conditions. Wesselink et al. (2014) examined the risk of adverse pregnancy outcomes in relation to TCDD concentrations (measured in 1996) in the SWHS among 1,211 post-chemical-explosion pregnancies through the 2008–2009 follow-up assessment. The birth outcomes that were examined included gestational age, pre-term delivery, and birth weight. Of note, only 35% of women in the analysis were age 21 or older at the time of explosion, thereby limiting the inference of this work to female veterans who served in Vietnam.

Several publications from the SWHS have been reviewed by the committee that examined health outcomes in addition to reproductive and pregnancy outcomes. These have included risk for the development of diabetes and metabolic syndrome 30 years after the accident (Warner et al., 2013), thyroid hormone levels (and disruption) in relation to TCDD concentrations measured over time (Chevrier et al., 2014), and TCDD concentrations measured in 1996 versus the bone mineral density of the spine and hip measured in 2008 (Eskenazi et al., 2014). A study of neurocognitive and physical functioning among the SWHS participants is reviewed in the current volume (Ames et al., 2018).

Japanese Environmental Studies

Several population-based studies of relevant exposures in Japan have been considered by VAO committees, including investigations of serum concentrations of PCDDs, PCDFs, and dioxin-like PCBs relative to the prevalence of diabetes (Uemura et al., 2008a), distributions with respect to various demographic characteristics (Uemura et al., 2008b), and the prevalence of metabolic syndrome (Uemura et al., 2009). In a separate analysis representative of the Japanese population, Nakamoto et al. (2013) gathered fasting blood samples from a cross-sectional sample of 1,063 men and 1,201 women (aged 15–76 years) who were living in 125 areas of 45 prefectures throughout Japan and who were not occupationally exposed to dioxins (including TCDD). The full WHO 2005 set of dioxin-like PCDDs, PCDFs, and PCBs were measured in the samples and assessed in relation to a range of self-reported history of diseases, including allergic diseases, hypertension, diabetes, hyperlipidemia, gout, thyroid disease, kidney disease, gastric ulcer, and gynecological disease. Fukuda et al. (2003) examined mortality and incinerator dioxin emissions in municipalities in Japan. The associations of adverse pregnancy outcomes and proximity of maternal residence (10 kilometers or fewer) with municipal solid waste incinerators with high dioxin emission levels at the time of birth have also been examined (Tango et al., 2004).

The emphasis of environmental studies in Japan has been on the long-term follow-up of the 1968 Yusho rice oil poisoning accident. The accident occurred in western Japan, where the rice oil intended for cooking was contaminated during processing with PCBs and PCDFs, exposing more than 1,900 people (Kuratsune et al., 1972). Based on unusual symptoms following exposure, a total of 1,961

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cases of "Yusho Disease" were registered and subsequently followed for more than 40 years. Because of changes in the symptoms and advances in analytic techniques, the diagnostic criteria have changed several times since they were first published in 1968 (Akahane et al., 2017). Several studies have considered exposure status based on designation as a Yusho case, but VAO committees have generally not reviewed such studies unless the concentrations of dioxins and dioxin-like chemicals were presented, such as in Tsukimori et al. (2012, 2013) (reviewed in *Update 2012*), and M. C. Li et al. (2015) and Mitoma et al. (2015), which are both reviewed in the current update.

Hokkaido Study on Environment and Children's Health

The Hokkaido Study on Environment and Children's Health is a prospective birth cohort study that began in 2002 and includes two cohorts (Kishi et al., 2017). The first is the Sapporo (Toho Hospital) cohort with one obstetric hospital, and the second cohort is the much larger Hokkaido cohort, which has 37 hospitals and clinics. The primary study goals are to examine the effects of low-level environmental chemical exposures on birth outcomes; to follow the development of allergies, infectious diseases, and neurobehavioral developmental disorders and perform a longitudinal observation of child development; to identify high-risk groups based on genetic susceptibility to environmental chemicals; and to identify the additive effects of various chemicals, including tobacco smoking (Kishi et al., 2017).

The Sapporo cohort consists of 514 women who enrolled at 23–35 weeks of gestation and who had planned to deliver at Toho Hospital in Sapporo city between July 2002 and October 2005 (participation rate of 26.6%). A self-administered questionnaire was completed at the time of enrollment to obtain parental baseline information. Various specimens, including maternal and cord blood, maternal hair, and breast milk, were collected for the assessment of exposures to 29 dioxin and dioxin-like PCB congeners, 58 other PCB congeners, and 5 hydroxylated PCB congeners. Maternal medical and infant birth records were obtained from the hospital. Follow-ups and the administration of neurobehavioral developmental tests were conducted at ages 6 and 18 months and 3.5 and 7 years. The focus of investigations using the Sapporo cohort are on child neurobehavioral development, but the development of asthma, allergies, and infectious diseases is also examined.

The Hokkaido cohort enrolled 20,926 pregnant women before 13 weeks of gestational age who visited one of the associated hospitals or clinics in the Hokkaido prefecture between February 2003 and March 2012 (participation rate of 55%). A simultaneous analysis of 11 perfluorinated alkyl substances in maternal plasma collected during the third trimester of pregnancy was conducted. The Hokkaido cohort focuses on rare diseases such as birth defects and developmental disorders as well as on the prevalence of complicated pregnancies and birth

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outcomes, such as miscarriage, stillbirth, low birth weight, preterm birth, and small for gestational age. Follow-ups of the children were conducted at 18 months and 3 years of age and began in October 2013 and January 2015, respectively. The follow-ups of 5- and 6-year-old participants started in October 2014 (Kishi et al., 2017).

Previous VAO volumes have reviewed publications from the Sapporo and Hokkaido cohorts. Four publications using subsets of the cohort data were identified and reviewed in this volume. Kobayashi et al. (2017) obtained maternal blood samples during the third trimester or within a week of delivery. The samples were tested for total dioxin levels (as the sum of 29 congeners) and genotyping for genes coding three enzymes involved in dioxin metabolism. The genotype status was previously shown to be related to birth weight in 484 children in the Hokkaido cohort. Miyashita et al. (2015a,b) investigated 70 PCB congeners, including dioxin-like PCBs, in the blood of 367 mother–child pairs from the Hokkaido Study cohort and the relationship with newborn anthropometric measurements of birth weight (small for gestational age), length, chest circumference, and head circumference. Finally, Nakajima et al. (2017) examined sex-specific differences in the effect of prenatal exposure to dioxin-like chemicals on neurodevelopment in children who were participants in the Sapporo cohort; 190 mother–infant pairs in the 6-month-old group and 122 mother–child pairs in the 18-month-old group were studied.

Russian Environmental Studies

Chapaevsk

Several studies in the Samara region of Russia have identified the Middle Volga Chemical Plant (also known as SZVH or Khimprom) in Chapaevsk, about 950 kilometers southeast of Moscow, as a major source of TCDD pollution (Revazova et al., 2001; Revich et al., 2001). From 1967 to 1987 the plant produced γ-hexachlorocyclohexane (lindane) and its derivatives, and many of the workers experienced chloracne. Since then, it has produced various chlorinated products. Dioxins were detected in the small number of air, soil, drinking-water, and cow's-milk samples gathered in the region, but no description of how these media were sampled was given. When Revich et al. (2001) compared the samples with measurements from four other Russian cities that had industrial facilities, the TCDD concentrations observed in Chapaevsk exceeded all reported maximums. Revich et al. (2001) presented rudimentary comparisons of cancer incidence and mortality and reproductive outcomes with regional and national rates; residence in the city of Chapaevsk was used as a surrogate for exposure, and no attempt was made to create exposure categories based on factors that might have influenced the degree of TCDD exposure. The analyses of chromosomal aberrations and other cytologic indicators of genetic damage partitioned the women studied

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into three groups on the basis of worker status or distance of residence from the factory (Revazova et al., 2001).

Chapaevsk Children's Study Later research efforts on Chapaevsk residents have focused on quantifying the serum concentrations of dioxins and TEQs associated with furans and PCBs. Akhmedkhanov et al. (2002) reported on a convenience sample of 24 volunteers. The Russian Children's Study was designed to assess the effect of in utero and childhood exposure on development. Although 516 peripubertal boys (identified through health insurance and clinic records) were enrolled, the final cohort consisted of 499 boys and 449 mothers. This prospective longitudinal study enrolled boys at age 8–9 years (in 2003–2005) who then underwent a physical exam and blood sampling and who, together with the mother or guardian, completed a questionnaire. Annual follow-up examinations were also conducted (9-year retention rate of 73%), blood is collected biennially, urine is collected annually, and semen collection began in 2012. Serum samples were used to measure 7 PCDDs, 10 PCDFs, 4 non-ortho-substituted PCBs, 6 mono-ortho-substituted PCBs, and 31 other non-dioxin-like PCBs.[2] Although the design does not allow researchers to isolate possible in utero exposure and postnatal exposure and its utility is further limited by the fact that subjects were exposed to dioxins in a different period of their lives (infancy, childhood, and adolescence) than Vietnam veterans, the exposures are well characterized. The information generated by this cohort will be relevant to VAO reports only in conjunction with effects in offspring after maternal exposure to the extent that the consequences of gestational and childhood exposure can be distinguished. The published findings have detailed the characterizations of serum concentrations in the boys (J. S. Burns et al., 2009) and their mothers (Humblet et al., 2010), and the first papers that examine semen parameters are detailed in Chapter 8 (Mínguez-Alarcón et al., 2017). The committee also heard an invited presentation from the lead researcher, Dr. Russ Hauser, and is able to offer a bit more detail regarding initial findings based on his presentation.

Swedish Environmental Studies

Most Swedish environmental studies have focused on results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Participants were recruited within 2 months after their 70th birthdays randomly from the registry of residents of the community of Uppsala, Sweden, between April 2001 and June 2004. The primary aim was to investigate cardiovascular disease (CVD) in an elderly population. Of the 2,025 subjects who were invited to participate, 1,016 were included, for a participation rate of about 50%; half

[2]Dr. Russ Hauser, Harvard T.H. Chan School of Public Health, presentation to the committee November 30, 2017.

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of the participants were female. All participants answered a questionnaire about their medical history, medications, diet, and smoking habits. The burden of POPs, including several dioxin-like PCBs, was assessed from blood serum or plasma. However, the results are limited by the fact that participants were recruited in the 2-month period after their 70th birthday. This potentially imparts a survival bias, meaning that persons from the catchment area with very high levels of POPs may have been disproportionately excluded from the study sample. In addition, results are limited by the relative non-specificity of the POPs examined, although the battery of congeners did include octachlorodibenzo-*p*-dioxin, which is relevant to Vietnam veterans.

VAO committees have reviewed several studies from the PIVUS data or subsets of it. The subjects of these studies have included sex differences in the concentrations of 17 of 21 POPs (Salihovic et al., 2012a); a new method for extracting POPs from human blood (Salihovic et al., 2012b); and associations between POPs and type 2 diabetes (D. H. Lee et al., 2011), fat mass and abdominal obesity (D. H. Lee et al., 2012a; Rönn et al. 2011), stroke (D. H. Lee et al., 2012b), and carotid atherosclerosis (Lind et al., 2012). Many other studies from the PIVUS cohort have examined relationships between POPs and surrogate health outcomes, including CVD measures and risk factors; examples include left ventricular systolic and diastolic dysfunction (Sjöberg Lind et al., 2013b), left ventricular hypertrophy (Sjöberg Lind et al., 2013a), and carotid atherosclerosis (Lind et al., 2012). In addition to these health outcomes, changes in weight (Lind et al., 2013), inflammatory markers, complement system, and oxidative stress (Kumar et al., 2014a,b,c) have also been studied. These studies augment previous publications from PIVUS that examined a range of indicators related to CVD and cardiovascular health. The topics of new publications from the PIVUS cohort reviewed in this volume include outcomes of hypertension (Lind et al., 2016), circulating lipid levels (Penell et al., 2014), and cognitive impairment (D. H. Lee et al., 2014).

Other studies of Swedish cohorts have also been included in the VAO series when the exposures have been relevant.

Taiwanese Environmental Studies

Two populations with environmental exposures in Taiwan have been examined by VAO committees: residents of contaminated areas and mother–child studies. The first type of studies primarily examined people who resided near a closed factory in the An-nan District of Tainan City in southwestern Taiwan that had manufactured PCP, which left the area highly contaminated with dioxin. Na-PCP, a widely used pesticide, had been used in the production process at the abandoned factory, and after the factory shut down, a large quantity was improperly stored and later released into the environment. A cross-sectional study was conducted in 2005–2007 using subjects recruited from a health center near the

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factory, and the final sample consisted of about 80% of the invited residents of the community. A series of publications using these data have been reviewed by prior VAO committees, an overview of which is provided here. The general limitations for all those studies include an unknown age at first exposure to PCDDs and PCDFs; an unknown duration of exposure; the cross-sectional design; an adjustment for obesity as one element of metabolic syndrome, rather than BMI; and some arbitrary choices inherent in factor analysis. J. W. Chang et al. (2010) used these data to report on the relationship between exposure to PCDDs and PCDFs and hypertension in metabolic syndrome in 1,490 non-diabetic people residing in this area. In addition, an analysis of the association between each congener and the prevalence of metabolic syndrome was conducted. J. W. Chang et al. (2011a) then reported on the same cross-sectional study, restricted to 1,449 non-diabetic residents, to investigate the joint effects of exposure to dioxins (from the factory) and mercury (from eating contaminated seafood from the reservoir near the factory) on pancreatic endocrine function; the committee noted several limitations with the study's analysis and methods for assessing insulin-resistance. Using the same cross-sectional study with enrollment extended to December 2009, J. W. Chang et al. (2011b) investigated the association between PCDD and PCDF exposure and continuous measures of CVD within 10 years as measured by the Framingham Risk Score, a formula for combining established risk factors into a single number, using a sample of 914 residents who did not have CVD and who were 30–45 years old. One limitation is the use of the Framingham score; other factors are associated with risk but were not included in the score, such as socioeconomic position, genetics, and imaging biomarkers. A fourth publication using this cross-sectionally collected data involved 1,167 residents who were more than 50 years old and had fasting blood samples available to investigate the biochemical profiles of those exposed to PCDDs and PCDFs (J. W. Chang et al., 2012). For this analysis, there were three exposure groups of retired Na-PCP workers: those who still lived locally, those who lived locally but did not knowingly eat polluted fish, and those who had moved away. Three control groups did not include any Na-PCP workers and consisted of local residents who had eaten polluted fish, local residents who had not eaten polluted fish, and "background participants" in Taiwan's general population. The first two of the control groups made up the 1,167 in the study population. The limitations of the study include the unknown PCDD and PCDF concentrations in retired workers who moved away and knowledge about when the exposure ceased. There may also be important unmeasured confounders related to which workers moved away and which ones did not.

Three new studies among the residential population near this factory were identified and reviewed in the current volume. The outcomes examined are type 2 diabetes (C. Y. Huang et al., 2015), chronic kidney disease (C. Y. Huang et al., 2016), and hyperuricemia (J. W. Chang et al., 2013).

The second type of relevant environmental exposure studies conducted in the Taiwanese population are mother-and-child studies, which have been reviewed

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in Chapter 8. These have included findings from a prospective study of healthy Taiwanese mothers and their children recruited during the mothers' pregnancy to study the associations between exposures to PCDDs, PCDFs, and PCBs and health outcomes (Chao et al., 2004, 2007; P. H. Su et al., 2010, 2012; S. L. Wang et al., 2004, 2005). The study enrolled pregnant women who had no clinical complications, were 25–35 years old, and delivered in the period December 1, 2000, to November 30, 2001, in a medical center in central Taiwan, the location of a solid-waste incinerator. Participants completed a questionnaire concerning maternal age, occupation, disease history, cigarette smoking, alcohol consumption, dietary habits, and the baby's stature. Biologic samples (including placenta, umbilical cord blood, mother's venous blood, and breast milk) were collected for analysis of PCDDs, PCDFs, and PCBs. A total of 610 women were enrolled (80% of those invited). The placenta was collected from and the questionnaire completed by 430 participants. Of those, 250 provided sufficient venous blood for the chemical analyses. Of the 250, 175 provided adequate breast-milk samples. S. L. Wang et al. (2004) reported on PCDDs, PCDFs, and PCBs in the biologic samples and on correlations among specimens. Chao et al. (2004) reported on PCDDs, PCDFs, and PCBs in breast milk and the cumulative dose derived for infants exclusively breastfed versus those formula-fed. In a follow-up analysis, S. L. Wang et al. (2005) examined the association between high and low in utero exposures to PCDDs, PCDFs, and PCBs and the thyroid and growth hormones in the newborns overall and by sex. S. L. Wang et al. (2006) examined the association between PCDDs, PCDFs, and PCBs measured in the placenta and estrogens and metabolites measured in mothers' blood. P. H. Su et al. (2010) reported on the 2-year and 5-year follow-ups of the mother–child pairs of S. L. Wang et al. (2005). Children's anthropomorphic measures were obtained, including height, weight, BMI, head circumference, chest girth, bone age, and the ratio between bone age and chronologic age. Thyroid, sex-hormone, and growth-factor concentrations were measured in venous blood samples obtained from those children whose mothers' serum PCDD and PCDF TEQs were available. The anthropomorphic measures and the thyroid, sex-hormone, and growth-factor concentrations were compared in children with high (≥15 pg-TEQ/g of lipid) versus low (< 15 pg-TEQ/g of lipid) in utero PCDD and PCDF concentrations. Comparisons were made by sex and by pooling all children. P. H. Su et al. (2012) reported on the 8-year follow-up of the same cohort in a subset of 23 boys and 33 girls. In addition to anthropomorphic measures used in previous waves, reproductive development (breast, genital, and armpit stages) was assessed. P. H. Su et al. (2015) conducted a study of 56 children from the cohort stratified into high- and low-exposure groups based on maternal PCB and PCDD/F concentrations to examine hormone levels and other measures of blood chemistry.

Other studies of health effects in offspring prenatally exposed to the COIs in Taiwan have been reviewed, but they have been from different population samples.

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Vietnamese Environmental Studies

Various epidemiologic studies have been conducted in subsets of the Vietnamese population who were exposed to the herbicide spraying that occurred during the Vietnam War. In a review paper, Constable and Hatch (1985) summarized the unpublished results of studies conducted by researchers in Vietnam. They also examined nine reports that focused primarily on reproductive outcomes (Can et al., 1983a,b; Huong and Phuong, 1983; Khoa, 1983; Lang et al., 1983a,b; Nguyen, 1983; Phuong and Huong, 1983; Trung and Chien, 1983). Vietnamese researchers later published the results of four additional studies: two on reproductive abnormalities (Phuong et al., 1989a,b), one on mortality (Dai et al., 1990), and one on hepatocellular carcinoma (Cordier et al., 1993). Ngo et al. (2006) published a meta-analysis that addressed an association between exposure to herbicides in Vietnam and birth defects and that covered some reports reviewed previously by Constable and Hatch (1985), some new Vietnam studies, and studies on U.S. and Australian veterans who served in Vietnam.

In general, three types of environmental studies have been conducted in Vietnam and reviewed by VAO committees: those that have measured environmental concentrations of contaminants in soil or animals (food) in "hot spot" areas such as Bien Hoa City (Schecter et al., 2003), studies of mother–child pairs and concentrations of dioxins in breast milk, and surrogate measures of health outcomes in adults in contaminated areas. In total, 10 new studies of outcomes in the Vietnamese population were identified and reviewed for the current volume.

C. H. Nguyen et al. (2017) examined serum TCDD levels and the expression of AHR and a variety of pro-inflammatory cytokines in people living in areas that were sprayed with herbicides and in areas that were not. Two studies (S. Hansen et al., 2009; Nhu et al., 2009) examined maternal concentrations of organochlorine chemicals and dioxins in communities known to have been sprayed with herbicides during the war compared with communities that were not sprayed. However, no results were reported on associations between the concentrations of these chemicals in mothers and health status in mothers or infants. Two new studies of mothers and their children in different herbicide-contaminated and non-contaminated areas in Vietnam were reviewed in the current volume (Anh et al., 2017; Van Tung et al., 2016).

Several studies have examined mother–infant pairs who were living near the Da Nang airbase, the site of a former U.S. airbase, which is an area of documented high exposures to TCDD and other PCDD/Fs. The Da Nang Birth Cohort consists of 216 mother–infant pairs recruited in 2008–2009. The recruitment and residence area includes two districts in a surrounding area of 10 kilometers from the former air base. This is because the residents outside the immediate area of the airbase have also been shown to have high dioxin levels suspected to have been caused by the ingestion of contaminated food and water originating from the airbase. Breast-milk samples were collected from each nursing mother 1 month after

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she gave birth in order to quantify the levels of 17 different 2,3,7,8-substituted PCDD and PCDF congeners, and TEQs were calculated.

Tai et al. (2013) examined 216 mother–infant pairs and relationships between the dioxin levels in breast milk and infant neurodevelopment parameters (based on the Bayley Scales of Infant and Toddler Development) at 4 months of age. Tai et al. (2016) followed the birth cohort longitudinally for neurodevelopment and physical growth during the first 3 years of life. In Pham et al. (2015), the Bayley Scales of Infant Development, 3rd edition, was administered at age 12 months to examine differences in overall cognition, language composite, receptive language and expressive language, and motor skills. Nishijo et al. (2014) examined 153 mother–infant pairs in the Da Nang birth cohort to evaluate potential associations between perinatal dioxin exposure and autism spectrum disorders in the children. Nishijo et al. (2015) focused on urinary metabolite levels in 26 children who were part of the study cohort and examined associations with dioxin-exposure-induced neurodevelopmental deficits. Using this same birth cohort, Tran et al. (2016) investigated effects of early life exposure to dioxins in 176 children. Dioxins were measured at birth and 5 years of age and compared with outcomes of the Movement Assessment Battery for Children-2 test and other tests of pattern reasoning, planning ability, and neurodevelopmental skills.

Although they are not a primary health outcome of interest per se, X. Sun et al. (2013) compared prostate-specific antigen levels (which may be indicative of a risk for prostate cancer, but is not a surrogate of prostate cancer) in a cross-sectional study of men over the age of 50 years residing in the Phu Cat district (a presumed contamination hot spot, n = 101) with those residing in the Kim Bang district (presumed non-sprayed, n = 97). Analyses were adjusted for age and included stratification by occupation, including farmers and other non-farm occupations. Results of this study are limited by its cross-sectional design and, in particular, the relatively crude measurement of exposure assessment many years after the time when herbicide spraying would have occurred. In a second study by X. L. Sun et al. (2014) that used similar methodology and surrogate indicators of health (and thus has similar limitations), serum dioxin and steroid hormone levels were compared between 48 men in the presumed hot spot area (Phu Cat district) and 36 men in the non-sprayed area (Kim Bang district). Five dioxin congeners expressed as TEQs were calculated along with the levels of nine serum steroid hormones, including testosterone, cortisol, estradiol, and others. Multiple linear-regression analyses were conducted with statistical adjustments made for age, BMI, employment status, and tobacco use.

Using the same study population as in prior studies (X. L. Sun et al., 2013, 2014), two new publications compared steroid hormone levels and other blood chemistry between men residing in the presumed hot spot area and men residing in the non-sprayed area (X. L. Sun et al., 2016, 2017). However, these studies are somewhat limited in that these measures do not serve as indicators or even surrogates of health conditions or diseases of primary concern to Vietnam veterans.

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CASE-CONTROL STUDIES

Numerous case-control studies have been reviewed in previous updates, some of which have produced multiple publications on the same population or dataset. For example, several case-control studies of specific cancers, such as STS, HL, and NHL, in Sweden were carried out to investigate exposure to phenoxyacetic acids and other relevant COIs (Eriksson et al., 1979, 1981, 1990; Hardell, 1977, 1979, 1981; Hardell and Bengtsson, 1983; Hardell and Eriksson, 1988, 1999; Hardell and Sandström, 1979; Hardell et al., 1980, 1981, 2002; Olsson and Brandt, 1988; Persson et al., 1989, 1993; Wingren et al., 1990). Similarly, overlapping case-control studies have been conducted among New Zealanders exposed to phenoxy herbicide and chlorophenols examining incidence and mortality from specific cancers (Pearce et al., 1985, 1986a,b, 1987; Smith and Pearce, 1986; A. H. Smith et al., 1983, 1984). Other case-control studies conducted internationally have addressed the connection between the COIs and various cancers in England (Balarajan and Acheson, 1984; Magnani et al., 1987), France (Aras et al., 2014; Orsi et al., 2009), Italy (Amadori et al., 1995; Donna et al., 1984; LaVecchia et al., 1989; Musicco et al., 1988; Nanni et al., 1996; Vineis et al., 1986), Canada (McDuffie et al., 1990; Ng et al., 2010; Spinelli et al., 2007), Australia (Smith and Christophers, 1992), and Denmark (Mellemgaard et al., 1994).

Case-control studies have been conducted in various U.S. populations looking for associations of herbicides with cancers. Studies have included leukemia mortality among white farmers in Nebraska (Blair and Thomas, 1979; Blair and White, 1985), Iowa (Burmeister, 1981; Burmeister et al., 1982), and in Iowa and Minnesota (L. M. Brown et al., 1990). Another study investigated associations of leukemia and NHL with 2,4-D in eastern Nebraska (Zahm et al., 1990). Other lymphohematopoietic cancer outcomes investigated as case-control studies in U.S. populations include NHL (Cantor, 1982; Cantor et al., 1992; Czarnota et al., 2015; Hartge et al., 2005; Tatham et al., 1997; Zahm et al., 1993), multiple myeloma (Boffetta et al., 1989; L. M. Brown et al., 1993; Morris et al., 1986), and NHL and HL (Dubrow et al., 1988). Other studies have assessed, generally using occupation or residence as surrogates for exposure, multiple cancer outcomes, such as gastric cancer, prostate cancer, NHL, and multiple myeloma (Burmeister et al., 1983); STS, HL, and NHL (Hoar et al., 1986); and STS and NHL (Woods and Polissar, 1989; Woods et al., 1987). In a subset of participants in the Hartge et al. (2005) study, De Roos et al. (2005a) studied associations between the overall TEQs of PCBs, furans, and dioxins but not TCDD alone.

Non-cancer health outcomes have also been investigated in case-control studies: birth defects and congenital anomalies (Blatter et al., 1997; García et al., 1998; Nurminen et al., 1994), spontaneous abortion (Carmelli et al., 1981), mortality from neurodegenerative diseases associated with occupational risk factors (R. M. Park et al., 2005; Schulte et al., 1996), and Parkinson disease (Firestone et al., 2005, 2010; Liou et al., 1997; Seidler et al., 1996; Semchuk et al., 1993).

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Again, as this chapter is not intended to be a compendium of every study ever reviewed in the VAO series, only those cohorts and groups that have contributed more than one study for review by VAO committees and for which new information is available are considered here. A full account of every study ever reviewed in the VAO series that has provided evidentiary weight for an association between exposure to the COIs and a health outcome can be found in the supplementary tables (available at www.nap.edu/catalog/25137), and details of the studies can be found in the corresponding report for which they were first identified and reviewed by a VAO committee. Some additional information may be found in the subsections of "Conclusions from VAO and Previous Updates" for an outcome throughout the chapters.

National Birth Defects Prevention Study

The National Birth Defects Prevention Study (NBDPS) is a population-based case-control study conducted cooperatively by CDC and eight monitoring centers throughout the United States (in Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, and Texas) using a standardized study methodology (Yoon et al., 2001). Starting in October 1, 1997, the individual centers began monitoring births in their respective areas for the occurrence of more than 30 types of birth defects (excluding cases attributable to single-gene conditions or chromosomal abnormalities) for comparison with randomly selected sets of live-born babies without malformations. Information about demographics and possible exposures is abstracted from an extensive telephone interview that the mothers complete within 24 months of delivery. On the basis of the work histories, job classifications are assigned by an industrial hygienist and processed using a job-exposure matrix and expert opinion used to derive occupational exposures. Buccal epithelial samples are gathered for DNA testing from the infant and its parents. Major limitations of the study are the lack of exposure specificity (e.g., using general categories of insecticides, herbicides, and fungicides), crude exposure histories (ever/never), lack of exposure concentrations or durations, and the use of self-report, which may introduce recall bias especially among mothers whose child has been diagnosed with a birth defect. Despite those limitations and the fact that the results are only potentially relevant to child-bearing female Vietnam veterans, several publications using data from all of the NBDPS or just subsets of the NBDPS have been reviewed by VAO committees.

Rocheleau et al. (2011) reported on the association between maternal occupational pesticide exposure and the risk of hypospadias in the NBDPS using a sample of 647 cases of hypospadias and 1,496 controls with estimated delivery dates of October 1997–December 2002. Most exposure was to insecticides only or to three types of pesticides (insecticides, herbicides, and fungicides), but there was generally a low level of occupational pesticide exposure in the study population. The lack of exposure specificity made it difficult to make an association

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between the birth defects and any pesticide exposure or an individual pesticide exposure, and other exposures of the population could have contributed to the outcome in question. In a separate analysis of NBDPS data, Kielb et al. (2014) analyzed the occurrence of isolated craniosynostosis, gastroschisis, diaphragmatic hernia, or transverse limb deficiencies in children born to employed women with due dates between October 1, 1997, and December 31, 2002. Cases included 871 live-born, stillborn, or electively terminated fetuses, which were compared to 2,857 live-born control infants. The odds of the appearance of these musculoskeletal malformations were examined in relation to periconceptional maternal occupational exposure to insecticides, herbicides, or fungicides (classified as yes/no) for each job held during the period of 1 month pre-conception through 3 months post-conception.

Several papers from the California Center (in the San Joaquin Valley) of the NBDPS have been published, although many of the same limitations apply as for analyses with the full NBDPS. The center has monitored deliveries from 1997 to 2006 and has invested considerable effort toward developing time-specific estimates of exposure to individual pesticides by women residing in the area at the time of delivery. Carmichael et al. (2014) evaluated 569 medically confirmed congenital heart defect cases (8 different types) and 785 non-malformed controls born during 1997–2006. Maternal pesticide exposure was crudely classified as "any" versus "no exposure" based on the commercial application of pesticides within a 500-meter radius of the mother's address during a 3-month periconception window (determined by data obtained from the California Pesticide Use Report record system). Exposure to individual pesticides was examined, including the dimethylamine salt of 2,4-D. Analogous investigations were conducted on neural tube defects and orofacial clefts (W. Yang et al., 2014) and on gastroschisis (Shaw et al., 2014).

Several new publications from the NBDPS were identified for the current update. Makelarski et al. (2014) and Pettigrew et al. (2016) both examined associations of spina bifida with parental non-specific herbicide exposure. Rocheleau et al. (2015) examined the association between maternal occupational exposure to fungicides, insecticides, and herbicides and the risk of congenital heart defects among offspring. Specific publications from the California Center of the NBDPS included studies of gastroschisis (Shaw et al., 2014) and anotia/microtia, anorectal atresia/stenosis, transverse limb deficiency, craniosynostosis, and diaphragmatic hernia (Carmichael et al., 2016).

Upper Midwest Health Study

The Upper Midwest Health Study (UMHS) was initiated by NIOSH as a population-based case-control study of cancer risk in a non-metropolitan Midwestern U.S. population. Several reports from the study were reviewed in previous updates. Chiu et al. (2004) and W. J. Lee et al. (2004a) conducted pooled

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analyses of two earlier case-control studies of NHL carried out by the UMHS in Iowa and Minnesota (Cantor et al., 1992) and in Nebraska (Zahm et al., 1990). Chiu et al. (2004) examined the association of NHL with agricultural pesticide use and familial cancers, and W. J. Lee et al. (2004a, 2006) looked at NHL in asthmatic people who reported pesticide exposure. Data from Nebraska (B. C. Chiu et al., 2006, based on Zahm et al., 1990, 1993) were used to determine if the risk of NHL was driven by any specific subtypes. Tissue samples were analyzed from 172 of 385 cases for the presence of a specific chromosomal translocation (t(14;18)(q32;q21)). Two studies focused on pesticide use and the risk of adenocarcinomas of the stomach and esophagus (W. J. Lee et al., 2004b) and the risk of gliomas (W. J. Lee et al., 2005) in white Nebraska residents over 21 years old who were identified from the Nebraska Cancer Registry and matched to controls drawn from an earlier study by Zahm et al. (1990). Other publications evaluated farm pesticide exposure in men (Ruder et al., 2004) and women (Carreon et al., 2005) in Iowa, Michigan, Minnesota, and Wisconsin in relation to gliomas as part of the UMHS. Ruder et al. (2006) reported a follow-up of Ruder et al. (2004) that evaluated gliomas in UMHS participants, but the new analyses provided no evidence of a greater use of pesticides in cases than in controls, and agents were not specified. Ruder et al. (2009) reported another follow-up in the same group, which had similar findings and, again, provided no specificity of agents. Finally, Yiin et al. (2012) has reported findings from new analyses of the UMHS sample that incorporated more detailed exposure information that was not used in previous analyses, including years of use and estimated cumulative exposures to categories of pesticides, including phenoxy herbicides, and the use of specific agents, including 2,4-D and dicamba.

Cross-Canada Study of Pesticides and Health (Rare Tumors Study)

The Cross-Canada Study of Pesticides and Health (Rare Tumors Study) was designed as a full population-based case-control study of men in six Canadian provinces to address the relation of four relatively uncommon malignancies—HL, NHL, multiple myeloma, and STS—with occupational and domestic exposure to pesticides (McDuffie et al., 2001). A target number of cases of each cancer type was preset for each province. Researchers gathered incident cases that were diagnosed starting on September 1, 1991, from the provincial cancer registries (or hospital records) in Québec until the end of 1994 or until the target number was reached. People who had Kaposi sarcoma or who were HIV positive were excluded. Physician consent was obtained, and diagnoses were confirmed with pathology reports and a review of preserved tissues. Consent forms and questionnaires were sent to the cases. The controls were men at least 19 years old identified in the health-insurance records of Alberta, Manitoba, Saskatchewan, and Québec; from telephone listings for Ontario; and from voter lists in British Columbia. The controls were selected randomly to obtain a stratified age distribution matching

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that of the cases, and they were sent consent forms and questionnaires. All 1,506 controls who responded were used in comparisons for each of four cancer groups: 316 HL cases, 517 NHL cases, 342 multiple myeloma cases, and 357 STS cases.

The postal questionnaire gathered standard demographic information, personal and family medical histories, employment history, smoking behavior, and basic data on pesticide exposure. The pilot study tested the reliability of self-reported pesticide use by comparison with purchase records. Any subject who reported at least 10 hours of pesticide exposure per year was asked to complete a telephone questionnaire on the details of the pesticide exposure; in addition, 15% of the remaining subjects were randomly selected to answer the telephone survey. A conditional logistic regression stratified on age and province and adjusted for all covariates found to be associated with the outcome at the 0.05 level of significance was used to estimate odds ratios for specific active ingredients, including dicamba and the phenoxy herbicides 2,4-D, Mecoprop, MCPA, and diclofop-methyl. Dose–response relationships were investigated for the cumulative time spent in mixing or applying particular products.

A series of publications have addressed the relationship between each of the cancers and various risk factors. Those pertaining to herbicides overall or to the particular ones of interest are as follows:

- HL: Karunanayake et al. (2012); P. Pahwa et al. (2003)
- NHL: Hohenadel et al. (2011); McDuffie et al. (2001)
- Multiple myeloma: P. Pahwa et al., (2003, 2012)
- STS: P. Pahwa et al., (2003, 2011)

A number of other publications arising from that dataset have addressed topics somewhat more tangential to the interests of the VAO reports. For instance, McDuffie et al. (2005) and P. Pahwa et al. (2006) considered the possible role of exposure to insect repellents, particularly DEET and phenoxy herbicides, in the genesis of the malignancies in question. McDuffie et al. (2009) examined family histories of cancers in first-degree relatives of the study participants to assess the interaction between family history and pesticide exposure. Hohenadel et al. (2011) investigated how various combinations of pesticide exposures influenced the occurrence of NHL. Ghosh et al. (2011) investigated the association of occupational exposures other than pesticides with the occurrence of multiple myeloma. M. Pahwa et al. (2012) examined the interactions between self-reported pesticide exposures and self-reported measures of immune suppression (asthma, allergies, hay fever) and the risk of NHL, but this analysis is limited by the use of self-reported measurements of immunologic conditions and the non-specific and crude self-report classification of pesticide use, which did not characterize exposure use by duration, intensity, or frequency. Similarly, Navaranjan et al. (2013) examined HL relative to the number of pesticides to which an individual reported exposure and to estimates of work-related exposure and home-related

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exposure, grouped by class (herbicides, insecticides, and fungicides). This study is also limited by the relatively nonspecific and crude self-report classification of pesticide use, which significantly limits direct inference to the effects of herbicide exposure during military service in Vietnam.

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6

Immune System Disorders

Chapter Overview

Based on new evidence and a review of prior studies, the current committee did not find any new associations between the relevant exposures and immune outcomes that warranted a change in level of evidence of association. Current evidence supports the findings of earlier studies that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the chemicals of interest (COIs) and any specific diseases involving immune suppression, allergy, autoimmunity, or inflammation.

Immune-system disorders affect more than 23.5 million Americans (NIEHS, 2012), with some sources estimating that as many as 50 million Americans are currently affected by immune-system disorders (AARDA, 2018). The causal factors for immune-system disorders are mainly unknown; however, it has been hypothesized that they most likely reflect both genetic and environmental factors. The immune system plays three important roles in the body:

- It defends the body against infectious pathogens, including viruses, bacteria, and other disease-producing microorganisms.
- It helps defend against cancer by destroying mutated cells that may otherwise develop into tumors and providing immunity against tumors.
- It provides resident immune cells that are specially adapted for different tissues and organs (such as microglia in the central nervous system and Kupffer cells in the liver) to help regulate the functional activity and integrity of those tissues.

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This chapter begins with an overview of the various types of health problems that can arise as a result of a malfunction of the human immune system, such as immune suppression, allergic diseases, autoimmune diseases, and inflammatory diseases. Outcomes related to infectious agents would be included in this chapter, but no studies had specific enough information on exposure to warrant inclusion. Following the brief description of the types of immune dysfunction, the findings from previously reviewed literature and the conclusions from prior updates are summarized regarding the epidemiologic evidence concerning an association between exposure to any one of the COIs, that is, 2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); picloram; dimethylarsinic acid (DMA or cacodylic acid); and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). As described in Chapter 3, studies of exposure to polychlorinated biphenyls (PCBs) and other dioxin-like chemicals were also considered informative if their results were reported in terms of TCDD toxic equivalents (TEQs) or as concentrations of specific congeners of dioxin-like chemicals. Studies that report TEQs based only on mono-ortho PCBs (which are PCBs 105, 114, 118, 123, 156, 157, 167, and 189) are considered even though their TEQs are several orders of magnitude lower than those of the non-ortho PCBs (77, 81, 126, and 169), based on the revised World Health Organization (WHO) toxicity equivalency factor (TEF) scheme of 2005 (La Rocca et al., 2008; van den Berg et al., 2006). Epidemiologic findings from five newly identified studies are reviewed. Biologic plausibility data on the effects of the COIs on the immune system are summarized. The chapter ends with a synthesis of the studies and what they contribute to the evidence base and then the committee's conclusion regarding the association of exposure to the COIs and effects on the immune system. The studies reviewed in this chapter are limited to those investigating effects on the immune system from exposures that occurred to adults. Immune effects stemming from perinatal exposures are discussed in Chapter 8. Table 1, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies reviewed in the VAO series related to immune disorders.

CATEGORIES OF IMMUNE DYSFUNCTION

There are four major categories of immune dysfunction, which are not mutually exclusive: immune suppression, allergy, autoimmunity, and inflammatory dysfunction (inappropriate or misdirected inflammation). Most times, immune suppression manifests itself as an increased incidence of infections or an increased risk of neoplasia. Allergic, autoimmune, and inflammatory disorders can be manifested as diseases that affect virtually any tissue. It is often difficult to diagnose such diseases, so they may not always be medically categorized as immune disorders.

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Immune Suppression

The suppression of immune responses can reduce resistance to infectious disease and increase the risk of cancer. Infection with the human immunodeficiency virus (HIV) is a well-recognized example of an acquired immune deficiency in which a specific type of lymphocyte (CD4+ T cell) is the target of the virus (Okoye and Picker, 2013). The decline in the number of CD4+ T cells after HIV infection correlates with an increased incidence of infectious diseases, including fatal opportunistic infections, and with an increased incidence of several types of cancer (Davis, 1998). The treatment of the cancer with toxic chemotherapeutic drugs suppresses the immune system by inhibiting the generation of new white blood cells by the bone marrow and blocking proliferation of lymphocytes during an immune response. Both of those examples represent severe immune suppression in which the adverse outcome is easily detected with clinical measurements.

Immune suppression can also result from exposure to chemicals in the workplace or in the environment and can manifest as recurrent infections, opportunistic infections, a higher incidence of a specific category of infections, or a higher incidence of many forms of cancer (Saberi Hosnijeh et al., 2013; Warren et al., 2000). However, unless the immune suppression is severe (as occurs in rare cases of genetic disorders of immunity), it is often difficult to obtain clinical evidence that directly links chemically induced changes in immune function to increases in infectious diseases or cancers because many confounding factors and effect modifiers can influence a person's ability to combat infection. These factors include age, vaccination status, the virulence of the pathogen, the presence of other diseases (such as diabetes), stress, smoking, and the use of drugs or alcohol. Therefore, immunotoxicology studies are often conducted in laboratory animals to understand the scope and mechanism of chemical-induced immune suppression. The results of such studies can be used to develop biomarkers to assess the effects in human populations. Infectious disease models in animals can also be used to determine whether the pattern of disease changes with chemical exposure.

Allergic Diseases

The immune system sometimes responds to a foreign substance that is not pathogenic; such immunogenic substances are called allergens. Like most immune-based diseases, allergic diseases have both environmental and genetic risk factors. Their prevalence has increased in many countries in recent decades (Linneberg et al., 2000; Simpson et al., 2008; Sly, 1999). The major forms of allergic diseases are asthma, allergic rhinitis, atopic dermatitis, and gastrointestinal responses. In immediate hypersensitivity, the response to some allergens, such as pollen and bee venom, results in the production of immunoglobulin E (IgE) antibodies. Once produced, IgE antibodies bind to mast cells, which are

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specialized cells that are found in tissues throughout the body, including the lungs, the intestinal wall, and blood-vessel walls. When a person is exposed once again to the allergen, it binds to the antibodies on the mast cells and causes them to release histamine and leukotrienes, which produce the symptoms associated with an allergic response. In delayed-type hypersensitivity reactions, also known as cell-mediated immunity, other allergens, such as poison ivy and nickel, activate allergen-specific lymphocytes (memory T cells) at the site of contact (usually the skin) that release substances that cause inflammation and tissue damage. Some allergic responses, such as those to food allergens, may involve a combination of allergen-specific lymphocyte-driven and IgE-driven inflammation. Allergic responses may manifest in specific tissues (such as the skin, eyes, airways, and gastrointestinal tract) or may result in a system-wide response called anaphylaxis.

Autoimmune Diseases

The National Institutes of Health's Autoimmune Diseases Coordinating Committee recognizes 80 different autoimmune diseases and conditions that affect the cardiovascular, respiratory, nervous, endocrine, skin, gastrointestinal, hepatic, and excretory systems (NIH Autoimmune Diseases Coordinating Committee, 2005). These diseases affect both men and women, but most of them affect more women than men (Fairweather et al., 2008). Genetic predisposition, age, hormone status, and environmental factors, such as the presence of infectious diseases and stress, are known to affect the risk of developing autoimmune diseases. Different autoimmune diseases can occur in the same person and tend to cluster in families. The development of one autoimmune condition is also a risk factor for the development of other immune-related diseases and for some types of cancer (Landgren et al., 2010).

Autoimmunity occurs when an individual's immune system fails to recognize self and attacks tissues as though they were foreign. Inappropriate immune responses that cause autoimmunity originate with either cell-mediated or humoral-mediated immune systems and can be directed against a wide variety of tissues or organs. For example, the autoimmune reaction in multiple sclerosis targets the myelin sheath of nerve axons; in Crohn's disease, the intestinal epithelium; in type 1 diabetes mellitus, the insulin-producing islet cells of the pancreas; and in rheumatoid arthritis, the joint synovium and other proteins associated with connective tissue.

Other systemic autoimmune diseases also occur. Systemic lupus erythematosus is an autoimmune disease in which multiple organs are targeted by a variety of autoantibodies. Patients display a variety of non-specific signs and symptoms such as joint pain or fatigue that makes timely diagnosis challenging. A characteristic rash across the cheeks and nose and a sensitivity to sunlight are common symptoms, but oral ulcers, arthritis, pleurisy, proteinuria, and neurologic signs may also be present. Almost all of those affected with systemic lupus erythematosus test positive for antinuclear antibodies, specifically antibodies directed at

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double-stranded DNA. The cause of systemic lupus erythematosus is unknown, but environmental and genetic factors have been implicated. The environmental factors that are thought to trigger it include infections, antibiotics (especially those in the sulfa and penicillin groups) and some other drugs, ultraviolet radiation, extreme stress, and hormones (Kamen, 2014). Occupational exposures to such chemicals as crystalline silica, solvents, and pesticides have also been associated with systemic lupus erythematosus (Cooper and Parks, 2004; Parks and Cooper, 2005).

Inflammatory Diseases

Inflammatory diseases (also referred to as auto-inflammatory diseases) make up a more recently identified category of immune-related disorders and are characterized by exaggerated, excessively prolonged, or misdirected dysfunctional inflammatory responses (usually involving immune cells). Tissue disease can result from this inappropriate inflammation, which can affect virtually any organ. Examples of the diseases and other conditions that are most often included in other disease categories but that are also considered to be inflammatory diseases are: coronary artery disease, asthma, eczema, chronic sinusitis, hepatic steatosis, psoriasis, celiac disease, and prostatitis. Inflammatory diseases often co-occur with one another, which has resulted in the categorizing of different but linked inflammatory diseases together as a single chronic inflammatory disorder (Borensztajn et al., 2011). Ordinarily, inflammation is advantageous in fighting infection. It is one component of the normal host response to infection and is mediated by innate and adaptive immunity. Innate inflammatory responses involve the rapid mobilization of macrophages, granulocytes, and natural killer cells to the area of infection, where they produce toxic metabolites that kill pathogens. The adaptive immune response follows with specific antibodies and cell-mediated immunity that add to the inflammatory process. Interactions among innate immune cells and epithelial and endothelial cells are important in regulating the magnitude of inflammation, and improperly regulated inflammation can contribute to diseases that arise in non-lymphoid tissues, such as the lungs, skin, nervous system, endocrine system, and reproductive system.

Inappropriate inflammation also appears to play a role in promoting the growth of neoplasms (Bornschein et al., 2010; Hillegass et al., 2010; Landgren et al., 2010; Porta et al., 2011; Winans et al., 2010); examples can be seen in the higher prevalence of specific cancers in patients who have such inflammatory diseases as inflammatory bowel disease (Lucas et al., 2010; Viennot et al., 2009; Westbrook et al., 2010), prostatitis (Sandhu, 2008; W. Wang et al., 2009), and psoriasis (Ji et al., 2009).

CONCLUSIONS FROM *VAO* AND PREVIOUS UPDATES

The studies reviewed in this section are limited to effects on the immune system due to exposures that occurred to adults. Immune effects stemming from

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perinatal exposures are discussed in Chapter 8: Reproductive Health Effects and Effects on Descendants.

Two studies of Vietnam veterans reported a statistically significant difference of single immune measures of veterans exposed to Agent Orange compared with veterans without diseases and with age-matched healthy controls. Among Air Force Health Study (AFHS) veterans, Ranch Hands with the highest TCDD levels had statistically significantly elevated absolute counts of CD20 cells, the sum of natural killer (NK) cell antigens (CD16 and CD56) and total CD3+ cells compared with the AFHS controls (Michalek et al., 1999b). H. A. Kim et al. (2003) conducted an immunotoxicologic study of Korean Vietnam veterans who had been deployed to areas that were known to be sprayed with Agent Orange and found elevated levels of IgE and IgG1 among exposed veterans compared with age-matched healthy controls. Other studies of Vietnam veterans and populations exposed to the COIs did not find similar elevations for these immune markers. Thus, there were no consistent findings indicative of immunosuppression, an increased risk of autoimmunity (usually as measured with autoantibodies), or biomarkers of atopy or allergy (such as increased IgE concentrations). Much of the focus of the studies was on measuring CD4+:CD8+ T-cell ratios (T4:T8). The T4:T8 ratio is an effective biomarker of the progression of HIV-induced AIDS (acquired immune deficiency syndrome), but the TCDD-exposure animal data indicate that it is not an immunologic index that is expected to be altered. The results of a survey of Australian Vietnam veterans (O'Toole et al., 2009) showed purportedly significant increases in the prevalence of a number of conditions in which immune function may play a prominent role, but the study's methods were deemed unreliable.

The occupational exposure studies evaluated by VAO committees have examined the concentrations of lymphoid populations in circulation, such as CD4+ T cells, CD8+ T cells (and their ratio), and NK cells; cell-mediated immunity (the delayed-hypersensitivity response); serum concentrations of immunoglobulins, such as IgM, IgG, and IgA; concentrations of complement, such as C3 and C4; and concentrations of cytokines, such as IL-1, IL-2, interferon-γ, IL-4, IL-6, and tumor necrosis factor (TNF)-α. A few studies also included disease or condition endpoints, such as rheumatoid arthritis, systemic lupus erythematosus, immune suppression, and sensitivity to fungal infection. Ex vivo analyses included measures of NK activity, lymphoid mitogen-induced proliferation, and the mixed lymphocyte response against allogeneic cells. Some studies identified one or more dioxin-related shifts in immune measures, but many reported no significant differences in the same measures. Saberi Hosnijeh et al. (2012a) reported a positive correlation between plasma TCDD concentrations and decreased levels of cytokines, chemokines, and growth factors in Dutch workers who produced and formulated chlorophenoxy herbicides. In additional studies, Saberi Hosnijeh et al. (2012b, 2013a) assessed TCDD levels with respect to several other immunological parameters and found no differences in hematologic

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measurements other than an increase in the T4:T8 ratio ($p = 0.05$) when high- and low-exposure groups were compared. In the third study, Saberi Hosnijeh et al. (2013a) reported on the levels of interleukin 1 receptor antagonist (IL-1RA) as well as of the soluble forms of CD27 and CD30, immunomodulatory members of the TNF receptor superfamily. In this case they found no association of TCDD level with CD27 or CD30; however, IL-1RA was significantly decreased in those with higher TCDD levels after adjusting for concurrent chronic disease. This result is also consistent with a degree of immune system impairment being associated with high exposure to TCDD. Similarly, the occupational exposure studies that examined NK concentrations reported the full spectrum of results: no alterations (Halperin et al., 1998), a decrease (Faustini et al., 1996), and even an increase in NK numbers (Jennings et al., 1988) in different populations of people exposed to dioxin.

Several environmental exposure studies have been published, with inconsistent findings. Some studies reported alterations in immune measures associated with dioxin exposure. For example, Van den Heuvel et al. (2002) reported a negative correlation between increased serum TEQ levels and eosinophil counts, NK-cell counts, and levels of cat dander, house dust mite, and grass pollen IgEs (measured by radioallergosorbent tests); but a positive correlation with IgA levels. These alterations, however, were not seen consistently in other studies. Baccarelli et al. (2002) found no changes in IgA levels but saw changes in IgG levels in the Seveso population. Svensson et al. (1994) found that NK-cell numbers were reduced with increasing concentrations of persistent organic chemicals, but Lovik et al. (1996) found no difference in NK numbers or activity.

Some early studies of the Quail Run Mobile Home Park population who were exposed to TCDD-contaminated soil reported that dioxin exposure was associated with a reduction in a specific type of cell-mediated immune response, the delayed type hypersensitivity response (Andrews et al., 1986; Hoffman et al., 1986; Knutsen et al., 1987; Stehr-Green et al., 1987). However, several studies of the Times Beach population, another dioxin-exposed population, did not find any alteration of the delayed type hypersensitivity response (Knutsen, 1984; Stehr et al., 1986; Webb et al., 1987). An analysis of National Health and Nutrition Examination Survey (NHANES) data found that exposure to dioxin-like PCBs was associated with an increase in self-reported arthritis (D. H. Lee et al., 2007a), but De Roos et al. (2005b) did not find this association. Spector et al. (2014) assessed immune function in 109 postmenopausal women who participated in a year-long study of exercise and health in Seattle. At baseline, the concentrations of mono-ortho PCBs 105, 118, and 156 were not associated with changes in lymphocyte proliferation assays; after 1 year, however, a decrease in lymphocyte proliferation was associated with increased levels of this group of PCBs ($p = 0.039$).

Nakamoto et al. (2013) gathered fasting blood samples to assess environmental exposure to dioxin-like polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and PCBs and found an association of

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exposure to dioxins with differential leukocytes and a significantly decreased incidence of reported atopic dermatitis. Gallagher et al. (2013) examined the link between environmental exposures to the COIs and autoimmunity in women. Among women, after adjustments for mercury blood level, race, menopausal status, diet, and body mass index (BMI), the total TEQs for PCBs were significantly associated with antinuclear antibody positivity (intensity ≥ 3) for each higher quartile compared with the lowest and as an overall trend ($p < 0.001$). After adjusting for mercury blood level, race, menopausal status, diet, and BMI, total TEQs for PCBs were significantly associated with antinuclear antibody positivity for each quartile compared with the referent group, and the overall trend was significant ($p < 0.001$). Turunen et al. (2012) derived the total toxic equivalence for 17 PCDD/F and 37 PCB congeners in blood samples from 123 men and 132 women from a population with high fish consumption and analyzed their relationship with C-reactive protein, an indicator of inflammation. No evidence of a trend across the tertiles of overall TEQ concentration was seen for either the men or the women.

Based on these mixed results, the variety of endpoints assessed, and the poor methods used in some studies, VAO committee have concluded that epidemiologic data were either insufficient or inadequate to determine an association between exposure to the COIs and immunosuppression, allergic disease, or autoimmune disease.

UPDATE OF THE EPIDEMIOLOGIC LITERATURE

Since *Update 2014*, five new published studies have been identified: one focused on New Zealand Vietnam veterans, three among occupational cohorts, and one on environmental exposures in Vietnam.

Vietnam-Veteran Studies

Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify prevalent health conditions in 2,783 male New Zealand veterans who served in Vietnam during 1964 to 1972. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% confidence intervals (CIs) were calculated for the veteran cohort and the general population for several noncancerous conditions and a standardized hospitalization ratio (SHR) was calculated using the two rates. Results were presented for three categories of arthritis: rheumatoid arthritis, infective arthritis, and osteoarthrosis. A statistically significant increased risk was observed for rheumatoid arthritis (n = 25; SHR = 1.70, 99% CI 1.03–2.36) and osteoarthrosis (n = 179; SHR = 1.32, 99% CI 1.12–1.51) but not for infective arthritis (n = 26; SHR = 1.39, 99% CI 0.85–1.92). However, arthritis is not usually

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a condition that requires hospitalization, so this study likely underestimates the prevalence or detects only persons with an unusually complicated disease process. In addition, hospitalization for systemic lupus erythematosus was not statistically significantly increased among veterans (n = 5; SHR = 1.82, 99% CI 0.22–3.41). Exposure to the COIs was not validated through serum measurements, and the study did not control for smoking or ethnicity or other potentially important risk factors.

Occupational Studies

Parks et al. (2016) used data collected from the prospective, longitudinal Agricultural Health Study (AHS) to examine the associations between rheumatoid arthritis and pesticide exposures among the spouses of licensed pesticide applicators from Iowa and North Carolina who were part of the AHS. Specific pesticide exposures included exposure to herbicides 2,4-D and dicamba and to several insecticides and fungicides; only 2,4-D and dicamba are considered relevant to committee's charge. Details of the AHS study design and data collections are found in Chapter 5. Cases of rheumatoid arthritis were identified based on self-report. Cases in which disease-modifying antirheumatic drugs or other medications for rheumatoid arthritis were identified were considered to be probable instances of rheumatoid arthritis, and those individuals for whom a medical record review allowed confirmation of the diagnosis were considered to be definite cases of rheumatoid arthritis. Surveys collected information on age, tobacco use, menopausal status, childhood farm exposure, non-specific household and specific farm pesticide exposure, farm activities related to pesticides and other activities such as sun exposure, exposure to solvents and exposure to pesticides through mixing or application. A total of 275 cases of rheumatoid arthritis were identified among the 24,018 women participants, of which 132 were incident cases (that is, they developed after the start of the study, having been reported in phase II or III surveys but not phase I). Cases reported in phase I were considered prevalent, that is, as having developed before the start of the study. After adjusting for age, state of residence, and smoking history, the researchers found a statistically significant association between all-cases rheumatoid arthritis (incident and prevalent) and any specific pesticide exposure (odds ratio [OR] = 1.4; 95% CI 1.0–1.8; $p < 0.05$). Incident cases of rheumatoid arthritis were not associated with any exposure (OR = 1.4; 95% CI 0.93–2.1). A decreased association was observed for rheumatoid arthritis and exposure to 2,4-D with all cases (OR = 0.75; 95% CI 0.51–1.1) and incident cases (OR = 0.69; 95% CI 0.39–1.2). Likewise, a decreased association was observed for rheumatoid arthritis and exposure to dicamba based on 7 cases (OR = 0.68; 95% CI 0.32–1.5). The effect estimate was not presented for incident cases of rheumatoid arthritis and dicamba exposure because there were fewer than 5 cases. The only individual pesticide to demonstrate a statistically significant association with rheumatoid arthritis was glyphosate (OR = 1.4; 95%

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CI 1.0–2.1; $p < 0.05$). Thus, this study does not support an association of 2,4-D in the development of rheumatoid arthritis. The strengths of the study are the sample size, duration of follow-up, and specific pesticide data. The self-reported nature of the exposure without quantification could lead to under- or over-estimation as well as a misclassification of the agents of exposure. Para-occupational exposure and agricultural drift were not considered. Though the statistical methods for handling missing data were sound, there was a variety of missing data.

't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and workers were potentially exposed to 2,4,5-T, intermediates of trichlorophenol and other chlorophenols, and TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by the International Agency for Research on Cancer (IARC; Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. The 't Mannetje et al. (2018) study extended the follow-up period of these workers to approximately 30 years from their last 2,4,5-T production exposure. From the original cohort of 1,025 workers, 631 were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current follow-up, 430 of the 631 workers were randomly selected and invited to participate in the morbidity survey, of which 245 (57%) participated. The survey, which was administered in 2007–2008 by face-to-face interview, collected information on demographic factors and health information, including doctor-diagnosed conditions and year of diagnosis. A blood sample was also collected at that time to be analyzed for TCDD, lipids, thyroid hormones, and other parameters. For 111 of the participants, a neurological examination was also conducted. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two methods: (1) working in a TCDD-exposed job (based on occupational records), and (2) serum TCDD concentration ≥10 pg/g lipid (18%). Mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy or trichlorophenol production and 6 pg/g lipid in 141 men and 43 women who worked in other parts of the plant. Compared with the 124 people in the non-highly exposed jobs, the 121 people who had ever worked in a highly exposed job were no more likely to have doctor-diagnosed nasal allergies, including hay fever (n = 21; OR = 1.00; 95% CI 0.48–2.08). When compared by serum TCDD concentration ≥10 pg/g lipid, no difference in nasal allergies was found (n = 7; OR = 0.80; 95% CI 0.27–2.37).

Cappelletti et al. (2016) performed a retrospective study of 331 male electric arc foundry workers at a single plant in Trentino, Italy, to determine if they had experienced excess mortality from all causes or were at an increased risk for several other diseases, including rheumatoid arthritis, due to occupational exposures to foundry dust. An analysis of the dust found that it contained metals (including

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iron, aluminum, zinc, manganese, lead, chromium, nickel, cadmium, mercury, and arsenic), polycyclic aromatic hydrocarbons (PAHs), PCBs, and PCDD/Fs (reported as TEQs). Therefore, the authors could not determine which of the agents were associated with a specific outcome or to what extent. Each of the men had worked at the factory for at least 1 year, and, for the rheumatoid arthritis analysis, they were compared with 32 presumed non-exposed workers (clerks, managers, and watchmen) or the standardized general population of Region Trentino-Alto Adige (where the factory was located) because there were few non-exposed foundry workers and high attrition rates. Company and medical records were used to determine vital status; cause of death was determined from death certificates or other registries. Requests for exemption health care fees were used as a surrogate measure to identify the most prevalent morbid conditions in the general population, which were then applied to the cohort to compute relative risks for each of the conditions. The workers were followed from March 19, 1979 (or their first day of employment), through December 31, 2009, or their date of death. The analysis for rheumatoid arthritis was limited to 235 workers, and effect estimates were calculated using Mantel-Haenszel tests. Although a statistically significant increase of rheumatoid arthritis was found among the workers compared with the age-adjusted provincial population, it was based on three cases, which resulted in an imprecise effect estimate (RR = 6.18, 95% CI 2.00–19.02). This study is most limited by the fact that foundry dust is a complex mixture, which results in an inability to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those exposed workers. Estimates were only adjusted for age group and were not adjusted for other risk factors such as tobacco use, BMI, or other jobs or activities that could also influence health outcomes. The exposure to foundry dust by the general population that was used for comparison was not discussed, although the foundry appears to be in the local vicinity, and emissions from it were reported to be present within a 2-kilometer radius of the foundry.

Environmental Studies

C. H. Nguyen et al. (2017), studied serum TCDD levels, the expression of AHR, and a variety of pro-inflammatory cytokines in Vietnamese who were either exposed or not exposed to TCDD-like chemicals. The exposed individuals (36 women and 24 men) had lived near the Da Nang Air Base for more than 10 years. The controls were healthy men and women recruited from unsprayed areas in northern Vietnam. Serum levels of TCDD and "other" types of dioxins were measured using a chemical-activated luciferase gene-expression bioassay. RNA was isolated from whole blood for quantitative real-time polymerase chain reaction assays that were used to examine gene expression for AHR, IL-1β, TNFα, IL-6, IL-22, and β-actin. Dioxin levels, presented as bioanalytic equivalents (BEQ)/g fat (1.5 interquartile range), were significantly higher in the TCDD-exposed participants than in the controls—62.03 (40.74–89.42) versus 17.45 (12.05–35.57),

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respectively. AHR expression was 16.39 times higher in the exposed versus unexposed controls, which was statistically significant. To determine if there was an association between AHR expression and rheumatoid arthritis in the dioxin-exposed subjects, AHR expression was measured and the 6 people with rheumatoid arthritis were compared to the 54 people without rheumatoid arthritis. While the dioxin-exposed group had higher levels of AHR expression than controls, there were no differences in AHR expression levels between the exposed subjects with and without rheumatoid arthritis. Because of the role they play in the pathogenesis of autoimmune conditions, IL-1β, TNFα, IL-6, and IL-22 were expression levels measured in TCDD-exposed and unexposed populations. The median increase and 1.5-interquartile range for IL-1β was 11.18-fold (6.76–20.72, $p < 0.05$); for TNFα, 6.64-fold (3.43–13.67, $p < 0.05$); and for IL-6, 2.93-fold (1.80–5.18, $p < 0.05$). IL-22 was decreased 0.05-fold (0.01–0.11, $p < 0.01$). There was, however, no association between cytokine levels and dioxin levels. There was a weak positive correlation between AHR expression and IL-6 and IL-22 expression.

Next the investigators looked at the range of conditions reported in the exposed subjects. Cardiac disease was the most common, affecting 18%. Rheumatologic conditions affected 16.7%, and rheumatoid arthritis affected 10%. The rate for rheumatoid arthritis in the general Vietnamese population was 0.05%. The main strength of this study of Vietnamese individuals living near the Da Nang Air Base is the availability of serum dioxin levels. While the levels of pro-inflammatory cytokines were significantly elevated in the exposed population, they did not correlate with serum dioxin levels. While there were more cases of rheumatoid arthritis in the exposed population than in the general population, neither the dioxin levels nor pro-inflammatory cytokines were compared between exposed people with and without rheumatoid arthritis. The study demonstrates an increased expression of pro-inflammatory cytokines in persons exposed to dioxins, but there is no information on lifestyle habits, tobacco, obesity, or other rheumatologic disorders or family history that may confound the findings.

Other Identified Studies

Several other studies were identified by the committee but either lacked sufficient exposure specificity or examined biologic markers of effect on the immune system that do not relate to a diagnosable health outcome; these studies were not considered further. Akahane et al. (2017) examined the prevalence of a variety of self-reported conditions, including several connective tissue disorders, in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil following the Yusho accident. Because neither TEQs nor any other quantification of relevant exposures was presented, this study was not considered further.

A study of people who either worked at a transformer and capacitor recycling plant in Dortmund, Germany, or lived in the immediate area and might

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have been exposed to dioxin-like (PCBs 105, 114, 118, 156, 157, 167, and 189) and non-dioxin-like PCBs as a result of contamination of the area by the facility confirmed that exposure can modify lymphocyte profiles, but this was not linked with specific health outcomes (Haase et al., 2016). The results are consistent with the literature suggesting that PCB exposure may alter the immune profile, but these data do not provide any evidence of any consistent abnormality. This study did not detect any functional immunodeficiency, and the problems with its design and analysis (detailed in Chapter 5) seriously limit its contribution to the scientific evidence on the effects of exposure to the COIs.

Serdar et al. (2014) used data from the 2003–2004 cycle of NHANES to examine the association of serum, lipid-adjusted PCB levels, and organochlorine pesticide levels (including PCDDs, PCDFs, and dioxin-like PCBs) with hematology and blood chemistry profiles that measured electrolytes, liver and renal function, globulin, total protein, and other factors. Although some statistically significant differences were found between counts and levels in the highest- and lowest-exposed quartiles, all were within the normal ranges and limits for those markers. These measures are indicators, not health outcomes, thus limiting their interpretability concerning immune system conditions.

BIOLOGIC PLAUSIBILITY

Given the growing recent understanding of the role of AHR in immune functions (Abe et al., 2014; Biljes et al., 2017; Bock et al., 2017a; Chinen et al., 2015; Huai et al., 2014; Kado et al., 2016; Kimura et al., 2014; Y. H. Lee et al., 2015; Liao et al., 2017; Murray and Perdew, 2016; Stockinger et al., 2014), it is not surprising that there is an extensive body of evidence from experimental studies in cultured cells and animal models indicating that TCDD and other dioxin-like chemicals are immunotoxic to a variety of leukocytes (Kerkvliet, 2009, 2012; Kreitinger et al. 2016). Given that most of the cell types involved in the immune system express the AHR, there are many potential pathways to immunotoxicity. TCDD-induced immunotoxicity is due primarily to changes in adaptive immune responses resulting in the suppression of both antibody-mediated and cell-mediated immunity. Dioxin and other AHR agonists may also reduce the clearance of infections and promote tumor growth through alterations in immune function. TCDD exposure alters macrophages and neutrophils so as to exacerbate some types of inflammation during infections, and it may contribute to the development of chronic inflammatory lung disease (Teske et al., 2005; P. S. Wong et al., 2010). Although there are many examples of dioxin and dioxin-like chemicals having immunosuppressive effects, these chemicals also appear to influence autoimmune diseases, which are viewed as an inappropriate increase in immune function. Therefore, these chemicals may be best described as immunomodulatory.

TCDD has been shown to be a potent immunosuppressive chemical in laboratory animals and cell culture models. Data show that the relative potencies

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of TCDD and dioxin-like chemicals on leukocytes are predicted by their TEFs (Frawley et al., 2014; Smialowicz et al., 2008). The exposure of animals to dioxin not only suppresses some adaptive immune responses, but also has been shown to increase the incidence, progression, and severity of various infectious diseases and to increase the development of cancers (Choi et al., 2003; Elizondo et al., 2011; Fiorito et al., 2010, 2011, 2014; Head and Lawrence, 2009; Jin et al., 2010; Sanchez et al., 2010). Also, developmental exposure to TCDD (in utero and via suckling) in mice reduces immune response capacity to the influenza virus in adulthood (Boule et al., 2014). Consistent with its immunosuppressive effects, TCDD exposure suppresses the allergic immune response of rodents; this in turn results in decreased allergen-associated pathologic lung conditions and has been shown to suppress the development of experimental autoimmune disease (Quintana et al., 2008), to induce the suppression of autoimmune uveoretinitis (L. Zhang et al., 2010), and to affect colitis (Ji et al., 2015; Takamura et al., 2011), arthritis (Nakahama et al., 2011), and inflammatory lung diseases, such as silicosis (Beamer et al., 2012).

Experimental studies indicate that the AHR pathway plays an integral role in B-cell maturation, and that exposure to TCDD and other dioxin-like chemicals may alter B-cells and result in critical changes in the immune response (Baba et al., 2012; Sibilano et al., 2012; Simones and Shephard, 2011; Singh et al., 2011). Feng et al. (2016) found that chronic TCDD exposure impaired both B- and T-cell differentiation in a mouse model. Working with human B cells in vitro, Allan and Sherr (2010) demonstrated a new AHR-dependent mechanism by which exposure to environmental PAHs suppressed humoral immunity by blocking the differentiation of B cells into plasma cells. This finding was confirmed by data from human hematopoietic stem cells and knockout *Ahr* mouse models showing that the *Ahr* is critical in the maturation and differentiation of hematopoietic stem cells (Bock, 2017b; Fracchiolla et al., 2011; J. Li et al., 2017; Singh et al., 2011; Vaidyanathan et al., 2017). Furthermore, data from a B-cell specific *Ahr* knockout showed that the receptor pathway is required for efficient B-cell proliferation (Villa et al., 2017). Using a novel pluripotent stem cell–based culture system, B. W. Smith et al. (2013) demonstrated that AHR expression and activity can direct human hematopoietic progenitor cell proliferation and differentiation. These data show that pluripotent hematopoietic human cells express AHR and that AHR agonists enhance erythroid differentiation, whereas the antagonism of AHR favors the expansion of megakaryocyte cells. This finding supports previous work indicating that B-cell activation results in increased AHR expression and that an exposure of B-cells to benzo[*a*]pyrene, a PAH, suppresses B-cell differentiation (Allan and Sherr, 2010). H. Lu et al. (2010) demonstrated that although human B cells appeared less responsive to TCDD in increasing the expression of AHR battery genes, TCDD's ability to decrease IgM production was similar in both mouse and human B cells. Data from Q. Zhang et al. (2013) suggest that this decrease in IgM production is the result of a TCDD-mediated decrease in B-cell

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terminal differentiation, which results in fewer IgM-producing cells. Recent data from Kovalova et al. (2017) identify other species-specific gene expression in TCDD-exposed mouse, rat, and human B cells. In addition, Kovalova et al. (2016) found that certain AHR polymorphisms altered the sensitivity of human B cells to TCDD-mediated suppression of IgM secretion, consistent with AHR levels mediating this suppression.

TCDD not only alters hematopoietic stem cell maturation but also alters activation, proliferation, and migration in vivo and in vitro (Casado et al., 2011; Fader et al. 2015; Phadnis-Moghe et al., 2016), which indicates that exposure to it may have multiple effects on immune-cell function. Recent data have linked TCDD activation of the AHR with altered regulation of BCL-6 in human B-cells, which in turn is linked to non-Hodgkin lymphoma and diffuse large B-cell lymphomas (Phadnis-Moghe et al., 2015, 2016), providing a suggestive mechanistic link between TCDD exposure and some lymphomas.

Cellular immunity, which is mediated by the thymus and T cells, is also a target of TCDD and dioxin exposure and the AHR pathway (Baricza et al., 2016; Feng et al., 2016; Kuwatsuka et al., 2014). Early evidence indicated that dioxin and dioxin-like chemicals alter cellular immunity because it was observed that exposure to these chemicals resulted in thymic involution and suppressed cytotoxic T-lymphocyte activity (Hanieh, 2014). Recently attention has focused on the ability of the AHR to induce regulatory T cells, or Tregs (Bruhs et al., 2015; Kerkvliet, 2012; Marshall and Kerkvliet, 2010; Mohinta et al., 2015). A recent paper elucidates one potential molecular mechanism of AHR activation of Tregs, using real-time imaging to show the migration of AHR-Tr1 Tregs in the small intestine and the colon (Ehrlich et al., 2017). Tregs have potent suppressive activity in the immune system, and their inappropriate induction by TCDD could account for much of the immune suppression (Funatake et al., 2008; Kerkvliet, 2012; Marshall et al., 2008; Quintana et al., 2008; Stockinger et al., 2011; Yamamoto and Shlomchik, 2010). AHR activation in dendritic cells has also been shown to promote the development of Tregs by inducing tryptophan metabolism. Furthermore, a recent study shows a role for the AHR pathway in promoting gut Tregs (Ye et al., 2017).

One ultimate effect of the dysregulation of the immune system is an alteration in autoimmunity. Data from animal models and cell cultures indicate that exposure to dioxin and dioxin-like chemicals alters the development of autoimmune disorders. Boule et al. (2015) found that developmental exposure to TCDD exacerbated the severity of autoimmune disease in a genetically susceptible mouse model. In another example, antagonism of the AHR repressed the expression of cytokines and chemokines in primary human synovial fibroblasts (Lahoti et al., 2013), indicating a potential contribution to the inflammatory process of rheumatoid arthritis. N. T. Nguyen et al. (2013) hypothesized that the inflammatory process may occur when AHR stimulation of IL-17 production in Th17 cells overwhelms the immune suppressive effects of the inhibition of Treg

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differentiation. TCDD has also been shown to induce apoptosis in rabbit chondrocytes, which supports a potential role of TCDD in contributing in a novel way to arthritis (Yang and Lee, 2010). A recent study indicates that sub-chronic low-dose TCDD exposure can show immunomodulatory effects in a mouse model of experimental autoimmune encephalitis (E. J. Yang et al., 2016). Recent work by L. Cheng et al. (2017) in a hospital-based study of Han Chinese patients suggests that a polymorphism in the AHR repressor gene may increase an individual's susceptibility to rheumatoid arthritis. Exposure to TCDD was also shown to induce the reactivation of the latent form of the Epstein Barr virus in 19 patients with Sjögren syndrome, an autoimmune disease, when compared with 19 healthy patients (Inoue et al., 2012). AHR activation by ultraviolet light may play a role in the exacerbation of systemic lupus erythematosus symptoms by reducing DNA methylation in CD4+ T cells (Z. Wu et al., 2017). Taken together these studies suggest mechanisms by which TCDD may alter the incidence and progression of autoimmune disorders.

Allergies and allergic-induced asthma are also linked to TCDD exposure. A study of 18 people who had allergic asthma, 17 people whose asthma was controlled, and 12 controls showed that the plasma concentrations of IL-22 and the expression of the AHR in peripheral blood mononuclear cells were associated with the severity of allergic asthma; this finding strengthened the possibility that the AHR is involved in allergic asthma, thereby implying a role for dioxin exposure in this condition (Zhu et al., 2011). X. M. Li et al. (2016) studied a mouse model of non-allergic asthma and found that TCDD exposure reduced the airway infiltration of neutrophils and airway hyperresponsiveness and inhibited Th17 differentiation. Thus, depending on the disease, TCDD exposure could exacerbate or ameliorate symptoms.

SYNTHESIS

Previous VAO committees have concluded that the data were inadequate or insufficient to support an increased risk of immune suppression, allergy, or autoimmune disease. The studies reviewed by these committees were at times poorly designed and often inconsistent and used a variety of biomarkers, making comparisons difficult. Most of the studies used biomarkers rather than health outcomes as endpoints.

The new studies reviewed here do not change this conclusion, as the results continue to be inconsistent and inconclusive. Cox et al. (2015) used hospital discharge records from New Zealand Vietnam veterans to examine the prevalence of inflammatory and autoimmune conditions. Although there was an increase in the standard hospitalization rate for rheumatoid arthritis but not systemic lupus erythematosus among veterans, no serum or tissue levels of dioxin-like chemicals were provided to confirm exposure. Rheumatoid arthritis incidence and prevalence were examined by Parks et al. (2016) in the female spouses of licensed

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pesticide applicators in the AHS and found to be associated only with glyphosate, not 2,4-D or dicamba. Again, exposure was not confirmed with blood or tissue levels. Cappelletti et al. (2016) studied electric arc foundry workers exposed to the COIs. Results showed a statistically significant increase in rheumatoid arthritis among workers exposed to foundry dust. In a comparison of high- and low-exposure areas in Vietnam, C. H. Nguyen et al. (2017) showed that persons living in the high-exposure area had higher levels of AHR expression and some pro-inflammatory cytokines. They also had a higher prevalence of rheumatoid arthritis, but no data were provided linking the higher levels of pro-inflammatory cytokines in persons with rheumatoid arthritis. Among New Zealand workers in a plant that produced 2,4,5-T, comparisons of high- versus low-exposed workers by job and by serum measurements showed no difference in doctor-diagnosed nasal allergies, including hay fever.

It is biologically plausible for dioxins to influence immune dysfunction, but because of the variety of methods and endpoints used in studies to date, it is hard to confirm specific mechanisms by which the COIs induce immune suppression and auto-immunity. TCDD has been shown to suppress both limbs of the adaptive immune response, reduce the clearance of infection, and promote tumor growth (Bruhs et al., 2015; Kerkvliet, 2009, 2012; Kreitinger et al., 2016; Marshall and Kerkvliet, 2010). Exposure to dioxin-like chemicals has been shown to induce immune suppression via T regulatory cells (Bruhs et al., 2015; Kerkvliet, 2012; Marshall and Kerkvliet, 2010; Mohinta et al., 2015) in animal models. Animal models also support the development of inflammatory conditions, especially rheumatoid arthritis, following exposure to dioxin, through the AHR and downstream increased production of IL-17 (N. T. Nguyen et al., 2013). AHR polymorphisms have been associated with an increased susceptibility to rheumatoid arthritis in exposed persons (L. Cheng et al., 2017) and AHR activation by ultraviolet light may exacerbate systemic lupus erythematosus via reduced methylation in CD4+ T cells (Z. Wu et al., 2017). The data are inconsistent for allergic asthma.

CONCLUSION

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and any specific diseases involving immune suppression, allergy, autoimmunity, or inflammation.

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7

Cancer

Chapter Overview

*Based on new evidence and a review of prior studies, the current committee determined that epidemiologic results concerning an association between exposure to any one of the chemicals of interest (COIs; 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, dimethylarsinic acid [DMA or cacodylic acid], and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]) and monoclonal gammopathy of undetermined significance (MGUS) met the criteria for sufficient evidence of an association. No other changes in association level between the relevant exposures and other cancer types were made as either there were no published studies or the new evidence supported the findings of earlier updates. Thus, the current findings on cancer can be summarized as follows:*

- *There is sufficient evidence of an association between the COIs and soft tissue sarcomas, B-cell lymphomas (Hodgkin lymphoma, non-Hodgkin lymphomas, chronic lymphocytic leukemia, hairy-cell leukemia), and MGUS.*
- *There is limited or suggestive evidence of an association between the COIs and bladder cancer; laryngeal cancer; cancers of the lung, bronchus, or trachea; prostate cancer; multiple myeloma; and AL amyloidosis.*
- *There is inadequate or insufficient evidence to determine whether there is an association between the COIs and any other specific type of cancer.*

Cancers are the second-leading cause of death in the United States (CDC, 2017a), with heart disease being the leading cause of death. However, among

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men 55–79 years old, the group that includes most Vietnam veterans, the risk of dying from cancer exceeds the risk of dying from heart disease (CDC, 2017b). According to estimates from the National Cancer Institute (NCI), 1,735,350 new cases of cancer were expected to be diagnosed and 609,640 people of all ages were expected to die from cancer in the United States in 2018 (NCI, 2018a).

The objective of this chapter is to provide an assessment of whether the occurrence of cancers in Vietnam veterans may be associated with exposures to herbicides that they may have experienced during their military service. This chapter summarizes and presents conclusions about the strength of the evidence from epidemiologic studies regarding associations between exposure to the COIs and various cancer types. As described in Chapter 3, studies of exposure to polychlorinated biphenyls (PCBs) and other dioxin-like chemicals were also considered informative if their results were reported in terms of TCDD toxic equivalents (TEQs) or concentrations of specific congeners of dioxin-like chemicals. Studies that report TEQs based only on mono-ortho PCBs (which are PCBs 105, 114, 118, 123, 156, 157, 167, and 189) are considered even though their TEQs are several orders of magnitude lower than those of the non-ortho PCBs (77, 81, 126, and 169), based on the revised World Health Organization (WHO) toxicity equivalency factor (TEF) scheme of 2005 (La Rocca et al., 2008; van den Berg et al., 2006). The lower TEQs of the mono-ortho PCBs, however, may be counterbalanced by their abundance, which is generally many orders of magnitude higher than the non-ortho PCBs (H.-Y. Park et al., 2010).

A compendium of all of the studies reviewed by the various Veterans and Agent Orange (VAO) and VAO Update committees can be found at www.nap.edu/catalog/25137. In this update, if a new study reported on only a single type of cancer and did not revisit a previously studied population, then its design information is summarized here with its results; design information on studies that are updates of or new analyses on populations or cohorts that have been previously studied can be found in Chapter 5. Studies of childhood cancers in relation to parental exposure to the COIs are discussed in Chapter 8, which addresses possible adverse effects in the veterans' offspring. Studies that consider only childhood exposure are not considered relevant to the committee's charge.

In evaluating possible connections between herbicide exposure and the risk of cancer, the approach used to assess the exposure of study subjects is of critical importance in determining the overall relevance and usefulness of findings. There is great variation in the detail and the accuracy of exposure assessments among studies, which can distort the true relationship between exposure and disease. A few studies used biologic markers of exposure, such as the presence of a chemical in serum or tissues; others developed an index of exposure from employment or activity records; and still others used other surrogate measures of exposure, such as an individual's presence in a locale when herbicides were used.

Each section on a specific cancer type opens with background information, including data on its incidence in the general U.S. population and its known

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or suspected risk factors. Cancer incidence in the general U.S. population is included in the background material to provide a context for consideration of the cancer risk in Vietnam veterans; the numbers presented are estimates of incidence in the entire U.S. population, not predictions for the Vietnam veteran cohort. The data on the expected numbers of new cases and deaths for specific types of cancerin 2017 are based on estimates from NCI's Surveillance, Epidemiology, and End Results (SEER) program or, if estimates for a particular cancer type were not available from NCI, on estimates by the American Cancer Society. Using the most recent SEER data available when this was written (SEER-18; 2000–2014), incidence data were derived for all races combined and separately for blacks and whites using age groups likely to include most Vietnam-era veterans. Incidence data are presented by sex, age, and race, all of which can have profound effects on risk (NCI, n.d.a). For example, the incidence of prostate cancer is about 2.6 times higher in men who are 70–74 years old as in men 60–64 years old and about 75% higher in blacks 60–64 years old than in whites in the same age group (NCI, 2015). Many other factors can influence cancer incidence, including screening methods, tobacco and alcohol use, diet, obesity, genetic predisposition, and medical history. Those factors can modify the risk of developing a given kind of cancer; they also need to be taken into account in epidemiologic studies of the possible contributions of the COIs.

Each section of this chapter pertaining to a specific type of cancer includes a summary of the findings described in the previous 10 *VAO* reports. That is followed by a discussion of the most recent scientific literature, and, when appropriate, the literature is discussed by exposure type (service in Vietnam, occupational exposure, or environmental exposure). A summary of biologic plausibility, which corresponds to the third element of the committee's congressionally mandated Statement of Task, follows the description of newly identified epidemiologic studies. In fact, the degree of biologic plausibility itself influences whether the committee perceives positive findings to be indicative of an association or the product of statistical fluctuations (chance) or bias. Following a synthesis of the material reviewed, each section ends with the committee's conclusion regarding the strength of the evidence from epidemiologic studies. The categories of association and the committee's approach to categorizing the health outcomes are discussed in Chapter 3.

Chapter 4 contains information on the general biologic mechanisms by which exposure to TCDD and the other COIs contribute to malignant transformation. Toxicology studies use a variety of methods and animal or cellular models to derive results on the interactions of the COIs with the cellular machinery known to be important in the development of cancer at any site. When biologic plausibility is discussed in each section, this generic information is implicit, and only experimental data specific to carcinogenesis at the site in question are presented. There is increasing evidence that TCDD and the COIs may disturb cellular processes through epigenetic mechanisms, and reference to this evidence as it applies to specific cancers is included where it exists.

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Considerable uncertainty remains about the magnitude of the risk posed by exposure to the COIs. Many of the veteran, occupational, and environmental studies reviewed by the committee did not fully control for important confounders. There is not enough information about the exposure experience of individual Vietnam veterans to permit combining exposure estimates for them with any potency estimates that might be derived from scientific research studies to quantify risk. The committee therefore cannot accurately estimate the risk to Vietnam veterans that is attributable to exposure to the COIs. The significant challenges in deriving useful quantitative estimates of the risks of various health outcomes in Vietnam veterans are explained in Chapter 2 of this report.

ORGANIZATION OF CANCER GROUPS

Consistent with the previous report in this series, the organization of cancer groups follows the major and minor categories of cause of death related to cancer sites established by the National Institute for Occupational Safety and Health (NIOSH; Robinson et al., 2006). For the present update, the committee gave more attention to the WHO's classification of lymphohematopoietic neoplasms (WHO, 2008), which stresses partitioning of the disorders first by the lymphoid or myeloid lineage of the transformed cells, rather than categorizing into lymphomas and leukemias.

The system of organization used by the committee simplifies the process for locating a particular cancer type for readers. For any cancer type for which no epidemiologic research findings have been identified, the default category has always been "inadequate or insufficient evidence" of association with exposure to the COIs. A failure to review a specific cancer or other condition separately reflects the paucity of information concerning that cancer, so there is indeed inadequate or insufficient information to categorize an association with such a disease outcome.

BIOLOGIC PLAUSIBILITY

The studies considered by the committee that speak to the biologic plausibility of associations between human cancers and exposure to 2,4-D, 2,4,5-T, picloram, cacodylic acid, and TCDD have been performed primarily in laboratory animals (rats, mice, hamsters, and monkeys) or in cultured cells.

The animal studies examining the carcinogenicity of 2,4-D, 2,4,5-T, and picloram have, in general, produced negative results, although some bioassays used in those studies would not meet current standards. For example, there is a question of whether the highest doses (generally 30–50 mg/kg) used in some of the experiments reached a maximum tolerated dose or represented the doses that are capable of inducing carcinogenesis. Therefore, it is not possible to have absolute confidence that these chemicals have no carcinogenic potential at higher

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doses. Additional evidence of a lack of carcinogenic potential comes from negative findings on the genotoxic effects of assays conducted primarily in vitro that indicate that 2,4-D and 2,4,5-T are genotoxic only at very high concentrations.

There is evidence in laboratory animals that cacodylic acid is carcinogenic, based on studies that have shown that it can induce neoplasms of the kidney (Yamamoto et al., 1995), bladder (Arnold et al., 2006; Cohen et al., 2007b; A. Wang et al., 2009; Wei et al., 2002; Yamamoto et al., 1995), liver, and thyroid gland (Yamamoto et al., 1995). Treatment with cacodylic acid induced the formation of neoplasms of the lung when administered to mouse strains that are genetically susceptible to developing those tumors (Hayashi et al., 1998; Yamanaka et al., 2009). Other studies have used the two-stage model of carcinogenesis in which animals are exposed first to a known genotoxic agent and then to a suspected tumor-promoting agent; with this model, cacodylic acid has been shown to act as a tumor promoter with respect to lung cancer (Yamanaka et al., 1996). These studies are further discussed in Chapter 4.

Collectively, the evidence obtained from studies of TCDD supports a connection between human exposure and cancers. The effects of TCDD on cellular function make carcinogenesis biologically plausible, and evidence from model systems indicate that TCDD can enhance carcinogenesis. This will be discussed in a generic sense below and more specifically in the biologic plausibility sections on individual cancers. Several reviews have affirmed the well-established mechanistic roles of the aryl hydrocarbon receptor (AHR) in TCDD-induced cancers (S. Ahmed et al., 2014; Androutsopoulos et al., 2009; Barouki and Coumoul, 2010; Dietrich and Kaina, 2010; Ide et al., 2017; Murray et al., 2014; Ray and Swanson, 2009; Rysavy et al., 2013; Tsay et al., 2013). The effect can be both cancer promoting—by activating oncogenes (Gardella et al., 2016) or blocking apoptosis (Bekki et al., 2015)—or protective, depending on the tissue type and timing of the exposure (Y. Li et al., 2014; Moore et al., 2016). The role of the AHR is further established by:

- its activation of several proteins of the P450 system of enzymes that play crucial roles in detoxification and drug metabolism (Al-Dhfyan et al., 2017a,b);
- activation of the paraoxanase antioxidant enzymes (Shen et al., 2016); and
- activation of the transforming growth factor (TGF)-β pathway (Silginer et al., 2016);

all of which are important in oncogenesis. TCDD can disrupt circadian rhythms via the AHR, and chronic disruption of circadian rhythms is associated with an increased incidence of cancer, suggesting a potential additional pathway by which TCDD increases cancer risk (C. Wang et al., 2014; C. X. Xu et al., 2013). TCDD increases the incidence or progression of human cancers through a variety of cellular mechanisms, and the biologic plausibility of an association between TCDD

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exposure and cancer has been firmly established in a mechanistic sense. Data also indicate that AHR can play a protective role in cancer (reviewed in Kolluri et al., 2017; Murray et al., 2014), and its role as a therapeutic target for cancer therapy is being investigated. However, cancer therapies were considered beyond the scope of the committee's charge, and not included in this report.

TCDD is considered a non-genotoxic carcinogen, as reviewed by Hernández et al. (2009), because it does not produce changes is DNA sequences. However, because of the oxidative stress it produces, TCDD does have some genotoxic potential. In vitro work with mouse hepatoma cells has shown that activation of the Ahr results in increased concentrations of 8-hydroxy-2-deoxyguanosine (8-OHdG), a product of DNA-base oxidation and a marker of DNA damage. The induction of cytochrome P4501A1 (CYP1A1) in these cells by TCDD or indolo (3,2-b) carbazole is associated with oxidative DNA damage (Park et al., 1996). In vivo experiments in mice corroborated those findings by showing that TCDD caused a sustained oxidative stress, as determined by measurements of urinary 8-OHdG (Shertzer et al., 2002), and that it involves AHR-dependent uncoupling of mitochondrial respiration (Senft et al., 2002). Mitochondrial reactive-oxygen production depends on the AHR. Electronics-dismantling workers who experienced complex exposures, including exposure to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), were shown to have increased concentrations of urinary 8-OHdG, indicative of oxidative stress and genotoxicity; however, this finding cannot be ascribed confidently to these compounds (Wen et al., 2008). Other than these observations of 8-OHdG formation and oxidative stress, there is little evidence that TCDD is genotoxic, and it appears likely that some of its mechanisms of action may involve epigenetic modifications of the DNA or chromatin (described in Chapter 4).

The ability to induce oxidative stress contributes to TCDD's recognized activity as a potent tumor promoter and as a weak initiator in two-stage initiation–promotion models for ovarian cancer (Davis et al., 2000), liver cancer (Beebe et al., 1995), and skin cancers (Wyde et al., 2004). Work with a mouse lung cancer model suggests that in addition to increasing cell division, the tumor-promoting activities of TCDD include blocking apoptosis (R. J. Chen et al., 2014a). Early studies demonstrated that TCDD is two orders of magnitude more potent than the "classic" promoter tetradecanoyl phorbol acetate and that its skin-tumor promotion depends on AHR (Poland et al., 1982).

Laboratory animals exposed to TCDD show an increase in the incidence of several neoplasms, most notably of the liver, lungs, thyroid, and oral mucosa (Kociba et al., 1978; NTP, 2006). In long-term bioassays in both sexes of several strains of rats, mice, hamsters, and fish, TCDD increases the incidence of tumors, including those at sites distant from the site of treatment, at doses well below the maximum tolerated dose (Rysavy et al., 2013). TCDD exposure has also been shown to cause hyperplasia or metaplasia of epithelial tissues. In both laboratory animals and cultured cells, TCDD has been shown to exhibit a wide array of effects

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on growth regulation, hormone systems, and other factors associated with the regulation of cellular processes that involve growth, maturation, and differentiation, in most cases via its interaction with AHR (Murray et al., 2014; Rysavy et al., 2013). In rat liver, TCDD downregulates reduced folate carrier (Rfc1) mRNA and protein, whose normal levels are essential in maintaining folate homeostasis (Halwachs et al., 2010). Reduced Rfc1 activity and a functional folate deficiency may contribute to the risk of carcinogenesis posed by TCDD exposure, perhaps via an epigenetic effect of interfering with DNA methylation levels (Davis and Uthus, 2004; Williams, 2012).

Tissue-specific protective cellular mechanisms may also be important to the response to TCDD and may complicate our understanding of its site-specific carcinogenic effects. For example, studies reviewed by this committee (and further described in specific cancer outcome sections below) include investigations of TCDD and other AHR ligands that found anti-proliferative and anti-metastatic activity in cell lines of different cancers, including breast, ovarian, and prostate (Hanieh, 2015; Hanieh et al., 2016; Ide et al., 2017; Y. Li et al., 2014).

In humans, the cancer-causing effects of TCDD (and the other COIs) have to be evaluated with respect to inherent genetic susceptibility or resistance, which can vary considerably across human beings. Several polymorphisms in the AHR gene have been identified in humans, although the functional significance remains uncertain. One genome-wide association study found a weak association with between the *AHR* locus and cutaneous squamous cell carcinoma (Chahal et al., 2016), and another study (Spink et al., 2015) found that the (GGGGC)n repeat polymorphism in the human *AHR* was overrepresented in a small sample size of lung cancers, compared to a neonatal population in New York thought to represent a the incidence in the general population. Variants of the DNA repair gene *XRCC1* have been associated with urothelial cancer risk and this risk was increased with arsenic exposure (Chiang et al., 2014). Some of these genes exert their effect by modulating the cellular exposure to COIs, such as the effects of the *CYP1A1*, *GSTM1*, and *p53* on polycyclic aromatic hydrocarbon (PAH) exposure (Gao et al., 2014). Genetic variants can also be associated with non-neoplastic health effects; for example, common polymorphisms in some cytochrome P450 genes (*CYP1A1*, *CYP1B1*, *CYP17*) are associated with benign prostatic hyperplasia related to organochlorine pesticide exposure (V. Kumar et al., 2014) and may have an impact on chronic kidney disease and dioxin levels in an endemic area of exposure (C. Y. Huang et al., 2016). Thus, identical exposures can have non-identical effects in different individuals, making it challenging to perform genotype/phenotype assessments of TCDD effects for specific cancers.

Several potential pathways for TCDD carcinogenesis have been proposed. TCDD may contribute to tumor progression via the inhibition of p53 tumor suppressor activity induced by genotoxic agents (Gardella et al., 2016). This inhibition may occur through AGR2 (Ambolet-Camoit et al., 2010) or through interaction with the AHR and FHL2 (four and a half LIM protein domains 2)

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(Kollara and Brown, 2009). A study in fish by Calò et al. (2018) suggests that dioxin-like PCB 126 may promote the degradation of tumor suppressor p53 through the proteasome ubiquitin system. (In related work, Luecke-Johansson et al. [2017] explored the mechanism of activating AHR function as E3 ubiquitin ligase.) Borlak and Jenke (2008) demonstrated that AHR is a major regulator of c-Raf and proposed that there is cross-talk between AHR and the mitogen-activated protein kinase signaling pathway in chemically induced hepatocarcinogenesis. TCDD inhibits ultraviolet-C radiation-induced apoptosis in primary rat hepatocytes and in Huh-7 human hepatoma cells, supporting the hypothesis that TCDD acts as a tumor promoter by preventing exposed cells from undergoing apoptosis (Bekki et al., 2015; R. J. Chen et al., 2014b; Chopra et al., 2009). AHR activation by TCDD in human breast and endocervical carcinogenic cell lines induces sustained high concentrations of the cytokine interleukin (IL)-6. IL-6 has tumor-promoting effects in numerous tissues—including breast, prostate, and ovary—which opens the possibility that TCDD may promote carcinogenesis in these and possibly other tissues (Hollingshead et al., 2008). However, recent work in normal mammary cells indicates that AHR may function as inhibitors of mammary tumors (Hall et al., 2010; Hanieh, 2015; Hanieh et al., 2016; S. Zhang et al., 2009, 2012), supporting work indicating that TCDD's effect is cell-type specific. More recent work has shown an interaction between the AHR and *ADM* (adrenomedullin) oncogene in cell lines and lung tissue (Portal-Nunez et al., 2012), and AHR repression experiments in gastric and head and neck cancers suggest that AHR expression leads to increased cancer cell growth and invasion (DiNatale et al., 2012; X. F. Yin et al., 2013). In cell culture studies of mechanisms of cancer progression and metastasis, TCDD exposure increased the epithelial-to-mesenchymal transition and the loosening of cell–cell contacts (Diry et al, 2006; Gao et al., 2016).

Genetic disturbances arising from confirmed exposure to herbicides were evaluated by analyzing sister-chromatid exchanges in lymphocytes from a group of 24 New Zealand Vietnam War veterans and 23 matched control volunteers (Rowland et al., 2007). The results showed a highly significant difference ($p < 0.001$) between the veterans and the control group in the mean frequency of sister-chromatid exchanges, which is thought to be an indicator of genetic damage. The distribution was skewed left, and the Vietnam veterans also had a much higher proportion of cells with sister-chromatid exchanges frequencies above the 95th percentile (≥17 sister chromatid exchanges per cell) than the controls (11.0% versus 0.07%). A study of sister-chromatid exchanges frequencies in blood samples taken from Vietnamese women from high- and moderate-TCDD-sprayed areas also showed increased sister-chromatid exchanges of 2.40 per cell and 2.19 per cell, respectively, compared with Vietnamese women from unexposed areas (1.48 per cell, $p < 0.001$) (Suzuki et al., 2014).

The weight of evidence that TCDD and dioxin-like PCBs make up a group of chemicals with carcinogenic potential includes unequivocal animal carcinogenesis

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and biologic plausibility based on mechanistic mode-of-action data. Although the specific biological and genetic mechanisms by which dioxin causes cancer remain to be elaborated, the intracellular factors and mechanistic pathways involved in dioxin's cancer-promoting activity all have parallels in animal and human studies. Nevertheless, the extrapolation of animal studies to humans should be viewed with caution since there are many biological differences among these species. The International Agency for Research on Cancer (IARC) has classified TCDD in group 1 as carcinogenic to humans. The strongest evidence for carcinogenicity was observed when all cancers sites were aggregated, but a positive association between TCDD exposure and soft-tissue sarcomas, non-Hodgkin lymphomas, and lung cancer has also been found (IARC, 2012b), which likely contributes to the strong association of all cancers combined. Risks for specific cancers in reports of TCDD-exposed workers and in the TCDD-exposed population in Seveso have been sporadic and inconsistent (J. Xu et al., 2016b), diluting the strength of the evidence for anything more than an aggregation of all cancers.

Thus, the toxicologic evidence indicates that a connection of TCDD and perhaps cacodylic acid with cancer in humans is, in general, biologically plausible. However, as discussed in the next section, whether such carcinogenic potential contributes to an individual type of cancer must be evaluated on a case-by-case basis. Experiments with 2,4-D, 2,4,5-T, and picloram in animals and cells have not provided a strong biologic basis for the presence or absence of carcinogenic effects for those COIs.

CURRENT VIEWS OF CANCER MECHANISMS

To address its charge, the committee weighed the scientific evidence linking the COIs to specific individual cancer sites. Before considering each site individually, it is important to address the concept that cancers share some characteristics among organ sites. All cancers share phenotypic characteristics: unregulated cell proliferation, increased cell survival, invasion outside normal tissue boundaries, and eventual metastasis. The current understanding of cancer development holds that a cell must acquire a series of specific genetic mutations that release it and its progeny from regulated growth in order to establish growth independence. These mutations can occur in a variety of genes that positively (oncogenes) or negatively (tumor suppressor genes) control cell growth, cell death (apoptosis), or the repair of genes when mutations do occur (Hanahan and Weinberg, 2000). Hanahan and Weinberg further add that for a tumor to survive, four other changes are necessary: changes in metabolism that give cells a selective growth advantage, evasion of the immune system, genetic instability leading to additional mutations, and local inflammation. In addition to mutational events, non-mutational or epigenetic events contribute to malignant transformation by altering the expression of genes that contribute to malignant transformation. However, some researchers have hypothesized that whatever the triggers, the earliest

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cancer cells have recognizable somatic mutations and establish clonality (Burgio and Migliore, 2015). Also, angiogenesis, or the formation of new blood vessels, allows a developing malignancy to obtain nutrients and enable the cells of that malignancy to invade the local normal tissue. Recent work has drawn attention to the interaction of cancer cells and the tumor microenvironment. Derbal (2017) has described how dysregulation of cellular metabolism locks cancer cells into a "state of mutual dependence with the tumor microenvironment and deepens the tumor's inflammation and immune-suppressive state," therefore making it more difficult to treat.

Both genetic (mutational) and epigenetic (non-mutational) effects of carcinogenic agents can further contribute to and stimulate oncogenesis. Genotoxic damage by environmental exposures, such as the committee's COIs, can affect tumor establishment through many non-carcinogenic processes, such as those that take place in the the metabolic and immune systems. As discussed above and in Chapter 4, 2,4-D, 2,4,5-T, and picloram have shown little evidence of genotoxicity in laboratory studies, except at very high doses, and little ability to induce carcinogenesis in laboratory animals. However, cacodylic acid and TCDD—acting more like promoters than genotoxic initiators—have been shown to induce tumors in laboratory animals. Extrapolating organ-specific results from animal experiments to humans is problematic because of important differences among species in the overall susceptibility of various organs to cancer development and in organ-specific responses to putative carcinogens. While experiments using animal models can be carefully designed to control for confounding risk factors, this is often not possible in human studies. Therefore, conclusions about the potential carcinogenicity of a chemical in humans rely heavily on the results of epidemiologic studies that examine evidence of an excess cancer risk for individual or multiple organ sites. As the evaluations of specific types of cancer in the remainder of this chapter indicate, the committee finds that TCDD appears to be a multisite carcinogen. That finding is in agreement with IARC, which has placed TCDD as a category 1 "known human carcinogen" (Baan et al., 2009; IARC, 2012b); with the U.S. Environmental Protection Agency (EPA), which has concluded that TCDD is "likely to be carcinogenic to humans" (EPA, 2004); and with the National Toxicology Program (NTP), which regards TCDD as "known to be a human carcinogen" (NTP, 2011). It is important to emphasize that the goals and methods of IARC and EPA in making their determinations were different from those of the present committee: Those organizations focus on anticipating hazards to minimize future exposure, whereas this committee focuses on risk after exposure. Furthermore, the recognition that TCDD and cacodylic acid are multisite carcinogens does not imply that they cause human cancer at every organ site.

The distinction between a general carcinogen and a site-specific carcinogen is more difficult to make because of the common practice of beginning analyses of epidemiologic cohorts with a category of "all malignant neoplasms," a routine first screen for increased cancer activity in a study population without any

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test of a biologically based hypothesis. When the distribution of cancers among anatomic sites is not provided in the report of a cohort study, a statistical test for an increase in all cancers is not meaningless, but it is usually less scientifically supportable than analyses based on specific sites for which more substantial biologically based hypotheses can often be developed. The size of a cohort and the length of the observation period often constrain the number of cancer cases that are observed and which specific cancers have enough observed cases to permit analysis. For instance, an analysis of the cumulative results on diabetes and cancers in the prospective Air Force Health Study (AFHS; Michalek and Pavuk, 2008) produced important information summarizing previous findings on diabetes, a fairly common condition, but the cancer analysis does not go beyond "all cancers." The committee does not interpret the cancer findings as an indication that exposure to herbicides increases the risk of every variety of cancer, but rather as an indication that the agent is carcinogenic to humans. For example, the committee acknowledges that the results of the highly stratified analyses conducted in the AFHS found an increased incidence of certain cancers as well as all cancers combined in the Ranch Hand subjects. It views the result of all cancers combined as a conglomeration of information on individual malignancies. However, it also recognizes that melanoma and prostate cancer are two malignancies for which increased risk has been published (Akhtar et al., 2004; Pavuk et al., 2006), and therefore, that these conditions merit continued individual longitudinal analysis to resolve outstanding questions and to confirm the association with TCDD.

OVERVIEW OF STUDIES THAT REPORT MULTIPLE CANCER OUTCOMES

To avoid needless redundancy, the current committee made the decision to summarize those studies that reported separate results on five or more individual cancer outcomes here, at the beginning of the chapter. Two studies met this criterion: Collins et al. (2016) and Coggon et al. (2015). The discussion of the relevant study in each individual cancer section only includes the study population and specific effect estimates as well as any nuances of which the reader should be aware.

Collins et al. (2016) provides additional follow-up to a retrospective analysis of a cohort of 2,192 workers (only 5 of whom were female) exposed to dioxins during trichlorophenol (TCP) and pentachlorophenol (PCP) production at a Dow chemical manufacturing plant in Midland, Michigan (see Chapter 5). The U.S. population was used as the comparator for standardized mortality ratio (SMRs). Work history records were used to determine the length of exposure. Serum samples to measure levels of six types of dioxins were collected for 431 TCP and PCP workers. Historic concentrations for each dioxin congener were calculated from the median concentrations from the serum samples and the known half-lives associated with each congener. A job exposure matrix was created for both the TCP

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and PCP production facilities based on the measured concentrations for workers in different jobs. A pharmacokinetic model was applied to job-specific concentrations based on the work history of each member of the study group to estimate their time-dependent serum concentration profiles for each dioxin congener (i.e., TCDD as well as Hexa-CDD, Hepta-CDD, and Octa-CDD). Complete vital status follow-up through December 2011 was achieved for the cohort, and there were 1,198 decedents through the entire study period (1979–2011); 1,615 TCP workers and 773 PCP workers (196 workers were exposed to both TCP and PCP and were included in both groups). SMRs were reported for more than 20 types of cancer and other health outcomes. Estimates were reported for all workers, TCP workers (196 of whom were also exposed to PCP), and PCP workers (196 who also had TCP exposure). This study is referred to throughout the chapter as the Dow Midland, Michigan, plant workers.

Coggon et al. (2015) extended the follow-up period of a large IARC-sponsored study and examined the carcinogenicity of phenoxy herbicides and their associations with, primarily, Hodgkin lymphoma (HL), STS, and chronic lymphocytic leukemia, but other types of cancers were also included as outcomes. The original IARC study, a nested case-control study within a large international cohort study included 36 subcohorts, 6 of which were made up of men who worked at 5 factories in the United Kingdom manufacturing or formulating a variety of phenoxy herbicides or else were contract workers spraying the compounds. The IARC study followed workers from 1947 through 1990/1991, and Coggon et al. (2015) extended the follow-up of the six UK cohorts to December 2012. Data were derived from individual employment and health care system records as well as from cancer registries and death records to detect additional cases. SMRs were reported, and the effect estimates were reported for all workers, workers exposed to herbicide levels above background, and workers exposed for more than 1 year at levels above background. The many results on specific cancer mortality in this group are referenced as the UK phenoxy herbicide manufacturers and sprayers.

STUDIES OF OVERALL CANCER MORTALITY OR INCIDENCE

The literature search for this update identified a number of publications on populations with relevant exposures that included risk estimates for overall mortality from any cancer (Cappelletti et al., 2016; Coggon et al., 2015; Collins et al., 2016; Kashima et al., 2015; S. A. Kim et al., 2015) or overall cancer incidence or prevalence (Ljunggren et al., 2014; Van Larebeke et al., 2015). However, grouping all cancer incidences or deaths is not informative for determining specific health effects that may be due to an exposure to the COIs versus those attributable to many other factors. This method also imposes the assumption of homogeneity of association across the combined deaths or cancer types. For example, Van Larebeke and colleagues (2015) assessed the prevalence of all cancer based on a self-reported affirmative response to the question, “Do you suffer or have

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you suffered from one or another form of cancer?" The committee believed that this was not specific enough to be useful for the assessment of cancer and the potential effects from the COIs and excluded it from further review in the cancer chapter. Likewise, Ljunggren et al. (2014) assessed the distribution of dioxin-like chemicals in the lipoprotein fractions of cancer patients and controls but did not distinguish the specific types of cancer diagnoses.

The committee identified four studies that examined the association between the COIs and cancer mortality. Among the Dow Midland, Michigan, plant workers exposed to TCP or PCP or both, Collins et al. (2016) found that there was no difference in mortality for all cancer when the 1,615 TCP workers were compared with the standardized U.S. population (SMR = 0.98, 95% confidence interval [CI] 0.86–1.11). Similarly, there was no difference found for mortality from all cancer sites for the 773 PCP workers (SMR = 1.04, 95% CI 0.86–1.24). Additional results for site-specific cancer mortality are covered in each applicable section.

S. A. Kim et al. (2015) used serum concentrations of persistent organic pollutants, including dioxin-like and non-dioxin-like PCBs (n = 633) and organochlorine pesticides (n = 675) collected within the 1999–2004 National Health and Nutrition Examination Survey (NHANES) and adjusted for fat mass to make associations with overall mortality, mortality from all cancers combined, and mortality from cardiovascular diseases in people aged 70 years and older. Models were adjusted for age, sex, race, cigarette smoking, and physical activity. When fat mass was not included in the analysis, no association was found between any of the persistent organic pollutants and total mortality. When fat mass was included in the analysis, PCBs were inversely associated with total mortality in persons with high fat mass, but not in those with low fat mass. Organochlorine pesticides were found to be positively associated with total mortality for low fat mass, but the association was weaker with higher fat mass. Cancer mortality was highest among persons with fat mass less than the 25th percentile and who had the highest tertile concentration of PCB and organochlorine pesticides. None of the hazard ratios for cancer mortality were statistically significant. The analysis is limited by the low numbers of deaths in the follow-up period, which reduces the power to calculate cause-specific mortality. One possible explanation for the observed association may be that persistent organochlorine pesticides influence disease pathogenesis but not mortality, which may be influenced by a number of other factors.

The extended follow-up study of UK phenoxy herbicide manufacturers and sprayers found that of the total 4,093 deaths reported among this cohort of workers, 1,205 deaths were attributable to cancer (Coggon et al., 2015). However, neither overall mortality nor cancer-specific mortality were elevated among all workers (SMR = 1.0, 95% CI 0.97–1.03 and SMR = 0.99, 95% CI 0.94–1.05, respectively) or among workers potentially exposed to phenoxy herbicide levels above background (SMR = 1.02, 95% CI 0.99–1.06 and SMR = 1.02, 95% CI

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0.96–1.09, respectively). Additional results for site-specific cancer mortality are covered in each applicable section.

Cappelletti et al. (2016) performed a retrospective study of 331 male electric arc foundry workers at a single plant in Trentino, Italy, to determine if they experienced excess mortality from all causes, all cancers, and specifically respiratory cancers, or if they experienced increased risk for other morbidities. An analysis of the dust emissions found that the dust contained metals (including iron, aluminum, zinc, manganese, lead, chromium, nickel, cadmium, mercury, and arsenic), PAHs, PCBs, and PCDD/Fs (reported as TEQs). Therefore, the authors could not determine which of the agents were associated with a specific outcome or to what extent. The men had worked at the factory for at least 1 year and, for the mortality analysis, were compared with the standardized general population of Region Trentino-Alto Adige (where the factory was located) because there were few non-exposed foundry workers and high attrition rates. Company and medical records were used to determine vital status; the cause of death was determined from death certificates or other registries. The workers were followed from March 19, 1979 (or their first day of employment), through December 31, 2009, or the date of death. No difference between exposed workers and the general population was found for all causes of mortality (SMR = 1.13, 95% CI 0.76–1.62, p = 0.53) or for all deaths from cancer (SMR = 1.36, 95% CI 0.75–2.29, p = 0.238). No differences in the mortality rates of all causes or all cancers were found when the cause of death was stratified by years of employment or time since first exposure. This study is most limited by the fact that foundry dust is a complex mixture, which makes it difficult to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those exposed workers. Estimates were adjusted only for age group and not for other risk factors such as tobacco use, body mass index (BMI), or other jobs or activities that could result in similar exposures. Exposure to foundry dust by the general population, which was used for comparison, is not discussed, although the foundry appears to be in the local vicinity and emissions were reported to be present within a 2-kilometer radius.

The remainder of this chapter deals with the committee's review of the evidence on each individual cancer site in accordance with its charge to evaluate the statistical association between exposure and cancer occurrence, the biologic plausibility and potential causal nature of the association, and the relevance to U.S. veterans of the Vietnam War. For each outcome, the relevant studies are presented for populations of Vietnam veterans and then for other exposed, non-veteran subjects (occupational cohort studies, environmental studies, and case-control studies).

A number of studies of populations that received potentially relevant exposures were identified in the literature search for this review but did not characterize exposure with sufficient specificity for their results to meet the committee's criteria for inclusion in the evidentiary database (see Chapter 3). For instance, this rubric would apply to the occupational study conducted by Ruder et al.

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(2014) in which 24,865 eligible workers from capacitor manufacturing, repair, and maintenance sites in the United States were exposed to arochlor 1254, 1242, and 1016, among others (mixed PCBs), and the authors sought to examine the relationship between PCB exposure and different causes of mortality. However, specific dioxin-like PCBs were not named, and no TEQs or other quantification of relevant exposures was presented. Similarly, the hospital-based case-control study by Niu et al. (2016) that examined hepatocellular carcinoma and risk factors including environmental exposures such as exposure to pesticides (not further defined) did not measure the levels of dioxins in serum samples and, as a result, lacked the necessary specificity to contribute to the weight of the evidence of an association between the COIs and hepatobiliary cancer; it was therefore excluded from further consideration. In previous updates as well as in the current update, numerous cancer studies have been identified that used case-control design and had exposure characterizations that were no more specific than job titles, farm residence, or herbicide exposure. The committee acknowledges that those studies were identified and presents briefly the reasons that they were not further considered and did not contribute to the evidentiary weight for an outcome under the heading of "Other Identified Studies."

ORAL, NASAL, AND PHARYNGEAL CANCERS

Oral, nasal, and pharyngeal cancers develop in anatomical sites of the head and neck: the structures of the oral cavity (inside lining of the lips, cheeks, gums, tongue, and hard and soft palate: *International Classification of Diseases*, 9th Revision (ICD-9) codes 140–145; ICD-10 codes C00–C08), oropharynx (ICD-9 146; ICD-10 C09–C10), nasopharynx (ICD-9 147; ICD-10 C11), hypopharynx (ICD-9 148; ICD-10 C13), other buccal cavity and pharynx (ICD-9 149; ICD-10 C14), and nasal cavity and paranasal sinuses (ICD-9 160; ICD-10 C30–C31). The salivary glands may or may not be included. The oropharynx includes the soft palate, the tonsils, the side walls, and the posterior tongue. The nasopharynx is made up of the structures from the part of the throat that is behind the nose, whereas the hypopharynx consists of the area from the hyoid bone to the cricoid cartilage. The larynx refers to only the laryngeal structures and is covered separately. Although the above cancers are classified together in the same category, the epidemiological risk factors for cancers that occur in the oral cavity and oropharynx are different from the risk factors for cancer of the nasopharynx.

Tobacco and alcohol use are well-established risk factors that contribute synergistically to the incidence of oral cavity and oropharyngeal cancers and, to a certain degree, nasopharyngeal cancers. Infection with human papilloma virus (HPV), particularly HPV16, is a relatively newly recognized major risk factor for oropharygeal cancers (Gillison and Shah, 2001; Gillison et al., 2012; Hashibe et al., 2007, 2009; Kreimer et al., 2013; Marur et al., 2010; Michaud et al., 2014; Oliveira et al., 2012; Szentirmay et al., 2005). Some evidence has also been found

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linking HPV to tonsillar and base-of-tongue cancers (Ramqvist et al., 2015). The risk factors for nasal cavity cancer include occupational exposure to nickel and chromium compounds (d'Errico et al., 2009; Feron et al., 2001; Grimsrud and Peto, 2006), wood dust (d'Errico et al., 2009), leather dust (Bonneterre et al., 2007), and high doses of formaldehyde (Nielsen and Wolkoff, 2010). Nasopharyngeal cancer is a very specific malignancy, and although alcohol, tobacco, and other environmental pollutants are risk factors, infection with the Epstein–Barr virus in combination with certain genetic predispositions and the consumption of poorly preserved food (Chang and Adami, 2006) constitutes the biggest attributable risk factor, especially in Africa, China, and other Asian countries.

Ecological studies in the United States have shown that between 2001 and 2010 the incidence of cancers of the oral cavity decreased (possibly because of the decreasing prevalence of smoking), whereas the incidence rates for oropharyngeal cancers increased annually by 2.9%, which has been attributed to HPV infection (Chaturvedi et al., 2011). In the United States in 2018 there were an estimated 51,540 new cases of and 10,030 deaths from oral cavity and pharyngeal cancers (NCI, n.d.b). Nasopharyngeal cancers occur very rarely in the United States (less than 1 case per 100,000 individuals); an estimated 3,200 cases were reported in 2015 (ACS, 2016). Most oral, nasal, and pharyngeal cancers are squamous-cell carcinomas. Nasopharyngeal carcinoma is the most common malignant epithelial tumor of the nasopharynx and can be further classified into one of three types: keratinizing squamous-cell carcinoma, nonkeratinizing carcinoma, and undifferentiated carcinoma.

The median age of diagnosis of oral cavity and pharynx cancers is 63 years, and 30.8% of new cases are diagnosed among people 55–64 years old, and 24.5% of cases are diagnosed among people 65–74 years old. Men of all races and ethnicities are at greater risk than women. Age-adjusted incidence rates were highest among white males and females and lowest among Hispanic men and women.

Conclusions from *VAO* and Previous Updates

The committee responsible for the original VAO report concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to any of the COIs and oral cavity, nasal, and pharyngeal cancers. Additional information available to the committees responsible for *Update 1996* through *Update 2014* did not change that conclusion.

In *Update 2006*, a separate evaluation of tonsil cancer cases was performed. In the United States an estimated 70% of oral cavity and oropharyngeal cancers are caused by HPV infection. Therefore, if herbicide exposure had inhibitory or suppressive effects on cell-mediated or humoral-mediated immunity, then Vietnam veterans with HPV16 infection might be at increased risk for the development of these cancers through the persistence of a high-risk oncogenic virus. The Update 2006 committee concluded that, based on the three identified studies

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that provided the number of tonsil cancer cases in their populations, there was not sufficient evidence to determine whether an association existed between exposure to the COIs and tonsil cancer. No new published studies have offered any important additional insight into this specific question. The present committee strongly reiterates the recommendation repeatedly made in *Updates 2006*, *2008*, *2010*, *2012*, and *2014* that the Department of Veterans Affairs (VA) develop a strategy that uses existing databases to evaluate tonsil cancer in Vietnam-era veterans.

Subsequent committees reviewed studies of U.S. and international cohorts of Vietnam veterans. No statistically significant increase in oral cavity and pharyngeal cancers was found between deployed and nondeployed Vietnam-era Army Chemical Corps veterans (Cypel and Kang, 2010); such findings were consistent with a prior report on mortality through 1991 (Dalager and Kang, 1997). Among the cohort of 2,783 New Zealand veterans who served in Vietnam and were followed prospectively beginning in 1988 for cancer incidence and mortality, no statistically significant increased risk of head and neck cancers overall and specifically cancers of the oral cavity, pharynx, and larynx was observed compared with the general population of New Zealand. Based on 11 cases each, statistically significant increased risks of death from head and neck cancers and from cancers of the oral cavity, pharynx, and larynx were observed among the New Zealand Vietnam veteran cohort compared with the general New Zealand population (McBride et al., 2013). However, that study had several limitations, including the lack of observation until 15 years post-conflict and missing information on (and therefore the inability to adjust for) known confounding factors, including smoking, drinking habits, and HPV status, which limits the interpretation of the data. The Update 2014 committee concluded that the greater than two-fold excess risks of mortality from head and neck cancers as well as from cancers of the oral cavity, pharynx, and larynx cannot be completely attributed to confounding by smoking because excess risks were not found in this cohort for deaths from other smoking-related diseases such as lung cancer, chronic obstructive pulmonary disease, or coronary artery disease.

The Korean Veterans Health Study followed 185,265 male Vietnam veterans who were alive in 1992 for cancer incidence through 2003 (Yi, 2013; Yi and Ohrr, 2014) and for mortality through 2005 (Yi et al., 2014b) from cancers of the oral cavity, nasal cavity, and pharynx. For the internal comparison analysis of high- versus low-exposure categories derived from the exposure opportunity index (EOI) scores generated by the EOI model, Yi and Ohrr (2014) found a 2.54 increase in hazard ratio for cancers of the mouth and a relative risk of nearly 7.0 for salivary glands (though the estimate was very imprecise) as well as a non-statistically significant increase in the risk of oropharyngeal cancer. No difference between the high- and low-exposure groups was found for tonsil cancer, and no differences in incidence were observed for the other head and neck cancers analyzed separately: lip, tongue, nasopharynx, hypopharynx, and nose and sinuses. Yi et al. (2014b) reported only on head and neck cancers as a group defined by

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ICD-10 codes C00–C14 and found no association when comparing the high- versus low-exposure categories, nor in the analysis based on the logarithms of the individual EOI scores.

Several studies of occupational cohorts that reported on cancers of the oral cavity or pharynx were examined by previous committees, but the evidence was inconsistent. Specifically, studies of workers at Dow's plant in Midland, Michigan, and in the NIOSH PCP cohort reported no increases in incidence (C. J. Burns et al., 2011) or mortality (Ruder and Yiin, 2011) from oral cavity and pharyngeal cancers. Likewise, McBride et al. (2009a) reported on mortality through 2004 in the New Zealand cohort of 1,599 workers who had been employed in manufacturing phenoxy herbicides from TCP; picloram was also produced in the plant. The researchers reported a non-significant excess in mortality from buccal cavity and pharyngeal cancers, but there were no deaths from nasopharyngeal cancers in either group. By contrast, Manuwald et al. (2012) reported a more than two-fold increase in mortality from cancers of the lip, oral cavity, or pharynx in a cohort of male and female chemical plant workers versus Hamburg's general population. Squamous cell oral cancer risk was also found to be elevated, but the estimate was imprecise, in Sweedish workers who worked for the pulp industry and with wood or wood products and workers who were exposed to phenoxyacetic acids (Schildt et al., 1999).

Update of the Epidemiologic Literature

No new studies of Vietnam veterans or published environmental or case-control studies of exposure to the COIs and oral, nasal, or pharyngeal cancers were identified for the current update. Reviews of the relevant studies are presented in the earlier reports. Table 2, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to oral, nasal, and pharyngeal cancer.

Occupational Studies

Cancers of the lip, tongue, and mouth were addressed by Coggon et al. (2015) in an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers. No deaths were due to cancer of the lip. Tongue cancer mortality was not statistically significant, and effect estimates were imprecise (wide CIs) for all workers (n = 8; SMR = 1.93, 95% CI 0.83–3.80), for workers exposed to herbicide levels above background (n = 5; SMR = 1.63, 95% CI 0.53–3.80), or for persons exposed for more than 1 year at levels above background (n = 3; SMR = 2.16, 95% CI 0.45–6.32). The estimates for mouth cancer showed a decreased risk that was likewise not statistically significant, with even more imprecise estimates for each of the groups: all workers (n = 2; SMR = 0.53, 95% CI 0.06–1.90), workers exposed to herbicide levels above background (n = 1; SMR

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= 0.36, 95% CI 0.01–1.99), and workers exposed for more than 1 year at levels above background (n = 0; SMR = 0.00, 95% CI 0.00–2.89). A decreased risk of pharynx cancer was found, but the estimates were imprecise and not statistically significant across the three groups of workers: all workers (n = 4; SMR = 0.49, 95% CI 0.13–1.25), workers exposed to herbicide levels above background (n = 4; SMR = 0.66, 95% CI 0.18–1.68), and workers exposed for more than 1 year at levels above background (n = 0; SMR = 0.00, 95% CI 0.00–1.34). These data do not support an association between exposure to phenoxy herbicides and cancer of the lip, tongue, or mouth.

Other Identified Studies

Although Ruder et al. (2014) examined U.S. workers exposed to mixed PCBs and reported SMRs from all buccal cavity and pharynx neoplasms overall as well as those specifically for the tongue, pharynx, and other parts of buccal cavity, the authors did not state the specific dioxin-like PCBs at issue, and no TEQs or other quantification of relevant exposures were presented. Akahane et al. (2017) examined the prevalence of many long-term health effects, including tongue cancer, of people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with a group of age-, sex- and residential-area-matched individuals. Because TEQs or other quantification of relevant exposures were not presented, the study was not considered further.

Biologic Plausibility

Long-term animal studies have examined the effects of exposure to the COIs on tumor incidence (Charles et al., 1996; Stott et al., 1990; Walker et al., 2006; Wanibuchi et al., 2004). The National Institute of Environmental Health Sciences conducted a 2-year study of female Harlan Sprage Dawley rats treated with TCDD and other dioxin-like PCBs (Nyska et al., 2005; Yoshizawa et al., 2005a). Yoshizawa et al. (2005a) reported an increase in the incidence of gingival squamous-cell carcinoma in the rats treated orally (by gavage) with TCDD only at doses as low as 3 ng/kg and average severities increased with higher dosing levels. In the groups receiving 46 ng/kg or greater of TCDD, the incidence of oral squamous-cell carcinoma increased, and a statistically significant difference occurred in the highest dosed group (incidence rate: 19%) compared to the control group (2%). In the 100 ng/kg for 5 days/week for 104 weeks stop group, the incidence of oral gingival squamous hyperplasia was also increased significantly, and increased occurrence of squamous-cell carcinoma was observed (incidence rate 10% versus 2% among controls). When a mixture of TCDD, PCB 126, and 2,3,4,7,8-pentachlorodibenzofuran was administered, all doses (ranging from 6 ng/kg to 200 ng/kg) induced gingival squamous hyperplasia significantly with

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no differences in severities, but the incidence of oral squamous-cell carcinoma, however, did not increase. A second publication from this study examined olfactory epithelial metaplasia and hyperplasia outcomes (Nyska et al., 2005). Squamous-cell carcinoma of the oral mucosa of the palate was increased. This study did not, however, find any pathologic effect of TCDD on nasal tissues (Nyska et al., 2005). Increased neoplasms of the oral mucosa had previously been observed and described as carcinomas of the hard palate and nasal turbinates (Kociba et al., 1978). Kociba et al. (1978) also reported a small increase in the incidence of tongue squamous-cell carcinoma.

DiNatale et al. (2012) used head and neck squamous-cell carcinoma cell lines to investigate mechanisms for tumor progression associated with AHR activation. This tumor type typically produces large amounts of cytokines, and its IL6 expression levels correlate with disease aggressiveness. In this model, AHR activation by TCDD enhances IL-6 production induced by another cytokine (IL-1β), so TCDD may play a role in head and neck squamous-cell oncogenesis. The potential impact of AHR activation on oral squamous cell carcinoma was recently described in a study by Stanford et al. (2016), which demonstrated that exposure to AHR ligands resulted in enhanced stem-cell-like properties of the human oral cells in culture and which used a novel orthotypic xenograft model to demonstrate the ability of AHR inhibitors to inhibit oral squamous-cell carcinoma progression.

Synthesis

Tonsil cancers, or more generally squamous-cell carcinomas of the oropharynx, remain of interest to Vietnam veterans and the committee, but no new information on them with respect to possible herbicide exposure was available for this update. Previous studies on Vietnam veterans from the Korean Veterans Health Study did not find an association between herbicide exposure and the risk of tonsillar cancers. Several previous studies have reported on oropharyngeal cancers broadly, but few have examined tonsil cancer as a distinct outcome.

The existing evidence from all published studies conducted among Vietnam veterans or various occupational cohorts reporting on the incidence of or mortality from cancers of the nose, oral cavity, or pharynx is largely inconclusive. Most of these studies have reported no association or else non-significant modest excesses in risk, while not characterizing exposure as specifically as needed for the committee's decision making. The one new study that extended the follow-up period of men who worked at five factories in the United Kingdom manufacturing or formulating a variety of phenoxy herbicides or who were contract workers spraying the compounds also found no association with exposure to phenoxy herbicides and mortality from cancer of the lip, tongue, or mouth (Coggon et al., 2015).

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The small numbers of oral, nasal, or pharyngeal cancer cases reported, in combination with a general lack of information on the smoking and drinking habits or HPV status of the tumors, limit the interpretation of the data. The other issue affecting the interpretation of the data is that this group of cancers is often grouped with respiratory cancers, most of which are cancers of the trachea, lung parenchyma, or bronchus. Because of the relatively small numbers of head and neck cancers, no meaningful conclusions can be drawn. Thus, in combination with the previously reviewed literature, the new information does not support an association between the cancers of oral cavity, nose, or pharynx with the herbicides sprayed in Vietnam.

Conclusion

Given the lack of new evidence, the committee concurs with the conclusion in *Update 2014* and concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and oral, oropharyngeal, or nasopharynx cancers.

CANCERS OF THE DIGESTIVE ORGANS

Esophageal cancer (ICD-9 150; ICD-10 C15), stomach cancer (ICD-9 151; ICD-10 C16), colon cancer (ICD-9 153; ICD-10 C15), rectal cancer (ICD-9 154; ICD-10 C19–C21), hepatobiliary cancers (ICD-9 155; ICD-10 C22), and pancreatic cancer (ICD-9 157; ICD-10 C25) are the major cancers arising in the digestive organs. NCI estimated that 17,290 people would receive diagnoses of esophageal cancers in the United States in 2018 and that 15,850 people would die from esophageal cancers (NCI, n.d.c). The corresponding 2018 estimates of U.S. diagnoses and deaths for the other digestive organ cancers are: stomach cancer (incident diagnoses, 26,240; deaths, 10,800) (NCI, n.d.d), colon and rectal cancer (incident diagnoses, 140,250; deaths, 50,630) (NCI, n.d.e), pancreatic cancer (incident diagnoses, 55,440; deaths, 44,330) (NCI, n.d.f), and hepatobiliary cancers (incident diagnoses, 42,220; deaths, 30,200) (NCI, n.d.g), with other digestive cancers—for example, small intestine and anal cancers—adding an estimated 19,050 new diagnoses and 2,610 deaths (NCI, n.d.h, n.d.i). Collectively, tumors of the digestive organs were expected to account for 17.4% of new cancer diagnoses and 25% of cancer deaths in 2017.

The incidences of esophageal, stomach, colon, rectal, and pancreatic cancers increase with age. In general, the incidences are higher in men than in women and higher in blacks than in whites (NCI, 2018a). Risk factors for the cancers vary but always include a family history of the same form of cancer, some diseases of the affected organ, and diet. Tobacco use is a risk factor for pancreatic cancer and possibly for stomach cancer (Maisonneuve and Lowenfels, 2015; Stewart et al., 2008). An infection with the bacterium *Helicobacter pylori* increases the risk of

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stomach and pancreatic cancers. Type 2 diabetes is associated with an increased risk of colorectal and pancreatic cancers (Berster and Göke, 2008).

Some studies of digestive cancers combine and report statistics on all digestive organ cancers rather than separating the data by types. For example, in a study of disease-related mortality through 2005 in Army Chemical Corps (ACC), veterans who handled or sprayed herbicides in Vietnam were compared with their non-Vietnam veteran peers or with U.S. men in general, with all gastrointestinal cancers reported collectively (Cypel and Kang, 2010). Adjusted estimates did not show any statistically significant excess in mortality from all cancers of the digestive tract in ACC Vietnam veterans compared with non-Vietnam veterans. Several other studies identified in *Update 2014* also combined several digestive cancers for their analyses, making the results not particularly informative for individual cancers in the group (Boers et al., 2012; C. J. Burns et al., 2011; Manuwald et al., 2012).

Esophageal Cancer

Epithelial tumors of the esophagus (squamous-cell carcinomas and adenocarcinomas) are responsible for more than 95% of all esophageal cancers; 17,290 newly diagnosed cases and 15,850 deaths were estimated for 2018 in the United States (NCI, n.d.c). In the United States, adenocarcinoma of the esophagus has slowly replaced squamous-cell carcinoma as the most common type of esophageal malignancy; although squamous-cell carcinoma continues to be the most common form of esophageal cancer worldwide (Rubenstein and Shaheen, 2015). The incidence of esophageal cancer is higher among men than women, higher in black women than white women for all age groups from 60 to 74 years, but higher for white men than black men for all age groups from 65 to 74 years.[1]

Smoking and heavy alcohol ingestion are associated with the development of squamous-cell carcinoma (Dong and Thrift, 2017; Matejcic et al., 2017). For esophageal adenocarcinoma, smoking is an established risk factor, but alcohol consumption does not appear to be strongly associated (Dong and Thrift, 2017). Some data suggest that gastroesophageal reflux disease and Barrett esophagus are associated with an increased risk of esophageal adenocarcinoma. The rapid increase in obesity in the United States has been linked to increasing rates of gastroesophageal reflux disease, and the resulting rise in chronic inflammation has been hypothesized as explaining the link between gastroesophageal reflux disease and esophageal adenocarcinoma (Rubenstein and Shaheen, 2015).

[1]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by choosing SEER 18 dataset, age-adjusted rates, esophageal cancer, and age $\geq$ 50 years. Data were displayed by choosing race and sex then separately by age.

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Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* explicitly excluded esophageal cancer from the group of gastrointestinal tract tumors, and it was not separately evaluated or categorized with this group until *Update 2004*, when it was formally placed into the inadequate or insufficient category. No additional studies of esophageal cancer were reviewed until *Update 2010*.

Most evidence on the potential effects of the COIs and esophageal cancer had come from occupational cohorts until *Update 2014*, when three studies of international Vietnam veteran cohorts were published. The strongest evidence of an association between the COIs and esophageal cancer came from an occupational cohort of workers at a chemical plant in Hamburg, Germany, which reported a statistically significant increase in esophageal cancer mortality relative to men in the general population of Hamburg (SMR = 2.56, 95% CI 1.27–4.57) (Manuwald et al., 2012). Several papers on mortality in TCP and PCP workers employed by Dow Chemical Company in Midland, Michigan, from 1937 to 1980 have been reviewed. Collins et al. (2009b) followed 1,615 workers who worked at least 1 day in a department that had potential TCDD exposure; 5 esophageal cancer deaths were observed but no statistically significant associations were found. Among the 773 PCP workers who were exposed to chlorinated dioxins that did not include TCDD, there were two observed deaths from esophageal cancer (Collins et al. 2009c). In the Agricultural Health Study (AHS), Koutros et al. (2010a), found a statistically significant decrease in the incidence of esophageal cancer among the private applicators (52 cases, standardized incidence ratio [SIR] = 0.64, 95% CI 0.48–0.85) compared with the general population, which could indicate a healthy worker effect.

Other than the study of chemical plant workers in Hamburg (Manuwald et al., 2012), studies conducted outside the United States found no statistically significant associations between the COIs and esophageal cancer. McBride et al. (2009a) reported on a mortality follow-up of the workers in the Dow AgroSciences plant in New Plymouth, New Zealand, who were potentially exposed to TCDD; neither the SMR for esophageal cancer deaths in exposed workers nor the never-exposed group was statistically significant compared with the general New Zealand population. A follow-up analysis on cancer incidence in the men and women exposed to dioxin in the Seveso accident found no esophageal cancers in the high-exposure zone and no exposure-related pattern in the occurrence of esophageal cancer in the medium- and low-exposure areas (Pesatori et al., 2009).

Among studies of esophageal cancer in Vietnam veterans, two were related to incidence (Yi, 2013; Yi and Ohrr, 2014), and one examined cancer-specific mortality (Yi et al., 2014b) in the Korean Veterans Health Study, a large prospective cohort of 185,265 male Vietnam veterans alive in 1992, who were followed for cancer incidence through 2003 and for mortality through 2005. Comparing the

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Vietnam veterans to the general Korean population, Yi (2013) reported a statistically significant decrease in the incidence of esophageal cancer (SIR = 0.70, 95% CI 0.64–0.85). However, in the internal comparison of those with high versus low EOI scores, Yi and Ohrr (2014) reported a statistically significant increased risk for esophageal cancer (hazards ratio [HR] = 1.36, 95% CI 1.00–1.85). This result was based on 184 incident esophageal cancers observed during follow-up, of which 113 cases were among veterans in the high-exposure category. Yi et al. (2014b) reported a non-statistically significant increase in mortality from esophageal cancer when comparing those in the higher exposure category (n = 98) with those with lower estimated exposure (n = 64). Similarly, mortality from esophageal cancer was not found to be associated with the individual, log-transformed EOI scores (Yi et al., 2014b). Information on smoking and alcohol consumption was not available, leading to concerns that some of the association could be due to confounding.

Update of the Epidemiologic Literature

No studies of Vietnam veterans or published environmental or case-control studies of exposure to the COIs and esophageal cancer were identified for the current update. Reviews of the relevant studies are presented in the earlier reports. Table 3, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to esophageal cancer.

Occupational Studies Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016) found no differences in mortality for esophageal cancer for the TCP workers (n = 8; SMR = 1.09, 95% CI 0.47–2.14) or the PCP workers (n = 5; SMR = 1.52, 95% CI 0.49–3.54).

Cancers of the digestive organs were addressed by Coggon et al. (2015) in an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers. No statistically significant associations between exposure to phenoxy acids and esophageal cancer were found for all groups of workers: all workers (n = 55; SMR = 0.99, 95% CI 0.74–1.28), workers exposed to herbicide levels above background (n = 46; SMR = 1.11, 95% CI 0.81–1.48), and workers exposed for more than 1 year at levels above background (n = 17; SMR = 0.89, 95% CI 0.52–1.43). These data do not support an association with phenoxy herbicides and cancer of the esophagus.

Other Identified Studies Two other studies of esophageal cancer were identified but were limited by a lack of exposure specificity (Ruder et al., 2014; Yildirim et al., 2014). A third study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including esophageal cancer) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the

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ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

Long-term animal studies have examined the effect of exposure to the COIs on tumor incidence (Charles et al., 1996; Stott et al., 1990; Walker et al., 2006; Wanibuchi et al., 2004), and no increase in the incidence of esophageal cancer has been reported in laboratory animals after exposure. A previous biomarker study analyzed esophageal-cell samples from patients who had been exposed to indoor air pollution of different magnitudes and who did or did not have high-grade squamous-cell dysplasia or a family history of upper gastrointestinal-tract cancer (Roth et al., 2009). AHR expression was higher in patients who had a family history of upper gastrointestinal-tract cancer, but it was not associated with indoor air pollution, esophageal squamous-cell dysplasia category, age, sex, or smoking. These results might be interpreted to suggest that an enhanced expression of AHR in patients who had a family history of upper gastrointestinal-tract cancer may contribute to upper gastrointestinal-tract cancer risk associated with AHR ligands—such as PAHs, which are found in cigarette smoke—and with TCDD.

In a small series of studies, AHR expression was found to be higher in esophageal tumors than in corresponding normal mucosa and, somewhat surprisingly, played a role in the suppression of metastatic potential, in contrast to many other cancers (Safe et al., 2013). The significance of these observations and the mechanism underlying increased AHR expression was not determined (J. Zhang et al., 2012). No new mechanistic or biologic plausibility studies on esophageal cancer have been published since *Update 2014*.

Synthesis

In this update, two studies were reviewed that increased the follow-up period of workers exposed to dioxins (Collins et al., 2016) and phenoxy herbicides (Coggon et al., 2015) and examined the relationship of a variety of cancers, including esophageal, with mortality. Neither study provided additional evidence for a potential association between esophageal cancer overall and exposure to the COIs. Because the risk factors and etiologies for adenocarcinomas and squamous-cell carcinomas differ to some extent, it would have been more informative if the analyses were stratified by type. In combination with the studies reviewed previously, findings from the additional follow-up times do not provide adequate new evidence of the relationship between exposure to the COIs and esophageal cancer. No toxicologic studies provide evidence of the biologic plausibility of an association between the COIs and tumors of the esophagus.

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Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and esophageal cancer.

Stomach Cancer

The incidence of stomach cancer increases with age. NCI estimated that 26,240 people would receive a diagnosis of stomach cancer in the United States in 2018 and that 10,800 people would die from it. The incidence is almost twice as high in men than in women and is higher among all other race and ethnicity groups than in whites (NCI, n.d.d). Other risk factors include a family history of this cancer, some diseases of the stomach, and diet. Infection with *Helicobacter pylori* increases the risk of stomach cancer. Tobacco or alcohol use and the consumption of nitrite- and salt-preserved food may also increase the risk (Ang and Fock, 2014; Brenner et al., 2009; Key et al., 2004). The incidence rate of stomach cancer has been decreasing since 1975 when SEER began tracking it, and is estimated to be 7.2 per 100,000 men and women per year (NCI, n.d.d). Among men over age 65 years (the age group of Vietnam veterans), the age-adjusted modeled incidence rate of stomach cancer for all races combined was 36.7 per 100,000 for 2000–2014.[2]

Conclusions from *VAO* and Previous Updates

Stomach cancer was first considered independently in *Update 2006*, and that committee concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and stomach cancer. That conclusion has been maintained by the committees responsible for subsequent updates.

Case-control studies reviewed in previous updates examined agricultural exposures and stomach cancer. Both Ekström et al. (1999) and Mills and Yang (2007) found an association with herbicides and with phenoxy herbicides in particular. A study that compared mortality from stomach cancer among Iowa farmers versus other occupations found that the proportional mortality ratio of farmers was significantly higher (Burmeister et al., 1981, 1983). Occupational cohort studies reported little evidence of an exposure-related increase in stomach cancer. Updated mortality findings from Seveso concerning TCDD exposure

[2]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by choosing SEER 18 dataset, age-adjusted rates, stomach cancer, all races, male, and over age 65.

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(Consonni et al., 2008; Pesatori et al., 2009) found no evidence of an increase in stomach cancer.

Update 2014 reviewed cohort studies of Vietnam veterans from New Zealand and Korea that reported on stomach cancer. Stomach cancer mortality (Yi et al., 2014b) and incidence (Yi and Ohrr, 2014) were assessed among Korean veterans who had served in Vietnam between 1964 and 1973. Yi and Ohrr (2014) reported a modestly increased risk of incident stomach cancer in the internal comparison of the high- and low-exposure groups based on the EOI scores. Similarly, for stomach cancer mortality, Yi et al. (2014b) reported a similar modestly increased risk for the high- versus low-exposure groups and a positive association with the individual log-transformed EOI scores. Among 2,783 New Zealand Vietnam veterans who served in Vietnam between 1964 and 1975, McBride et al. (2013) reported that stomach cancer mortality was slightly elevated in the cohort, while stomach cancer incidence was slightly less than expected; however, neither estimate was statistically significant.

Update of the Epidemiologic Literature

No studies of Vietnam veterans or published environmental or case-control studies of exposure to the COIs and stomach cancer were identified for the current update. Reviews of the relevant studies are presented in the earlier reports. Table 4, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to stomach cancer.

Occupational Studies Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016) found no differences in mortality from stomach cancer for the TCP workers (n = 11; SMR = 1.58, 95% CI 0.79–2.83) or the PCP workers (n = 5; SMR = 1.30, 95% CI 0.42–3.04).

Cancers of the digestive organs were addressed by Coggon et al. (2015) in an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers. Mortality from stomach cancer was lower than expected for all groups of workers: all workers (n = 66; SMR = 0.78, 95% CI 0.61–1.00), workers exposed to herbicide levels above background (n = 43; SMR = 0.73, 95% CI 0.53–0.99), and workers exposed for more than 1 year at levels above background (n = 21; SMR = 0.75, 95% CI 0.46–1.15).

Other Identified Studies Two other studies of stomach and gastric cancer were identified but lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effects of the COIs (Ruder et al., 2014; Yildirim et al., 2014). A third study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including stomach cancer) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals

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through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

Long-term animal studies have examined the effect of exposure to 2,4-D and TCDD on tumor incidence (Charles et al., 1996; Stott et al., 1990; Walker et al., 2006; Wanibuchi et al., 2004). No increase in the incidence of gastrointestinal cancers has been reported in laboratory animals. However, studies of laboratory animals have observed dose-dependent increases in the incidence of squamous-cell hyperplasia of the forestomach or fundus of the stomach after an administration of TCDD (Hebert et al., 1990; Walker et al., 2006). Similarly, in a long-term TCDD-treatment study in monkeys, hypertrophy, hyperplasia, and metaplasia were observed in the gastric epithelium (Allen et al., 1977). A transgenic mouse bearing a constitutively active form of the Ahr has been shown to develop stomach tumors (Andersson et al., 2002); the tumors are neither dysplastic nor metaplastic, but are indicative of both squamous-cell and intestinal-cell metaplasia (Andersson et al., 2005). The validity of the transgenic-animal model is indicated by the similarities in the phenotype of the transgenic animal (increased relative weight of the liver and heart, decreased weight of the thymus, and increased expression of Ahr target gene *CYP1A1*) and of animals treated with TCDD (Brunnberg et al., 2006). Recent cell culture work consistent with the in vivo studies showed that decreased AHR expression in two human gastric cancer cell lines was associated with decreased cell growth, migration, and invasion, all of which are hallmarks of malignant potential (X. F. Yin et al., 2013). In a biomarker study of cancer patients, AHR expression and nuclear translocation were significantly higher in stomach-cancer tissue than in precancerous tissue (Peng et al., 2009a). The results suggest that AHR plays an important role in stomach carcinogenesis. AHR activation in a stomach-cancer cell line has also been shown to enhance stomach-cancer cell invasiveness, potentially through a c-Jun-dependent induction of matrix metalloproteinase-9 (Peng et al., 2009b). No new mechanistic or biologic plausibility studies on gastrointestinal cancers have been published since *Update 2014*.

Synthesis

Studies that examined the association between the COIs and stomach cancer that were reviewed in previous VAO Updates reported mixed findings. Among the studies of Vietnam veterans, analyses from the AFHS did not report a statistically significant association of increasing serum levels of TCDD and stomach cancer (Pavuk et al., 2005). Other mortality studies of U.S. Vietnam veterans also did

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not report an increased risk of death from stomach cancer. A modestly increased risk of stomach cancer was reported in Korean veterans, but there was inconsistent evidence in New Zealand Vietnam veterans. Whereas case-control studies of agricultural exposures reported evidence of an association with stomach cancer, studies of occupational cohorts—including the two reviewed above—found little evidence of an exposure-related increase in stomach cancer. Moreover, Collins et al. (2016) reported an increased but not statistically significant risk of stomach cancer among the U.S. TCP and PCP manufacturing workers, but Coggon et al. (2015) reported a statistically significant decreased risk of stomach cancer among the entire cohort and short-term UK phenoxy herbicide workers or sprayers.

There is some evidence of biologic plausibility in animal models, but overall the epidemiologic studies do not support an association between exposure to the COIs and stomach cancer.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and stomach cancer.

Colorectal Cancers

Colorectal cancers include malignancies of the colon (ICD-9 153; ICD-10 C18) and of the rectum and anus (ICD-9 154; ICD-10 C19–C21); less prevalent tumors of the small intestine (ICD-9 152; ICD-10 C17) are often included. Colorectal cancers account for 8% of all incident cancers; NCI estimated that 140,250 people would receive diagnoses of colorectal cancer in the United States in 2018 and that 50,630 would die from it. Colon and rectum cancer is the second leading cause of cancer death in the United States (NCI, n.d.e).

The incidence of colorectal cancers increases with age; the median age of diagnosis is 67 years. Incidence is higher in men than in women, and highest in blacks and lowest in Hispanics and Asians/Pacific Islanders. Between 2000 and 2013, incidence rates in adults aged 50 years and older declined by 32%, with the drop largest for distal tumors in people aged 65 years and older. Over this same period, colorectal cancer incidence rates increased by 22% among adults aged less than 50 years, driven solely by tumors in the distal colon and rectum (Siegel et al., 2015). (Screening can affect the incidence, and screening is recommended for all persons over 50 years old.) Among men over age 65 years (the age group of Vietnam veterans), the age-adjusted modeled incidence rate of colorectal cancer for all races combined was 203.6 per 100,000 for 2000–2014.[3] Other risk factors

[3]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by choosing SEER 18 dataset, age-adjusted rates, colorectal cancer, all races, age ≥ 65 years, and male sex.

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include a family history of this form of cancer, obesity, a lack of physical exercise, and diet (Johnson et al., 2013; Kamangar et al., 2006). Type 2 diabetes is associated with an increased risk of colorectal cancers (Berster and Göke, 2008). Additional risk factors that have been studied include having a personal history of chronic ulcerative colitis or Crohn disease for 8 years or longer, smoking cigarettes, and having three or more alcoholic drinks per day (NCI, 2018c).

Conclusions from *VAO* and Previous Updates

Until *Update 2006*, colorectal cancers were considered as a group with gastrointestinal-tract cancers. Beginning with *Update 2006*, colorectal cancers were considered independently, but each update committee has concluded that the available evidence has not been sufficient to reassign the association between exposure to the COIs and colorectal cancers from inadequate or insufficient evidence.

Studies of veterans from New Zealand and Korea who served in Vietnam and reported colorectal cancer outcomes, in general, found no statistically significant associations. McBride et al. (2013) reported on mortality among 2,783 male New Zealand veterans who had served in Vietnam between 1964 and 1975, were alive in 1988, and were followed through 2008. Based on 20 deaths from colorectal cancer, the effect estimate (SMR) was not statistically significantly elevated compared with the general New Zealand population, and the all-cause mortality estimate was statistically significantly lower in the cohort. Colorectal cancer incidence, based on 63 cases, was not statistically significantly different than the general population.

Among Korean veterans who served in Vietnam, Yi and Ohrr (2014) found a lower incidence of colon cancer among the more highly exposed compared with the less exposed as well as a small excess of rectal cancer, but neither was statistically significant. Regarding mortality from colorectal cancers, Yi et al. (2014b) reported no evidence of an increase in mortality from these cancers combined for high- versus low-exposure opportunity groups or in association with the individual log-transformed EOI scores.

Update of the Epidemiologic Literature

No studies of Vietnam veterans or published environmental or case-control studies of exposure to the COIs and colorectal cancer were identified for the current update. Reviews of the relevant studies are presented in the earlier reports. Table 5, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to colon cancer.

Occupational Studies Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016)

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reported SMRs for colon and rectal cancers separately. Only 4 total deaths from rectum cancer were reported (3 among the TCP workers and 1 among the PCP workers), making the risk estimates unreliable. Compared with the standardized U.S. population, no statistically significant difference in mortality for colon cancer was found for the TCP workers (n = 22; SMR = 1.11, 95% CI 0.69–1.67) or the PCP workers (n = 12; SMR = 1.23, 95% CI 0.64–2.15).

Coggon et al. (2015) extended the follow-up of a cohort of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides. Mortality from colon and rectal cancers was reported separately. No difference in colon cancer mortality was found for all workers (n = 77; SMR = 0.97, 95% CI 0.77–1.22), for workers exposed to herbicide levels above background (n = 50; SMR = 0.87, 95% CI 0.65–1.15), or for persons exposed for more than 1 year at levels above background (n = 23; SMR = 0.86, 95% CI 0.54–1.29), although the all effect estimates indicated decreased risk. The estimates for rectal cancer were similarly not statistically significant and lower than expected for each of the groups: all workers (n = 39; SMR = 0.76, 95% CI 0.54–1.04), workers exposed to herbicide levels above background (n = 31; SMR = 0.84, 95% CI 0.57–1.19), and workers exposed for more than 1 year at levels above background (n = 14; SMR = 0.81, 95% CI 0.44–1.36).

Other Identified Studies Three other studies of colon and rectal cancers were identified. One lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Ruder et al., 2014). The second study examined disparities in colorectal cancer incidence in communities around Ontario, Canada, but did not collect information on the COIs (Sitharan et al., 2014). A third study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including bowel cancer) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

Long-term animal studies examining the effect of exposure to the COIs on tumor incidence (Charles et al., 1996; Stott et al., 1990; Walker et al., 2006; Wanibuchi et al., 2004) have reported no increase in the incidence of colorectal cancers. Xie et al. (2012) reported that AHR activation by TCDD induces robust proliferation in two human colon-cancer cell lines through Src-mediated epidermal growth factor receptor activation. That novel finding suggests that TCDD and other AHR ligands may contribute to an increased proliferation of colonic cells, but more studies are needed to understand the relation of increased proliferation of these cells to colorectal cancers, if any, and the potential role of AHR

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activation in colorectal and intestinal carcinogenesis. Yin et al. (2016a) found keratinocyte growth factor (KGF), AHR, and CYP1A1 are overexpressed in colorectal cancer tissues. KGF promoted colon cancer cell growth in vitro and also upregulated and activated AHR. Furthermore, KGF promoted cell proliferation through the AHR–cyclin D1 pathway in colon cancer cells.

In another study, Yin et al. (2016b) found that an endogenous AHR agonist (6-formylindolo [3, 2-b] carbazole, or FICZ) inhibited LoVo colon cancer cell proliferation by inducing cell cycle arrest. Ahr activation by TCDD was anti-inflammatory in a mouse model of colon cancer by inducing acetylcholinesterase-targeting micro RNA-132, which suppressed production of TNF-α, IL-1β and IL-6 (Alzahrani et al., 2017). These studies support a plausible mechanism by which TCDD may influence colorectal cancers.

Synthesis

Epidemiologic findings for colorectal cancers have not been particularly suggestive of an association with exposure to the COIs. None of the studies of U.S. Vietnam veterans found an elevated risk of colorectal cancer, and similar results have been reported for Vietnam veterans from Australia, Korea, and New Zealand. In line with previous follow-up studies of the U.S. Dow chemical plant workers, Collins et al. (2016) found few deaths from rectal cancer, and colon cancer was not found to be elevated in either TCP or PCP workers compared with the standardized U.S. population. Coggon et al. (2015) reported decreased, but not statistically significant risks among the UK factory workers or sprayers of phenoxy herbicides. Limited evidence is available on the biologic plausibility of an association between exposure to any of the COIs and tumors of the colon or rectum. Overall, the available evidence does not support an association between the COIs and colorectal cancers.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and colorectal cancers.

Hepatobiliary Cancers

Hepatobiliary cancers include cancers of the liver (ICD-9 155.0, 155.2; ICD-10 C22) and the intrahepatic bile duct (ICD-9 155.1; ICD-10 C22.1). NCI estimated that 42,220 men and women would receive diagnoses of liver cancer or intrahepatic bile duct cancer in the United States in 2018 and that 30,200 people would die from these cancers (NCI, n.d.g). Gallbladder cancer and extrahepatic

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bile duct cancer (ICD-9 156; ICD-10 C23–C24) are uncommon and, when they are addressed, are often grouped with liver cancer.

In the United States, liver cancers account for 2.4% of new cancer cases and 4% of cancer deaths. Misclassification of metastatic cancers as primary liver cancer can lead to an overestimation of the number of deaths attributable to liver cancer (Chuang et al., 2009). Liver cancer is the second most common cause of death from cancer worldwide and it is estimated that it will be responsible for nearly 782,000 deaths in 2018 (Globocan, 2018). Liver cancers are most common and are among the leading causes of death in less developed countries and regions, especially those in Northern Africa, Micronesia, and Eastern and Southeastern Asia (Globocan, 2018). Known risk factors for liver cancer include chronic infection with the hepatitis B or hepatitis C virus and exposure to the carcinogens aflatoxin and vinyl chloride. Alcohol cirrhosis and obesity-associated metabolic syndrome may also contribute to the risk of liver cancer (Chuang et al., 2009; Farazi et al., 2006). In the general population, the incidence of liver and intrahepatic bile duct cancers is higher in men than in women and higher in blacks than in whites but highest among Asian/Pacific Islanders and American Indians/Alaska Natives (NCI, n.d.g). Among males of all races aged 65 years and older, the age-adjusted modeled incidence rate of hepatobiliary cancers was 53.3 per 100,000 for 2000–2014.[4]

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and hepatobiliary cancers. Additional information available to the committees responsible for subsequent updates did not change that conclusion.

The committee for *Update 2014* reviewed two studies of Korean veterans who served in Vietnam and were part of the Korean Veterans Health Study. When compared to the general Korean population, there was no evidence of increased liver cancer risk (Yi, 2013). In the internal comparison of the high- versus low-exposure-opportunity group, Yi and Ohrr (2014) reported a nonstatistically significant elevation in liver cancer for the high-exposure group. The risk of liver cancer mortality was slightly increased in the internal comparison and from the analysis of the individual EOI scores (Yi et al., 2014b). The committee for Update 2014 also reviewed an occupational study of 3,529 employees of a Chinese automobile foundry that reported a statistically significantly elevated risk of liver cancer mortality, based on 32 cancer deaths (L. Wang et al., 2013). However, these studies did not change the committee's conclusion that there is

[4]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by choosing SEER 18 dataset, age-adjusted rates, hepatobiliary cancer, all races, age ≥ 65 years, and male sex.

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inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and hepatobiliary cancers.

Update of the Epidemiologic Literature

No environmental or case-control studies of the COIs and hepatobiliary cancers have been identified since *Update 2014*. Reviews of the relevant studies are presented in the earlier reports. Table 6, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to hepatobiliary cancer.

Vietnam-Veteran Studies Krishnamurthy et al. (2016) conducted a retrospective study in the Dayton, Ohio, Veterans Administration Medical Center to investigate all patients carrying dual diagnoses of both cirrhosis and hepatitis C virus (verified by viral genotype) at this facility from January 2000 to December 2013. Of the 509 patients with both diagnoses, 119 did not have genotype results and were excluded from the analysis. A total of 390 patients confirmed to have both cirrhosis and hepatitis C virus were identified, and 311 had follow-up information; there were 79 confirmed hepatocellular carcinomas among the 390 patients with dual diagnoses. Exposure to Agent Orange was determined by self-report. Effect estimates were adjusted for smoking, alcohol "addiction" (ill defined), and race. Alcohol addiction and African American race were associated with hepatocellular carcinoma among the patient sample (OR = 2.17, 95% CI 1.07–4.43 and OR = 2.07, 95% CI 1.22–3.51, respectively). The association between self-reported exposure to Agent Orange and hepatocellular carcinoma was not statistically significant (OR = 1.76, 95% CI 0.85–3.64).

Occupational Studies Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, only four deaths from hepatobiliary cancers were reported during the entire follow-up period (Collins et al., 2016). All four deaths occurred in the TCP workers, and although the estimate showed decreased risk, it was not statistically significantly different from the comparison population (SMR = 0.62, 95% CI 0.17–1.58).

Cancers of the digestive organs were addressed by Coggon et al. (2015) in an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers. Mortality from liver and gall bladder cancers was reported separately, but the effect estimates from gall bladder cancer (two deaths among all workers) are unreliable because of the small number of cases. The risk for liver cancer mortality was not different for any of the groups of workers: all workers (n = 20; SMR = 1.24, 95% CI 0.76–1.91), workers exposed to herbicide levels above background (n = 14; SMR = 1.14, 95% CI 0.62–1.91), or for workers exposed for more than 1 year at levels above background (n = 4; SMR = 0.72, 95% CI 0.20–1.85).

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Other Identified Studies Four other studies of hepatobiliary cancers were identified but lacked sufficient exposure specificity (Niu et al., 2016; Ruder et al., 2014; VoPham et al., 2015) and either did not collect serum samples to measure levels of dioxins (Ruder et al., 2014; VoPham et al., 2015) or did not measure the dioxin levels in collected samples (Niu et al., 2016). Therefore, these studies were not considered as contributing to the evidence base of the potential effect of the COIs. A fourth study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including liver and gallbladder cancers) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident), compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

Long-term animal studies have examined the effect of exposure to the COIs on tumor incidence (J. F. Brown et al., 2007; Charles et al., 1996; Stott et al., 1990; Walker et al., 2006; Wanibuchi et al., 2004). Studies performed in laboratory animals have consistently demonstrated that long-term exposure to TCDD results in the formation of liver adenomas and carcinomas (Knerr and Schrenk, 2006; Walker et al., 2006). Furthermore, TCDD increases the growth of hepatic tumors that are initiated by treatment with a complete carcinogen. Pathologic liver changes have been observed after exposure to TCDD, including nodular hyperplasia and massive inflammatory cell infiltration (Kociba et al., 1978; NTP, 2006; Walker et al., 2006; Yoshizawa et al., 2007); inflammation can be important for the development and progression of many cancers, including liver cancers (Mantovani et al., 2008). In monkeys treated with TCDD, hyperplasia and an increase in cells that stain for alpha-smooth muscle actin have been observed (Korenaga et al., 2007). Positive staining for alpha-smooth muscle actin is thought to indicate a process (the epithelial–mesenchymal transition) that is associated with the progression of malignant tumors (Weinberg, 2008). Zucchini-Pascal et al. (2012) showed that TCDD exposure induced an epithelial-to-mesenchymal transition in primary cultured human hepatocytes.

Bile duct hyperplasia (but not tumors) has been reported in rodents following chronic treatment with TCDD (Knerr and Schrenk, 2006; Walker et al., 2006; Yoshizawa et al., 2007). Similarly, monkeys treated with TCDD developed metaplasia, hyperplasia, and hypertrophy of the bile duct (Allen et al., 1977). Hollingshead et al. (2008) showed that TCDD-activated AHR in human breast and endocervical cell lines induces sustained high concentrations of the IL-6 cytokine, which has tumor-promoting effects in numerous tissues, including cholangiocytes; thus, TCDD might promote carcinogenesis in biliary tissue.

TCDD may contribute to tumor progression by inhibiting p53 regulation (phosphorylation and acetylation) triggered by genotoxicants through the increased

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expression of the metastasis marker AGR2 (Ambolet-Camoit et al., 2010) and a functional interaction between the AHR and FHL2 (Kollara and Brown, 2009). AHR has also been shown to be a regulator of c-Raf and proposed cross-talk between AHR and the mitogen-activated protein kinase signaling pathway in chemically induced hepatocarcinogenesis (Borlak and Jenke, 2008). TCDD inhibits ultraviolet-C radiation-induced apoptosis in primary rat hepatocytes and Huh-7 human hepatoma cells, which supports the hypothesis that TCDD acts as a tumor promoter by preventing damaged cells from undergoing apoptosis (Chopra et al., 2009). TCDD inhibited the proliferation of isolated mouse oval cells, which are liver precursor cells, via an Ahr-dependent pathway, suggesting that these cells are not the precursor for TCDD-induced tumors in the mouse (Faust et al., 2013a).

Elyakim et al. (2010) found that human microRNA miR-191 was upregulated in hepatocellular carcinoma and that miR-191 was also upregulated after TCDD treatment, which may contribute to the mechanism of the carcinogenic activity of TCDD. Ovando et al. (2010) used toxicogenomics to identify genomic responses that may contribute to the development of hepatotoxicity in rats treated chronically with the AHR ligands, TCDD, or PCB 126. The researchers identified, respectively, 24, 17, and 7 genes that were differentially expressed in the livers of rats exposed to those Ahr ligands and in human cholangiocarcinoma, human hepatocellular adenoma, and rat hepatocellular adenoma. These findings may help elucidate the mechanisms by which dioxin-like chemicals induce their hepatotoxic and carcinogenic effects.

In rodents, TCDD may promote hepatocarcinogenesis through cytotoxicity, chronic inflammation, and liver regeneration and through hyperplastic and hypertrophic growth due to the sustained activation of Ahr (Köhle and Bock, 2007; Köhle et al., 2008). For example, TCDD exposure has been reported to increase liver fibrosis in mice via an Ahr-dependent pathway (Andreola et al., 2004). Kennedy et al. (2014) used transgenic mouse strains to measure dioxin-induced liver cancers in a model in which TCDD was used as a tumor promoter. One set of experiments showed that the number of TCDD-induced liver tumors was significantly higher in mice that expressed Ahr with high-binding affinity to TCDD than in an isogenic strain that expressed a low-binding-affinity Ahr. A second set of experiments showed that the genetic ablation of inflammatory cytokines significantly reduced TCDD-induced liver tumors. Likewise, the genetic ablation of AHR reduced TCDD-induction of the inflammatory cytokines (Pierre et al., 2014). Species differences governing AHR activation are demonstrated by the divergence in the transcriptomic responses to TCDD in mouse, rat, and human liver (Boutros et al., 2008, 2009; Carlson et al., 2009; S. E. Kim et al., 2009), but it should be noted that the in vitro human hepatocyte studies may not reflect the in vivo response of human liver to TCDD. In vitro studies with transformed cell lines and primary hepatocytes cannot replicate the complexity of a tissue response that is important in eliciting the toxic responses

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observed in vivo (Dere et al., 2006). Finally, AHR expression was shown to be significantly elevated in human liver cancers, although the absolute level of increase is only about 30% to 40%, but the biological significance of this observation is not known (Z. Liu et al., 2013).

In a study of gene-expression changes in adult female primary human and rat hepatocytes exposed to TCDD in vitro, Black et al. (2012) used whole-genome microarrays to show that TCDD produced different gene-expression profiles in rat and human hepatocytes, both on an ortholog basis (conserved genes in different species) and on a pathway basis. For commonly affected orthologs or signaling pathways, the human hepatocytes were about one-fifteenth as sensitive as rat hepatocytes. Such findings are consistent with epidemiologic studies that show humans to be less sensitive to TCDD-induced hepatotoxicity. A study of gene-expression changes in cultured rat liver cells (the WB-F344 cell line) showed that AHR agonist PCB 126 identified hundreds of dysregulated genes that increased in number as a function of time after exposure from 6 to 72 hours; these included the Wnt and TGF-β signaling pathways, which are involved in tumorogenesis (Faust et al., 2013b). Peyre et al. (2014) studied TCDD activity in human HepG2 hepatocarcinoma cells and demonstrated pro-carcinogenic activity (anti-apoptosis and induction of epithelial to mesenchymal transition) via dysregulation of the TGF-β pathway. Reyes-Reyes et al. (2016) described another mechanism of cancer progression in HepG2 cells involving TGF-β; the long interspersed nuclear element-1 (L1) damages DNA and was activated in HepG2 cells via TGF-β signaling in a study of AHR activation by benzo[*a*]pyrene. L. T. Wang et al. (2017) found a high correlation between histone deacetylase 8 (HDAC8) and AHR expression as mRNA and protein levels. Expression of both HDAC8 and AHR was significantly upregulated in hepatocellular carcinoma cell lines and tumor tissues compared with normal hepatocytes; inhibition of HDAC8 inhibited hepatoma cell proliferation and transformation.

The chronic exposure of rats to TCDD was associated with fatty liver degeneration and necrosis (X. Chen et al., 2012). Another group reported that the hepatotoxic effects of TCDD were exacerbated in mice that had glutathione deficiency (Y. J. Chen et al., 2012). The combined exposure to PCBs and TCDD induced significant hepatotoxicity in rats (C. Lu et al., 2010). Brown et al. (2007) carefully evaluated the mechanisms by which dioxin-like PCBs caused liver cancer in rats suggesting a process involving the net activity of multiple mixed-function oxidases, redox cycling quinones, and reactive oxygen species.

Cacodylic acid (DMA^{III} and DMA^{V}) is carcinogenic and has been shown to induce renal cancer. In F344/DuCrj rats treated with a mixture of carcinogens for 4 weeks, a subsequent exposure to DMA (not indicated whether this was DMA^{III} or DMA^{V}) via their drinking water for 24 weeks caused tumor promotion in the liver, kidney, urinary bladder, and thyroid gland but inhibited induction of tumors of the nasal passages (S. Yamamoto et al., 1995). More recent studies have also

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found that oral exposure of adult mice to 200 ppm DMA^V in addition to fetal arsenic exposure can act as a promoter of renal and hepatocellular carcinoma, markedly increasing tumor incidence beyond that produced by fetal arsenic exposure alone (Tokar et al., 2012).

Synthesis

Epidemiologic findings for hepatobiliary cancers have not suggested an association with exposure to the COIs. Since the previous update, one new study of U.S. Vietnam veterans who attended a VA Medical Center examined hepatocellular carcinoma comorbid with dual diagnoses of cirrhosis and hepatitis C virus and exposure to Agent Orange (determined by self-report) (Krishnamurthy et al., 2016). Although the risk estimate was elevated, no statistically significant association was found between exposure to Agent Orange and hepatocellular carcinoma. Such null findings are consistent with studies of other cohorts of U.S. Vietnam veterans as well as those from Australia and Korea. Two occupational cohort studies that extended the long-term follow-up period of their cohorts were reviewed. Similar to findings from previous follow-ups, few deaths from hepatobiliary cancers were reported among the U.S. Dow chemical plant workers, and the risk estimate was lower for the workers than in the general U.S. population (Collins et al., 2016). Among the UK factory workers or sprayers of phenoxy herbicides that was followed since 1947, Coggon et al. (2015) also reported a decreased, but not statistically significant risk of hepatobiliary cancer.

Although one new study (Krishnamurthy et al., 2016) reported modest evidence of excess liver cancer among Vietnam veterans using VA medical center services, the weak design, nonspecific exposure, and confounding remain a concern for interpreting its results. The lack of evidence of association between exposure and hepatobiliary cancers in the well-designed and exposure-characterized occupational studies does not support an association. Despite the evidence of TCDD's activity as a hepatocarcinogen in animals, the evidence from epidemiologic studies remains inadequate to link the COIs with hepatobiliary cancers, which has a relatively low incidence in Western populations. Overall, the available evidence does not support an association between the COIs and hepatobiliary cancers.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and hepatobiliary cancers.

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Pancreatic Cancer

The incidence of pancreatic cancer (ICD-9 157; ICD-10 C25) increases with age, and the median age of diagnosis is 70 years. NCI estimated that there would be 55,440 new diagnoses of pancreatic cancer in the United States in 2018, and that 44,330 people would die from it (NCI, n.d.f). The incidence is higher in men than in women. The age-adjusted modeled incidence rate of pancreatic cancer for men 50–64 years old of all races combined was 23.1 per 100,000 in 2014 and increased to 62.5 for 65- to 74-year-olds and 100.2 for men over 75 years.[5] Blacks have the highest incidence rates for both men and women, and American Indians/Alaska Natives and Asians/Pacific Islanders have the lowest incidence rates. Risk factors include chronic pancreatitis (Yadav and Lowenfels, 2013), family history, diet, tobacco use, obesity, and type 2 diabetes (Huxley et al., 2005).

Conclusions from *VAO* and Previous Updates

Like other digestive organ cancers, pancreatic cancer was considered independently for the first time in *Update 2006*. In reviewing the existing evidence concerning an association between herbicide exposure and pancreatic cancer, the committee for *Update 2006* noted a report of increased rates of pancreatic cancer in U.S. female Vietnam nurse veterans (Dalager et al., 1995a) but concluded that it alone did not constitute limited or suggestive evidence of an association. That increase persisted in the follow-up study of the American female veterans cohort (Cypel and Kang, 2008), but committees for subsequent updates have concurred with the decision of the committee for *Update 2006*, which concluded that there is inadequate or insufficient evidence of an association of exposure to any of the COIs and pancreatic cancer.

Four studies of Vietnam veterans were reviewed in *Update 2014*: a follow-up study of U.S. women veterans (H. K. Kang et al., 2014a), the cohort from New Zealand (McBride et al., 2013), and two studies of Korean veterans (Yi, 2013; Yi and Ohrr, 2014). Pancreatic cancer incidence was lower among the New Zealand and Korean veterans than in their respective general populations, but the difference was not statistically significant. Among the mortality analyses, deaths from pancreatic cancer were lower, but not statistically significantly so, among the New Zealand cohort veterans compared with the standardized general population of New Zealand. The risk of death from pancreatic cancer was higher among the other Vietnam veteran cohorts that were followed, but again the differences were not statistically significant. Among the U.S. women veterans, deployed women were compared with Vietnam-era women who remained in the United States, and no statistically significant differences in pancreatic cancer mortality were

[5]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using the SEER 18 dataset and choosing age-adjusted rates, pancreatic cancer, all races, age ≥ 65 years, and stratifying the results by sex.

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observed overall or when the analyses were limited to deployed and nondeployed nurses. Among Korean veterans, neither pancreatic cancer incidence nor risk of mortality from pancreatic cancers in association with herbicide exposure from either the internal comparison of the high- and low-exposure-opportunity groups or the analysis of the individual EOI scores was statistically significant. However, none of the veteran studies controlled for smoking status, a known risk factor of pancreatic cancer.

Update of the Epidemiologic Literature

No studies of Vietnam veterans or environmental studies of the COIs and pancreatic cancer have been identified since *Update 2014*. Reviews of the relevant studies are presented in the earlier reports. Table 7, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to pancreatic cancer.

Occupational Studies Two occupational cohort studies were identified since *Update 2014* that examined the relationship between phenoxy herbicides and pancreatic cancer. Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, no differences in mortality for pancreatic cancer were found for the TCP workers (n = 7; SMR = 0.55, 95% CI 0.22–1.14) or the PCP workers (n = 6; SMR = 1.00, 95% CI 0.37–2.18) (Collins et al., 2016).

Mortality from pancreatic cancers was one of the outcomes addressed by Coggon et al. (2015) in an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers. No difference in risk of mortality from pancreatic cancer was found for any of the groups of workers: all workers (n = 54; SMR = 1.04, 95% CI 0.78–1.35), workers exposed to herbicide levels above background (n = 39; SMR = 1.03, 95% CI 0.73–1.40), or workers exposed for more than 1 year at levels above background (n = 13; SMR = 0.74, 95% CI 0.39–1.26).

Case-Control Studies One population-based case-control study of occupational exposure to pesticides, including phenoxy herbicides, and pancreatic cancer has been published since *Update 2014*. Fritschi et al. (2015) recruited 504 confirmed pancreatic cancer cases and 643 controls between January 2007 and June 2011 as part of the Queensland [Australia] Pancreatic Cancer Study. All participants were Queensland residents and at least 18 years old. Controls were randomly selected from the Australian electoral roll and frequency matched to cases by sex and 5-year age group at diagnosis. Participants completed face-to-face or telephone interviews that collected information about sociodemographic and lifestyle factors (including detailed smoking behaviors and history), medical history, history of cancer in first-degree relatives, and detailed lifetime job histories (including job title, industry, location, main tasks, and ages at start and finish). Additional questions were asked for people who reported ever working in

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industries that are associated with nitrosamine exposure and the occupational or direct use of pesticides on animals or crops. An occupational hygienist, who was blinded to case status, reviewed the job history information to assess the likelihood of exposure to N-nitrosamines and pesticides and estimated level and frequency of such exposures. Pesticide assessments were made for organochlorine insecticides, organophosphate insecticides, phenoxy herbicides, other herbicides, fumigants, fungicides, and other pesticides. Risk estimates were adjusted for age and sex. No statistically significant associations were found with exposure to any of the individual pesticide groups. Specifically examining ever versus never exposure to phenoxy herbicides (19 cases and 24 controls reported ever exposure), no statistically significant association with pancreatic cancer was found (OR = 0.93, 95% CI 0.50–1.74). Among non-smokers the odds of pancreatic cancer with exposure to pesticides was 1.00 (95% CI 0.48–2.06), and among ever smokers it was 0.82 (95% CI 0.51–1.32).

Other Identified Studies Two other studies of pancreatic cancer were identified. The first lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Ruder et al., 2014). The second study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including pancreatic cancer) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

Long-term animal studies have examined the effect on tumor incidence of exposure to each of the COIs: 2,4-D and 2,4,5-T (Charles et al., 1996), TCDD (Walker et al., 2006), picloram (Stott et al., 1990), and DMA (Wanibuchi et al., 1996, 2004). No increase in the incidence of pancreatic cancer in laboratory animals after the administration of cacodylic acid, 2,4-D, or picloram has been reported. A 2-year study of female rats reported increased incidences of pancreatic adenomas and carcinomas after treatment at the highest dose of TCDD (100 ng/kg per day) (Nyska et al., 2004). Other studies have observed chronic active inflammation, acinar-cell vacuolation, and an increase in the proliferation of the acinar cells surrounding the vacuolated cells (Yoshizawa et al., 2005b). As previously discussed, chronic inflammation and hyperproliferation are closely linked to the formation and progression of cancers, including cancers of the pancreas (Hahn and Weinberg, 2002; Mantovani et al., 2008). Also, metaplastic changes in the pancreatic ducts were observed in female monkeys treated with TCDD (Allen et al., 1977). No new mechanistic or biologic plausibility studies on pancreatic cancer have been published since *Update 2014*.

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Synthesis

Although prior studies of Vietnam veterans from the United States and Australia had reported an excess of pancreatic cancer, studies of New Zealand and Korean veterans who served in Vietnam did not report an association between service and pancreatic cancer, although none of them controlled for smoking. In the current update, two studies of occupational cohorts that produced phenoxy herbicides or were exposed to TCDD did not find elevated risks of pancreatic cancer mortality and often did not report consistent directions of any effect. The population-based case-control study of Fritschi et al. (2015) explored exposure to organochlorine insecticides and herbicides and also found no evidence for an association of exposure to these compounds with pancreatic cancer. Limited evidence is available on the biologic plausibility of an association between exposure to any of the COIs and tumors of the pancreas; long-term animal studies of exposure to each of the COIs have not found increased incidence of pancreatic cancer. Overall, the existing evidence does not support a conclusion that exposures to the COIs are associated with the occurrence of pancreatic cancer.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and pancreatic cancer.

Other Digestive Cancers

Findings on cancers of the small intestine (ICD-9 152; ICD-10 C17), ill-defined intestine, rectosigmoid junction, gallbladder (ICD-9 156; ICD-10 C23), retroperitoneum (ICD-9 158; ICD-10 C48), and other unspecified digestive organs (ICD-9 159; ICD-10 C26.8, C26.9, C48.8) are considered in this category.

Some studies have reported the risk of small intestine cancer separately, and others have grouped it under "other digestive cancers." Epidemiologic findings for cancer of the small intestine and the COIs have not been encountered in any of the VAO updates except in analyses of the Korean Veterans Health Study, reviewed by the Update 2014 committee. In a mortality analysis, Yi et al. (2014b) found a statistically significant elevated risk for the internal comparison but not for the analysis of the individual EOI scores of mortality from cancer of the small intestine. Yi and Ohrr (2014) found a statistically significant increase in incidence of cancers of the small intestine when comparing the high- versus low-exposure opportunity groups, but the estimate was imprecise.

Since *Update 2014*, the committee has identified three studies, one that reported on mortality from small intestine cancer specifically, one that reported

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mortality from "other digestive sites" that would include the small intestine and others as defined above, and a third that presented "malignant neoplasms of the peritoneum and other and unspecified of digestive organs" as a single group.

Among the Dow Midland, Michigan, worker cohort that used the standardized U.S. population for comparison, only four deaths were reported from other digestive cancers during the follow-up period (Collins et al., 2016). The small number of deaths resulted in a slightly elevated but imprecise risk estimate for all reported categories of workers: all TCP/PCP workers (SMR = 1.37, 95% CI 0.37–3.51), TCP workers (n = 3; SMR = 1.91, 95% CI 0.52–4.89), and PCP workers (n = 1; SMR = 0.94, 95% CI 0.02–5.25).

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers examining the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) reported that there were four deaths due to cancers of the small intestine over the entire follow-up period. The risk estimates were not statistically significant and were imprecise for all three groups of workers presented: all workers (SMR = 1.67, 95% CI 0.65–4.28), workers exposed to phenoxy herbicides above background (n = 4; SMR = 2.26, 95% CI 0.62–5.80), and workers exposed to phenoxy herbicides above background levels for 1 year or longer (n = 2; SMR = 2.48, 95% CI 0.30–8.96).

A third identified occupational study (Ruder et al., 2014) reported SMRs from many types of malignant neoplasms in the digestive organs using a category of "malignant neoplasms of the peritoneum and other and unspecified of digestive organs" but lacked the necessary exposure specificity to be considered further.

Given the small number of studies and, in some cases, non-specific categorization of the outcomes, these data do not allow the committee to reach any definitive conclusions regarding the association of exposure to the COIs and other digestive cancers, including cancers of the small intestine.

LARYNGEAL CANCER

The larynx is a part of the throat between the base of the tongue and the trachea, and it contains the vocal cords, epiglottis, supraglottis, glottis, and subglottis. NCI estimated that in the United States in 2018, 13,150 people would receive a new diagnosis of and 3,710 men and women would die from laryngeal cancer (ICD-9 161; ICD-10 C32) (NCI, n.d.j). It is the 20th most common cancer diagnosis. The incidence of laryngeal cancer increases with age; the age-adjusted modeled incidence rate of laryngeal cancer for men 65 years and older (the age of Vietnam veterans) for all races combined was 25.1 per 100,000 for 2000–2014.[6] It is more common in men than in women and in blacks than in whites. Exposure to tobacco smoke, paint fumes, metalworking fluids, and asbestos have been associated with laryngeal cancer, as has alcohol and occupational exposures

[6]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using SEER 18 dataset, choosing age-adjusted rates, laryngeal cancer, all races, male sex, and age ≥ 65 years.

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to wood dust and employment in the petroleum, plastics, and textile industries (ACS, 2012a; IOM, 2006a).

Conclusions from *VAO* and Previous Updates

The original VAO committee reviewed five studies that presented data on laryngeal cancers separately and concluded that "although the numbers are too small to draw strong conclusions, the consistency of a mild increase in relative risk is suggestive of an association for laryngeal cancer." The weight of evidence regarding laryngeal cancer has increased since the original VAO committee review, particularly with the addition of epidemiological studies of workers employed in manufacturing herbicides potentially contaminated with TCDD. An elevated rate of laryngeal cancer deaths in workers who were exposed to any phenoxyacetic acid herbicide or chlorophenol was found (SMR = 1.6, 95% CI 1.0–2.5, based on 21 deaths), especially in workers who were exposed to TCDD or higher-chlorinated dioxins (SMR = 1.7, 95% CI 1.0–2.8, based on 15 deaths) (Kogevinas et al., 1997). Ongoing updates have continued to indicate an increase in laryngeal cancer in the occupational cohorts making up this IARC cohort. An environmental study of residents of Chapaevsk, Russia, which was heavily contaminated by many industrial pollutants, including dioxin, showed an association with laryngeal cancer in men (relative risk [RR] = 2.3, 95% CI 1.2–3.8) (Revich et al., 2001).

Among the studies of Vietnam veterans, a positive association was found in the study of veterans in Australia that compared mortality from laryngeal cancer with that in the general population (ADVA, 2005a) but not in the study that compared Australian veterans who served in Vietnam with non-deployed soldiers (ADVA, 2005c). In contrast, Watanabe and Kang (1996) found a statistically significant 40% excess of mortality from laryngeal cancer in U.S. Army personnel deployed to the Vietnam theater. The AFHS did not have sufficient power to detect whether an association existed. The New Zealand cohort of 2,783 Vietnam veterans reported a total of five incident cases and two deaths from larynx cancers, but the study was insufficiently powered to provide reliable estimates (McBride et al., 2013). The Korean Veterans Health Study identified a large number of incident cases (n = 157) and deaths (n = 82) from larynx cancer during a 20-year follow-up (Yi, 2013; Yi and Ohrr, 2014; Yi et al., 2014a,b). Despite the large sample size, the modestly increased risks of both incidence and mortality from larynx cancer were not statistically significant. Although additional studies of laryngeal cancers and exposure to the COIs have been reviewed, the update committees have continued to conclude that there is limited or suggestive evidence of an association between exposure to at least one COI and laryngeal cancer.

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Update of the Epidemiologic Literature

No studies of Vietnam veterans and laryngeal cancer have been published since *Update 2014*. Furthermore, no environmental or case-control studies of the COIs and laryngeal cancer have been identified since *Update 2012*. Reviews of the relevant studies are presented in the earlier reports. Table 8, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to larangeal cancer.

Occupational Studies

Coggon et al. (2015) reported on mortality from laryngeal cancer as part of their follow-up study that examined the carcinogenicity of phenoxy herbicides among UK phenoxy herbicide manufacturers and sprayers. Fewer deaths than expected from laryngeal cancer were found for all groups of workers, but none of the estimates were statistically significantly different from the comparison population: all workers (n = 7; SMR = 0.66, 95% CI 0.26–1.35), workers exposed to herbicide levels above background (n = 7; SMR = 0.91, 95% CI 0.36–1.87), and workers exposed for more than 1 year at levels above background (n = 3; SMR = 0.84, 95% CI 0.17–2.44). These data do not support an association with phenoxy herbicides and cancer of the larynx.

Other Identified Studies

One other study of laryngeal cancer was identified. However, it lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Ruder et al., 2014).

Biologic Plausibility

Long-term animal studies have examined the effect of exposure to the COIs on tumor incidence (Charles et al., 1996; Stott et al., 1990; Walker et al., 2006; Wanibuchi et al., 2004). No increase in the incidence of laryngeal cancer in laboratory animals after the administration of any of the COIs has been reported.

Synthesis

Overall, most reports reviewed in previous updates suggested an increased risk of laryngeal cancer, although the individual studies often were based on small numbers of cases and did not control for smoking. In addition, there is evidence of an excess risk of laryngeal cancer among those who experienced chloracne—a marker of high exposure to dioxins. The literature provides a reasonable level of consistency regarding evidence of a moderate increase in the relative risk of

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laryngeal cancer. In larger occupational studies with good exposure characterizations that focus on the COIs, the associations are generally strong for laryngeal cancer; however, in the extended follow-up time of the UK phenoxy herbicide workers and sprayers, Coggon et al. (2015) found decreased risk estimates of mortality from laryngeal cancer for all categories of exposure in workers. Studies of Vietnam veterans have provided modest, generally not statistically significant, associations.

Conclusion

Based on one additional study reviewed for the current update, the committee concurs with prior VAO committees and concludes that there is limited or suggestive evidence of an association between exposure to at least one COI and laryngeal cancer.

LUNG CANCER

Lung cancer (carcinoma of the lung or bronchus, ICD-9 162.2–162.9; ICD-10 C34) is the second most common diagnosed cancer and the leading cause of cancer death (accounting for about 26% of all cancer deaths) in the United States. An estimated 234,030 people will receive diagnoses of lung cancer in the United States in 2018, and 154,050 people will die from it (NCI, n.d.k). The principal types of lung neoplasms are identified collectively as bronchogenic carcinoma and carcinoma of the lung. Cancer of the trachea (ICD-9 162.0) is often grouped with cancers of the lung and bronchus under ICD-9 162.2, but it is a rare cancer. The lung is also a common site of metastatic tumors from other organ sites, but only studies of primary cancer sites are reviewed.

The incidence of lung cancer increases with age, and the median age of diagnosis is 70 years. The age-adjusted modeled incidence rate of lung and bronchus cancers for men 50–64 years old of all races combined was 83.3 per 100,000 in 2014 and increased to 298.6 for 65–74 year olds and 447.5 for men over 75 years. The increased incidence rate with age is similar for women, though not as high as it is for men of the same age groups.[7] The incidence of lung cancer is consistently higher in black men than in white men, but slightly lower in black women compared with white women. It is lowest among Hispanic men and women (NCI, n.d.k).

Smoking is a major risk factor for lung cancer and increases the risk of all histologic types of this disease, but the associations with squamous-cell and small-cell carcinomas are the strongest. CDC's 2014 Surgeon General report estimated that 82% of lung cancer deaths are attributable to cigarette smoking (CDC,

[7]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using SEER 18 dataset and choosing age-adjusted rates, lung cancer, all races, age ≥ 65 years, and male sex.

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2014a). Other risk factors include exposure to asbestos, uranium, vinyl chloride, nickel chromates, coal products, mustard gas, chloromethyl ethers, gasoline, diesel exhaust, and inorganic arsenic. The risk posed by arsenic does not imply that cacodylic acid, which is a metabolite of inorganic arsenic, can be assumed to be a risk factor for lung cancer. Important environmental risk factors include exposure to secondary tobacco smoke and radon (Lantz et al., 2013; NRC, 1999; Samet et al., 2009).

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of an association between exposure to at least one COI and lung cancer. Additional information available to the committees responsible for subsequent Updates did not change that conclusion.

The most compelling evidence of an association with lung cancer has come from studies of heavily exposed occupational cohorts, including British 2-methyl-4-chlorophenoxyacetic acid (MCPA) production workers (Coggon et al., 1986), German production workers (Becher et al., 1996), a BASF cohort (Ott and Zober, 1996), a NIOSH cohort (Fingerhut et al., 1991; Steenland et al., 1999), and Danish production workers (Lynge, 1993). The methodologically sound AHS did not show any increased risk of lung cancer. Although there was substantial 2,4-D exposure in the AHS cohort (Blair et al., 2005b), the dioxin exposure of the contemporary farmers was probably negligible. In large part, the environmental studies have not been supportive of an association, although in the cancer-incidence update from Seveso (Pesatori et al., 2009), the highest risks of lung cancer occurred in the most exposed.

In studies of U.S. veterans, a significantly increased risk of lung-cancer risk was found in ACC veterans who used herbicides in Vietnam (Cypel and Kang, 2010; Dalager and Kang, 1997), and an increased risk of lung cancer was associated with increased serum TCDD concentrations in AFHS Ranch Hand veterans (Pavuk et al., 2005). The Australian cohort studies of Vietnam veterans (ADVA, 2005a,b,c), which presumably cover a large proportion of exposed soldiers, showed higher than expected incidence of and mortality from lung cancer. The main limitations of the Australian and American ACC studies are that there was no assessment of exposure and that some potential confounding variables, notably smoking, could not be accounted for. Additionally, the Korean Veterans Health Study (Yi, 2013; Yi and Ohrr, 2014; Yi et al., 2014b) found modestly elevated, but not statistically significant, relative risks of both lung cancer incidence and mortality compared with the general population and high- versus low-exposure groups. The results were not adjusted for smoking, but earlier self-reported information from a large portion of the cohort indicated that smoking behavior did not appear related to the extent of a veteran's exposure to herbicides.

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Despite evidence of an association with lung cancer incidence and mortality in the male Vietnam veteran studies, data from the U.S. veteran women showed no excess lung cancer mortality in comparison to the U.S. cohort of non-deployed women or those from the U.S. general population. Similar results were observed also among male Vietnam veterans in New Zealand. Despite their limitations, these studies of Vietnam veterans are largely suggestive of modest associations between herbicide exposure and lung cancer incidence and mortality.

Update of the Epidemiologic Literature

No new studies of Vietnam veterans (U.S. or international) and lung cancer have been identified since *Update 2014*. No environmental or case-control studies of the COIs and lung cancer have been identified since *Update 2012*. Reviews of the relevant studies are presented in the earlier reports. Table 9, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to lung, bronchus, or trachea cancer.

Occupational Studies

Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, two estimates were presented—all cancers of the respiratory system combined (ICD-10 C30–C39) and also a subgroup of bronchus, trachea, and lung cancers (ICD-10 C33–C34) (Collins et al., 2016). For all cancers of the respiratory system combined, no differences in mortality were found for the TCP workers (n = 77; SMR = 0.88, 95% CI 0.69–1.10) or the PCP workers (n = 42; SMR = 1.05, 95% CI 0.76–1.42). Likewise, after restricting the analysis to the subgroup of bronchus, trachea, and lung cancers, no differences in mortality was found for the TCP workers (n = 72; SMR = 0.86, 95% CI 0.67–1.08) or the PCP workers (n = 39; SMR = 1.02, 95% CI 0.73–1.40).

A recent analysis of lung cancer incidence was conducted using data collected from the U.S. AHS (Bonner et al., 2017). The sample included 57,310 pesticide applicators from Iowa and North Carolina who were enrolled in the study between 1993 and 1997; vital status was updated through 2011. Exposure was assessed by extensive questionnaire, allowing for estimates of intensity and duration of exposure, and the information was updated from 1999 to 2005. In the 43 pesticides chosen for assessment of risk, there was considerable variation in the risk estimates associated with exposure estimates, with dicamba exposure estimated to be inversely related to lung cancer risk when modeled both as quartiles of lifetime days of exposure and as quartiles of intensity-weighted lifetime days of exposure and compared with the non-exposed group (p trend = 0.007 and 0.001, respectively). Similar results were found for 5-year and 15-year lagged lifetime-days of dicamba exposure presented as quartiles and again compared with no exposure (p trend = 0.001 and < 0.001, respectively). Robust

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data collection allowed for an adjustment of confounders and common risk factors, including lag time from first exposure. However, the committee notes that the authors did not control for multiple comparisons (although there is some disagreement in the literature concerning such methods), the number of lung cancer cases is small, and the exposure data are based only on recall.

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) reported deaths from lung cancer. Their analyses were not adjusted for smoking status, but mortality from lung cancer was not elevated for any of the groups of workers: all workers (n = 392; SMR = 1.01, 95% CI 0.91–1.11), workers exposed to herbicide levels above background (n = 298; SMR = 1.07, 95% CI = 0.95–1.20), or workers exposed for more than 1 year at levels above background (n = 138; SMR = 1.05, 95% CI 0.88–1.24).

Cappelletti et al. (2016) performed a retrospective study of 331 male electric arc foundry workers at a single plant in Trentino, Italy, to determine if they experienced excess mortality from all causes, all cancers, and specifically respiratory cancers or if they experienced increased risk for other morbidities. Their analysis of the dust emissions found that the dust contained metals (including iron, aluminum, zinc, manganese, lead, chromium, nickel, cadmium, mercury, and arsenic), PAHs, PCBs, and PCDD/Fs (reported as TEQs). Therefore, the authors could not determine which of the agents were associated with a specific outcome or to what extent. The men had worked at the factory for at least 1 year and, for the mortality analysis, were compared with the standardized general population of Region Trentino-Alto Adige (where the factory was located) because there were few nonexposed foundry workers and high attrition rates. Company and medical records were used to determine vital status; the cause of death was determined from death certificates or other registries. The workers were followed from March 19, 1979 (or their first day of employment), through December 31, 2009, or date of death. Compared with the general population, workers exposed for more than 1 year were at increased risk of mortality from malignant tumors of the larynx, trachea, bronchi, and lungs (reported as a group) (n = 8; SMR = 3.35, 95% CI 1.45–6.60, p = 0.01). When workers were stratified by the number of years of exposure, there were three individuals or fewer in each of the strata, resulting in large and imprecise effect estimates, but none was statistically significant. This study is most limited by the fact that foundry dust is a complex mixture, which results in an inability to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those exposed workers. Estimates were only adjusted for age group and not adjusted for other risk factors such as tobacco use, BMI, or other jobs or activities that could result in similar exposures. Exposure to foundry dust by the general population that was used for comparison is not discussed, although the foundry appears to be in the local vicinity and emissions from it were reported to be present in a 2-kilometer radius of it.

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Other Identified Studies

Two other studies of respiratory cancers were identified. The first lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Ruder et al., 2014). The second study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including lung cancer) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

Long-term animal studies have examined the effect on tumor incidence of exposure to each of the COIs: 2,4-D and 2,4,5-T (Charles et al., 1996), TCDD (Walker et al., 2006), picloram (Stott et al., 1990), and DMA (Wanibuchi et al., 1996, 2004). As noted in previous VAO reports, there is evidence of an increased incidence of squamous-cell carcinoma of the lung in male and female rats exposed to TCDD at high concentrations (Kociba et al., 1978; Van Miller et al., 1977). A significant increase in neoplastic and non-neoplastic lung lesions was found in female rats exposed to TCDD for 2 years (Kociba et al., 1978; NTP, 1982a,b, 2006; Walker et al., 2006, 2007). The most common non-neoplastic lesions were bronchiolar metaplasia and squamous metaplasia of the alveolar epithelium. Cystic keratinizing epithelioma was the most commonly observed neoplasm. The lung was also identified as a target organ in an NTP tumor-promotion study, which examined the presence of tumors after 60 weeks of exposure to TCDD in ovariectomized female Sprague Dawley rats initiated with a single dose of diethyl-N-nitrosamine (Beebe et al., 1995; Tritscher et al., 2000). Those studies reported increased incidences of alveolar epithelial hyperplasia and alveolar–bronchiolar metaplasia—results that were similar to those found in the earlier NTP studies (Tritscher et al., 2000). A study with female mice in the lung-cancer-sensitive A/J strain background showed that estrogen exposure increased lung tumor incidence significantly in ovariectomized mice treated with a chemical carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), known to induce these tumors. However, TCDD exposure did not increase lung tumor formation further in these ovariectomized and estrogen-treated mice (R. J. Chen et al., 2014a). TCDD by itself had little lung tumor–promoting activity in intact female A/J mice, but it exhibited a significant synergistic effect when combined with a low dose of NNK. Cell-culture experiments suggested that the TCDD effect was via the inhibition of apoptosis (R. J. Chen et al., 2014b). The AHR has been implicated in both the chemical induction of lung tumors (Tsay et

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al., 2013) and the inhibition of their metastasis (Zhang et al., 2012) but has not been linked specifically at this time to TCDD or the other COIs.

Cacodylic acid (DMA^{III} and DMA^{V}) is carcinogenic, but results from studies of DMA exposure and lung cancer in laboratory animals have not been consistent. In the mouse lung, cacodylic acid (DMA^{V}) was been shown to act as a tumor initiator (Yamanaka et al., 1996, 2009) and as a tumor promoter (Mizoi et al., 2005). DMA^{V} can also act as a complete carcinogen, inducing lung tumors in susceptible strains of mice, including those with deficient DNA-repair activity (Hayashi et al., 1998; Kinoshita et al., 2007). However, a 2-year study of F344 rats exposed to cacodylic acid at 0–100 ppm and B6C3F1 mice exposed at 0–500 ppm failed to detect lung neoplasms at any dose (Arnold et al., 2006). 2,4-D causes lung damage, and a recent report provided evidence that this effect occurs via disruption of the microtubule network (Ganguli et al., 2014).

Synthesis

Epidemiologic findings for the incidence and mortality of lung, bronchus, and trachea cancers have been suggestive of an association with exposure to the COIs. The several toxicologic studies of mechanistic activity provide further support for the conclusion that the evidence of an association is limited or suggestive.

Since *Update 2014*, four occupational studies have been published. Three of them extended the follow-up period of their respective, well-characterized cohorts. Consistent with the prior follow-ups of the Dow Midland, Michigan, plant workers, TCP workers had a slightly decreased risk of all respiratory system cancers combined and also when restricted to only lung, bronchus, and trachea cancers, but neither estimate was statistically significant. PCP workers had slight but not statistically significant increased risks of all respiratory system cancers combined as well as for lung, bronchus, and trachea cancers (Collins et al., 2016). Among the UK factory workers or sprayers of phenoxy herbicides who have been followed since 1947, Coggon et al. (2015) reported a slightly increased, but not statistically significant, risk of lung cancer mortality for all groups of workers; however, the analyses were not adjusted for smoking status. Although it was a relatively small cohort of male steel workers, Cappelletti et al. (2016) found that workers exposed for more than 1 year were at increased risk of mortality from malignant tumors of the larynx, trachea, bronchi, and lungs compared with the general population of the area. However, neither smoking nor residential proximity to the plant was considered in the analysis.

An analysis of lung cancer incidence in the U.S. AHS found that dicamba exposure, measured as both lifetime days and years of lag time, showed an inverse relationship with lung cancer (Bonner et al., 2017). However, the analysis is limited by the small number of lung cancer cases and the fact that exposure data are based only on recall.

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Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one COI and carcinomas of the lung, bronchus, and trachea.

BONE AND JOINT CANCERS

Primary bone and joint cancers (ICD-9 170; ICD-10 C40–C41) are relatively rare, with an estimated 3,450 new diagnoses and 1,590 expected deaths in the United States in 2018 (NCI, n.d.l). Primary bone and joint cancers refer to malignancies that originate in the bone joint; cancers that metastasize from another site are excluded from the discussion. Bone and joint cancers are most commonly diagnosed in persons less than 20 years of age, and these cancers are rare in adults, including those in the youngest age group of Vietnam veterans (62–70 years). Risk factors for bone and joint cancers in adults are gender, ethnicity, genetic and familial factors, exposure to ionizing radiation in treatment for other cancers, and a history of some non-cancer bone diseases, including Paget disease (Chung and Van Hul, 2012; Ottaviani and Jaffe, 2009).

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and bone and joint cancers. Very few studies of bone and joint cancers were conducted in veteran populations, and none found statistically significant associations. Additional information available to the update committees, in general, did not have adequate power or detailed information concerning exposures to the COIs to change that conclusion.

Update of the Epidemiologic Literature

Two studies that examined the prevalence of osteosarcoma (Akahane et al., 2017) and the mortality outcomes of bone and joint cancers (Ruder et al., 2014) have been identified since *Update 2014*. The first study examined the prevalence of a variety of self-reported conditions, including osteosarcoma, in Yusho patients (known to be exposed to PCBs, PCDD/Fs, and dioxin-like chemicals) and an age-, sex-, and residence-matched comparison group. Because TEQs or other quantification of relevant exposures were not presented, the study was not considered further. The study by Ruder et al. (2014) lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. No environmental or case-control studies of exposure to the COIs and bone and joint cancers have

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been published since *Update 2012*. Reviews of the relevant studies are presented in the earlier reports. Table 10, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to bone and joint cancers.

Biologic Plausibility

No animal studies have reported an increased incidence of bone and joint cancers after exposure to the COIs.

Synthesis

There is a paucity of literature on bone and joint cancers to explain the biological plausibility or population-level risk from exposure to the COIs. This is not surprising, given the low frequency of these tumors in the adult population. The newly identified studies (Akahane et al., 2017; Ruder et al., 2014) either lacked exposure specificity or a quantification of exposure and, therefore, were not considered further by the committee.

Conclusion

Given the absence of new evidence for the current update, the committee concurs with prior VAO committees and concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to at least one COI and bone and joint cancers.

SOFT-TISSUE SARCOMAS

Soft-tissue sarcomas (STSs) (ICD-9 164.1, 171; ICD-10 C38.0, C47, and C49) arise within organs and in the tissue between organs, such as fat, muscle, nerves, fibrous tissues, blood vessels, or deep skin tissues, and can be found in any part of the body. There are more than 50 types of STSs, but the three most common types are undifferentiated pleomorphic sarcoma (malignant fibrous histiocytoma), leiomyosarcoma, and liposarcoma (ACS, 2018a). Because of the diverse histological features of STS, accurate diagnosis and classification can be difficult. The American Cancer Society estimated that 13,040 new diagnoses of STS and 5,150 deaths were expected in the United States in 2018 (ACS, 2018a).

The incidence of STS increases with age. The age-adjusted modeled incidence rate of STS for men 65 years and older for all races combined was 15.4 per 100,000 for 2000–2014 and increases to 19.9 for men over 75 years. The increase

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in incidence rate with age is similar for women, but it is not as high as it is for men of the same age groups.[8]

Among the risk factors for STS are exposure to ionizing radiation during treatment for other cancers, some inherited genetic conditions (including neurofibromatosis and Li-Fraumeni syndrome), chronic conditions including lymphedema and immune deficiency (Kaposi sarcoma), and exposure to chemicals such as phenoxyacetic acids (herbicides), and woods preservatives that contained chlorophenols (Cormier and Pollock, 2004).

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was sufficient epidemiologic data to support an association between exposure to the COIs and STS. Additional information available to the committees responsible for subsequent updates has reaffirmed that conclusion.

The available epidemiologic evidence suggests that phenoxy herbicides rather than TCDD may be associated with developing STS. Some of the strongest evidence of an association between STS and exposure to phenoxy herbicides comes from a series of case-control studies conducted in Sweden (Eriksson et al., 1979, 1981, 1990; Hardell and Eriksson, 1988; Hardell and Sandström, 1979; Wingren et al., 1990). The studies, involving a total of 506 cases, show an association between STS and exposure to phenoxy herbicides, chlorophenols, or both. The VAO committee concluded that although those studies have been criticized, there is insufficient justification to discount the consistent pattern of increased risks in these well-designed and conducted studies. Furthermore, a reanalysis of the data by Hardell (1981) to evaluate the potential influence of recall bias and interviewer bias confirmed the original results. Other studies among male Danish gardeners (Hansen et al., 2007) and forestry workers (Reif et al., 1989) also supported the association with STS. Case-control studies evaluating the association between phenoxy herbicide and chlorophenol exposure and STS incidence and mortality in New Zealand found the risk of STS to be elevated but not statistically significant so (A. H. Smith et al., 1983, 1984; Smith and Pearce, 1986). Other international case-control studies of STS and the COIs have been conducted in England (Balarajan and Acheson, 1984), northern Italy (Vineis et al., 1986), and Australia (Smith and Christophers, 1992).

Additional support for an association between exposure to the COIs and STS comes from a NIOSH study showing increased risk for STS in those production workers who were most highly exposed to TCDD (Fingerhut et al., 1991). Similarly, an increased risk of death 10–19 years after first exposure was seen in the

[8]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using the SEER 18 dataset and choosing age-adjusted rates, soft-tissue sarcoma, all races, age ≥ 65 years, and male sex, and using the modeled incidence rate.

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IARC cohort (Kogevinas et al., 1992; Saracci et al., 1991) according to a fairly crude exposure classification. An updated and expanded study of the IARC cohort by Kogevinas et al. (1997) found risk of STS was not statistically significantly increased when follow-up was extended to 1992. The NIOSH and IARC cohorts are among the largest and the most highly exposed occupational cohorts. Smaller studies of workers that are included in the multinational IARC cohort—Danish herbicide manufacturers (Lynge et al., 1985, 1993) and Dow production workers in Midland, Michigan (Collins et al., 2009b), and in New Zealand ('t Mannetje et al., 2005)—showed an increased risk of STS, but the results were often not statistically significant, possibly because of the small samples (related to the relative rarity of STS in the population). Several studies have reported on STS in relation to living near waste incinerators that release dioxin as a contaminant; each of those studies found a statistically significant excess of STS, but none showed any objective evidence of human exposure. No cases of STS have been reported in zones A (highest exposure) and B (intermediate exposure) in the Seveso cohort (Consonni et al., 2008); the incidence of STS was slightly, but not significantly, increased in Zone R (lowest exposure) (Pesatori et al., 2009).

Case-control studies have also been conducted in various U.S. populations looking for associations of herbicides with STS and other lymphohematopoietic cancers (Hoar et al., 1986; Woods and Polissar, 1989; Woods et al., 1987) but no statistically significant associations were reported for STS. Case-control studies conducted among international populations have, for the most part, reported null associations.

Studies of Vietnam veterans have not found significant increases in STS. No increase was seen in Ranch Hand veterans (AFHS, 1996, 2000; Michalek et al., 1990) or in VA studies of U.S. Vietnam veterans (Breslin et al., 1986, 1988; Bullman et al., 1990; Watanabe and Kang, 1995; Watanabe et al., 1991). A nonstatistically significant increase in mortality from STS was seen in state studies of veterans in Massachusetts, Michigan, and New York. A slight increase in the incidence of STS was seen in Australian Air Force veterans compared with the general Australian population but not in Army or Navy personnel (ADVA, 2005a), and no increase in mortality was seen in Australian veterans who served in any of the military branches (ADVA, 2005b). No differences in incidence of or mortality from connective and soft-tissue cancers was found in the New Zealand veteran cohort (McBride et al., 2013), nor among the Korean veteran cohort (Yi and Ohrr, 2014).

Update of the Epidemiologic Literature

No studies of Vietnam veterans or environmental studies of the COIs and STS have been identified since *Update 2014*. Reviews of the relevant studies are presented in the earlier reports. Table 11, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to STS.

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Occupational Studies

Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population (Collins et al., 2016), few deaths from STSs were reported (four among TCP workers and one among PCP workers), and risk estimates for the total worker population were imprecise (SMR = 2.31, 95% CI 0.63–5.91). When examined by the specific dioxin congener and exposure level, an increased risk for STS was found with increasing exposure level of TCDD, but the test for trend was not statistically significant (p = 0.32). None of the individual exposure-level risk estimates reached statistical significance, and all estimates were imprecise. The one caveat the authors present is that they knew that one of the four deaths at the highest exposure level of TCDD was determined to be misclassified and, in fact, was not an STS. Because this determination was discovered during the pathology review of an earlier study and the rest of the cases in this study were not reviewed, this finding was ignored, and the death was counted as an STS death. The authors concluded that any increased risk of STS with TCDD should be interpreted with caution, given the small numbers of cases of STS and the uncertainty of the diagnosis.

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides and their association primarily with HL, STS, and chronic lymphocytic leukemia, Coggon et al. (2015) reported four cases of STS among all the workers. The highest risk estimate for STS was in workers with exposure above background and for more than 1 year (n = 3; SMR = 2.05, 95% CI 0.42–5.98), while the effect for all workers regardless of the extent and duration of exposure was less than 1.0 (n = 4; SMR = 0.92, 95% CI 0.25–2.36). Neither estimate was statistically significant, and both were imprecise. A nested case-control analysis was performed within the cohort that showed a protective effect for workers exposed to above background levels for less than 1 year (OR = 0.77, 95% CI 0.17–3.50) but an increased risk for workers exposed above background for more than 1 year (OR = 1.30, 95% CI 0.30–5.62), but again both estimates were imprecise. Stratifying the workers with above-background levels into low- and high-exposure groups resulted in decreased and imprecise risk estimates (OR = 0.79, 95% CI 0.16–3.89 and OR = 0.95, 95% CI 0.19–4.88, respectively). No clear link between phenoxy herbicides and STS was observed in this study. The authors concluded that if there is an association, it is very small, and it is unlikely that phenoxy herbicides were responsible for the increased risk of STS.

Case-Control Studies

In a Finnish study, Tuomisto et al. (2017) compared the odds ratios for STS calculated from survey data versus tissue TEQs for dioxin congeners. Previously, the authors had performed a prospective study in which patients undergoing

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STS surgery or appendectomy (no cancer) consented to have a subcutaneous fat sample taken at the time of surgery. Fat samples were tested for 17 PCDD/F congeners. All participants filled out a survey regarding exposure history to wood preservatives, fungicides and herbicides, and insecticides. The data were collected prospectively, and this analysis included 87 STS patients age-matched to 308 controls who underwent an appendectomy. When exposure information from questionnaires was used to compute effect estimates, wood preservatives (n = 8; OR = 6.7, 95% CI 1.4–33), fungicides and herbicides (n = 7; OR = 16.0, 95% CI 1.9–138), and reported exposure to any of the chemical groups (n =15; OR = 7.0, 95% CI 2.2–22) were statistically significant but not precise. However, when the exposure assessment was based on the actual levels of PCDD/Fs in the same patients, none of the classes of chemicals were statistically significantly associated with STS. One reason for the differences between the survey results and the objective measures in the fat samples may be that cancer patients are more prone to recall bias than controls. A second factor for the difference in results between questionnaires and tissue samples may be that the risk for STS is not the contaminant dioxin, but rather the primary chemical exposure. Dioxin is poorly absorbed and, in order to accumulate a high level, an individual is also likely to have accumulated a high level of the primary chemical, which perhaps is more likely to be the real carcinogen. The authors concluded that the number of cases of STS was so low that it is unlikely that chlorophenols or phenoxy herbicides were major risk factors for STS. While this study contradicts the earlier literature supporting an association between exposure to the COIs and STS (Eriksson et al., 1981, 1990; Hardell and Eriksson, 1988; Hardell and Sandström, 1979), it has too few cases of STS to reverse the previous VAO committees' conclusions. This study does, however, demonstrate the strength of using tissue levels versus questionnaires to overcome recall bias among cancer patients.

Other Identified Studies

One other study that reported on mortality of STSs was identified, but it lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Ruder et al., 2014).

Biologic Plausibility

In a 2-year study, dermal application of TCDD to Swiss-Webster mice led to an increase in fibrosarcomas in females but not in males (NTP, 1982b). There is some concern that the increase in fibrosarcomas may be associated with the treatment protocol rather than with TCDD. The NTP gavage study (NTP, 1982a) also found an increased incidence of fibrosarcomas in male and female rats and in female mice. No new mechanistic or biologic plausibility studies on STS have been identified by VAO committees.

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Synthesis

Previous committees have concluded that the occupational, environmental, and Vietnam-veteran studies showed sufficient evidence to link herbicide exposure to STS. Mechanistic data from animal studies continue to be sparse and not compelling. Two new occupational studies that extended the follow-up period of established cohorts (Coggon et al., 2015; Collins et al., 2016) reported small numbers of STS cases and imprecise risk estimates, which do not provide enough evidence to refute the previous conclusion that there are sufficient data to link STS with one of the COIs. The findings from Tuomisto et al. (2017), which show sizable differences in risk estimates when using questionnaires versus tissue levels to determine exposure to the COIs, raise important methodological issues for future studies.

STS is a rare tumor that is frequently misclassified. In a small study, even a single death can have a large impact on effect estimates. The findings from the three studies reviewed for the current update are not as supportive of an association between STS and the COIs. This may be due to flaws in design in earlier studies, or it may reflect the possibility that the risk for this malignancy occurs within a shorter latency period than other malignancies. Therefore, the conclusion of sufficient evidence of an association between exposure to at least one of the COIs and STS remains unchanged.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is sufficient evidence of an association between exposure to at least one of the COIs and STS.

SKIN CANCERS

Skin cancers are generally divided into two broad categories: malignant melanoma (or simply melanoma) and non-melanoma skin cancers. The two most common non-melanoma skin cancers are squamous-cell carcinomas, which are derived from the squamous epithelium, and basal-cell carcinomas, which are derived from stem cells. Melanomas are derived from melanocytes. Non-melanoma skin cancers have a far higher incidence than melanoma but are less likely to metastasize and are more easily cured with primary resection.

The committee responsible for *Update 1998* first chose to address melanoma studies separately from those of non-melanoma skin cancers. Some researchers report results by combining all types of skin cancers without specifying type. Although there is a general supposition that high mortality figures refer predominantly to melanoma and high-incidence figures refer to non-melanoma skin cancers, the committee believes that combined information is not interpretable,

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and therefore, it is interpreting data only when the results specify melanoma or non-melanoma skin cancers.

Melanoma

Melanoma is the fifth most commonly diagnosed cancer; with an estimated 91,270 new diagnoses (ICD-9 172; ICD-10 C43) expected in the United States in 2018 accounting for 5.3% of all new cancer diagnoses. It is estimated that 9,320 people will die from melanoma in 2018 (NCI, n.d.m). Because non-melanoma skin cancers are not required to be reported to registries, the estimated number of cases is not as precise as those of other cancers. Although melanoma accounts for less than 5% of skin cancer cases, it is responsible for about 75% of skin cancer deaths (Siegel et al., 2017). The incidence of melanoma is higher in men than in women and increases with age. The age-adjusted modeled incidence rate of melanoma for men 50–64 years old of all races combined was 47.5 per 100,000 for 2000–2014 and increased to 118.3 for 65–74 year olds and 184.7 for men over 75 years. The increasing incidence rate with age is similar for women, though not as high as it is for men of the same age groups.[9]

Melanoma occurs more frequently in fair-skinned people than in dark-skinned people; the risk in whites is roughly 20 times higher than it is in blacks. Other risk factors include the presence of large numbers of moles (> 50) or dysplastic nevi (also called atypical moles), having a suppressed immune system, and excessive exposure to ultraviolet (UV) radiation, typically from the sun but also from artificial sources, such as tanning beds. A family history of the disease has been identified as a risk factor, but it is unclear whether that is attributable to genetic factors or to similarities in skin type and sun-exposure patterns (Rastrelli et al., 2014). There is a genetic predisposition to melanoma, known as BK Mole syndrome (also called familial atypical multiple mole melanoma syndrome), which is characterized by a mutation in the *CDKN2A* gene. In addition to the dermal forms of melanoma, these tumors occur much more infrequently in various tissues of the eye.

Conclusions from *VAO* and Previous Update

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and skin cancers. The Update 1998 committee considered the literature on melanoma separately from that of non-melanoma skin cancers and found that there was inadequate or insufficient information to determine

[9]Modeled incidence rate as calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output using SEER 18 dataset and by choosing age-adjusted rates, melanoma of the skin, all races, age ≥ 50 years, and male sex.

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whether there is an association between the COIs and melanoma. Additional evidence reviewed by update committees has not changed the conclusion.

Few studies of melanoma among veteran populations have been conducted. In a comparison of cause-specific mortality between the deployed and the non-deployed veterans who served in the U.S. ACC, a moderate but not statistically significant increase in the risk of malignant skin cancer was observed in the deployed cohort (Cypel and Kang, 2010). Few cases of melanoma were reported among the AFHS participants, and when stratified by serum TCDD exposure level, the risk estimates were elevated but imprecise and not statistically significant (Pavuk et al., 2005). An analysis using quartiles of years of service in Southeast Asia also failed to find an association with melanoma among Ranch Hands (Akhtar et al., 2004). Data from the final AFHS examination cycle indicate that many more melanoma cases were diagnosed in the comparison veterans than in the Ranch Hand subjects. Consequently, the committee responsible for *Update 2006* (the report in which these studies were reviewed) recommended that a uniform TCDD-based analysis be performed on the on the most recent melanoma data for all subjects in the AFHS (Pavuk, 2005). These data would possibly support or contradict the suggestive findings of the Ranch Hand cohort (Akhtar, 2004). Such a comprehensive analysis of the most current melanoma data from the AFHS has not yet been published.

In a study of 2,783 New Zealand veterans who served in Vietnam, both melanoma mortality and incidence risk estimates were less than 1.0 but neither was statistically significant (McBride et al., 2013). Likewise, findings from the Korean Veterans Health Study showed that the incidence of melanoma in high-exposed veterans was lower, but not to the point of statistical significance, than in the low-exposed group, based on the calculated EOI scores (Yi and Ohrr, 2014). No statistically significant difference was observed for melanoma mortality between high- and low-exposed groups.

Studies of mortality from melanoma related to occupational exposures have also been reviewed. In TCP workers in New Zealand (McBride et al., 2009a) and in the Dow cohort in Midland, Michigan (Collins et al., 2009b), no evidence of an association between the COIs and melanoma was found. In evaluating the use of specific pesticides and melanoma in the AHS, Dennis et al. (2010) found that only an exposure to arsenic-based pesticides, among the COIs, was correlated with any increase in risk, which was weak and not statistically significant. Updates of cancer incidence in the Seveso cohort for the period 1977–1996 (Pesatori et al., 2009) continued to provide evidence that melanoma is associated with exposure to TCDD.

Update of the Epidemiologic Literature

No new studies of Vietnam veterans (U.S. or international) and melanoma or environmental studies of melanoma and the COIs have been identified since *Update 2014*. Reviews of the relevant studies are presented in the earlier reports.

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Table 12, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to melanoma.

Occupational Studies Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016) found only two deaths from malignant melanoma during the follow-up period. Although the calculated estimate showed decreased risk, because of the few cases it is unreliable and imprecise (SMR = 0.36, 95% CI 0.04–1.30), and no conclusions should be made based on this finding.

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) reported a total of seven deaths from melanoma among all the workers. Melanoma mortality was lower than expected for each of the three groups of workers presented, but no estimates were statistically significant: all workers (n = 7; SMR = 0.66, 95% CI 0.26–1.35), workers exposed to herbicide levels above background (n = 6; SMR = 0.73, 95% CI 0.27–1.59), or workers exposed for more than 1 year at levels above background (n = 2; SMR = 0.55, 95% CI 0.07–2.00). This study does not support an association between pheonxy herbicide exposure and melanoma.

Other Identified Studies Three other studies were identified that reported on the outcomes of melanoma or skin cancer. The first was a mortality study of an occupational cohort of capacitor manufacturers exposed to mixed PCBs as well as several other chemicals and metals; this study lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs and melanoma (Ruder et al., 2014). The second study was a well-designed case-control study to examine the association of pesticide use and the risk of cutaneous melanoma in Brazil (Segatto et al., 2015). Although a validated structured questionnaire was used to collect detailed information on exposure factors (including domestic and occupational exposures to pesticides and herbicides, frequency, types, and commercial names), only 3.5% of pesticide exposures were to organochlorines, which were not specified further, and a small number of participants (7 cases and 3 controls) reported exposure to herbicides, for which an effect estimate was not provided. A third study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects, including skin cancer without further differentiation, in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

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Biologic Plausibility

TCDD and related herbicides have not been found to cause melanoma in animal models. IARC considers DMA to be a Group 2B compound indicating that it is possibly carcinogenic to humans (IARC, 2012a). In general, rodents, which are used in most toxicology studies, are not a good model for studying melanoma. As discussed elsewhere in this chapter, TCDD and DMA are known tumor promoters and could act as a promoter for skin cancer initiators, such as UV radiation (Morikawa et al., 2005). Ikuta et al. (2009) examined the physiologic role of the AHR in human skin and theorized that over-activation can lead to skin cancers, but they provided no evidence that melanoma incidence is increased after TCDD exposure. Ahr has been shown to mediate UVB-induced skin tanning in a murine model through an action on melanocytes (Jux et al., 2011), which suggests that TCDD may affect skin pigmentation and potentiate other pathways in the developmet of melanoma. Studies of human cells have also confirmed a role of the AHR in the regulation of keratinocytes and melanocytes. Kalmes et al. (2011) showed that AHR signaling in immortalized HaCaT cells is associated with cell-cycle progression. Borland et al. (2014) found that peroxisome proliferator-activated receptor beta/delta is involved in AHR-mediated tumorigenic activity in keratinocytes. In human melanocytes, Luecke et al. (2010) demonstrated that TCDD exposure induced tyrosinase and tyrosinase-related protein 2 gene expression—an indication that AHR signaling after TCDD exposure modulates melanogenesis. O'Donnell et al. (2012) further showed that the activity of AHR was associated with the proliferation of melanoma cells. Finally, a study of a Han Chinese population (X. W. Wang et al., 2012) has shown that normal genetic variants of AHR are associated with the occurrence of vitiligo. Studies reviewed here strongly suggest that AHR is associated with melanocyte function and number in humans.

Synthesis

No association between the COIs and melanoma was observed in occupational studies that extended the follow-up period for their cohorts of interest (Coggon et al., 2015; Collins et al., 2016). For both studies, the total number of melanoma deaths in each cohort was less than five, leading to imprecise effect estimates. Mechanistic data from animal studies are sparse and have not found associations between the COIs and melanoma. As such, the committee maintains the previous conclusion of inadequate or insufficient evidence to determine whether there is an association between exposure to any one of the COIs and melanoma.

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Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and melanoma (dermal or ocular).

Basal-Cell and Squamous-Cell Cancers (Non-Melanoma Skin Cancers)

Basal- and squamous-cell skin cancers are the most common type of cancer, with an estimated 5.4 million basal and squamous cell skin cancers diagnosed each year (occurring in about 3.3 million Americans since some people have more than one). About 80% of these are basal-cell cancers. The incidence of these cancers has been increasing for several years, and some possible explanations for the increase are better skin cancer detection, people getting more sun exposure, and people living longer (ACS, 2018b).

Excessive exposure to UV radiation is the most important risk factor for non-melanoma skin cancers (ICD-9 173; ICD-10 C44); radiation exposure, HPV, immune suppression, and a family history of non-melanoma skin cancers have also been identified as potential risk factors (Dubas and Ingraffea, 2013). Although exposure to inorganic arsenic is recognized as a risk factor for non-melanoma skin cancers (Bailey et al., 2010; Gilbert-Diamond et al., 2013), this does not imply that exposure to cacodylic acid (DMA), which is a metabolite of inorganic arsenic, can be assumed to be a risk factor. The relevance of aresenicals and DMA to the committee's charge are discussed in Chapter 4.

Conclusions from *VAO* and Previous Updates

Until *Update 1998*, all skin cancers were considered together, and the evidence of an association between the COIs and skin cancers was determined to be inadequate or insufficient. *Update 1998* and all subsequent updates through *Update 2014* concluded that there was inadequate or insufficient information, based on new evidence of studies of veterans, occupational cohorts, and case-control studies, to determine whether there is an association between exposure to the COIs and basal-cell or squamous-cell cancers.

Update of the Epidemiologic Literature

Since *Update 2014*, no additional environmental or case-control studies of non-melanoma skin cancers and exposure to the COIs have been published. Reviews of the relevant studies are presented in the earlier reports. Table 13, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to non-melanoma skin cancers.

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Vietnam-Veteran Studies Nosrati et al. (2014) performed a retrospective medical chart review of 1,499 non-melanoma skin lesions from 1,024 patients seen at a VA medical center from 2003 to 2013 in order to determine the rates of spontaneous regression of residual tumor left behind after biopsy. In patients with positive margins, the absence of tumor tissue in the re-resection sample implies spontaneous regression. The authors hypothesized that Agent Orange–induced tumors would behave more aggressively and be less likely to undergo spontaneous regression. Agent Orange exposure was determined by participation in the Agent Orange Registry (n = 100). The overall regression rates for all non-melanoma subtypes was 43% for the Agent Orange exposed and 41% for unexposed; there was no difference between groups (p = 0.28).

Occupational Studies In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) reported four deaths from "other skin" (not melanoma) cancers among all the workers (SMR = 1.15, 95% CI 0.31–2.93). Two deaths were reported among workers exposed to herbicide levels above background (SMR = 0.81, 95% CI 0.10–2.91), and no deaths were reported among workers exposed for more than 1 year at levels above background. Because of the small number of deaths, the effect estimates are imprecise, which limits their interpretation. This study does not support an association between pheonxy herbicide exposure and non-melanoma/other skin cancer.

Other Identified Studies One other study that reported on the mortality of non-melanoma skin cancers was identified, but it lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Ruder et al., 2014).

Biologic Plausibility

There are no new studies on animal models of skin cancers that are relevant to this update. TCDD and DMA have been shown to produce non-melanoma skin cancers in animal models (Morikawa et al., 2000; Wyde et al., 2004). As discussed elsewhere in this chapter, TCDD and DMA are known tumor promoters and each could act as a promoter for skin cancer initiators, such as UV radiation.

Synthesis

The two new studies of non-melanoma skin cancer do not support any change in the conclusion of inadequate or insufficient evidence to determine whether an association with exposure to at least one of the COIs exists. In the study of veterans seen at a VA medical center, exposure was based on self-report and participation in the Agent Orange Registry (Nosrati et al., 2014). Tumors

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among the Agent Orange–exposed participants were not more aggressive or less likely to undergo spontaneous regression than those in the unexposed group. In a study extending the follow-up period of UK men who worked in factories manufacturing or formulating a variety of phenoxy herbicides or who were contract workers spraying the compounds, Coggon et al. (2015) reported small numbers of non-melanoma skin cancer deaths (n = 4) among all workers, yielding unstable and imprecise risk estimates that do not support an association between pheonxy herbicide exposure and non-melanoma skin cancer. Although TCDD has been previously shown to produce non-melanoma skin cancers in animal models (Wyde et al., 2004), no new studies on animal models of skin cancers have been published.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and basal-cell or squamous-cell skin cancers.

BREAST CANCER

Breast cancer (ICD-9 174 for females, ICD-9 175 for males; ICD-10 C50 for both males and females) is the second most common type of cancer (after non-melanoma skin cancers) in women in the United States (ACS, 2018c). For 2018, NCI estimated that in the United States, there would be 266,120 incident cases of breast cancer and 40,920 deaths from it (NCI, n.d.n). Overall, those numbers represent 15% of all new cancer diagnoses and 6.8% of all cancer deaths. Breast cancer in men is much less common; the American Cancer Society estimated that in 2018 in the United States, there would be 2,550 incident cases of and 480 deaths from breast cancer in men (ACS, 2018d).

Breast cancer incidence generally increases with age; the median age of diagnosis is 62 years for females. In the age groups of most Vietnam veterans, the incidence in men is higher in blacks than in whites, while in women the incidence is generally higher in whites than blacks (NCI, n.d.n). The age-adjusted modeled incidence rate of breast cancers for women 50–64 years old of all races combined was 265.5 per 100,000 for 2000–2014 and increased to 448.6 for 65- to 74-year-olds and drops to 412.1 for women over 75 years.[10] The roughly 5,000–8,000 female Vietnam veterans who were potentially exposed to herbicides in Vietnam would now be menopausal. Given the high incidence of breast cancer in older and

[10]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using the SEER 18 dataset, and choosing age-adjusted rates, breast cancer, female, all races, and the appropriate age range.

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postmenopausal women in general, it is expected on the basis of demographics alone that the breast cancer burden in female Vietnam veterans will be increasing in the near future.

The age-adjusted modeled incidence of breast cancers for men 65 years and older for all races combined was 6.4 per 100,000 for 2000–2014.[11] Breast cancer incidence in men has increased over the past 30 years (Kamińska et al., 2015). However, as the majority of breast cancer epidemiologic studies involve women, although instances of male breast cancer are noted below when they have been reported, the committee's conclusions are based on the studies in women.

Established risk factors for women other than age include a personal or family history of breast cancer, alcohol consumption, and some characteristics of reproductive history, specifically early menarche, late onset of menopause, and either no pregnancies or a first full-term pregnancy after the age of 30 years (Kamińska et al., 2015). In a meta-analysis of studies on alcohol consumption and female breast cancer, Corrao et al. (2004) reported that, in comparison to those who never drank, light drinkers (≤ 1 drink/day or 12.5 g/day) had an elevated pooled relative risk (RR = 1.25, 95% CI 1.20–1.29), and the risk was markedly increased (RR = 1.55, 95% CI 1.44–1.67) for heavy drinkers (≥ 4 drinks/day or 50 g/day). Other post-menopausal lifestyle risk factors for breast cancer include high BMI/obesity and physical inactivity. In addition, breast cancer risk is increased by the prolonged use of hormone-replacement therapy, particularly preparations that combine estrogen and progestins, whereas estrogen-only therapy (only applied in women without a uterus) slightly decreased the risk (Anderson et al., 2004; Chlebowski et al., 2003). The potential of other personal behavioral and environmental factors (including the use of exogenous hormones) to affect breast cancer risk is being studied extensively.

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and breast cancer. The additional information available to the committees through *Update 2014* did not change that conclusion. Several studies with positive, but mostly not statistically significant, findings in different populations and with different COI exposures have since been reviewed (for example, updates from the Seveso Women's Health Study, women employed at an insecticide/herbicide plant in Hamburg, Dow's Michigan plant cohort, the NIOSH PCP cohort). However, due to the null findings on mortality from breast cancer in the important cohorts of female Vietnam-era veterans (Cypel and Kang,

[11]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by choosing incidence by cancer site for the SEER 18 dataset and using age-adjusted rates, breast cancer, all races, male sex, and selecting 65 years and older.

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2008; Dalager et al., 1995a; H. K. Kang et al., 2000b, 2014a; Thomas et al., 1991) as well as other U.S. and foreign cohorts of male Vietnam veterans (ADVA, 2005b,c; CDVA, 1997; Yi and Ohrr, 2014) and the inconsistent findings of risk in studies of the incidence of breast cancer in several occupational cohorts and the Seveso study, the committees have maintained that breast cancer should remain in the category of inadequate or insufficient evidence to determine whether there is an association.

Update of the Epidemiologic Literature

No studies of breast cancer among Vietnam veterans or occupational cohorts of exposure to the COIs and breast cancer have been published since *Update 2014*. Reviews of the relevant studies are presented in the earlier reports. Table 14, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to breast cancer.

Environmental Studies

Morgan et al. (2017) used 1999–2004 NHANES data to examine the association between breast cancer and environmental exposure to PCBs, bisphenol A (BPA), and phthalates. The study sample included women, ages 20–85 years, who participated in NHANES by completing questionnaires and for whom data were available on serum concentrations of PCBs and urinary phthalate and BPA. Cases were defined as women who self-reported breast cancer, and controls were chosen as those who had specifically responded that they did not have breast cancer. The women were grouped by age for analysis (20–59 years, 60–74 years, and 75 years and above). Serum levels of PCBs 74, 99, 118, 138, 153, and 180 were measured, as were the sums of dioxin-like PCBs (PCBs 74 and 118) and non-dioxin-like PCBs for 2,007 women. Urinary BPA and seven urinary phthalate metabolites were also analyzed (but the information is not presented, as these chemicals are not related to the COIs). Concentrations of all individual PCBs and sums of PCBs increased with age. When cases were compared with controls, the age-adjusted geometric means for individual PCB concentrations were significantly higher in cases for the non-dioxin-like PCBs only. When stratified by age groups, non-dioxin-like PCBs were significantly higher in cases versus controls only for the 20- to 59-year age group. When examined by ethnic background, the women with breast cancer who identified race as "other" had significantly higher levels of all PCBs (including the dioxin-like PCBs) except PCB 180 in comparison to non-Hispanic whites with breast cancer, with a geometric mean (standard deviation) for PCB 74 of 27.4 (1.05) versus 13.5 (1.07), $p < 0.05$; and for PCB 118 of 32.8 (1.01) versus 16.4 (1.21), $p < 0.05$. To further examine the relationship of PCB concentration and breast cancer, cases and controls were divided into two groups, PCB levels ranging from below the limits of detection

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to the 50th percentile (reference) and those above the 50th percentile (no cases had PCB concentrations below the limits of detection). The geometric means of the PCB concentrations were significantly higher in cases versus controls for all PCBs, but when this model was adjusted for age, BMI, race/ethnicity, lactation, and age of menarche, the concentrations of non-dioxin-like PCBs 138 and 180 were imprecise, but significantly associated with breast cancer. For comparisons of the dioxin-like PCBs above the 50th percentile versus the reference, after full adjustment in the model, neither PCB 74 (OR = 2.64, 95% CI 0.59–12.0) or PCB 118 (OR = 2.01, 95% CI 0.48–8.44) or their sum (OR = 1.58, 95% CI 0.29–8.53) was statistically significantly associated with breast cancer. The authors then performed an analysis using the same reference group of women with PCB levels from below the limits of detection to the 50th percentile (reference) and comparing them with two groups of higher PCB exposure: those between 50th and 75th percentile and those above the 75th percentile. This model included adjuments for age, BMI, and race/ethnicity, and only non-dioxin-like PCB 138 was significantly associated with breast cancer in the 50th to 75th percentile (OR = 2.93, 95% CI 1.04–8.26) and the above 75th percentile groups (OR = 3.43, 95% CI 1.13-10.4). For the dioxin-like PCBs, neither the 50th to 75th nor the above 75th percentiles were statistically significant for PCB 74 (OR = 1.79, 95% CI 0.20–10.8 and OR = 1.65, 95% CI 0.24–11.4, respectively) or PCB 118 (OR = 1.27, 95% CI 0.21–7.79 and OR = 1.45, 95% CI 0.26–7.92, respectively). Likewise when the dioxin-like PCBs were summed and entered into the fully adjusted model, no statistically significant associations were found. Thus, in adjusted models that account for known risk factors for breast cancer, dioxin-like PCB were not associated with increased risk.

Danjou et al. (2015) reported findings from a prospective study of dioxin exposure through dietary intake and risk of breast cancer using a subset of women who participated in the French E3N study. Women in the E3N study were born between 1925 and 1950 and, when enrollment began in 1990, were members of a national teachers' health insurance plan. Participants completed a dietary survey in 1993 and were followed through 2008, with additional questionnaires on lifestyle, health status, and medical history completed every 2–3 years. The current analysis was limited to 63,830 women who did not have a cancer diagnosis (except non-melanoma skin cancer) and for whom follow-up data were available (including height and weight). Women who had never menstruated were excluded. The occurrence of breast cancer was ascertained by self-report from the health questionnaires, but 92% of cases were confirmed by pathology reports. The study team used the dietary questionnaires to calculate the amount and the frequency of various food groups (meat, seafood, fruits and vegetables, eggs, dairy) consumed by the individual women, and then dioxin levels in various foods were estimated using a database from a large public health study that had measured dioxin levels in various foods during a time period similar to that of the E3N study. During the 14.9 years of follow-up, there were 3,465 incident cases of breast cancer. The

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average daily dioxin exposure was 1.3+/– 0.4 pg TEQ/kg of bodyweight per day (range 0.1–5.7), which is below the acceptable WHO level of 2.3 pg TEQ/kg of bodyweight per day. Only 2.7% of the women in the study had higher levels of exposure. Hazard ratios were calculated for pre- and post-menopausal breast cancer risk per increased intake of 0.43 pg/kg/day (one standard deviation) and per quartiles of estimated dietary dioxin exposure. No increased risk of breast cancer was found. After adjusting for multiple factors, a decreased HR was found for all types of breast cancer in postmenopausal women at the highest quartile of exposure (HR = 0.77, 95% CI 0.54–1.06). For post-menopausal women in the highest quartile of exposure, the HR for ER+/PR+ (estrogen and progesterone receptor positive) tumors, corrected for multiple risk factors, was again decreased (HR = 0.91, 95% CI 0.75–1.10). Moreover, among postmenopausal women there was an inverse relationship between dioxin exposure and ER-/PR- breast cancer. This finding is not unique and has been reported by others. Signaling through AHR may have an antiproliferative effect, or the effect may depend on the timing of exposure. The analysis by Danjou et al. was well powered, had a long length of follow-up (which allowed for an adequate latency period), had few missing data, and used validated questionnaires. Limitations include recall bias for dietary history, the fact that the dioxin sampling did not include all foods consumed, and the source of the food was not considered. Some food could have been more contaminated if grown or produced near dioxin sources. Estimates of dietary dioxin exposure cannot be generalized to the French population as the exposure levels are highly dependent on the food groups consumed. There was no increased risk for breast cancer in this primarily postmenopausal group of women who were apporoximate the age of the female Vietnam veterans. Most of the women had low levels of exposure (below what WHO has reported as safe), and even the women in the highest quartile of exposure (> 1.52 pg/kg of bodyweight per day) did not have an increased risk for breast cancer.

Case-Control Studies

Arrebola et al. (2015) performed a case-control study of incident non-metastatic female breast cancer cases in two cancer centers in Tunisia. In all, 69 cases were recruited and age matched to 56 female visitors, blood donors, or staff from the two centers who served as the control group. Fasting serum was obtained before any therapeutic intervention, and concentrations of multiple chemicals including β-hexachlorocyclohexane (β-HCH), hexachlorobenzene, heptachlor (a metabolite of dioxin), and oxychlordane were measured by gas chromatography with micro-electron capture detection. Wet-basis models and lipid-basis models were both performed with no reported difference in results. Serum levels were presented in tertiles or, in the case of β-HCH, above or below the limits of detection. Adjustments were made for age, age at menarche, reproductive history, breastfeeding history, tobacco and alcohol use, and BMI. Data

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were collected on occupational status, urban-versus-rural residence, education levels, family history, and marital status. The cases were more likely to be less educated, rural dwellers, and postmenopausal than the controls. Serum concentration levels of β-HCH and heptachlor were significantly higher in the cases: β-HCH mean (standard deviation) 25.17 (57.74) ng/g lipid versus below limits of detection in controls, $p = 0.003$; heptachlor, 22.49 (15.12) ng/g lipid versus 14.48 (14.86) ng/g lipid in controls, $p = 0.001$. In adjusted models, β-HCH (OR = 3.44, 95% CI 1.30–9.72) and the third tertile of hepatachlor (OR = 1.06, 95% CI 1.00–1.15) were elevated, but the estimate for β-HCH was imprecise. When heptachlor, β-HCH, and p,p'-DDE were included in a model that was adjusted for covariates and used all three chemicals as continuous variables, only β-HCH remained associated with elevated odds of breast cancer (OR = 1.18, 95% CI 1.05–1.34). The results suggest a potential relationship between β-HCH levels and breast cancer, but the results should be interpreted with caution and require verification. Heptachlor has dioxin-like properties and is the chemical in this study most related to the COIs, but it was not associated with increased odds of breast cancer in the most rigorous model, and therefore, this analysis does not add sufficient data to the weight of the evidence regarding the association between exposure to the COIs and female breast cancer.

Yang et al. (2015) conducted a hospital-based case-control study of women in China to compare levels of organochlorine pesticides in serum and breast adipose tissue and infiltrating ductal carcinomas. The women were recruited over 19 months from 2005 through 2006, and the final study sample consisted of 75 women with infiltrating ductal carcinoma, 79 women with benign conditions, and 80 healthy women (no breast conditions); the latter two groups served as controls. Pesticide residues were measured and included 4 isomers of 1,2,3,4,5,6-HCH (α,β,γ,δ), which exhibit dioxin-like properties, and other organochlorine pesticides and metabolites. β-HCH was one of two residues detected in the serum, and the concentration was statistically significantly higher in the women with breast cancer than in the women with benign breast disease or the healthy controls (3.42 mg/L versus 0.60 mg/L and 0.58 mg/L, respectively, $p < 0.05$). β-HCH, pp'-DDE, and PCTA residues were detected in breast adipose tissue, with the concentrations of β-HCH statistically significantly higher in the women with breast cancer than in the women with benign breast tissue (219.34 mg/L versus 57.84 mg/L, $p < 0.05$). The serum concentration of β-HCH increased with differentiation and was significantly elevated for well-differentiated versus poorly-differentiated tumors (4.92 mg/L and 2.32 mg/L, $p < 0.05$, respectively). β-HCH was significantly elevated in breast adipose tissue in well-differentiated (303.6 mg/kg) compared with moderately-differentiated (214.0 mg/kg) and poorly-differentiated tumors (147.8 mg/kg) ($p < 0.05$). When examined by estrogen receptor status, ER+ tumors had significantly higher levels of β-HCH in the breast adipose: 238.78 mg/k versus 141.92 mg/k ($p < 0.05$). The authors propose that organochlorine pesticides acting as

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endocrine disruptors upset the normal estrogen–progesterone balance contributing to breast cancer. The higher levels of organochlorine pesticide residues in blood and breast adipose tissue imply an association with infiltrating ductal carcinoma, but further work is needed to determine causality.

Other Identified Studies

Four other studies that reported outcomes of breast cancer were identified, but all lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. The first study examined breast cancer mortality in an occupational cohort of both women and men who worked in capacitor manufacturing and who were exposed to mixed PCBs as well as several other chemicals and metals, but no additional information was provided regarding the specific PCBs or objective measures of exposure (Ruder et al., 2014). Benedetti (2017) conducted an ecologic analysis to determine breast cancer (and other cancer) incidence rates at 14 of Italy's national priority contaminated sites and compared the rates among those sites. Although the known exposures at these sites consisted of mixtures of PCBs, furans, benzene and other solvents, arsenic, and cadmium, they were not measured or specified in enough detail to indicate which of the COIs were present and to what extent. Finally, two studies reported on breast cancer outcomes following the 1968 Yusho accident in Japan, where people were exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil. Kashima et al. (2015) compared mortality rates from breast cancer using an ecologic design to define exposure (likely introducing selection bias) and did not use individual serum samples or previously published measurements. Akahane et al. (2017) examined the prevalence of self-reported long-term health effects, including breast cancer, in people exposed via the Yusho accident compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

The experimental evidence indicates that 2,4-D, 2,4,5-T, and TCDD are weakly genotoxic at most. However, TCDD is a demonstrated carcinogen in animals and is recognized as having carcinogenic potential in humans because of the mechanisms discussed in Chapter 4. There is no evidence from carcinogenicity bioassays that TCDD causes breast cancer in laboratory animals (Baan et al., 2009; IARC, 2012c). However, studies performed in laboratory animals indicate that TCDD may modify the carcinogenic process in the mammary gland and that the effect of TCDD may depend on the age of the animal. Toxicology studies using different rat models have demonstrated that the fetal mammary gland is highly sensitive to dioxin, and severe and persistent mammary-gland

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developmental abnormalities—including decreased ductal branching, delayed epithelial migration into the fat pad, and fewer differentiated terminal end buds—were evident after exposure to a single dose of dioxin during mammary bud development (N. M. Brown et al., 1998; Fenton et al., 2002; Lewis et al., 2001). For example, a single oral exposure of 50-day-old Sprague Dawley rats to 10 µg/kg TCDD 3 days prior to a single administration of the chemical carcinogen dimethylbenzanthracene (DMBA) was found to inhibit mammary-tumor induction (Holcombe and Safe, 1994), while a single 2.5-µg/kg dose of TCDD to 18-day-old rats slightly increased tumor induction when followed by a single injection of the carcinogen methylnitrosourea (MNU) at 21 days of age (Desaulniers et al., 2001).

In a 2015 review of the literature on TCDD and breast cancer, Fenton and Birnbaum suggested a mechanism that may be related to endocrine disruption and that might indicate a close association between the development of mammary cancers and mammary gland differentiation. Agents capable of disrupting the ability of the normal mammary epithelial cell to enter or maintain its appropriate status (a proliferative, differentiated, apoptotic state), to maintain its appropriate architecture, or to conduct normal hormone (estrogen) signaling are likely to act as carcinogens, co-carcinogens, or tumor promoters for the breast (Fenton, 2006; McGee et al., 2006). In that light, it is interesting that prenatal exposure of rats to TCDD was found to alter the proliferation and differentiation of cells in the mammary gland of the dams (Vorderstrasse et al., 2004) and of the offspring (Birnbaum and Fenton, 2003). There is evidence that TCDD directly targets mammary epithelial cells and the surrounding stromal fat cells during pregnancy-induced mammary gland differentiation; this points to interference with stromal–epithelial cross-talk as one of several underlying pathways (Lew et al., 2011). Jenkins et al. (2007) used a rat carcinogen-induced mammary cancer model to show that prenatal exposure to TCDD alters mammary gland differentiation and increases susceptibility to mammary cancers by altering the expression of estrogen-receptor genes and of genes involved in oxidative-stress defense. Thus, the effect of TCDD may depend on the timing of the exposure and on the magnitude of gene expression at the time of exposure; TCDD may influence mammary-tumor development only if exposure to it occurs during a specific window during breast development (Rudel et al., 2011). Susceptibility to breast cancer appears to peak in utero and at puberty, which would not be relevant for female Vietnam veterans, who were potentially exposed as adults. This finding would only be relevant to the female child of a female veteran exposed to the herbicides while pregnant, an unlikely scenario given that few women were stationed in areas where herbicides were known to be sprayed and that pregnant women were barred from duty in Vietnam. The breast is the only human organ that does not fully differentiate until it becomes ready for use; nulliparous women have less-differentiated breast lobules, which are presumably

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more susceptible to carcinogenesis. Pregnancy is protective, particularly if carried to full term.

Activation of AHR by dioxin or by the non-dioxin ligand indole-3-carbinol has also been shown to protect against experimental breast cancer by mechanisms that disrupt migration and metastasis (Bradlow, 2008; Hsu et al., 2007). The administration of TCDD to mice that harbored highly metastatic murine breast-cancer cells in the mammary fat pad reduced the rate of metastasis by 50% without suppressing primary tumor size—an indication that TCDD's protective effects are selective to the metastatic process (T. Wang et al., 2011). In addition, AHR agonists inhibit the formation of lung metastases by ER-negative breast cancer cells (S. Zhang et al., 2012). Hanieh (2015) and Hanieh et al. (2016) found that TCDD and other AHR agonists suppressed pro-metastatic SOX4 in breast cancer cell lines. However, Spink et al. (2013), using clones derived from the MCF-7 human breast cancer cell line that express different levels of AHR, showed that in nude mice, Ahr expression is not necessary for proliferation, migration, invasion, or tumor growth of ER-positive MCF-7 cells and that the knock-down of Ahr in wild-type MCF-7 cells did not affect the anti-proliferative effect of TCDD (Yoshioka et al., 2012). Also, knock-down of AHR in triple-receptor-negative MDA-MB-231 cells inhibited their in vivo growth and metastases (Goode et al., 2013). Recently Go et al. (2017) showed that 17b-estradiol promoted AHR-dependent CPY1A1 expression in MCF-7 clonal variant cells through an ER pathway. Collectively, these findings suggest that there may be species differences, ER-specific mechanisms, ER-independent mechanisms, or carcinogenic process-specific effects of AHR in breast carcinogenesis. It is possible that some protective effects may be mediated through the known cross-talk between AHR and ERα, which has been studied extensively at the molecular level for potential therapeutic benefit. There is evidence to indicate that AHR controls ERα-regulated gene expression through its effects on DNA methylation (Marques et al., 2013) or through the recruitment of receptor-interacting protein 140 (RIP140), which can both activate and repress ER actions (Madak-Erdogan and Katzenellenbogen, 2012). In the presence of dioxin, AHR can repress specific estrogen-dependent genes in MCF-7 breast cancer cells (Labrecque et al., 2012) and in triple-receptor-negative MDA-MB-231 cells (Goode et al., 2014). TCDD can also activate AHR-mediated G1cell-cycle arrest (Barhoover et al., 2010); however, in the presence of progesterone receptor, TCDD enriches the G2/M phase and stimulates the proliferation of MCF-7 cells (Y. J. Chen et al., 2012). Together, these results demonstrate a complicated interplay between the AHR and other nuclear transcription factors, including steroid hormone receptors, which can either stimulate or inhibit breast cancer growth in a manner that depends on cell context. The growth of MCF-7 cells as mammospheres appears to be negatively regulated by AHR (Zhao et al., 2012), but in the context of an inflammatory microenvironment and HER2 overexpression, the opposite effect has been reported (Zhao et al., 2013). Saito et al. (2017) found that TCDD-activated AHR stimulated the estrogen-dependent progression of breast

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carcinoma by inducing aromatase expression under some conditions; although AHR activation has also been shown to inhibit the ER pathway, the AHR-induced aromatase activity persisted for a longer duration than the ER inhibition in three breast carcinoma cell lines.

TCDD may affect breast carcinogenesis by silencing the Brca-1 tumor suppressor gene through promoter hypermethylation, thereby impairing DNA repair (Papoutsis et al., 2012). TCDD has also been shown to modulate the induction of DNA-chain breaks in human breast cancer cells by regulating the activity of the enzymes responsible for estradiol catabolism and generating more reactive intermediates, which might contribute to TCDD-induced carcinogenesis by altering the ratio of 4-OH-estradiol to 2-OH-estradiol, a marker of breast cancer risk (La Merrill et al., 2010; P. H. Lin et al., 2007, 2008). A similar imbalance in metabolite ratios has been observed in pregnant Taiwanese women, in whom the ratio of 4-OH-estradiol to 2-OH-estradiol decreased with increasing exposure to TCDD (S. L. Wang et al., 2006). The expression of *CYP1B1*, the cytochrome P450 enzyme responsible for 2-OH-estradiol formation, but not of *CYP1A1*, the one responsible for 4-OH-estradiol formation, was found to be highly increased in premalignant and malignant rat mammary tissues in which Ahr was constitutively active in the absence of ligand (X. Yang et al., 2008). On the basis of recent mechanistic data, it has been proposed that AHR contributes to mammary-tumor cell growth by inhibiting apoptosis while promoting the transition to an invasive, metastatic phenotype (Marlowe et al., 2008; Schlezinger et al., 2006; Vogel et al., 2011). There is also evidence showing that AHR activation by TCDD in human breast and endocervical cell lines induces sustained high concentrations of the IL-6 cytokine, which has tumor-promoting effects in numerous tissues, including breast tissue, suggesting that TCDD might promote carcinogenesis in these tissues (DiNatale et al., 2010; Hollingshead et al., 2008). Similarly, TCDD induced IL-8 expression in an AHR-dependent manner and may contribute to inflammatory breast cancer (Vogel et al., 2011). Degner et al. (2009) have shown that AHR ligands can upregulate the expression of COX-2, which may lead to a proinflammatory local environment that can support tumor development.

Synthesis

In the early 1990s it was suggested that exposure to some environmental chemicals, such as organochlorine compounds, might play a role in the etiology of breast cancer through estrogen-related pathways. The relationship between organochlorines and breast cancer risk has been studied extensively, especially in the past decade; TCDD and dioxin-like chemicals have been among the organochlorines so investigated.

Some well-designed environmental and case-control studies with good exposure assessment found statistically significant increased risk of breast cancer (Bertazzi et al., 1993; Pesatori et al., 2009; Reynolds et al., 2005; Viel et al.,

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2008a; Warner et al., 2011). On the other hand, no increased risk of breast cancer mortality was observed in the cohorts of female Vietnam-era veterans (Cypel and Kang, 2008; Dalager et al., 1995a; Kang et al., 2014a; Thomas et al., 1991) and Seveso residents (Consonni et al., 2008). The data on male breast cancer from the Korean veterans study are sparse and imprecise mainly due to the very low incidence of breast cancer in men (Yi and Ohrr, 2014). The findings of breast cancer risk in follow-up studies of cancer incidence in Seveso were inconsistent (Pesatori et al., 2009; Warner et al., 2011). An increase in the SMR for breast cancer was observed in the Hamburg cohort of workers exposed to dioxin (Manuwald et al., 2012), while the study of the Dow 2,4-D production workers had null findings (C. J. Burns et al., 2011).

The two new environmental studies do not support an association with exposure to dioxin and breast cancer incidence (Danjou et al., 2015) or exposure to dioxin-like PCBs and breast cancer (Morgan et al., 2017). The results of the case-control studies were mixed. Arrebola et al. (2015) did not find an association with dioxin-like chemicals, β-HCH and heptachlor, and breast cancer in adjusted models. But C. Y. Yang et al. (2015) showed higher levels of β-HCH in the serum of breast cancer patients versus controls as well as higher levels in ER+ versus ER- tumors and well-differentiated versus poorly differentiated tumors. The authors propose a likely association between the endocrine disrupter organochlorine pesticides in the breast adipose and serum and breast cancer. Furthermore, because several organochlorine residues, not considered relevant to this study, were also measured and also found to have statistically significant positive associations with ER+ and well-differentiated tumors, it is not clear which (or if all) of these agents are responsible for the increased risk of breast cancer.

The main strength of these studies was the availability of organochlorine pesticide levels in blood (Morgan et al., 2017; C. Y. Yang et al., 2015) and breast adipose tissue (C. Y. Yang et al., 2015). The data from C. Y. Yang and colleagues showing significantly elevated concentrations of β-HCH in well-differentiated ER+ tumors aligned with the role of endocrine disrupters in breast cancer oncogenesis. However, Morgan et al. (2017) found a greater association with non-dioxin-like PCBs than with dioxin-like PCBs and breast cancer. TEQ data were not presented for the dioxin-like PCBs, and of the dioxin-like PCBs measured, PCB 118, a mono-ortho is given low consideration as it contributes less than 10% to total TEQs.

Biological mechanistic data from cell lines and non-primate animal models have provided insight into a number of ways in which TCDD and related chemicals may interact with AHR, ER, aromatase enzyme, inflammatory cytokines, DNA repair genes, and the stroma to modulate events leading to breast cancer. Pre-clinical studies have shown that the timing of the exposure to the COIs is critical, with in utero and pubertal breast tissue being most sensitive to carcinogenic effects. Data also suggest that AHR agonists are protective against breast cancer via the modulation of signaling pathways involved in proliferation and

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metastases. Despite the advances in understanding the various effects of TCDD and related chemicals on breast cell metabolism, there is no experimental evidence to support the hypothesis that TCDD by itself is a breast tissue carcinogen or that it enhances breast carcinogenesis.

Conclusion

Having considered the new evidence and the results of studies reviewed in previous updates, the present committee concludes that there is inadequate or insufficient evidence to determine whether there is an association (either positive or negative) between exposure to the COIs and breast cancer.

CANCERS OF THE FEMALE REPRODUCTIVE SYSTEM

This section addresses cancers of the cervix (ICD-9 180; ICD-10 C53), endometrium (also referred to as the corpus uteri; ICD-9 182.0–182.1, 182.8; ICD-10 C54), and ovary (ICD-9 183.0; ICD-10 C56). Additional cancers of the female reproductive system that are infrequently reported separately are cancers of the uterus not otherwise specified (ICD-9 179; ICD-10 C55), placenta (ICD-9 181; ICD-10 C58), fallopian tube and other uterine adnexa (ICD-9 183.2–183.9; ICD-10 C57.0–C57.4), and other female genital organs (ICD-9 184; ICD-10 C57, C58); findings on these cancers are included in this section. NCI estimates of the numbers of new female reproductive-system cancers in the United States in 2018 are presented in Table 7-1; they represent roughly 12% of new cancer cases and 11% of cancer deaths in women (NCI, n.d.o–r).

Cervical cancer occurs more often in blacks than in whites, but endometrial and ovarian cancers occur more often in whites. The incidence of endometrial and ovarian cancers is higher in older women and in those who have family histories of these cancers. The use of unopposed (without progestogen) estrogen-hormone therapy and obesity, which increases endogenous concentrations of estrogen, increases the risk of endometrial cancer. HPV infection, particularly infection with HPV types 16 and 18, is the most important risk factor for cervical cancer (McGraw and Ferrante, 2014).

The age-adjusted modeled incidence rate of female genital system cancers (which includes the cervix uteri, corpus and uterus not otherwise specified, ovary, vagina, and vulva) for women 50–64 years old of all races combined was 114.2 per 100,000 in 2015 and increased to 169.1 for 65–74 year olds and dropped to 147.3 for women over 75 years.[12] The roughly 7,500 female Vietnam veterans who were potentially exposed to herbicides in Vietnam would now be menopausal.

[12]Modeled incidence rates as calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using the SEER 18 dataset and choosing age-adjusted rates, female genital system, all races, and age groups 50–64, 65–74, and ≥ 75 years.

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TABLE 7-1 Estimates of New Cases and Deaths from Selected Cancers of the Female Reproductive System in the United States in 2018

Site	Estimated Incidence	Estimated Deaths
Cervix	13,240	4,170
Endometrium	63,230	11,350
Ovary	22,240	14,070
Vagina and other female genital	6,190	1,200

SOURCES: NCI, n.d.o–r.

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and female reproductive cancers. Additional information available to the committees responsible for subsequent updates through *Update 2014* has not changed that conclusion.

Results from three cohorts of U.S. female veterans who were followed for overall and specific-cause mortality from 1992 through 2010 (H. K. Kang et al., 2014a) were reviewed in *Update 2014*. Among the three cohorts of U.S. women veterans—those who served in Vietnam (n = 4,734), those who served in countries near Vietnam (n = 2,062), and those who did not deploy (n = 5,313)—very few deaths from cervical cancer were observed: five among those who served in Vietnam, one among those who served near Vietnam, and six among those who were non-deployed. Compared with the general population of U.S. women, SMRs were lower for each cohort (SMRs between 0.27 and 0.65) but the estimates were imprecise due to the small number of cervical cancer deaths. In comparison with non-deployed female Vietnam-era veterans, those who served in Vietnam had no excess cervical cancer mortality. A further analysis restricted to female nurses, again using the non-deployed cohort as the referent, yielded virtually the same nonstatistically significant risk of mortality from cervical cancer. Similarly, there were also very few observed uterine cancer deaths of women who served in Vietnam, served near Vietnam, or were non-deployed, with 9, 4, and 12 deaths, respectively, and no excess risk of uterine cancer mortality was found in any of the three cohorts when compared with the general population. In the internal comparison to non-deployed Vietnam-era veterans, uterine cancer mortality was not associated with service in Vietnam or near Vietnam. Similar results were observed in the analysis restricted to only nurses. There were more deaths from ovarian cancer in the entire cohort, but no differences in the risk of ovarian cancer mortality were found among those who served in Vietnam, served near Vietnam, or were non-deployed in comparison with the general population of U.S. women. In the internal comparison with the non-deployed veterans, ovarian cancer

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mortality was increased among Vietnam veterans and among women who served near Vietnam, but neither was statistically significant. An analysis restricted to nurses revealed similar patterns of increased (albeit not statistically significant) ovarian cancer mortality, both for veterans who served in Vietnam and for veterans who served near Vietnam, when compared with non-deployed nurses.

Update of the Epidemiologic Literature

Relevant studies on cancers of the female reproductive system include the cervix, uterus, ovary, and vagina. No studies of female reproductive cancers among Vietnam veterans have been published since *Update 2014*. No occupational cohort, environmental, or case-control studies of exposure to the COIs and female reproductive cancers have been published since *Update 2012*. Reviews of the relevant studies are presented in the earlier reports. Tables 15, 16, and 17, which can be found at www.nap.edu/catalog/25137, summarize the results of studies related to female cervical, uterine, and ovarian cancer.

Other Identified Studies

Three studies that reported outcomes of uterine or ovarian cancer were identified, but all lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. The first study examined mortality from malignant neoplasms of female genital organs in an occupational cohort of capacitor manufacturers who were exposed to mixed PCBs as well as to several other chemicals and metals, but no additional information was provided regarding the specific PCBs or objective measures of exposure (Ruder et al., 2014). Two studies reported on mortality from (Kashima et al., 2015) or the prevalence of (Akahane et al., 2017) uterine or ovarian cancer outcomes related following the 1968 Yusho accident in Japan, where people were exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil. Both lacked the necessary exposure specificity to be considered further.

Biologic Plausibility

Yoshizawa et al. (2009) showed that the chronic administration of TCDD and other AHR ligands to adult female Harlan Sprague Dawley rats results in chronic inflammation and an increased incidence of reproductive-tissue preneoplasia and tumors, including cystic endometrial hyperplasia and uterine squamous-cell carcinoma. The mechanism of action might be related to endocrine disruption and chronic inflammation. Qu et al. (2014) observed increased mRNA and protein expression of AHR in human endometrial cancer tissue and human endometrial cancer cell lines (Ishikawa and ECC-1) compared with nonmalignant

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endometrium; increased AHR expression in human endometrial cancer tissue compared to nonmalignant tissue has also been reported by D. Li et al. (2013).

Qu et al. (2014) showed that a polycyclic hydrocarbon known to be an AHR ligand inhibited the proliferation of Ishikawa and ECC-1 cells mediated in part by AHR. Wormke et al. (2000) reported that TCDD inhibited the proliferation of Ishikawa endometrial cancer cells stimulated by estradiol and also reduced estrogen receptor activity, but increased AHR-mediated gene expression in these cells, suggesting that the estrogen receptor, not the AHR, mediates the anti-proliferative effect of TCDD in the Ishikawa endometrial cancer cells. Y. Li et al. (2014) showed that TCDD-AHR activation inhibited cell proliferation in human ovarian cancer (OVCAR-3) cells. Hollingshead et al. (2008) showed that TCDD activation of AHR in human breast and endocervical cancer cell lines induces sustained high concentrations of the IL-6 cytokine. It is noteworthy that the effects of TCDD treatment differed between MCF-7 breast cancer cells and ECC-1 endometrial carcinoma cells with respect to the activation and repression of genes; this illustrates the role of cell context and organ specificity in responses to TCDD by cancer cells (Labrecque et al., 2012).

Synthesis

Compared with other cancer types, relatively few studies have reported on associations between any of the COIs and female reproductive cancers. The most relevant evidence came from a follow-up study on mortality among female U.S. Vietnam-era veterans that was reviewed in *Update 2014*. For both cervical and uterine cancers there was no evidence of increased mortality risk; however, the small observed number of deaths for these outcomes in all three cohorts limited the statistical power of the associations. With regard to ovarian cancer, there was some evidence of a slightly elevated mortality in veterans who served either in or near Vietnam, but for both risks the CIs were large and their point estimates imprecise. However, because the rate of ovarian cancer mortality was similar between veterans who served in Vietnam (with potential exposure to herbicides) and those who served near Vietnam (who presumably were not so exposed), this evidence is equivocal.

Most findings from occupational cohorts and environmental studies for which exposure was well characterized have not found increased risks for cervical, uterine, or ovarian cancers. Follow-ups of the Seveso cohort (where residents were exposed to TCDD following an explosion of the chemical plant) have not found increased incidence of or mortality from uterine cancer (Bertazzi et al., 2001; Consonni et al., 2008; Pesatori et al., 2009). No new studies with sufficient exposure specificity were identified for the current update.

The results of mechanistic studies provide more plausibility for a reduced risk of female reproductive cancers than for an increased risk. Therefore, the conclusion

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of inadequate or insufficient evidence of an association between the COIs and uterine, ovarian, or cervical cancers remains unchanged.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and uterine, ovarian, or cervical cancers.

PROSTATE CANCER

The prostate gland lies just below the urinary bladder and surrounds the urethra. It makes fluid that forms part of semen. NCI estimated that 164,690 new cases of prostate cancer (ICD-9 185; ICD-10 C61) would be diagnosed in the United States in 2018 and that 29,430 would die from it (NCI, n.d.s). That makes prostate cancer the second most common cancer in men (after non-melanoma skin cancers); it is expected to account for about 9.6% of all new cancer diagnoses and 4.4% of all cancer deaths in 2017. The incidence of and mortality from prostate cancer varies widely with age and race. The incidence rate of prostate cancer for all races combined more than doubles from the ages of 50–64 years (207.3 per 100,000) to the ages of 65–74 years (579.5 per 100,000). The incidence rate of prostate cancer for men aged 75 years and older decreases slightly, but remains high (432.7 per 100,000). As a group, African American men have the highest recorded incidence of prostate cancer in the world (Jemal et al., 2011); their risk is roughly 40% higher than that of whites in the United States and three times that in "American Indians/Alaska natives" (NCI, n.d.s).

Little is known about the causes of prostate cancer. Other than race and age, the risk factors include a family history of the disease both in first- and second-degree relatives (Bruner et al., 2003; Zeegers et al., 2003). A review of occupational factors found heavy metals, PCBs, and PAHs to be associated with prostate cancer but did not specify particular pesticides (Doolan et al., 2014). There is some evidence that some elements of the Western diet, including a high consumption of red meat and saturated fats, may be a risk factor for prostate cancer, but these have not been conclusively identified. Of note, selenium and vitamin E supplementation did not reduce, but rather slightly increased, prostate cancer incidence in a large clinical trial (Klein et al., 2011; Kristal et al., 2014; Lippman et al., 2009), and soy protein supplementation did not prevent the recurrence of prostate cancer after surgical treatment in a randomized study (Bosland et al., 2013). The 5α-reductase inhibiting drugs finasteride and dutasteride, which are widely used to treat benign enlargement of the prostate, were found to decrease the prevalence of prostate cancer by about 25% in two major randomized trials

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(Andriole et al., 2010; Thompson et al., 2003); however, in the finasteride trial, the risk of high-grade prostate cancer was increased. Finasteride acts by decreasing the formation of the potent androgen metabolite 5α-dihydrotestosterone in the prostate.

Study of the incidence of and mortality from prostate cancer is complicated by various approaches to screening for the disease in different countries and populations. The widespread adoption of serum prostate-specific antigen (PSA) screening in the 1990s led to very large increases in reported prostate cancer incidence in the United States, which have recently subsided as exposure to the screening has become saturated. However, PSA screening has recently come under scrutiny and is no longer uniformly recommended or consistently applied in the United States, following a D grade recommendation from the U.S. Preventive Services Task Force in 2012 (Moyer et al., 2012).

Prostate cancer tends not to be fatal in many cases (NCI estimates a 98% 5-year survival rate), particularly for screening-detected (i.e., localized stage/well-differentiated grade) prostate cancer, so mortality studies may miss an increase in the incidence of the disease and thus potentially misclassify the outcome. In addition, findings that show an association between an exposure and prostate cancer mortality should be examined closely to determine whether the exposed group had poorer access to screening or treatment that would have decreased the likelihood of survival.

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of an association between exposure to the COIs and prostate cancer, based on positive associations observed in occupational and environmental studies. Additional information from various epidemiologic studies—including the AFHS and ACC, veterans seen in VA medical facilities, and occupational cohorts of phenoxy chemical factory workers and pesticide applicators from the AHS—reviewed in subsequent updates has not changed that conclusion.

Four studies of prostate cancer and Vietnam veterans were reviewed in *Update 2014*, two among veterans at VA medical facilities and two international cohort studies of male Vietnam veterans from New Zealand and Korea. A study of U.S. Vietnam veterans who were referred to the Portland VA Medical Center with an elevated serum PSA and who underwent an initial prostate biopsy reported a statistically significant association between herbicide exposure (7.5% of cases classified as herbicide-exposed) and the overall risk of prostate cancer after adjustment for age and the receipt of screening (Ansbaugh et al., 2013). Stratifying tumors by grade and characteristics led to a stronger association between herbicide exposure and intermediate- to high-grade prostate cancer and an even stronger association with more aggressive prostate cancer. Although this study had a relatively large sample size, it is limited by uncertainty about individual exposure

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levels and potential selection/referral bias because men who were referred for prostate biopsy probably had an elevated PSA and could possibly have had better access to health care in comparison with other veterans.

A small study using VA administrative databases to determine the relationship between herbicide exposure (determined by self-report and military records confirming that Vietnam veterans had served in an area in which herbicides had been sprayed) and a biochemical recurrence of prostate cancer during an average of 5.3 years of follow-up after prostatectomy was conducted (Q. Li et al., 2013). Subcutaneous adipose tissue was obtained during prostatectomy and assayed for dioxin, and the measured TEQ levels of the 37 men with self-reported herbicide exposure were statistically significantly higher than those of the 56 men who did not report exposure. The proportions of men with biochemical recurrence were not statistically significantly higher for the veterans with self-reported herbicide exposure than for those who did not report exposure or for those with higher TEQ levels compared with those with lower TEQ levels.

In a follow-up study of 2,783 male New Zealand veterans who had served in Vietnam and were still alive as of 1988, McBride et al. (2013) reported that the incidence of prostate cancer was slightly but not statistically significantly increased among the veterans; the difference from the general male population of New Zealand was not statistically significant, nor was the rate of prostate cancer–specific mortality. No information on potential confounding factors was included.

Among Korean veterans who served in Vietnam, a total of 125 incident cases and 53 deaths from prostate cancer were identified during the follow-up period in the cohort studied by Yi and colleagues (Yi, 2013; Yi and Ohrr, 2014; Yi et al., 2014b). When compared with the general Korean population, there was a 22% statistically significant excess prostate cancer risk in the entire cohort (Yi, 2013), which was mostly due to a significant 2.5-fold elevated prostate cancer incidence among officers. In the internal comparison analysis, Yi and Ohrr (2014) reported an inverse association between the EOI scores and prostate cancer incidence, which was based on 53 cases in the high-exposure category. Yi and Ohrr (2014) did not stratify incident prostate cancer cases according to tumor characteristics (low- versus high-grade tumors) as is usually done in studies of prostate cancer incidence. Similarly, Yi et al. (2014b) reported a similar inverse association when examining exposure potential and prostate cancer–specific mortality.

Update of the Epidemiologic Literature

Several new studies reporting associations of prostate cancer or surrogate measures and the COIs were identified. Reviews of the relevant studies are presented in the earlier reports. Table 18, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to prostate cancer.

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Vietnam-Veteran Studies

Ovadia et al. (2015) conducted an analysis of the relationship between self-reported Agent Orange exposure (veterans submit a claim of exposure that is reviewed by VA using service records to confirm whether the veteran was stationed in an area that was sprayed with herbicides during the service period) and long-term outcomes among prostate cancer patients. Data for this analysis were drawn from the Shared Equal Access Regional Cancer Hospital database of 1,882 men undergoing radical prostatectomy for prostate cancer between 1988 and 2011 at six VA health care facilities; 333 men (17.7%) were considered Agent Orange exposed. The clinical outcomes reported included pathologic Gleason Score, pathologic stage, and postoperative pathologic and treatment characteristics and prostate cancer–specific death. Cox proportional hazards regression modeling was used to assess the relationship between exposure to Agent Orange and biochemical recurrence, secondary treatment, metastases, and prostate cancer–specific mortality. Models were adjusted for age, race, clinical stage, PSA level, BMI, center, and biopsy Gleason sum. Agent Orange exposure was not found to be associated with biochemical recurrence (HR = 1.21, 95% CI 0.99–1.49), secondary treatment (HR = 1.21, 95% CI 0.97–1.5), metastases (HR = 0.93, 95% CI 0.30–2.66), or prostate cancer–specific mortality (HR = 0.89, 95% CI 0.46–1.85). The study was generally well conducted, with excellent clinical data and follow-up available within the VA system. Although Agent Orange exposure included an additional level of service location verification to self-report, this measure is still only a proxy for actual initial and subsequent exposure levels. However, a previous study by Q. Li et al. (2013) of 93 men showed good correlation between self-reported Agent Orange exposure and dioxin TEQ levels in adipose tissue. The study's negative results may be applicable to the relationship between Agent Orange and prostate cancer progression, but do not directly address initiation and incidence.

Occupational Studies

Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016) found no differences in mortality from prostate cancer for either the TCP workers (n = 21; SMR = 1.01, 95% CI 0.62–1.54) or the PCP workers (n = 11; SMR = 1.05, 95% CI 0.53–1.87).

In a well-designed and conducted study and analysis, Christensen et al. (2016) examined the interactions among pesticide exposure, single nucleotide polymorphisms (SNPs) of genes in the steroid hormone synthesis, metabolism, or regulation pathway, and the risk of prostate cancer. Interactions with 39 pesticides were examined using 776 cases and controls nested in the AHS cohort of private and commercial pesticide applicators. A total of 1,117 SNPs (in 56 genes) were evaluated. Cases were diagnosed from the period 1993–2004. Controls were

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pesticide applicators frequency-matched 2:1 to cases by age. Lifetime pesticide exposure information was obtained from multiple surveys. Several pesticide exposure metrics were constructed for each pesticide based on the duration and frequency of pesticide exposure. Estimates were adjusted for age and the study site; other covariates of smoking, BMI, and physical activity were also included but did not change the effect estimates. The final analysis included 39 pesticides. Only one multiple-comparisons corrected herbicide–SNP interaction was found—between the herbicide dicamba and a SNP in the testosterone metabolizing gene *SRD5A*. In the cohort, no association for 2,4-D or 2,4,5-T was found, and it appears there are no SNP interactions with these two herbicides. The results suggest that a genetic variation may decrease the risk of prostate cancer with exposure to dicamba. However, this finding requires replication and is not directly useful in drawing any conclusions about the significance of potential positive associations of COIs and prostate cancer.

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) reported deaths from several types of cancer. Prostate cancer was slightly elevated but was not statistically significant for any of the groups of workers: all workers (n = 120; SMR = 1.10, 95% CI 0.91–1.32), workers exposed to herbicide levels above background (n = 89; SMR = 1.14, 95% CI 0.92–1.14), or workers exposed for more than 1 year at levels above background (n = 43; SMR = 1.15, 95% CI 0.83–1.55).

Environmental Studies

In a well-designed and conducted nested case-control study, Koutros et al. (2015) used prospectively collected serum to estimate associations between organocholorine exposures and metastatic prostate cancer in a population-based cohort from Norway. The study sample was identified from the Janus Serum Bank cohort, a population-based research biobank consisting of almost 317,000 individuals with an average age at enrollment of 41 years. The Janus cohort was linked with to the Cancer Registry of Norway to identify new cases of prostate cancer. Only metastatic prostate cancer cases were included to avoid possible detection bias associated with PSA testing. Eligible cases consisted of incident metastatic prostate cancer cases with no history of cancer (except non-melanoma skin cancer) who were diagnosed from enrollment through December 31, 1999, and were diagnosed at least 2 years after serum collection. Controls (up to six per case) were randomly selected male members of the cohort who had no history of cancer (except for non-melanoma skin cancer) at the time of their matched case's diagnosis. Cases (n = 150) and controls (n = 314) were matched on date of blood draw (1-year strata), age at blood draw (2-year strata), and region. Sera concentrations of 11 organochlorine pesticide metabolites and 34 PCB congeners (including five dioxin-like congeners PCB 118, PCB 156, PCB 157, PCB 167,

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and PCB 189) were analyzed for cases and their matched controls in the same laboratory batch. Demographic data and other covariates, including BMI, as well as smoking habits were obtained from baseline questionnaire data at the National Institute of Public Health, and census data were obtained from Statistics Norway. No statistically significant pattern of association was found with regard to any of the dioxin-like PCBs analyzed. Comparing the highest exposure quartile to the lowest quartile, the odds ratios adjusted for country, age at collection, and date of collection, were: PCB 118 (OR = 1.07; 95% CI 0.56–2.02), PCB 156 (OR = 0.80; 95% CI 0.42–1.52), PCB 157 (OR = 0.86; 95% CI 0.43–1.71), PCB 167 (OR = 1.23; 95% CI 0.67–2.29), and PCB 189 (OR = 0.64; 95% CI 0.30–1.37). The power to detect more modest associations was limited in the higher exposure level categories.

Lim et al. (2017) conducted a case-cohort study to evaluate the relationship between serum concentrations of persistent organic pollutants and the incidence of prostate cancer using the Korean Cancer Prevention Study-II cohort. The case–cohort design consisted of 1,879 subjects who were randomly selected from the full Korean Cancer Prevention Study-II cohort of 270,514 individuals who visited 11 national health promotion centers from 1994 to 2013. After excluding women and men with missing data, the subcohort consisted of 831 subjects from which 256 controls and 110 incident cases of prostate cancer (identified through the National Cancer Registry, a nationwide hospital cancer registry covering 99% of all cases diagnosed in South Korea) were selected. Serum concentrations of 32 PCB congeners and 19 organochlorine pesticides were measured and were included individually and in sum in the analysis. The sum of dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) and the sum of non-dioxin-like PCBs was calculated. A TEQ was calculated based on the individual TEF of each PCB congener, and tertiles of concentrations were calculated. Hazard ratios for the association between the chemicals and incidence of prostate cancer were estimated using the weighted Cox regression models adjusted for age, BMI, smoking status, physical activity, and the age difference between age at enrollment and age at serum measurement. Elevated adjusted HRs were found for higher serum levels (tertile 3 versus tertile 1) of PCBs that have dioxin-like activity including PCB 118 (HR = 3.44, 95% CI 1.01–11.69), PCB 156 (HR = 2.26, 95% CI 0.84–6.10), and PCB 167 (HR = 1.75, 95% CI 0.71–4.29) but the estimates were imprecise. The model that used a continuous measure of chemicals (instead of grouping into tertiles) was statistically significant for PCB 156 (HR = 2.17, 95% CI 1.28–3.66) and PCB 167 (HR = 2.09, 95% CI 1.26–3.47). Using the sum of non-dioxin-like PCBs the upper tertile was statistically significantly elevated compared with the lowest tertile (HR = 3.47, 95% CI 1.21–9.98). The effect estimate using the sum of dioxin-like PCBs was elevated but not statistically significant (continuous HR = 1.39, 95% CI 0.89–2.19; tertile 3 versus tertile 1 HR = 1.73, 95% CI 0.70–4.27). The TEQ continuous measure was statistically significantly elevated (HR = 1.40, 95% CI 1.21–1.62) as was the tertile 3 versus

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tertile 1 comparison (HR = 1.83, 95% CI 0.75–4.46). The study is somewhat limited by the relatively small number of cases and by the fact that 89,169 of the full Korean Cancer Prevention Study-II cohort participants (56% of the original participating cohort) were excluded from selection because of insufficient serum samples for assay.

Case-Control Studies

Pi et al. (2016) conducted a hospital-based case-control study of prostate cancer with cases identified from a tertiary general hospital in Singapore. A total of 240 incident cases were identified, and 268 controls with other diseases (except cancer) were recruited and matched to cases on ethnicity and age. Sera samples from 60 cases and 60 controls were used to assess the presence of 74 organohalogens. Other information was collected by interview. The mean concentration of PCB 118 was significantly higher ($p < 0.05$) in the serum of patients than in that of controls. Results were reported for two PCBs with dioxin-like activity. PCB 118 at the highest tertile (> 67th) (OR = 1.71, 95% CI 0.79–3.71) had a significant trend test. For PCB 156 the trend test was significant, although the only elevated OR estimate was in the third tertile. Given that this is a small study that did not report information on case and control response rates, that control diagnoses were not known, and that it is not clear whether there was adjustment for potential confounders, this study is of limited utility.

Other Identified Studies

Several other studies of prostate cancer were identified. Among occupational cohorts, one examined mortality (Ruder et al., 2014), and one incidence (Lemarchand et al., 2016). Both lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. An Italian environmental study was also identified that performed an ecological analysis to determine prostate cancer (and other cancer) incidence rates at 14 of Italy's national priority contaminated sites and compare the rates among those sites. Although the known exposures at these sites consisted of mixtures of PCBs, furans, benzene and other solvents, arsenic, and cadmium, they were not measured or specified in enough detail to indicate which of the COIs were present and to what extent. Akahane et al. (2017) examined the prevalence of self-reported long-term health effects, including prostate cancer, in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

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X. L. Sun et al. (2017) conducted a study of dioxin levels and steroid hormone levels (including PSA, testosterone, estradiol, DHEA, DHT, and 3β-hydroxysteroid dehydrogenase and CYP17-lyase activity and 5α-reductase activity) among rural men residing in a dioxin "hot spot" located at a former U.S. Vietnam War–era air base and men in a non-sprayed region in the Kim Bang district (Ha Nam Province). Although serum dioxin levels were measured and the mean levels of most dioxins, furans, and non-ortho PCBs were significantly higher in the hot spot area group than in the non-sprayed area group, this study was not given further consideration because it was not designed to directly estimate the risk of prostate cancer with dioxin exposure. The small number of participants, uncertainty about length of residence in the study areas, unknown response rate, and uncertainty on how the blood draw 2 years apart might have affected the results further limit this study's utility to the committee.

Biologic Plausibility

In prostate cells and prostate cancer cell lines TCDD can lead to the induction of various genes, including those involved in drug metabolism. Simanainen et al. (2004) used different rats (TCDD-resistant Hannover/Wistar and TCDD-sensitive Long Evans) and found that TCDD treatment resulted in a significant decrease in the weight of prostate lobes; the effect did not appear to be rat strain–specific. Different responses to TCDD in the human prostate cancer cell lines LNCaP and PC3 have been reported, including increased proliferation or no growth and stimulation or repression of AHR activity, which may be a function of coactivator–corepressor concentrations in the cells (Kollara and Brown, 2009, 2010). In addition, AHR activation has been shown to interfere with androgen receptor binding to androgen response elements in LNCaP cells via the upregulation of AP-1, resulting in a reduced expression of PSA (Kizu et al., 2003). However, the number of CAG repeats in the androgen receptor gene, which affects androgen receptor activity, did not significantly affect the induction of CYP1A1 by TCDD in androgen receptor–negative prostate cells transfected with androgen receptor constructs with different CAG repeat lengths (Björk and Giwercman, 2013). In that study, TCDD altered androgen receptor activity in a CAG repeat length–dependent manner in PC3 cells, but not in a non-tumorigenic, immortalized epithelial prostate cell line. AHR is upregulated in androgen receptor–negative, hormone-independent prostate cancer cells compared with androgen receptor–positive, hormone-dependent LNCaP cells, and the treatment of these cells (PC3, PC3M, and DU145) with an AHR agonist suppressed their growth (Richmond et al., 2014). In contrast, even though AHR is upregulated in castration-resistant C4-2 cells compared with the LNCaP cells from which they have been derived, the silencing of AHR caused a growth inhibition of these cells, perhaps because they retained androgen-receptor expression and are androgen-sensitive (C. Tran et al., 2013). TCDD suppressed expression of

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genes associated with cell-cycle progression in LNCaP cells, but also suppressed DNA-repair genes and increased Wnt5a concentrations; these effects could lead to divergent responses in prostate cancer progression (Hruba et al., 2011). AHR overexpression and activation reduced induction of the expression of vascular endothelial growth factor in PC3 cells, raising the possibility of interference with angiogenesis by AHR ligands (P. Y. Wu et al., 2013). LNCaP cells under oxidative stress have reduced viability and migration, and greater induced apoptosis; AHR activation exacerbates those changes (Yu et al., 2017). TGF-β suppressed AHR expression via SMAD4 and possibly also interfered with AHR signaling in a non-tumorigenic, but immortalized, epithelial prostate cell line (BPH-1) (Staršíchová et al., 2012); whether this also occurs in prostate cancer cells and has a bearing on prostate carcinogenesis is not known.

In utero and lactational exposure to TCDD increases aging-associated cribiform hyperplasia in the murine prostate, which may be a pre-cancerous lesion (Fritz et al., 2005). In a follow-up, progeny of a genetic cross between Ahr-null mice and the transgenic adenocarcinoma of the mouse-prostate (TRAMP) strain that models prostate cancer showed that the presence of Ahr inhibited the formation of prostate tumors that have a neuroendocrine phenotype (Fritz et al., 2008). As with breast cancer, these studies suggest that the timing of an exposure may be critical, with early-life exposures increasing prostate cancer susceptibility or risk and adult Ahr activation reducing it (recently demonstrated in TRAMP mice by Moore et al. [2016]). Because male Vietnam veterans were exposed to herbicides after adolescence, toxicologic findings concerning early-life exposure are not particularly relevant to this population, although their exposure to herbicides could potentially influence risk of the prostate cancer later in life.

Taken together, there is some in vivo and in vitro laboratory evidence in support of a role of AHR in prostate cancer and suggesting that dioxin exposure could affect processes involved in prostate carcinogenesis or prostate cancer growth and progression. However, there is no substantial understanding of the importance of these mechanisms or how they could affect prostate cancer risk.

Synthesis

This update describes several newly published studies involving prostate cancer in Vietnam veterans being seen at VA medical centers, occupational cohorts, and several other populations outside the United States. Most of the effect estimates were either below 1.0 or barely above 1.0 and were not statistically significant.

One new study of U.S. veterans was identified. Ovadia et al. (2015) examined outcomes related to prostate cancer progression (not the incidence of prostate cancer) and found self-reported Agent Orange exposure was not associated with biochemical recurrence, secondary treatment, metastases, or prostate

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cancer–specific mortality after adjusting for age, race, clinical stage, PSA level, BMI, health care center, and biopsy Gleason sum. However, studies among U.S. Ranch Hands and Australian Vietnam veterans that used better exposure assessment support an association between exposure to the herbicides used in Vietnam and prostate cancer.

Several positive associations between exposure to specific herbicides or their contaminants and prostate cancer have been reported from previously reviewed occupational studies. In studies extending the follow-up period of workers who were exposed to dioxins during the manufacturing process of PCP and TCP (Collins et al., 2016) and workers who manufactured or sprayed phenoxy herbicides (Coggon et al., 2015), no statistically significant difference in mortality for prostate cancer was found between the workers and the corresponding standardized populations. Christiansen et al. (2016) conducted a well-designed nested case-control study of 776 cases and controls within the AHS that examined the interactions among exposure to 39 pesticides (including dicamba, 2,4-D, and 2,4,5-T); 1,117 SNPs from 56 genes in the steroid hormone synthesis, metabolism, or regulation pathway; and the risk of prostate cancer. Several pesticide exposure metrics were constructed for each pesticide based on the duration and frequency of pesticide exposure. After adjustment, the only interaction found was for a SNP with dicamba, which resulted in lower prostate cancer risk with dicamba exposure; no association for 2,4-D or 2,4,5-T was found, and it appears there are no SNP interactions with these two herbicides.

Environmental and case-control studies reviewed in this update each used sera samples to estimate TEQs for dioxins, organochlorine pesticide metabolites, or PCB congeners. A well-designed and conducted nested case-control study by Koutros et al. (2015) used prospectively collected serum to estimate associations between organocholorine exposures and 34 PCB congeners (including five dioxin-like congeners) and metastatic prostate cancer in a population-based cohort from Norway. After controlling for demographic factors, BMI, and smoking habits, no statistically significant pattern of association was found with any of the dioxin-like PCBs analyzed. A hospital-based case-control study of prostate cancer in Singapore (Pi et al., 2016) used sera samples to assess the presence of 74 organohalogens and found that the mean concentration of dioxin-like PCB 118 was significantly higher ($p < 0.05$) in the serum of patients than in that of controls. PCBs with dioxin-like activity (PCB 118 and PCB 156) showed increased, but generally not statistically significant, risk estimates. A Korean case-cohort analysis reported elevated hazard ratios for some dioxin-like PCBs and for the sum of dioxin-like PCBs (Lim et al., 2017).

There is some in vivo and in vitro laboratory evidence supporting the role of AHR in prostate cancer and suggesting that dioxin exposure could affect processes involved in prostate carcinogenesis or in prostate cancer growth and progression. However, there is no substantial understanding of the importance of these mechanisms or how they could affect prostate cancer risk.

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The existing body of epidemiologic evidence, including the new studies reviewed here, provide mixed findings on an association between exposure to the COIs and prostate cancer. The epidemiologic evidence is robust enough and has support through biologic mechanisms that this committee finds no justification for reversing the conclusion of prior VAO committees that there is limited or suggestive evidence of an association.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there remains limited or suggestive evidence of an association between exposure to at least one of the COIs and prostate cancer.

TESTICULAR CANCER

NCI estimated that 9,310 men would receive diagnoses of testicular cancer (ICD-9 186; ICD-10 C62) in the United States in 2018 and that 400 men would die from it (NCI, n.d.t). Other cancers of the male reproductive system that are infrequently reported separately are cancers of the penis (ICD-9 187.1–187.4; ICD-10 C60) and of other male genital organs (ICD-9 187.5–187.9; ICD-10 C63).

Testicular cancer occurs most often in men between the ages of 25 and 29. The modeled incidence rate of testicular cancer in 2014 for all races combined for men ages 65 years and over (which would include most Vietnam veterans) is 1.2 per 100,000.[13] On a lifetime basis, the risk in white men is about five times higher than in black men (Stevenson and Lowrance, 2015). Known risk factors for testicular cancer include cryptorchidism (undescended testes) and having a previous occurrence of testicular cancer, infertility, and HIV infection. Several other hereditary, medical, and environmental risk factors have been suggested, but the results of research are inconsistent (Michaelson and Oh, 2018; Mikuz, 2015; Stevenson and Lowrance, 2015).

Conclusions from *VAO* and Previous Updates

After a published study indicated a potential association between testicular cancer in dogs and their service in Vietnam (Hayes et al., 1990), Tarone et al. (1991) conducted a case-control study of testicular cancer in male veterans, but the committee responsible for VAO concluded that there was inadequate or insufficient information to determine whether there was an association between exposure to the COIs and testicular cancer.

[13]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using SEER 18 dataset and choosing age-adjusted rates, testicular cancer, all races, and age ≥ 65 years.

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Additional information available to the committees responsible for *Update 1996* through *Update 2012* did not change that conclusion. *Update 2014* reviewed a follow-up of the Korean Veterans Health Study through 2003 and found no difference in the incidence of testicular cancer (n = 5 cases) compared with the Korean general population (Yi, 2013) and maintained the conclusion of inadequate or insufficient evidence of an association between exposure to the COIs and testicular cancer.

Update of the Epidemiologic Literature

No studies of testicular cancer in Vietnam veterans (U.S. or those of other countries) or environmental studies of exposure to the COIs and testicular cancer have been published since *Update 2014*. Reviews of the relevant studies are presented in the earlier reports. Table 19, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to testicular cancer.

Occupational Studies

Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016) presented mortality for several cancers. For this outcome, results were presented as cancers of the testes and other male genital (excluding prostate), but only one death of a TCP worker was reported in this category. Therefore, given the single death from this outcome, no conclusions can be drawn.

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) found only five deaths in the cohort from testicular cancer. Mortality effect estimates from testicular cancer were elevated but imprecise and not statistically significant for any of the groups of workers: all workers (SMR = 2.00, 95% CI 0.65–4.67), workers exposed to herbicide levels above background (n = 4; SMR = 2.10, 95% CI 0.57–5.37), or workers exposed for more than 1 year at levels above background (n = 3; SMR = 4.03, 95% CI 0.83–11.78).

Case-Control Studies

Paoli et al. (2015) conducted a small case-control study to examine the association between occupational and environmental endocrine disruptor exposure and testicular cancer. They recruited 125 testicular cancer (seminoma and non-seminoma) patients attending the Laboratory of Seminology Sperm Bank at the University of Rome for semen cryobanking. All patients were studied about 1 month after orchiectomy and before beginning chemo- or radiotherapy. The control group consisted of 103 healthy men undergoing an andrological examination and semen analysis in the same department as part of a nationwide preventive

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screening campaign. Cases and controls completed an in-person interview to collect demographic information, residence prior to diagnosis and andrological medical history, occupational history, diet history, lifestyle, and other environmental factors involving activities with suspected exposure to organochlorines. Serum samples were assayed for nine PCB congeners (including the dioxin-like congeners 77, 126, 169) and hexachlorobenzene. The associations between the organochlorine exposure and testicular cancer were estimated by logistic regression with adjustment for age and educational level. In the analysis, PCB and hexachlorobenzene values were grouped as being below or above the level of detection (0.2 ng/ml). Analyses of potential occupational pesticide exposure and possible maternal occupational exposure to pesticides (from interview data, but pesticides were not specified) both found nonstatistically significant associations. A detectable concentration of the sum of the nine PCB congeners (none of the congeners were analyzed separately) was found in 16 testicular cancer cases and no controls ($p < 0.001$). No effect measure was presented for this comparison, making this study of limited utility for the committee.

Other Identified Studies

An Italian environmental study was identified that performed an ecological analysis of testicular cancer incidence rates at 14 Italian priority contaminated sites and compared the rates among those sites (Benedetti et al., 2017); however, the study lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs.

Biologic Plausibility

No animal studies of the incidence of testicular cancer after exposure to any of the COIs have been published since *Update 2012*. That is undoubtedly due to the lack of a valid animal model of testicular cancer. SNPs of uncertain functional significance in the human AHR gene (11 SNPs) and the AHR repressor gene (18 SNPs) were studied in a case-control study of 278 Swedish men and 89 Danish men with testicular germ cell cancers (mean age 31 years) and 214 Swedish men without testicular cancer (mean age 18 years) (Brokken et al., 2013). There was no association between the risk of testicular germ cell cancer and any of the SNPs analyzed, but four SNPs in the AHR repressor gene were significantly associated with the risk of metastatic cancer compared to localized cancer.

Synthesis

The evidence from epidemiologic studies is inadequate to link herbicide exposure and testicular cancer. The relative rarity of this cancer makes it difficult to develop risk estimates with any precision. Most cases occur in men 25 to 35 years

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old, and men who have received such a diagnosis could have been excluded from military service; this could explain the slight reduction in risk observed in some veteran studies. In the analysis extending the follow-up period of workers who were exposed to dioxins during the manufacturing process of PCP and TCP (Collins et al., 2016), only one case of testicular cancer was reported. Among UK workers who manufactured or sprayed phenoxy herbicides (Coggon et al., 2015), five cases were reported, resulting in mortality effect estimates that, although elevated, were imprecise and not statistically significant for any of the groups of workers.

The committee considered one other study of testicular cancer, but exposure characterization was nonspecific, making it of limited value to the evidence base for determining associations with testicular cancer. Paoli (2015) recruited patients with testicular cancer who had not begun treatment and controls undergoing an andrological examination to examine the association between occupational and environmental endocrine disruptor exposure and testicular cancer. Serum samples were assayed for nine PCB congeners and hexachlorobenzene; however, results for specific congeners were not presented. Analyses of potential occupational pesticide exposure (pesticides not specified) and possible maternal occupational exposure to pesticides found no statistically significant associations.

No valid animal model has been identified for testicular cancer, which has resulted in a paucity of biologic plausibility data for mechanisms relating exposure to one of the COIs with testicular cancer. Based on the findings from studies reviewed in the current and previous updates and the lack of supporting mechanistic data, the committee maintains the conclusion of inadequate or insufficient evidence for an association between exposure to at least of the COIs and testicular cancer.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and testicular cancer.

BLADDER CANCER

Urinary bladder cancer (ICD-9 188; ICD-10 C67) is the most common urinary tract cancer. Cancers of the urethra, and paraurethral glands and other or unspecified urinary cancers (ICD-9 189.3–189.9; ICD-10 C68) are infrequently reported separately; any findings on these cancers would be reported in this section. NCI estimated that 81,190 people would receive a diagnosis of bladder cancer in the United States in 2018 and that 17,240 people would die from it (NCI, n.d.u). For all races combined, the incidence of bladder cancer in males is four times higher than in females.

Bladder cancer risk rises rapidly with age. The median age of diagnosis is 73 years. The age-adjusted modeled incidence rate of bladder cancer for men

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50–64 years old of all races combined was 36.1 per 100,000 in 2014 and increased to 144.5 for 65–74-year-olds and 296.4 for men over 75 years.[14] In men in the age groups that characterize most Vietnam veterans, bladder cancer incidence is nearly twice as high in whites as in blacks. The most important known risk factor for bladder cancer is tobacco smoke inhalation, which accounts for about one-half of the bladder cancers in men and one-third of them in women (Cumberbatch et al., 2016; Ferrís et al., 2013a). Occupational exposure to hair dyes, aromatic amines (also called arylamines), PAHs, and some other organic chemicals used in the aluminum, rubber, leather, textile, paint-products, and printing industries is associated with higher incidence (Ferrís et al., 2013a,b). In some parts of Africa and Asia, infection with the parasite *Schistosoma haematobium* contributes to the high incidence (Ferrís et al., 2013a).

Exposure to inorganic arsenic is also a risk factor for bladder cancer. Although cacodylic acid is a metabolite of inorganic arsenic, as discussed in Chapter 4, the data are insufficient to conclude that studies of inorganic-arsenic exposure are directly relevant to exposure to cacodylic acid, so the literature on inorganic arsenic is not considered in this section. Cacodylic acid constituted about 30% of the approximately 4 million liters of Agent Blue mixtures sprayed in Vietnam (see Table 2-2), as compared with approximately 44 million liters of 100% phenoxy herbicide mixtures with various degrees of TCDD contamination. Moreover, other than studies of exposure in Vietnam, there have been no occupational or environmental epidemiologic studies investigating bladder cancer incidence or mortality involving direct exposure to cacodylic acid.

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of *no* association between exposure to the COIs and urinary bladder cancer. The conclusion of no increased risk of bladder cancer was based largely on the null results from the overarching IARC cohort study of phenoxy herbicide production workers and sprayers (Saracci et al., 1991) and the consistently inconclusive results from studies of additional occupationally exposed cohorts, environmentally exposed populations, and two small studies of Vietnam veterans. Updates of the IARC cohort, augmented with 12 additional cohorts and updated through 1992 (Kogevinas et al., 1997), led the committee responsible for *Update 1998* to move bladder cancer to the default category of inadequate or insufficient information to determine whether there is an association. The committees responsible for subsequent updates did not change that conclusion. A total of 264 incident cases and 61 deaths from bladder cancer were reported during follow-up of the Korean Veterans Health Study, and the internal comparison analysis of the

[14]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using SEER 18 dataset and choosing age-adjusted rates, urinary bladder cancer, all races, and male sex.

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groups with high- versus low-exposure-opportunity scores (Yi and Ohrr, 2014) revealed no difference in the risk of a bladder cancer diagnosis (RR = 0.99, 95% CI 0.77–1.28). By contrast, Yi et al. (2014b) reported a statistically significant two-fold increase in bladder cancer–specific mortality in this same cohort (RR = 2.04, 95% CI 1.17–3.55), comparing the high- and low-exposure groups without adjustment for smoking. On the basis of those studies and the evidence reviewed in previous VAO reports, the *Update 2014* committee concluded that there is limited or suggestive evidence of an association between exposure to the COIs and bladder cancer.

Update of the Epidemiologic Literature

No studies of U.S. or international cohorts of Vietnam veterans have been published since *Update 2014*. No environmental or case-control studies of exposure to the COIs and urinary bladder cancer have been published since *Update 2012*. Reviews of the relevant studies are presented in the earlier reports. Table 20, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to urinary bladder cancer.

Occupational Studies

Among the Dow Midland, Michigan, worker cohort, Collins et al. (2016) combined deaths from bladder and other types of urinary cancer (ICD-10 C66–C68). Compared with the standardized U.S. population, no difference in mortality for bladder or other urinary cancers was found for the TCP workers (n = 9; SMR = 1.26, 95% CI 0.57–2.38) or the PCP workers (n = 4; SMR = 1.13, 95% CI 0.31–2.90).

Koutros et al. (2016) used data collected by the AHS to report the association of general exposure to herbicides, insecticides, and pesticides with bladder cancer. This is a prospective study of 57,310 pesticide applicators from Iowa and North Carolina who were enrolled between 1993 and 1997, and whose vital status was followed through 2011. Exposure was assessed by an extensive questionnaire, allowing for estimates of intensity and duration of exposure, and the information was updated from 1999 to 2005. A total of 321 incident bladder cancers were reported. After adjusting for lifestyle and demographic factors (including age, race, state of residence, and smoking status), elevated, but not statistically significant, risks were associated with use of chlorphenoxy herbicides 2,4,5-T (n = 91; RR = 1.15, 95% CI 0.84–1.59), 2,4,5-TP (n = 40; RR = 1.07, 95% CI 0.74–1.56), 2,4-D (n = 245; RR = 1.46, 95% CI 0.98–2.18), and organochlorine insecticides. There was a decreased risk for exposure to dicamba after adjusting for the same factors as above (n = 125; RR = 0.84, 95% CI 0.62–1.14). Never smokers with the highest use of 2,4,5-T and 2,4-D had higher risks of bladder cancer (RR = 2.64, 95% CI 1.23–5.68 and RR = 1.88, 95% CI 0.94–3.77, respectively), and for both herbicides there was a

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statistically significant trend of increasing risk with increasing exposure (p = 0.02). As in other publications from the AHS, concerns included the lack of control for multiple comparisons, the small number of cases, and the assessment of exposure based only on recall.

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) found that mortality from bladder cancer was lower than expected but not statistically significant for any of the groups of workers: all workers (n = 44; SMR = 0.92, 95% CI 0.67–1.23), workers exposed to herbicide levels above background (n = 30; SMR = 0.87, 95% CI 0.59–1.25), and workers exposed for more than 1 year at levels above background (n = 16; SMR = 0.98, 95% CI 0.56–1.59).

Other Identified Studies

Two other studies of bladder cancer among occupational cohorts were identified: one examined mortality in U.S. workers exposed to mixed PCBs (Ruder et al., 2014), and the other examined risk factors among Egyptian agricultural workers (Amr et al., 2015). However, both lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. A third study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including bladder cancer) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

Cacodylic acid (DMA^{III} and DMA^{V}) is carcinogenic and has been shown to induce urinary bladder cancer in F344 rats (Arnold et al., 2006; Cohen et al., 2007b; A. Wang et al., 2009; M. Wei et al., 2002, 2005; S. Yamamoto et al., 1995). A study by Z. Lin et al. (2015) suggests a carcinogenic process of chronic inflammation, bladder epithelium lesions, and proliferation in rat bladder following DMA^{V} exposures. Cao et al. (2015) exposed Wistar rats to DMA^{V} and found increased TGF-β immunoreactivity in bladder epithelium and increased IL-1β secretion in urine.

No studies have reported an increased incidence of urinary bladder cancer in TCDD- or 2,4-D-treated animals. Working with tissues from urothelial cancer patients, Ishida et al. (2010) found that the activation of the AHR pathway by TCDD enhanced bladder cancer cell invasion through an upregulated expression of matrix metalloproteinases 1 and 9 and that reduced expression of AHR resulted in the inhibition of invasive behavior of urothelial cancer cells. They also found that the level of nuclear AHR expression in human upper urinary tract urothelial

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cancers was positively associated with cancer grade and stage and that it predicted poor prognosis. In contrast, transgenic mice that have a deletion of Ahr exhibit immune-cell infiltration in bladder submucosa and the loss of e-cadherin in some epithelial cells in aged mice (Butler et al., 2012); although direct studies with TCDD were not undertaken, these findings suggest a protective effect of AHR signaling in bladder cancer.

Synthesis

This update describes three new published studies extending the follow-up period of occupational cohorts. Many of the studies reviewed in previous updates were characterized by low precision because of the small numbers of exposed cases, low exposure specificity, and a lack of ability to control for confounding, particularly cigarette smoking, which is a major risk factor for bladder cancer. Studies of U.S. Vietnam veterans (including AFHS Ranch Hands, Army veterans in the CDC Vietnam Experience Study, and state-specific studies of veterans) have not reported statistically significant increased risks of bladder cancer. More recent analyses of the Korean Veterans Health Study were well powered but did not control for smoking; however, self-reported information on smoking among surviving Korean veterans revealed that the distribution of smoking habits was similar in high and low EOI-score groups, indicating that the results for bladder cancer mortality are unlikely to have been majorly confounded by smoking (Yi et al., 2013b). Although Yi and Ohrr (2014) did not observe an increased incidence of bladder cancer, Yi et al. (2014b) reported a statistically significant difference in mortality from bladder cancer among veterans in the high-exposure-opportunity group relative to those in the low-exposure group.

Several positive associations between exposure to specific herbicides or their contaminants and bladder cancer mortality have been reported from previously reviewed occupational studies (Boers et al., 2010; Manuwald et al., 2012; Steenland et al., 1999); however, several other studies of occupational cohorts have found minimal or no association with exposure to one of the COIs and bladder cancer (Boers et al., 2010; C. J. Burns et al., 2011; McBride et al., 2009a; Ruder and Yiin, 2011). Collins et al. (2016) extended the follow-up period of workers who were exposed to dioxins during the manufacturing process of PCP and TCP in Midland, Michigan, and found slightly elevated, but not statistically significant, differences in mortality for bladder or other urinary cancers compared with the standardized U.S. population. In contrast, additional follow-up of the UK workers who manufactured or sprayed phenoxy herbicides found decreased, though not statistically significant, risks for mortality from bladder cancer for all three groups of exposed workers (Coggon et al., 2015). An analysis of exposure to herbicides, insecticides, and pesticides relative to bladder cancer incidence, using data collected by the AHS, found that after an adjustment for lifestyle and demographic factors, elevated risks were associated with the use of chlorphenoxy

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herbicides and organochlorine insecticides (Koutros et al., 2016). Never smokers with the highest use of 2,4,5-T and 2,4-D had higher risks of bladder cancer, and for both of these herbicides there was a statistically significant trend in increasing risk with increasing exposure.

Toxicologic and mechanistic data to support an association between the COIs and bladder cancer are limited. Cacodylic acid (DMA^{III} and DMA^{V}) is carcinogenic and has been shown to induce urinary bladder cancer in F344 and Wistar rats, but no studies have reported an increased incidence of urinary bladder cancer in TCDD- or 2,4-D-treated animals. The three new occupational cohort studies reviewed by the committee in combination with the prior reviewed literature continue to support the conclusion of limited suggestive evidence for an association of bladder cancer with exposure to the COIs.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to the COIs and urinary bladder cancer.

RENAL CANCERS

Cancers of the kidney other than the renal pelvis (ICD-9 189.0; ICD-10 C64) and cancer of the renal pelvis (ICD-9 189.1; ICD-10 C65) are often grouped in epidemiologic studies; cancer of the ureter (ICD-9 189.2; ICD-10 C66) is also sometimes included. Although diseases of these organs have distinct characteristics and could have different risk factors, there is some logic to grouping them: the structures are all exposed to filterable chemicals, such as PAHs, that appear in urine. NCI estimated that 63,990 men and women would receive diagnoses of kidney or renal pelvis cancer in the United States in 2018 and that 14,400 men and women would die from it. The incidence of renal cancer increases with age, and median age of diagnosis is 64 years (NCI, n.d.v). Except for Wilms tumor, which is more likely to occur in children, renal cancers are more common in people over 50 years old.

In the age groups that include most Vietnam veterans, the age-adjusted modeled incidence rate of kidney and renal pelvis cancers for men 50–64 years old of all races combined was 45.3 per 100,000 in 2014 and increased to 93.1 for 65–74-year-olds and 105.1 for men over 75 years. The incidence rate for men is about twice as high as it is for women of the same race. Among men, blacks

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have the highest incidence rate, whereas among women, both black and American Indian/Alaska Natives have the highest incidence rates.[15]

Tobacco use is a well-established risk factor for renal cancers (Qayyum et al., 2013). Obesity is also another risk factor for renal cell carcinoma, and a recently published meta-analysis of 21 cohort studies reported an elevated risk for renal cancers (RR = 1.77, 95% CI 1.68–1.87) when comparing obese to normal weight participants (Wang and Xu, 2014). Some rare syndromes—notably, von Hippel–Lindau syndrome and tuberous sclerosis—are associated with an elevated risk of renal cancer. Other potential risk factors include acetaminophen or non-aspirin non-steroidal anti-inflammatory drug use, organic solvents, and, in men, a history of kidney stones (Cheungpasitporn et al., 2015; Choueiri et al., 2014; Qayyum et al., 2013). Firefighters, who are routinely exposed to numerous pyrolysis products, have a significantly increased mortality risk after 20 or more years of employment (Youakim, 2006).

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and renal cancers. Additional information available to the committees responsible for subsequent updates from *Update 1996* through *Update 2012* did not change that conclusion. *Update 2014* identified a single study of renal cancer in a follow-up of the Korean Veterans Health Study. Results were reported separately for kidney cancer (n = 186 cases) and renal pelvis cancer (n = 23 cases), but no excess cancer risk for the kidney or renal pelvis was found when compared with the general Korean population (Yi, 2013) or when internal comparisons of high- versus low-exposure-opportunity scores were made (Yi and Ohrr, 2014). An inverse association for renal cancer risk (HR = 0.74, 95% CI 0.55–1.00) was reported, but no association for cancer of the renal pelvis (HR = 1.05, 95% CI 0.44–2.50). A non-significant increased risk of ureter cancer (ICD-10 C66) was also reported. When kidney, renal pelvis, and ureter cancer deaths were combined for the internal cohort comparison of high versus low exposure, no excess cancer mortality was found (Yi et al., 2014b). Information on smoking or other lifestyle habits was not available for this cohort during the follow-up through 2003, and thus the modest associations could be due to confounding by smoking or obesity.

[15]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using the SEER 18 dataset and choosing age-adjusted rates, kidney and renal pelvis cancers, all races, age ≥ 50 years, and male sex.

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Update of the Epidemiologic Literature

Two new published studies were identified that addressed exposure to the COIs from occupational settings and mortality from renal cancers. No studies of renal cancers in Vietnam veterans have been identified since *Update 2014*. Furthermore, no environmental studies or case-control studies of exposure to the COIs and renal cancers have been published since *Update 2010*. Reviews of the relevant studies are presented in the earlier reports. Table 21, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to renal cancer.

Occupational Studies

Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016) found no differences in mortality for kidney cancers (C64–C65) for the TCP workers (n = 4; SMR = 0.63, 95% CI 0.17–1.61) or the PCP workers (n = 4; SMR = 1.37, 95% CI 0.37–3.51).

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine carcinogenicity of phenoxy herbicides, Coggon et al. (2015) found that mortality from kidney cancer was not statistically significant for any of the groups of workers: all workers (n = 23; SMR = 0.87, 95% CI 0.55–1.31), workers exposed to herbicide levels above background (n = 16; SMR = 0.81, 95% CI 0.46–1.32), or workers exposed for more than 1 year at levels above background (n = 11; SMR = 1.22, 95% CI 0.61–2.19).

Other Identified Studies

Two other studies that reported outcomes of renal cancer were identified, but both lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Akahane et al., 2017; Ruder et al., 2014).

Biologic Plausibility

Cacodylic acid (DMA^{III} and DMA^{V}) is carcinogenic and has been shown to induce renal cancer. In F344/DuCrj rats treated with a mixture of carcinogens for 4 weeks, subsequent exposure to DMA (not indicated whether this was DMA^{III} or DMA^{V}) via their drinking water for 24 weeks caused tumor promotion in the kidney, liver, urinary bladder, and thyroid gland, but it inhibited induction of tumors of the nasal passages (S. Yamamoto et al., 1995). More recent studies have also found that oral exposure of adult mice to 200 ppm DMA^{V} in addition to fetal arsenic exposure can act as a promoter of renal and hepatocellular carcinoma,

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markedly increasing tumor incidence beyond that produced by fetal arsenic exposure alone (Tokar et al., 2012).

No animal studies with exposure to the other COIs have reported an increased incidence of renal cancers.

Synthesis

The available analyses of an association between exposure to the COIs and renal cancer risk have been limited by the small number of cases and a lack of exposure specificity. Studies of Vietnam veterans have not found statistically significant associations between deployment and presumed exposure to the herbicides and incidence or mortality of renal cancers. Similarly, no increases of risk or mortality from renal cancers have been reported among the several occupational cohorts, for which exposure was often better characterized. Collins et al. (2016) extended the follow-up period of workers who were exposed to dioxins during the manufacturing process of PCP and TCP in Midland, Michigan, and reported four cases of renal cancer among PCP workers and four cases among TCP workers, but in neither group were the risk estimates statistically significant. Coggon et al. (2015) identified a total of 23 deaths from renal cancer in the additional follow-up of the UK workers who manufactured or sprayed phenoxy herbicides, but the direction of the risk estimates for mortality from renal cancer was not consistent or statistically significant for any of the three groups of exposed workers.

Cacodylic acid (DMA^{III} and DMA^{V}) is carcinogenic and has been shown to induce tumors in the kidneys of F344 rats, but no animal studies with exposure to the other COIs have reported an increased incidence of renal cancer. Results from two studies that extended the follow-up period of well-characterized occupational cohorts in combination with the prior reviewed literature continue to support the conclusion of inadequate or insufficient evidence for an association of renal cancers with exposure to the COIs.

Conclusion

Based on the epidemiologic evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and renal cancers.

BRAIN CANCER

Nervous-system cancers (ICD-9 191–192; ICD-10 C70–C72) involve the central nervous system (CNS) and include tumors of the brain and spinal cord, the cranial nerves, and the meninges (the outer coverings of the brain and spinal cord). Any of the cell types in the CNS can develop into cancer. Tumors of the

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peripheral nervous system and autonomic nervous system are considered soft-tissue tumors (ICD-9 171; ICD-10 C38.0, C47, C49). Most cancers that are found in the CNS are not primary tumors arising from nervous system tissues, but instead represent metastases from other primaries, such as the lung or breast. This section focuses on cancers that originate in the CNS. Cancer of the eye (ICD-9 190; ICD-10 C69) is included with the results on brain cancer because when cancer of the eye is reported, it is often grouped with brain cancer.

About 95% of primary CNS malignancies originate in the brain, cranial nerves, and cranial meninges. In people over 45 years old, about 90% of tumors that originate in the brain are gliomas—astrocytoma, ependymoma, oligodendroglioma, or glioblastoma multiforme. Although the committee was tasked with examining all health outcomes that may be associated with exposure to the COIs, VA specified that the committee should give particular attention to glioblastoma multiforme. Glioblastoma multiforme is the most common brain tumor and has the worst prognosis (Muth et al., 2016). Meningiomas make up 20% to 40% of CNS cancers; they tend to occur in middle age and are more common in women than in men. Most meningiomas are benign and can be removed surgically.

NCI estimated that about 23,880 people would receive diagnoses of brain and other nervous-system cancers in the United States in 2018 and that 16,830 people would die from them (NCI, n.d.w). Those numbers represent 1.4% of new cancer diagnoses and 2.8% of cancer deaths. An estimated 12,000 new cases of glioblastoma are diagnosed in the United States each year.[16] The incidence of brain and other CNS cancers increases with age, and the median age of diagnosis is 58 years. The incidence rate for men is higher than for women of the same race. By race and sex, whites have the highest incidence rate and American Indian/Alaska Natives have the lowest incidence rates (NCI, n.d.w). In the age groups that include most Vietnam veterans, the age-adjusted modeled incidence rate of brain and other nervous-system cancers for men 50–64 years old of all races combined was 11.5 per 100,000 in 2014 and increased to 20.2 for 65- to 74-year-olds and 26.2 for men over 75 years.[17]

In reviewing the descriptive epidemiology of these cancers, it is important to recognize the variation with which specific cancers are included in published reports, many of which distinguish between benign and malignant tumors. Another variation is whether cancers derived from related tissues (such as the pituitary or the eye) are included with CNS cancers. Several types of cancer are usually grouped together; although this may bias results in unpredictable ways, the most likely consequence is a dilution of risk estimates toward the null.

[16] Paul Mischel, head of Laboratory of Molecular Pathology at Ludwig Institute for Cancer Research San Diego, presentation to the committee, November 30, 2017.

[17] As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output using the SEER 13 dataset and choosing age-adjusted rates, brain and other nervous-system cancers, all races, age ≥ 50 years, and male sex.

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The only well-established environmental risk factor for brain tumors is exposure to high doses of ionizing radiation (ACS, 2012b; Wrensch et al., 2002). Other environmental exposures—such as to petroleum products, electromagnetic fields, and cell-phone use—are unproven as risk factors (Gomes et al., 2011; Ostrom et al., 2015). The causes of most cancers of the brain and other portions of the nervous system are unknown.

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was limited or suggestive evidence of *no* association between exposure to the COIs and brain cancer. The committees responsible for *Update 1996*, *Update 1998*, *Update 2000*, *Update 2002*, and *Update 2004* did not change that conclusion.

The committee responsible for *Update 2006* changed the classification for brain cancer (formally expanding it to include cancers of the eye and orbit) to inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and brain cancer. That committee considered one study that suggested a relationship between phenoxy acid herbicides and adult gliomas (W. J. Lee et al., 2005), studies that reported slightly but not statistically significantly higher risks of brain cancer in deployed versus non-deployed Australian Vietnam-era veterans (ADVA, 2005a,b) and in pesticide applicators in the AHS (Alavanja et al., 2005), and several studies that had essentially neutral findings (Carreon et al., 2005; Magnani et al., 1987; McLean et al., 2006; Ruder et al., 2004; Torchio et al., 1994).

The committees for *Update 2008*, *Update 2010*, and *Update 2012* reviewed several new occupational, environmental (including updates of the Seveso cohort), case-control, and Vietnam-veteran studies, but maintained that brain cancer should remain in the inadequate or insufficient category, given the largely null findings and that several studies did not specify the chemicals of exposure.

The Update 2014 committee reviewed two studies of Vietnam veterans. In a retrospective study of mortality outcomes in three cohorts of U.S. military women—4,734 who served in Vietnam, 2,062 who served in countries near Vietnam, and 5,313 who served primarily in the United States—no association was found between any cohort and brain or nervous-system cancers (H. K. Kang et al., 2014a). In a sub-analysis, nurses who served in Vietnam had a statistically significantly higher risk of brain cancer death than nurses who served in the United States (adjusted RR = 4.61, 95% CI 1.27–16.83), but nurses who served near Vietnam did not have an elevated risk (adjusted RR = 2.12, 95% CI 0.42–10.83) although both estimates were imprecise. None of the other studies of U.S. or international Vietnam veterans found statistically significant associations between exposure to the COIs and brain cancer. The U.S. Vietnam veterans nurses study is limited by the issue of multiple comparisons, the possibility of false positives, and imprecise risk estimates.

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Update of the Epidemiologic Literature

Because glioblastoma multiforme was specifically noted as an outcome of importance in the committee's Statement of Task, a targeted literature search for this outcome was undertaken. No date or language parameters were applied, and a total of 153 articles were found. Each was reviewed for relevance against the COIs and checked against the previous *VAO* reports. Using those criteria, only two epidemiologic studies were identified that had exposure to one of the COIs, were published before 2014, and had not previously been reviewed. Both studies are summarized in this section. No new published literature of Vietnam veterans that addressed exposure to the COIs and brain cancers was identified by the committee for the current update. Furthermore, no environmental studies of exposure to the COIs and brain cancer have been published since *Update 2012*. Reviews of the relevant studies are presented in the earlier reports. Table 22, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to brain tumors.

Occupational Studies

Although the results are not brain-cancer specific, but rather presented as cancers of the CNS (C70–C72), Collins et al. (2016) reported on the causes of mortality among the Dow Midland, Michigan, worker cohort. Few cases of CNS cancers were identified: three among TCP workers and one among PCP workers, again making reported risk estimates unreliable. Compared with the standardized U.S. population, no differences in mortality for CNS cancers were found for the TCP workers (SMR = 0.47, 95% CI 0.10–1.38).

In the Coggon et al. (2015) extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine carcinogenicity of phenoxy herbicides, no differences in mortality from brain and CNS cancers were found for any of the groups of workers when compared with the standardized population of Great Britian: all workers (n = 33; SMR = 1.15, 95% CI 0.79–1.62), workers exposed to herbicide levels above background (n = 25; SMR = 1.15, 95% CI 0.74–1.70), or workers exposed for more than 1 year at levels above background (n = 10; SMR = 1.04, 95% CI 0.50–1.92).

Case-Control Studies

Brownson et al. (1989) conducted a case-control study using data from the Missouri Cancer Registry to examine the association between employment in certain industries and specific occupations with brain cancer. A total of 312 cases (all white males diagnosed with histologically confirmed brain and other CNS cancer between 1984 and 1988) were studied. Each case was frequency matched to four controls, white males diagnosed with other cancers who were randomly selected from each of six age strata and information on occupation

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(usual or longest held) and tobacco smoking history (never, former, current). Odds ratios were calculated and adjusted for age and smoking status. Additional analyses were conducted for specific brain cancers, including unspecified astrocytomas, unspecified glioblastomas, anaplastic astrocytomas, and other (oligodendrogliomas, unspecified cell types, and unspecified ependymomas). Although specific exposures were not collected and many of the categories had small numbers of people, a statistically significant excess of brain cancer was associated with agricultural work (22 cases and 62 controls, OR = 1.5, 95% CI 1.0–2.4) and printing and publishing work (6 cases and 36 controls, OR = 2.8, 95% CI 1.0–8.3), although the effect estimate for the printing industry was quite imprecise. Comparisons by occupation found an excess risk associated with social science professionals (6 cases and 4 controls, OR = 6.1, 95% CI 1.5–26.1) and police and fire protection professionals (12 cases and 22 controls, OR = 2.2, 95% CI 1.0–4.7), but these estimates were imprecise and limited by specific exposure information and the small number of individuals reporting such occupations. Furthermore, no excess of brain cancer was found for farming (21 cases and 80 controls, OR = 1.1, 95% CI 0.6–1.7), food production (3 cases and 8 controls, OR = 1.6, 95% CI 0.3–6.8), or laborers other than construction (7 cases and 46 controls, OR = 0.6, 95% CI 0.2–1.4). Analyses by histologic type of brain cancer found elevated, but imprecise, risks of "other" cell types for workers in the agricultural industry (OR = 5.3, 95% CI 1.9–14.3) and for occupation of farmer (OR = 3.7, 95% CI 1.4–9.8). When cases and controls were compared by tobacco smoking status, no difference in risk for brain cancer was found. The study is limited by the lack of specific exposure information; industry and occupation information was incomplete in the registry (of the initially eligible subjects, 34% of cases and 38% of controls were excluded from the final sample due to missing information), restricted to "usual" or "longest held" job, and obtained from the medical record at the time of diagnosis and subject to misclassification. Therefore, while these data are consistent with some other studies that suggest an agricultural chemical exposure risk for brain cancer, they are very nonspecific and must be considered exploratory.

Other Identified Studies

Three other studies of brain cancer were identified, but all lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. One examined causes of mortality in U.S. workers exposed to mixed PCBs (Ruder et al., 2014). Bencko et al. (2009) was an ecological study that compared the 10-year incidence of selected cancers, including brain cancer, in known PCB/TCDD-contaminated regions of the Slovak Republic and the Czech Republic. A third study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects, including brain tumors, in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the

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ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Invited Presentations to the Committee

Given the dearth of new studies that examined exposure to the COIs and brain cancer (particularly glioblastoma multiforme), and because this outcome was specified in the committee's Statement of Task, the committee invited presentations by VA (to hear of their specific concerns, questions, and continuing research initiatives regarding glioblastoma multiforme), by experts on the epidemiology and molecular pathology of glioma, and by veterans and their families. Hearing from the scientific experts ensured that the committee had information that was as complete and current as possible regarding the science of glioblastoma, whereas hearing from the families of veterans who had been diagnosed with glioblastoma provided a reminder of the burden of the disease. The committee appreciates the efforts of all presenters and members of the public who attended the open session and their willingness to provide information for the committee's consideration.

Quinn Ostrom, Ph.D., from Case Western Reserve University, presented a review of the epidemiology of glioma, which included population estimates of its incidence and prevalence as well as its distribution by demographic factors. Although a relatively small number of all new cancer cases each year originate in the brain or nervous system for both men and women (1.6% and 1.2%, respectively), they account for 3% of deaths from cancer each year. Dr. Ostrom reported that the overall incidence of primary brain and CNS tumors is 22.6 per 100,000 and that almost one-third of these tumors (31.5%) are malignant. Gliomas are the most common type of malignant brain tumor, accounting for approximately 26.6% of all primary brain tumors and nearly 80% of malignant brain tumors. There are multiple subtypes of glioma, with glioblastoma being the most common (56.1%). The incidence of glioblastoma increases with age and peaks at 75–79 years for both males and females; the median age of diagnosis is 64 years. In the United States, incidence is about 50% higher in males than in females, highest for non-Hispanic whites, and associated with higher socioeconomic status. This review highlighted the stark fact that there has been little change in the incidence rate of glioblastoma since the 1990s and little progress eliciting clear risk factors. Consistent with the prior updates by VAO committees, this committee found that while numerous environmental risk factors have been studied, no environmental factors accounting for glioma have been identified. Of interest, about 25% of glioma risk is estimated to be genetic, and current research has identified 12 common genetic variants that explain approximately 27% of the genetic risk for glioblastoma. Accepted non-genetic risk factors include exposure to ionizing radiation and a history of respiratory allergies and atopic disease,

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specifically asthma and eczema. While associations with other exposures have been studied, including herbicide exposure, no additional accepted risk factors (including immune suppression arising from various exposures) have been found.

A presentation by Paul Mischel, M.D., which highlighted the fast-moving research into the basic science of glioblastoma, concentrated on both new discoveries and their implications for understanding controversies concerning the mechanisms responsible for the aggressive nature of this disease. Many fundamental characteristics of this tumor—one of the cancers that has been most well characterized genetically by NCI's Cancer Genome Atlas initiative—have been well described, and research has identified some of the discrete pathways involved in its development and elucidated some of the factors underlying its mutational diversity, including copy number alterations and point mutations, insertions and deletions, and the role of extrachromosomal DNA. These novel mechanisms may fundamentally change how we think about the evolution of this (and other) cancers. At the same time, this understanding of the basic biology has so far not directly led to new treatment options.

VA representatives, who reiterated the origin of the VAO series and the current committee's specific charge, presented data on overall brain cancer incidence from VA administrative databases and summarized the results of selected studies of brain cancer in different populations of veterans. All of the published studies they presented had been reviewed by previous VAO Update committees. In the final part of its presentation, VA representatives informed the committee of the department's current and planned studies of brain cancer in Vietnam veterans. The planned studies included an update of the causes of mortality of deployed and Vietnam-era veterans from 1979 through 2014 and an exploratory study of self-reported exposures and different types of brain cancer using information, in part, from the Agent Orange Registry. VA has completed a new national survey on the current health of deployed and nondeployed Vietnam-era veterans (VE-HEROeS); however, none of the results were published in time for the committee's consideration. The committee supports VA's continued research efforts and urges the department to continue to conduct rigorous and thorough epidemiologic investigations of veterans' health issues, but cautions that these are unlikely to yield information on the association between exposure to the COIs and brain cancers..

Finally, the committee also heard from family members and widows of veterans who died from brain cancer and who are working to have VA recognize the diagnosis of brain cancer, specifically glioblastoma, as a presumptive disease associated with exposure to the herbicides used in Vietnam. Their presentations were compelling and heartfelt. One such effort discussed was Sierra Valley Cancer Registry Services, which is a registry that collects self-reported information related to exposures and confirmed diagnosis of glioblastoma for Vietnam veterans. As of 2017, the registry contains information on 372 Vietnam veterans who have been diagnosed with glioblastoma.

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Biologic Plausibility

The committee did not identify any animal studies that have reported an association between exposure to the COIs and brain cancer.

In a study of the role of the *AHR–MYCN* oncogene in neuroblastoma, P. Y. Wu et al. (2014) reported that AHR was inversely correlated with MYCN expression in neuroblastoma tissues, but found AHR expression to be highly correlated with the histological grade of differentiation. This correlation was confirmed in a further study of 14 human neuroblastoma samples. Dever et al. (2012) found that AHR promotes proliferation in medulloblastoma cells. Silginer et al. (2016) identified a signaling network comprising integrins, AHR, and TGF-β in a human glioma cell line. AHR mediates integrin control of the TGF-β pathway; TGF-β contributes to the malignancy of glioblastoma by "promoting invasiveness and angiogenesis, maintaining tumor cell stemness and inducing profound immunosuppression" (p. 3260).

Recent work relevant to glioblastoma elucidates a role for AHR activation by endogenous ligand kynurenine (Beischlag et al., 2016; Platten et al., 2012a,b). The activation of AHR by kynurenine resulted in tumor growth, invasiveness, and immunosuppression (Platten et al., 2012a). Genes that may mediate the promotion of tumor growth and invasiveness include interleukins 1β, IL-6, IL-8, epiregulin and aldehyde dehydrogenase 1 family, member A3 (Platten et al., 2012b).

Synthesis

Studies of Vietnam veterans have not found statistically significant associations between deployment and presumed exposure to the herbicides and incidence or mortality of brain or other nervous-system cancers. Many of the studies conducted among U.S. Vietnam veteran cohorts were underpowered. Similarly, no increases of risk or mortality from brain and CNS cancers have been reported among the several occupational cohorts, for which exposure was often better characterized. Collins et al. (2016) extended the follow-up period of workers who were exposed to dioxins during the manufacturing process of PCP and TCP in Midland, Michigan, and reported four cases total of brain and other nervous-system cancers resulting in decreased risk estimates that were not statistically significant. Coggon et al. (2015) identified a total of 33 brain and nervous-system cancer deaths in the additional follow-up of the UK workers who manufactured or sprayed phenoxy herbicides, but no increased risk was observed for any of the three groups of exposed workers. Brownson et al. (1989), used a case-control design and reported some small but statistically significant elevations in risk associated with several occupations. However, the study lacked exposure estimates and was underpowered and potentially biased by missing data, and, ultimately, the committee considered it an exploratory analysis and did not give it full weight.

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Given the limited epidemiologic data available on glioblastoma, the committee heard invited presentations from two glioblastoma experts. While the presentations to the committee were helpful and impressive, demonstrating that the biological understanding of glioblastoma is rapidly advancing, they reinforced the absence of clear data suggesting that the COIs are associated with the occurrence of brain cancers.

The committee did not identify any animal studies that have reported an association between exposure to the COIs and brain cancer. AHR was found to be inversely correlated with *MYCN* expression but highly correlated with the histological grade of differentiation in neuroblastoma tissues in a small sample of human cases. Recent research has shown that AHR mediates integrin control of the TGF-β pathway and that TGF-β contributes to the malignancy of glioblastoma. The new epidemiologic and mechanistic data reviewed in this update along with the presentations to the committee from experts in the field were not sufficient to alter the committee's conclusion that the evidence is inadequate or insufficient to determine whether there is an association between exposure to the COIs and brain or other nervous-system cancers.

Conclusion

Based on the epidemiologic evidence from new and previously reported studies of populations that had potential exposure to the COIs, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and brain cancer or other nervous-system cancers.

The committee believes it is appropriate for VA to be mindful of the concerns raised about the possible association between Vietnam service and glioblastoma but observes that the outcome is so rare and the information concerning herbicide exposures so imprecise that is doubtful that any logistically and economically feasible epidemiologic study of veterans—no matter how well designed or executed—would produce meaningful results. **The committee therefore recommends that VA not seek to undertake such an epidemiologic study and instead to focus on fostering advancements in other areas that may be used to inform improved treatment options.**

ENDOCRINE CANCERS

Cancers of the endocrine system have a disparate group of ICD codes: thyroid cancer (ICD-9 193; ICD-10 C73) and other endocrine cancers including the thymus (ICD-9 164.0, 194; ICD-10 C37, C74, C75). According to NCI, in the United States in 2018 there would be an estimated 53,990 new diagnoses of and 2,060 deaths from thyroid cancer (NCI, n.d.x). NCI does not report estimates of other endocrine cancers, but the American Cancer Society estimated that 1,210

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men and 1,170 women would receive diagnoses of other endocrine cancers in 2017 and that 520 men and 480 women would die from them (Siegel et al., 2017).

Thyroid cancer is the most prevalent endocrine cancer. The thyroid contains two main types of cells: follicular cells, which synthesize and store thyroid hormones and synthesize thyroglobulin, and C cells, which synthesize the hormone calcitonin, which regulates calcium metabolism. Malignancy can develop from any cell type. The four main types of thyroid cancer are papillary cancer, follicular cancer, anaplastic cancer, and medullary carcinoma (Wiltshire et al., 2016). Papillary carcinoma is the most common and accounts for the majority of the increasing incidence rate (Lubitz and Sosa, 2016). It usually affects women of childbearing age; the most common variant of papillary carcinoma is the follicular subtype (also known as mixed papillary–follicular variant), which metastasizes slowly and is the least aggressive type of thyroid cancer. Follicular carcinoma (or follicular adenocarcinoma), which is associated with inadequate dietary iodine intake, accounts for about 10% of all cases and has greater rates of recurrence and metastasis. Medullary carcinoma, a cancer of the parafollicular cells in the thyroid, is less common (4% of all cases) and tends to occur in families. Anaplastic carcinoma (also called giant-cell cancer and spindle-cell cancer) is rare but is the most aggressive form of thyroid cancer; it does not respond to radioiodine therapy and metastasizes quickly, invading such nearby structures as the trachea and causing compression and breathing difficulties.

Thyroid cancer can occur in all age groups, but the median age of diagnosis is 51 years (NCI, n.d.x). As radiation exposure is recognized as a risk factor for thyroid cancer, increased incidence is being observed in people who received radiation therapy directed at the neck (a common treatment in the 1950s for enlarged thymus, adenoids, and tonsils and for skin disorders) or who were exposed to iodine-125, for example, from the Chernobyl nuclear power-plant accident. If the radiation exposure occurred in childhood, then the risk of thyroid cancer is further increased. In the age groups that include most Vietnam veterans, the age-adjusted modeled incidence rate of thyroid cancer for men 50–64 years old of all races combined was 13.4 per 100,000 in 2014 and increased to 21.5 for 65- to 74-year-olds before decreasing to 16.5 for men over 75 years.[18] The incidence rate of thyroid cancer is about three times higher in women than in men of the same race. Whites and Asian/Pacific Islanders have the highest incidence rates for both sexes (NCI, n.d.x). Other risk factors are a family history of thyroid cancer and chronic goiter. Adrenal and pituitary cancers are much less common than thyroid cancers. Benign adenomas are more common than malignancies in these endocrine glands.

[18]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using the SEER 13 dataset and choosing age-adjusted rates, thyroid, all races, age, and male sex.

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Conclusions from *VAO* and Previous Updates

The committees responsible for *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, *Update 2002*, and *Update 2004* did not report endocrine cancers as a separate outcome and therefore reached no conclusion as to whether there was an association between exposure to the COIs and endocrine cancers. The committees responsible for *Update 2006*, *Update 2008*, *Update 2010*, and *Update 2012* did consider endocrine cancers separately and concluded that there was inadequate or insufficient evidence to determine whether there is an association between the COIs and endocrine cancers. Analysis of incidence and mortality of cancers in the Korean Veterans Health Study was reviewed in *Update 2014*. There were no statistically significant differences in the incidence of or death from thyroid cancer when compared to the general Korean population; nor were there differences between the high- and low-exposure groups (Yi and Ohrr, 2014). However, based on 11 deaths, a statistically significant association between exposure and thyroid cancer–specific mortality was found both when analyzed in terms of log increments in the exposure opportunity scores and when comparing high- versus low-exposure groups (Yi et al., 2014b). The small number of cases and imprecise estimates did not change the conclusion that there was inadequate or insufficient evidence to determine whether there is an association between the COIs and endocrine cancers.

Update of the Epidemiologic Literature

No environmental or case-control studies of exposure to the COIs and thyroid or other endocrine cancers have been published since *Update 2012*. Reviews of the relevant studies are presented in the earlier reports. Table 23, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to endocrine cancers (thyroid, thymus, and other).

Vietnam-Veteran Studies

Le et al. (2016) performed a retrospective review of the VA Corporate Data Warehouse database from all VA sites from October 1, 1999, to December 21, 2013, to examine incident cases of thyroid cancer based on ICD-9 codes. No pathology was available, and no clinical information on the patients was reported. A total of 19,592 thyroid cancer cases were identified, 42% of which were among Vietnam-era veterans. Agent Orange exposure was obtained from VA records and was determined by self-report at first visit to the VA system. The authors found a statistically significantly higher proportion of self-reported Agent Orange exposure among thyroid cancer patients (10.0%) compared with the general VA health care population (6.2%) ($p < 0.0001$). However, this analysis is limited by the absence of pathology reviews of identified cases, no reporting of histological subtypes, and

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no adjustment or inclusion of additional information on comorbidities or other risk factors. Furthermore, the exposure to Agent Orange was based on self-report.

Occupational Studies

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) reported a total of 3 deaths from thyroid cancer. With limited deaths, mortality risk estimates were imprecise and not statistically significant for any of the groups of workers.

Other Identified Studies

Four other studies were identified that reported outcomes of thyroid cancer, but all lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. The first examined mortality from malignant neoplasms of the thyroid gland in an occupational cohort of capacitor manufacturers who were exposed to dioxin-like and non-dioxin-like PCBs (Ruder et al., 2014). The second study (Benedetti et al., 2017) was an Italian environmental study that performed an ecological analysis of thyroid cancer (and other cancer) incidence rates at 14 Italian priority contaminated sites and compared the rates among those sites. The third study looked for clusters of thyroid cancer in an Andean town in Colombia using a spatial analysis of aggregated data (smoothed standardized incidence ratios at census tracts) and point data (individual case location) to determine if clusters of thyroid cancer in this region were associated with industrial sources of air pollution, which included PCDD/Fs (Arias-Ortiz et al., 2018). The final study (Akahane et al., 2017) examined the prevalence of self-reported long-term health effects (including thyroid cancer) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

NTP conducted carcinogenesis bioassays in Osborne-Mendel rats and B6C3F1 mice that were exposed to TCDD by gavage (NTP, 1982a). The incidence of follicular-cell adenoma, but not of carcinoma, increased with increasing TCDD dose in male and female rats; the increase was significant in male but not in female rats. There was a significant increase in follicular-cell adenoma in female but not in male mice. NTP then carried out a similar study in female Sprague Dawley rats (NTP, 2006), and Walker et al. (2006) compared the data

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from that study and the results of the Dow Chemical assessment of TCDD carcinogenicity (Kociba et al., 1978). In the NTP and Dow studies, the incidence of C-cell adenoma and carcinoma decreased with an increasing dose of TCDD. However, an increased incidence of mild thyroid follicular-cell hypertrophy was noted in rats that were given TCDD at 22 ng/kg of body weight or more. A more recent 2-year NTP study (Yoshizawa et al., 2010) treated female Sprague Dawley rats with TCDD, 2,3,4,7,8-pentachlorodibenzofuran, dioxin-like PCB congeners (PCB 126 or PCB 118), a non-dioxin-like PCB (PCB 153), or mixtures of these chemicals; it did not find any increases in either thyroid adenoma or carcinoma. Thus, although human and animal studies showed that dioxin and dioxin-like chemicals alter thyroid hormones and increase follicular-cell hyperplasia, there is little evidence of an increase in thyroid cancer. There are some reports of therapeutic treatment with arsenic trioxide and later development of thyroid cancer (Au et al., 2014; Firkin, 2014), raising the possibility of an association between arsenic and a risk of this malignancy. DMA treatment via the drinking water for 24 weeks caused increases in the incidence of thyroid hyperplasia and adenoma, but not of adenocarcinoma, in male F344/DuCrj rats that were first exposed for 4 weeks to a mixture of five carcinogens to induce tumor initiation in a wide range of tissues (S. Yamamoto et al., 1995). These increases were statistically significant at DMA doses of 200 and 400 ppm, but not at 50 or 100 ppm. Animals treated with 200 and 400 ppm DMA but not given the carcinogens did not develop thyroid lesions, which is consistent with the absence of elevated incidences of endocrine organ tumors in other bioassays with DMA (see Chapter 4).

As indicated in Chapter 4, 2,4-D and 2,4,5-T are at most weakly mutagenic or carcinogenic, and no studies that addressed a possible association between exposure to those herbicides and thyroid cancer in animal models have been identified.

Synthesis

The studies of Vietnam veterans and of occupational cohorts reviewed in previous updates did not provide compelling evidence to determine whether there is an association between exposure to the COIs and cancers of the endocrine organs. Although Le et al. (2016) found an increased incidence of thyroid cancer among VA health care users who self-reported exposure to Agent Orange, the study had several limitations, including self-reported, unconfirmed Agent Orange exposure and a lack of pathology confirmation of thyroid carcinomas. The small number of thyroid cancer deaths and the imprecise risk estimates identified among the UK phenoxy herbicide manufacturers and sprayers limited those findings as well. Few animal studies have been conducted examining the association between TCDD, dioxin-like PCBs, or DMA and thyroid cancer, but the results were inconsistent. Consequently, given the limitations of the new epidemiologic studies and the inconsistent results of the animal studies, the committee maintains that

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the evidence is inadequate or insufficient to determine an association between exposure to the COIs and thyroid and other endocrine cancers.

Conclusion

Based on the epidemiologic evidence reviewed here, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and thyroid or other endocrine cancers.

LYMPHOHEMATOPOIETIC CANCERS

Lymphohematopoietic cancers (LHCs) constitute a heterogeneous group of clonal hematopoietic disorders including leukemias, lymphomas, and multiple myeloma. They are among the most common types of cancer induced by environmental and therapeutic agents. As with other cancers that are subject to evolving and complex grouping in reports of the results of epidemiologic studies (notably, head and neck cancers and gastrointestinal cancers), the conclusions that the VAO committees have drawn about associations between exposure to the COIs and specific LHCs have been complicated by the lack of specificity and by inconsistencies in groupings in the available evidence. For LHCs, that has been a function not only of epidemiologists seeking to combine related cancers to produce categories that have enough cases to permit statistical analysis, but also of a continuous evolution of the system used by the medical community to classify these malignancies. The categorization of cancers of the lymphatic and hematopoietic systems has changed over time, guided by growing information about somatic mutation, gene expression, and subclonal lineage of the cancer cells that characterize each of a broad spectrum of neoplasms arising in these tissues (Jaffe, 2009). The WHO categorization presented in the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue* (WHO, 2008) bases its primary partition on whether the cancer cells are of myeloid or lymphoid origin (see Figure 7-1). This classification was updated in 2016 and reviewed by several academics and clinicians (Arber et al., 2016; Swerdlow et al., 2016), but the revised "blue book" has not yet been published.

Stem cells arising in the bone marrow generate two major lineages of leukocytes: myeloid and lymphoid. Myeloid cells include monocytes and three types of granulocytes (neutrophils, eosinophils, and basophils). Lymphoid cells include T and B lymphocytes and a smaller set of cells called natural killer cells. All of these mature cells circulate in the blood and are collectively referred to as white blood cells or leukocytes. Monocytes move out of the bloodstream into inflamed tissues, where they differentiate into macrophages or dendritic cells. Progenitor cells that are destined to become T lymphocytes migrate from the bone marrow to the thymus, where they acquire antigen-specific receptors. Antigen stimulation induces the T cells to differentiate into several subtypes involved in cell-mediated

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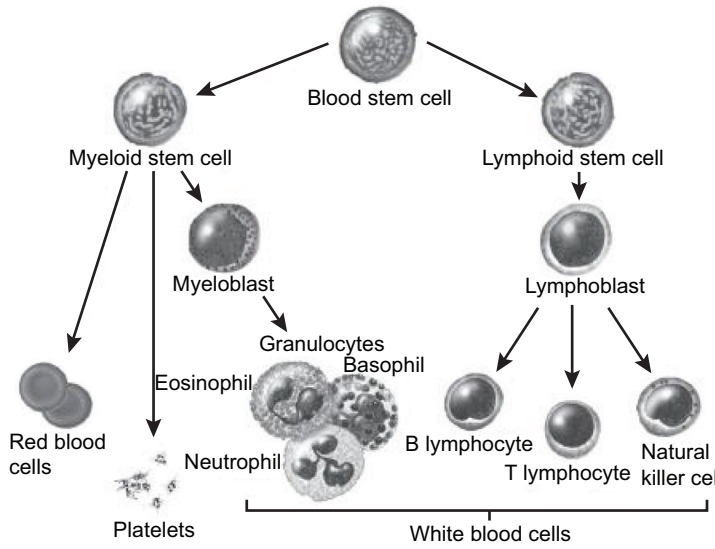

FIGURE 7-1 Hematopoiesis of stem cell differentiation.
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immunity, immune regulation, and the facilitation of B-cell function. Progenitor or pre-B cells mature in the bone marrow into antigen-specific B cells. On encountering their cognate antigens, B cells differentiate into antibody-secreting plasma cells involved in humoral immunity.

LHCs originate in specific pluripotent or lineage-restricted cells at different stages in hematopoiesis and immune-cell development. The normal cells are transformed into a malignant cell population through a multistep process that involves genetic and epigenetic alterations. Traditionally, LHCs have been divided into leukemias, lymphomas, myelomas, and so on, according to their cell type and site of origin (see Figure 7-1). Additional morphologic, cytochemical, and immunophenotypic data are used to characterize LHCs further and to further divide them into distinct subtypes.

Leukemias occur when a myeloid stem cell residing in the bone marrow becomes transformed, resulting in a failure of differentiation and a resistance to normal feedback on cellular proliferation. As the leukemic cells (blasts) fill the bone marrow, they actively secrete cytokines that prevent normal cellular proliferation, leading to reduced circulating normal blood cells. In addition, changes in adhesion molecules allow the release of these immature cells into the peripheral blood. Leukemias are generally classified as myeloid or lymphoid, depending on the lineage of the malignant cell population. If the original mutated cell of

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a cancer of the blood arises in a lymphoid progenitor, then the malignancy is termed lymphocytic leukemia; lymphocytic leukemias have been further partitioned into acute lymphoblastic leukemia (ALL) (also known as acute lymphoid leukemia or acute lymphocytic leukemia), which is derived from precursor B or T lymphoid stem cells, and chronic lymphocytic leukemia (CLL), which is a "mature B-cell neoplasm" arising from a transformed B lymphocyte. Myeloid leukemias arise from a myeloid hematopoietic stem cell and are classified into acute (AML) and chronic (CML) forms.

Lymphoma is a general term for malignancies that arise from lymphocytes (B, T, or natural killer cells). Lymphomas generally present as solid tumors at lymphoid proliferative sites, such as lymph nodes and the spleen. As stem cells mature into B or T cells, they pass through several developmental stages, each with unique functions. The developmental stage of the malignant cell defines the subtype of lymphoma. About 85% of lymphomas are of B-cell origin, and 15% are of T-cell or natural killer–cell origin (Jaffe et al., 2001; Liao et al., 2012). There are two major types of B-cell lymphomas: HL, previously referred to as Hodgkin disease, and NHL. B cells give rise to a wide array of neoplasms, which are characterized by the stage at which B-cell development was arrested, as well as by the surface protein expression and the genetic characteristics of the malignant cells. Follicular, large-cell, and immunoblastic lymphomas result when a malignancy develops after a B cell has been exposed to antigens. CLL is a tumor of antigen-experienced (memory) B cells (Chiorazzi et al., 2005); small lymphocytic lymphoma involves the same cells as CLL, but presents primarily in lymph nodes rather than in the bone marrow and blood and is therefore a variant of the same disease (Jaffe et al., 2008).

Multiple myeloma is a lymphohematopoietic malignancy derived from antibody-secreting plasma cells, which also have a B-cell lineage, that accumulate primarily in the bone marrow but may also infiltrate extramedullary sites. The related condition amyloid light chain (AL) amyloidosis also arises from B cell–derived plasma cells and reflects an abnormal deposition of antibody-derived light chains. It occurs as a complication in 5–15% of patients with multiple myeloma, and may also occur without evidence of frank multiple myeloma. Monoclonal gammopathy of undetermined significance (MGUS) is a clonal proliferation of plasma cells condition that may progress to multiple myeloma.

The ICD system partitions these malignancies into leukemias and lymphomas based primarily on whether the cancer cells circulate in the blood (disseminated) or appear in the lymphatic tissues, respectively, before subdividing by cell type. The emerging WHO classification of lymphohematopoietic malignancies (Campo et al., 2011; Jaffe, 2009) stratifies malignancies of the blood and lymph nodes into disease categories by their cell lineages—lymphoid or myeloid—as shown in Figure 7-1. It represents a substantial advance in understanding the biologic paths by which these malignancies develop. Since the new WHO classification has not yet been definitively approved, the committee decided that it

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would not be productive to reformulate this entire section to correspond to the new WHO categories. In practice, LHCs have routinely been reported in a variety of groupings, so it is a continuing challenge to parse out results, noting when results for broader groupings are presented in the supplementary tables for several more specific diagnoses, while recognizing that the specific results may be confounded by being "misclassified" with other entities. Most epidemiologic studies already in the evidentiary database that specifed diseases precisely used codes from ICD-9 or earlier versions, but some recent studies have applied ICD-10. Furthermore, the existing records that will serve as the basis of many current and even future studies will use earlier and evolving classifications, so a confounding of classification is likely to remain, even in new literature. The nomenclature has become more uniform in recent studies, but the possibility of ambiguity remains if earlier researchers did not use a unique code in accordance with some established system.

On occasion, the observed number of cases is so small that researchers cannot perform useful analyses for each type of LHC and will instead provide summary statistics for the entire group of them. In updating mortality in the Hamburg cohort in 1952–2007, Manuwald et al. (2012) found non-significant increases in mortality from LHC in both men and women, which combined to give a significant association between TCDD and all LHC deaths in the whole cohort (SMR = 1.61, 95% CI 1.03–2.40). In a Dutch cohort of workers in two phenoxy-herbicide plants, Boers et al. (2012) assessed plasma TCDD concentrations at the time of the assumed last exposure and reported a modest but not statistically significant increase in the HR for LHC in the total cohort, but there was no increase in plant A, where workers were occupationally exposed to TCDD.

Because VAO committees aim to address disease entities as specifically as possible with the available data, the overall results on the broader grouping of LHCs are of little consequence for the conclusions of association that have been drawn for the more specific entities. The committee for *Update 2010* noted, however, that the common biologic origin of LHCs that have been judged to have a substantial amount of evidence supporting association with the COIs (HL, NHL, CLL, hairy-cell leukemia [HCL], multiple myeloma, and AL amyloidosis) means that the WHO approach is supportive of and consistent with these decisions on the part of VAO committees. The Update 2014 committee familiarized itself with the classification systems that have been used for lymphoid malignancies, including hearing a presentation from the International Lymphoma Epidemiology Consortium (InterLymph) describing a proposed classification of these cancers into subtypes that are particularly appropriate for epidemiologic research, including methods to harmonize data, standardized definitions of disease entities and rigorous quality control of these subtype assessments, and attempts to understand the implications of etiologic heterogeneity (Morton et al., 2014a,b). At the same time, as has been recognized by others (Saberi Hosnijeh et al., 2012c), given the type and quality of the historical data that constitute the majority of the material

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available to the committee for review and judgement, little of that impressive effort can be applied to the committee's assessment of association.

VA asked previous VAO committees to address CLL, AML, and HCL individually. A scrutiny of the entire body of epidemiologic results on leukemias for findings on particular types (as had been the most common manner of grouping) revealed several studies that showed increased risks specifically of CLL but that did not provide support for an association of AML with exposure to the COIs. The committee for *Update 2002* advised VA that CLL is recognized as a form of NHL, which is already recognized as a service-related condition, whereas the committee for *Update 2006* did not recognize an association between the COIs and AML. Later, the committee responsible for *Update 2008* advised VA that, like CLL, HCL should be grouped as a mature B-cell neoplasm. For the current update, VA has tasked the committee to specifically address myeloproliferative neoplasms (MPNs). In light of the history and in accord with the current WHO classification, the committee for this update has incorporated data specifically on CLL and HCL into the section on NHL. After a brief synopsis of biologic plausibility of the LHCs overall, the more common cancers of the lymphatic system are described in the sections below on HL, NHL, and multiple myeloma (with a section on the related condition, AL amyloidosis), and then evidence on leukemias in general is discussed, with a focus on information regarding leukemias of myeloid origin.

Biologic Plausibility

Studies in animal models have indicated that the AHR pathway plays an integral role in B-cell maturation and that TCDD and dioxin-like chemical exposure may alter the function of these cells and lead to critical changes in the immune response. The suppression of the immune response by TCDD and similar chemicals in rodents and primates has been known for more than 30 years, but the effect on human cells is less clear. Some reports indicate that TCDD and dioxin-like chemicals elicit similar effects in humans. The activation of non-transformed human B cells results in an increase in the expression of AHR, and additional data indicate that this pathway has a role in normal B-cell function (Allan and Sherr, 2010; Sherr and Monti, 2013). Furthermore, treating these cells with benzo[*a*]pyrene suppresses B-cell differentiation. H. Lu et al. (2010) demonstrated that although human B cells appeared less responsive to TCDD in terms of increasing the expression of AHR pathway genes, the ability of TCDD to decrease immunoglobulin (Ig) M production is similar in both mouse and human B cells. Research that modeled the mode by which TCDD suppresses the terminal differentiation of B cells offers distinct pathways whose action can be altered by exposure (Q. Zhang et al., 2013). Data on human hematopoietic stem cells and from the use of knockout *Ahr* mouse models show that Ahr is critical in hematopoietic stem cell maturation and differentiation (Ahrenhoerster et al.,

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2014; Fracchiolla et al., 2011; K. P. Singh et al., 2011, 2014; B. W. Smith et al., 2013). TCDD not only alters hematopoietic stem cell maturation, but also alters proliferation and migration in vivo and in vitro (Casado et al., 2011). Finally, emblematic of the potential pleotropic effects of TCDD, Hughes et al. (2014) demonstrated that AHR plays a critical role in promoting lymphocyte differentiation into mature natural killer cells. Reviews have highlighted the complex and varied nature of the interaction of TCDD with the immune system (Gasiewicz et al., 2014; Lindsey and Papoutsakis, 2012).

Saberi Hosnijeh et al. (2012b, 2013a) assessed both the immune profile and the levels of soluble immune signaling proteins in TCDD-exposed workers. Consistent with data published on U.S. ACC veterans, in 47 highly TCDD-exposed and 38 low TCDD-exposed workers they found no effect of TCDD on major leukocyte subsets or on white blood cell counts. They did note a non-significant decrease in most lymphocyte subsets, which was most prominent for B cells. In these same workers, a study of soluble CD27 and soluble CD30 in sera found no clear dose–response relationship of TCDD with the level of these signaling proteins. However, there was a significant negative association of the serum IL-1RA level with the TCDD serum level among workers without chronic disease. Taken together, these data indicate that exposure to TCDD (and the alteration of normal AHR function) may have multiple effects on immune cell differentiation and function.

No new mechanistic or biologic plausibility studies regarding lymphohematopoietic cells have been identified by the committee since *Update 2014*.

Hodgkin Lymphoma

HL (ICD-9 201; ICD-10 C81), also known as Hodgkin disease, is distinguished from NHL primarily based on its neoplastic cells, mononucleated Hodgkin cells, and multinucleated Reed–Sternberg cells, derived from germinal-center B cells (Küppers et al., 2002). NCI estimated that 8,500 people would receive a new diagnosis of HL in the United States in 2018 and that 1,050 men and women would die from it; it ranks 25th in most common cancer diagnoses (NCI, n.d.y).

The incidence of HL increases with age; the average age of diagnosis is 39 years. Although the incidence rate is slightly higher in men than in women of the same race, it is about the same in whites and blacks (NCI, n.d.y). In the age groups that include most Vietnam veterans, the age-adjusted modeled incidence rate of HL for men 50–64 years old of all races combined was 3.2 per 100,000 in 2014 and increased to 4.7 for 65–74-year-olds and 5.7 for men over 75 years.[19]

[19]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output by using the SEER 13 dataset and choosing age-adjusted rates, HL, all races, age ≥ 50 years, and male sex.

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The possibility that HL has an infectious etiology has been a topic of discussion since its earliest description. A higher incidence in people who have a history of infectious mononucleosis has been observed in some studies, and a link with Epstein–Barr virus has been proposed (Balfour et al., 2015; Murray and Bell, 2015). In addition to the occupational associations discussed below, higher rates of the disease have been observed in people who have suppressed or compromised immune systems.

Conclusions from *VAO* and Previous Updates

Based on the 32 studies it reviewed, the committee responsible for *VAO* determined that there were sufficient epidemiologic data to support an association between exposure to the COIs and HL. Additional studies available to the committees responsible for subsequent updates have not changed that conclusion.

Two well-conducted Swedish studies with good exposure characterization reviewed in *VAO* provide the most comprehensive information on the association between exposure to phenoxy herbicides (2,4-D and 2,4,5-T), picloram, or chlorophenols and HL. Hardell et al. (1981) considered NHL and HL together, and Hardell and Bengtsson (1983) considered HL separately; they found statistically significant associations with exposure to phenoxy acids (after excluding people who were exposed to chlorophenols) and with exposure to chlorophenols. In a study of 54 HL cases, Persson et al. (1989) found a large but imprecise and not statistically significant risk associated with exposure to phenoxy acids. Several of the other case-control and occupational-cohort studies in U.S. and international populations reviewed in *VAO* showed an increased risk of HL, but only a few of the results were statistically significant. As with NHL, even the largest studies of production workers who were exposed to TCDD did not indicate an increased risk. The few studies of HL in Vietnam veterans tended to show increased risks, but only one (Holmes et al., 1986) was statistically significant.

Among the studies of veterans reviewed throughout the VAO series, no statistically significant increased risk of HL was found. Publications from the AFHS showed no statistically significant increases in HL or lymphopoietic cancers (AFHS, 2000; Akhtar et al., 2004). Other populations of Vietnam-era veterans likewise did not find an association (Anderson et al., 1986a,b; Holmes et al., 1986; Lawrence et al., 1985; Visintainer et al., 1995). A proportionate mortality ratio analysis that compared the experience of 33,833 U.S. Army and Marine Corps Vietnam veterans who died during 1965–1988 with that of 36,797 deceased non-Vietnam veterans found a statistically significant increase of deaths from HL in Marine Corps veterans—although not Army veterans—who had served in Vietnam (Watanabe and Kang, 1996). Cypel and Kang (2008) compared mortality from lymphopoietic cancers in female Vietnam veterans with that of female era

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veterans and the U.S. population; deaths from lymphopoietic cancers were not higher in those who served in Vietnam.

Studies of Australian, New Zealand, and Korean veterans who served in Vietnam have also been reviewed. The incidence of HL was found to be statistically significantly higher than the general population when Australian veterans from all branches were combined (ADVA, 2005a), but when veterans were stratified by service branch, the only statistically significant association was between HL and service in the Army. However, no statistically significantly increased mortality from HL was found for all service branches combined, nor for any single branch (ADVA, 2005b). A comparison of deployed and non-deployed Vietnam-era Australian conscripted Army National Service veterans found no association between deployment and the incidence of or mortality from HL (ADVA, 2005c). Among a cohort of male Vietnam veterans from New Zealand, McBride et al. (2013) reported one death from and three incident cases of HL resulting in imprecise and unstable effect estimates. Among the Korean veterans who had served in Vietnam, Yi and Ohrr (2014) reported no statistically significant difference in the risk of HL in the internal comparison of the high- and low-exposure groups based on the EOI scores.

Several occupational cohorts of workers from several countries who were exposed to phenoxy herbicides and other related chemicals have been followed long term. Studies of the IARC phenoxy-herbicide cohort showed no excess of HL incidence or mortality compared with national rates (Kogevinas et al., 1993). Additional follow-up showed a non-statistically significant increase in HL in workers who were exposed to TCDD or higher chlorinated hydrocarbons but no association was found between phenoxy herbicides or chlorophenols and HL (Kogevinas et al., 1997). In a multinational IARC cohort of 60,468 pulp-and-paper-industry workers, McLean et al. (2006) found that death from HL was significantly higher in those who had ever been exposed to nonvolatile organochlorine compounds (which would include TCDD) but not in those who had never been exposed compared with the national standardized populations.

In a retrospective cohort study of Dutch production and contract workers who were exposed to phenoxy herbicides, chlorophenols, and contaminants during 1950–1976, Hooiveld et al. (1998) reported a non-significant increase in HL. Rix et al. (1998) compared mortality in a cohort of Danish paper mill workers with mortality in the general Danish population and found a statistically significant increase in men but not women. Swaen et al. (2004) extended the follow-up of mortality by 13 years in a cohort of Dutch herbicide appliers and observed no additional deaths, causing the earlier mortality risk estimate of HL in the cohort to no longer be statistically significant (Swaen et al., 1992). Studies of German manufacturing workers found no association between exposure to TCDD and HL (Becher et al., 1996). McBride et al. (2009a) examined mortality in TCP manufacturing workers in the Dow AgroSciences plant in New Zealand, but a single observed HL death yielded inconclusive results. A French,

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hospital-based, case-control study of lymphoid neoplasms (Orsi et al., 2009) did not find a statistically significant increase in the risk of HL after occupational exposure to herbicides in general or after occupational exposure to phenoxy herbicides in particular.

Among U.S. production workers, the low numbers of deaths from HL and imprecise estimates limited the findings. In an update and expansion of cohorts involved in the NIOSH study, Steenland et al. (1999) observed three deaths attributed to HL, which was not statistically different from the comparison population. No deaths from HL were identified in Dow PCP workers in Midland, Michigan (Collins et al., 2009c; Ramlow et al., 1996), and only two deaths from HL occurred among the TCP workers, resulting in imprecise risk estimates (Collins et al., 2009b). Additional updates of these occupational cohorts noted very small numbers of additional cases of HL, which did not produce substantive changes in prior findings. Burns et al. (2011) reported an additional case among Dow 2,4-D production workers. Ruder and Yiin (2011) likewise reported one additional HL death in the NIOSH cohort of PCP workers.

Studies of the Seveso cohort have found few cases and no increased incidence or mortality of HL among men or women in zones A, B, or R. These findings remained consistent at the 10-year follow-up (Bertazzi et al., 1993), 15-year follow-up (Bertazzi et al., 1997), 20-year update (Bertazzi et al., 2001; Pesatori et al., 2009), and 25-year update (Consonni et al., 2008).

Several studies of HL among agricultural workers have also been reviewed by the update committees. Whereas Persson et al. (1993) reported a significant increase in the odds of HL prevalence in Swedish farmers who were exposed to phenoxy acid herbicides, no excess of HL deaths was found in a death certificate review of U.S. farmers from 23 states (Blair et al., 1993). In a nonspecific analysis of exposure to "herbicides" in a Michigan farming community, Waterhouse et al. (1996) demonstrated a statistically significant increase in the combined incidence of lymphopoietic neoplasms in a prospective study. Two reports from the AHS found no excess risk of HL in pesticide applicators, commercial applicators, or their spouses, but herbicide exposures were not specific (Alavanja et al., 2005; Blair et al., 2005a). Updates of cancer incidence (Koutros et al., 2010a) and mortality (Waggoner et al., 2011) among participants in the AHS also did not find increases of HL in private applicators or their spouses.

In the Cross-Canada Study of Pesticides and Health, P. Pahwa et al. (2006) found no association of any exposure to phenoxy herbicides, 2,4-D, Mecoprop, or MCPA and HL. Nested case-control studies of data from the Cross-Canada study found no statistically significant associations of exposure to COIs with HL (Karunanayake et al., 2012; P. Pahwa et al., 2003). A follow-up analysis of the Cross-Canada study (Navaranjan et al., 2013) that examined the incidence of STS, NHL, multiple myeloma, and HL in men ages 19 years and older found no increased risk of HL in those exposed to one, two, or three or more phenoxy

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herbicides after adjusting for age and the province of residence. However, exposure specificity remains a major issue for this study.

Update of the Epidemiologic Literature

No new published literature of Vietnam veterans, environmental studies, or case-control studies that addressed exposure to the COIs and HL was identified by the committee for the current update. Reviews of the relevant studies are presented in the earlier reports. Table 24, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to HL.

Occupational Studies Among the Dow Midland, Michigan, worker cohort that was compared with the standardized U.S. population, Collins et al. (2016) found only two deaths from HL were reported during the follow-up period and both were among TCP workers, making the reported risk estimates unreliable.

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides, Coggon et al. (2015) reported only three deaths from HL, and all were among the workers potentially exposed to phenoxy acids above background levels and for more than 1 year (SMR = 1.71, 95% CI 0.35–4.99).

Other Identified Studies One other study that reported outcomes of mortality from HL was identified, but it lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effects of the COIs (Ruder et al., 2014).

Biologic Plausibility

HL arises from the malignant transformation of a germinal-center B cell and is characterized by malignant cells that have a distinctive structure and phenotype; these multinucleate cells are known as Reed–Sternberg cells (Jaffe et al., 2008). No animal studies have shown an increase in HL after exposure to the COIs. Reed–Sternberg cells have not been demonstrated in mice or rats, so there is no good animal model of HL. Thus, there are no specific animal data to support the biologic plausibility of an association between the COIs and HL.

Synthesis

The relative rarity of HL complicates the evaluation of epidemiologic studies because their statistical power is generally low. Earlier studies (Eriksson et al., 1992; Hardell et al., 1981; Holmes et al., 1986; LaVecchia et al., 1989; Persson et al., 1993; Rix et al., 1998; Waterhouse et al., 1996; Wiklund et al., 1988a) were generally well conducted and included excellent characterizations of exposure,

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and they formed the basis of previous VAO committees' conclusions. Subsequent findings have not contradicted those conclusions, especially given that most studies have had low statistical power, as was seen in the current extended follow-ups of occupational cohorts that reported two (Collins et al., 2016) and three (Coggon et al., 2015) deaths from HL. Although it has not been demonstrated in any animal models, a positive association between the COIs and the development of HL is biologically plausible because of the common lymphoreticular origin of HL and NHL (which has been demonstrated in animal models) and their common risk factors.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is sufficient evidence of an association between exposure to at least one of the COIs and HL.

Non-Hodgkin Lymphoma

NHL (ICD-9 200.0–200.8, 201, 202.0–202.2, 202.8–202.9; ICD-10 C82–85, C96.3) is a general name for malignancies of the lymphatic system other than HL or plasma cell dyscrasias. NHL consists of a large group of lymphomas that can be partitioned into acute and aggressive (fast-growing) or chronic and indolent (slow-growing) types of either B-cell or T-cell origin. B-cell NHL includes Burkitt lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, large-cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle-cell lymphoma. T-cell NHL includes mycosis fungoides and anaplastic large-cell lymphoma. Precursor T-lymphoblastic lymphoma is not considered a type of NHL and is considered instead part of T-lymphoblastic lymphoma/leukemia, a precursor lymphoid neoplasm included with the broad group of "acute lymphoid leukemias," which can be of either T-cell or B-cell origin.

In response to requests from VA to address CLL, HCL, and AML specifically and individually, CLL and HCL have been recognized as subtypes of NHL (demonstrating B-cell origin and immunohistochemical properties consistent with B-cell lymphoma). The proposed WHO classification of NHL notes that CLL (ICD-9 204.1; ICD-10 C91.1) and its lymphomatous form, small lymphocytic lymphoma, are both derived from mature B cells (Chiorazzi et al., 2005; IARC, 2001). The committee for *Update 2012* determined that it is more appropriate to consider those lymphatic malignancies with other forms of NHL. Therefore, the discussion of CLL and HCL has been moved into the NHL grouping.

NCI estimated that 74,680 people would receive a new diagnosis of NHL in the United States in 2018 and that 19,910 men and women would die from it; it ranks as the seventh most common cancer diagnosis (NCI, n.d.z). The incidence of NHL increases with age; the average age of diagnosis is 67 years. The incidence rate is about 50% higher in white and black men than in women of the

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same race and is highest for whites. In the age groups that include most Vietnam veterans, the age-adjusted modeled incidence rate of NHL for men 50–64 years old of all races combined was 36.3 per 100,000 in 2014 and increased to 91.1 for 65–74-year-olds and 147.8 for men over 75 years.[20]

In addition, NCI estimated that 20,940 men and women would receive a diagnosis of CLL in the United States in 2018 and that 4,510 people would die from it. Nearly all cases occur after the age of 50 years, and the median age of diagnosis is 70 years. The incidence rate is about two times higher in men than in women of the same race and is highest for whites (NCI, n.d.aa).

The causes of NHL are poorly understood. People who have suppressed or compromised immune systems are known to be at higher risk, and some studies show an increased incidence in people who have HIV, human T-cell leukemia virus type I, Epstein–Barr virus, surface antigen positive hepatitis B, or gastric *Helicobacter pylori* infections. The human retrovirus HTLV-1 causes adult T-cell lymphoma, but early reports that HTLV-2 might play a role in the etiology of HCL have not been substantiated. A broad spectrum of behavioral, occupational, and environmental risk factors have been proposed as contributors to the occurrence of NHL, but given the diversity of malignancies included under this name and the evolving classification of the subtypes (to date, minimal exploration of etiologic heterogeneity has been done), it is not too surprising that—aside from infectious agents, immune problems, and particular chemotherapies—specific risk factors have not been definitively established (Morton et al., 2008, 2014a,b; Wang and Nieters, 2010).

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* concluded that there was sufficient evidence to support an association between exposure to at least one of the COIs and NHL. Additional information available to the committees responsible for later updates has not changed that conclusion. *Update 2002* was the first to discuss CLL separately from other leukemias. The epidemiologic studies indicated that farming, especially with exposure to 2,4-D and 2,4,5-T, is associated with significant mortality from CLL. Many more studies support the hypothesis that herbicide exposure can contribute to NHL risk. Most cases of CLL and NHL reflect the malignant transformation of germinal-center B cells, so these diseases could have a common etiology.

As with HL, the epidemiologic data reviewed by previous VAO committees suggest that it is the phenoxy herbicides (including 2,4-D) rather than TCDD that may be associated with NHL. The original VAO committee concluded that a

[20]As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output using the SEER 13 dataset and by choosing age-adjusted rates, non-Hodgkin lymphoma, all races, age groups 50–64, 65–74, and ≥ 75 years, and male sex.

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positive association existed between exposure to herbicides and the development of NHL, and studies reviewed by later committees have continued to support that finding. A large, well-conducted case-control study in Sweden by Hardell (1981) examined NHL and HL together and found, on the basis of 105 cases, a statistically significantly increased risk associated with exposure to phenoxy acids or chlorophenols. Those results were replicated in further investigations of the validity of the exposure assessment and potential biases (Hardell, 1981). Hardell et al. (1994) also examined the relationship between occupational exposure to phenoxyacetic acids and chlorophenols and various characteristics related to NHL—including histopathologic measures, stage, and anatomic location—on the basis of the NHL cases in a previous study (Hardell et al., 1981). Similar data by Persson et al. (1989) showed, based on a logistic regression analysis of 106 cases, an increased risk of NHL in those exposed to phenoxy acids. Other case-control studies of NHL conducted in Sweden found a statistically significantly elevated risk of NHL (Eriksson et al., 2008; Hardell and Eriksson, 1999; Hardell et al., 2001, 2002; Olsson and Brandt, 1988).

Several case-control studies of NHL incidence, risk, or mortality in other exposed populations have also been reviewed by VAO committees, including studies from New Zealand (Pearce et al., 1985, 1986b, 1987), Italy (Amadori et al., 1995; Nanni et al., 1996), and Canada (Ng et al., 2010; Spinelli et al., 2007). Various U.S. populations have also been studied for associations of herbicides with NHL (Cantor, 1982; Cantor et al., 1992; Colt et al., 2009; Hartge et al., 2005; Tatham et al., 1997; Zahm et al., 1993); NHL and multiple myeloma (Burmeister et al., 1983); and STS and NHL (Woods and Polissar, 1989; Woods et al., 1987).

Studies of production workers have shown some association between TCDD exposure and NHL. A larger study of 21,863 workers in the IARC phenoxy-herbicide cohort followed from 1939 to 1992 found a non-significant increase in NHL risk (Kogevinas et al., 1997). Other studies of Danish and Dutch phenoxy-herbicide workers who were part of the IARC cohort have shown a non-significant increased risk of NHL (Boers et al., 2010; Bueno de Mesquita et al., 1993; Hooiveld et al., 1998; Lynge, 1993). A cohort of 2,479 workers in four plants in Germany with exposure to phenoxy herbicide and contaminants (dioxins and furans) had a significantly increased risk of NHL on the basis of five cases (Becher et al., 1996). Non-statistically significant increases in risk have also been found in the NIOSH mortality study (Steenland et al., 1999). Risks were not significantly increased among the Dow Chemical Company Midland, Michigan, or Plymouth, New Zealand, chemical production workers, phenoxy-herbicide sprayers, or 2,4-D production workers (Bloemen et al., 1993; Bodner et al., 2003; C. J. Burns et al., 2001; Collins et al., 2009b,c; McBride et al., 2009a,b; Ramlow et al., 1996; 't Mannetje et al., 2005). A multinational IARC cohort study of paper-and-pulp workers found a statistically significant increase in workers who were exposed to chlorophenols (McLean et al., 2006).

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Studies of farmers and agricultural workers have been generally positive for an association between herbicides or TCDD and NHL; however, only a few were statistically significant. A meta-analysis of several studies of the association between employment as a farmer in the central United States and NHL showed a statistically significant risk (Keller-Bryne et al., 1997). All of the studies of U.S. agricultural workers that were reviewed showed increased risk ratios, and two NCI studies of farmers in Kansas and Nebraska (Hoar et al., 1986; Zahm et al., 1990) showed patterns of an increased risk linked to use of 2,4-D. A third NCI study of NHL was conducted in four Surveillance, Epidemiology and End Results program centers (Detroit, Iowa, Los Angeles, and Seattle) from 1998 to 2000 (Pronk et al., 2013). The researchers used residential history from 15 years prior to diagnoses to link residence to EPA databases of dioxin-emitting facilities, studying 969 cases and 749 controls. Proximity to any dioxin-emitting facility was not associated with NHL. A study of a subcohort of Hispanic workers in a larger cohort of 139,000 California members of the United Farm Workers of America (Mills et al., 2005) and a population-based case-control study in Italy of NHL and CLL cases (combined) identified during 1991–1993 (Miligi et al., 2006) both showed statistically significant associations with 2,4-D. An additional occupational exposure study using the EPI-LYMPH multicenter study (conducted in the Czech Republic, France, German, Ireland, Italy, and Spain from 1998 to 2004) assessed pesticide use in applying a crop-exposure matrix with lymphoma diagnoses in a case-control design (Cocco et al., 2012). No statistically significant association was observed between any kind of pesticide or herbicide use and lymphomas or its subtypes, including between exposure to phenoxy acids and CLL.

The Cross-Canada Study of Pesticides and Health, a large, well-conducted, population-based, case-control study, reported on pesticide use and NHL incidence in men identified from cancer registries of six Canadian provinces from 1991 to 1994. Statistically significant associations were found between exposure to phenoxy herbicides, 2,4-D, or Mecocrop (MCPA) and NHL. A reanalysis of the data from that study confirmed the findings on phenoxy herbicides but found that the association with 2,4-D, although still increased, was no longer statistically significant (McDuffie et al., 2001). A study of the participants who had a first diagnosis of STS, NHL, multiple myeloma, or HL during the original study period were followed through mailed and telephone interviews to examine the joint effects of asthma, allergies, or asthma and allergies and hay fever combined with pesticide exposure in the genesis of NHL. Incident NHL cases (n = 513) diagnosed between 1991 and 1994 were compared with the experience of 1,506 controls. Subjects with asthma, allergies, or hay fever had elevated risks that were not statistically significant associated with the use of phenoxy herbicides, MCPA, or 2,4-D. The results overall were not supportive of any major effect modification by these immune conditions.

A population-based case-control study conducted in 2000–2001 of men and women 20–74 years old living in New South Wales, Australia, found an

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increased risk of NHL associated with "substantial" exposure to phenoxy herbicides (Fritschi et al., 2005). Spinelli et al. (2007) reported on a population-based case-control study in Vancouver and Victoria, British Columbia, which found strong monotonic increases in serum concentrations of two dioxin-like PCBs (PCB 118 and PCB 156). Chiu et al. (2004) and W. J. Lee et al. (2004a) conducted a pooled analysis of two case-control studies that were carried out in three midwestern U.S. states—Iowa and Minnesota (Cantor et al., 1992) and Nebraska (Zahm et al., 1990)—and found that NHL risks were increased in farmers by the use of herbicides, including 2,4-D and 2,4,5-T. In a study of NHL incidence in people who lived near 13 French municipal waste incinerators, Viel et al. (2008b) found a small but statistically significant increase in the risk of NHL and evidence of a dose–response relationship with increased exposure to dioxin. A case-control study of NHL rates in people who lived near a municipal solid-waste incinerator in Bensaçon, France, found that the incidence of NHL was significantly increased in the area determined to have the highest dioxin contamination, but no increases were found in the low and intermediate categories (Floret et al., 2003). A French hospital-based case-control study of lymphoid neoplasms (Orsi et al., 2009) did not find the occurrence of NHL to be associated with occupational or domestic use of pesticides or phenoxy herbicides in particular. With 25 years of follow-up of the Seveso population and a relatively small number of observed cases, there is no evidence of an increased incidence of NHL or mortality in any of the exposed zones (Bertazzi et al., 1989b, 1993, 1997, 2001; Consonni et al., 2008; Pesatori et al., 1992, 2009).

The findings of several PCB-focused studies (Bertrand et al., 2010; Engel et al., 2007; Laden et al., 2010) are consistent with the associations with NHL repeatedly observed in connection with the COIs in the VAO series. However, the extent of intercorrelation of these persistent organic pollutants greatly curtails the degree to which any effect can be specifically attributed to dioxin-like activity.

Evidence of an association between the COIs and NHL in Vietnam veterans, the primary population of interest in the VAO updates, has been lacking. The CDC Selected Cancers Study (CDC, 1990a,b) showed a significantly increased risk of NHL in all Vietnam veterans; however, in an analysis that considered branch of service, Army and Air Force personnel were found not to be at increased risk. Marine Corps veterans had a higher mortality in the CDC Selected Cancers Study and significantly increased risks in several other studies (Breslin et al., 1988; Burt et al., 1987; Watanabe and Kang, 1996; Watanabe et al., 1991), but the implications of these findings are unclear. No increased risk has been found in Ranch Hand veterans (AFHS, 2000; Akhtar et al., 2004; Michalek et al., 1990; Wolfe et al., 1990) or in members of the ACC (Boehmer et al., 2004).

In studies of Vietnam veterans from New Zealand and Korea, no statistically significant increased risk of NHL was found in either group. The New Zealand study also reported on lymphoid leukemia (which would include any cases of CLL) and found a statistically significant increase (McBride et al., 2013). Yi et

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al. (2014b) reported a modestly increased risk for NHL mortality between the high- versus low-exposure groups of the Korean veterans and a small increased risk with the individual log-transformed EOI scores, but neither was statistically significant. Lymphoid leukemia showed a nonstatistically significant decreased risk based on nine low-exposure and five high-exposure deaths.

Update of the Epidemiologic Literature

Several new studies of exposure to the COIs and NHL were identified including two studies of Vietnam veterans and CLL. Reviews of the relevant studies are presented in the earlier reports. Table 25, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to NHL.

Vietnam-Veteran Studies A retrospective study of veterans diagnosed with CLL who attended the Minneapolis VA at least one time between 2001 and 2010 was conducted to determine whether exposure to Agent Orange alters features of CLL disease presentation or its prognostic features, including stage at diagnosis, lymphocyte doubling time, or cytogenetics (Baumann Kreuziger et al., 2014). Of the 195 veterans identified as having a diagnosis of CLL, 33 (17%) were determined to have been exposed to Agent Orange. (Veterans submit a claim of exposure that is reviewed by VA using service records to confirm whether the veteran was stationed in an area that was sprayed with herbicides during the service period.) Except for age at diagnosis, demographic factors and laboratory counts were similar between the veterans determined to have been exposed and those reported as unexposed. Compared with veterans who were reported as unexposed to Agent Orange, the exposed patients showed a statistically significantly lower age of presentation (61 versus 72 years; $p = 0.001$), and the time to first treatment was also shorter in the exposed patients (9.6 versus 30 months, $p = 0.02$), but the number of therapies and the rate of transformation were not different between exposed and unexposed patients. However, providers were not blinded to exposure status, and, because patients were younger, these factors may have influenced the therapy paradigm used. Overall, survival was not statistically different between exposed and unexposed patients, and this remained the case after adjusting for age (HR = 1.8, 95% CI 0.7–4.5, $p = 0.24$).

A second retrospective study among U.S. Vietnam veterans was identified that examined CLL prognosis, treatment, and survival and their association with exposure to Agent Orange (determined by benefits and compensation officers who use service records to confirm if the locations and timeframes of deployment correspond to sprayed areas). Mescher et al. (2018) identified 2,052 Vietnam veterans (20.4% determined to have been exposed to Agent Orange) who were diagnosed with CLL between 2009 and 2013 from the National VA Tumor Registry. Information on demographics, laboratory and disease-related parameters at the time of the initial diagnosis, and the type and number of treatments received was taken from

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medical records. Overall survival was defined as time from CLL diagnosis to death from any cause or else to date of last follow-up. The Cox proportional hazards model included age, Rai stage, and baseline laboratory parameters. Prognostic factors did not differ based on exposure. Compared with the unexposed, veterans exposed to Agent Orange were diagnosed at younger age (63.2 versus 70.5 years, $p < 0.0001$) and had longer overall survival ($p < 0.001$). CLL prognostic factors and cytogenetics did not differ between the groups, although more of the exposed group had cytogenetics available. Time to initial treatment was shorter in exposed patients. Median overall survival was longer in exposed patients—quite possibly because they were treated more aggressively because they were young.

Occupational Studies Among the Dow Midland, Michigan, worker cohort, Collins et al. (2016) found that compared with the standardized U.S. population, no differences in mortality for NHL were found for the TCP workers (n = 10; SMR = 1.08, 95% CI 0.52–1.99) or the PCP workers (n = 8; SMR = 1.92, 95% CI 0.83–3.79).

In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers examining the carcinogenicity of phenoxy herbicides and their association primarily with HL, STS, and CLL, Coggon et al. (2015) calculated standardized mortality ratios using person-years and the general populations of England and Wales as the comparison group. Nested case-control analyses compared men with incident or fatal NHL/CLL (n = 74) and matched controls (up to 10 per case). A total of 29 NHL/CLL deaths were reported. The risk of death from NHL/CLL was not statistically significantly elevated among all workers (SMR = 0.96, 95% CI 0.64–1.38) or among workers exposed to herbicide levels above background (n = 26; SMR = 1.15, 95% CI 0.75–1.68). However, death from NHL was statistically significantly higher for men who were exposed to phenoxy acids above background for 1 year or longer (n = 19; SMR = 1.85, 95% CI 1.12–2.89). No other statistically significant associations or trends were reported for mortality from other types of cancers. Although a statistically significant excess of deaths from NHL was reported, the authors believe that greater weight should be given to the nested case-control analysis, which included both non-fatal and fatal cases and men with CLL as well as solid lymphomas. That analysis found no statistically significant increased risk with exposure to phenoxy herbicides; the highest risk estimate was for workers with high exposure (OR = 1.22, 95% CI 0.61–2.46). The committee believes that the caveat here is that the researchers seem to use CLL as their surrogate for NHL, which may or may not be correct. A cleaner analysis would have been to look specifically at other lymphomas (diffuse large B cell, follicular lymphomas, etc.). However, as the data linking exposure to phenoxy herbicides and NHL have been inconsistent, the committee agrees with the authors that if there really is an increased risk of NHL/CLL, it is likely very small.

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Case-Control Studies Czarnota et al. (2015) conducted an analysis of 27 correlated chemicals (5 PCBs, 7 PAHs, and 15 pesticides) measured in house dust samples and NHL risk using data collected from 1998 to 2000 as part of the population-based case-control study of NHL in four SEER study sites (Detroit, Iowa, Los Angeles, and Seattle). Eligible cases were 20–74 years of age, diagnosed with a first primary NHL, and uninfected with HIV. The study recruited 1,321 people with NHL who were frequency-matched to 1,057 controls by sex, age (within 5-year groups), race, and study site. Computer-assisted personal interviews were conducted in the home of each participant and included questions on demographics, house characteristics, pesticide use in the home and garden, and residential and occupational histories. Dust samples were collected and analyzed from vacuum cleaners for participants who had used their vacuum cleaner within the past year and owned at least half of their carpets or rugs for 5 years or more. The chemicals relevant to the work of the VAO committee that were analyzed were PCB 105, 2,4-D, and dicamba. Pairwise comparisons were performed to obtain a weighted quantile sum for an index of 27 environmental chemicals. The 27 chemicals were not weighted equally in the index, overall or by site. The weighted quantile sum index was statistically significantly associated with NHL (OR = 1.30, 95% CI 1.08–1.56). Concentrations of 2,4-D and dicamba were found to be elevated in the Iowa site. The only individual chemical that was associated with NHL was non-dioxin-like PCB 180. However, associations with NHL were negative and statistically significant for 2,4-D (OR = 0.36, 95% CI 0.19–0.68) and dicamba (OR = 0.48, 95% CI 0.26–0.90) in the Iowa site and 2,4-D (OR = 0.53, 95% CI 0.29–0.97) and dicamba (OR = 0.41, 95% CI 0.22–0.76) in the Seattle site.

Kelly et al. (2017) used data from the EnviroGenoMarkers study, which is nested within two prospective cohorts in Italy and Sweden, to determine the association between NHL and prediagnositic blood plasma concentrations of 10 environmental pollutants, including two dioxin-like PCB congeners (118 and 156). During 16 years of follow-up, 270 incident cases of B-cell NHL (including 76 cases of multiple myeloma) were diagnosed. Cases were matched to 270 healthy controls by center, age, gender, and date of blood collection. Cases were categorized into ordered quartiles of exposure for each persistent organic pollutant based on the distribution of exposure in the control population. No difference in median exposure levels was observed between cases and controls for any of the chemical exposures ($p > 0.05$) or, specifically, for the dioxin-like PCBs: PCB 118 ($p = 0.991$) and PCB 156 ($p = 0.186$). The quartile analysis similarly did not show an association between the dioxin-like PCBs and NHL (only the comparison of cases and controls in the third quartile was statistically significant, but there was no significant association in the test for trend). These were not heavily exposed individuals, so the levels may be uniformly low. Overall, this study does not provide evidence that higher body burden of the two dioxin-like PCBs (or

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the other persistent organic pollutants measured) increases the risk of subsequent NHL diagnosis.

Other Identified Studies Six other studies of NHL in occupational cohorts were identified. Two examined participants of the AHS: in the first study none of the COIs were included in the analysis of exposures (Alavanja et al., 2014), and the second paper (Goodman et al., 2017) was an updated meta-analysis of 2,4-D and NHL that included 10 studies of the AHS but did not contribute original data. A third study examined cause-specific mortality, including NHL, among U.S. workers exposed to mixed PCBs (Ruder et al., 2014), the fourth study analyzed health outcomes among Brazilian agricultural workers exposed to unspecified pesticides (Boccolini et al., 2017), and the fifth study was a small case-control study designed to investigate the effects of exposure to pesticides (determined by occupation as a farm worker) on NADPH (nicotinamide adenine dinucleotide phosphate, a cofactor used in biochemical reactions), including its level and relationship with the Th1/Th2 ratio, in patients diagnosed with NHL in Algeria (Zahzeh et al., 2015). A sixth study (Aras et al. 2014) was a case-control study conducted at a cancer center in France to determine whether patient diagnosed with NHL had reported exposure to pesticides (types of insecticides and herbicides, if known; frequency; and duration), and other known or suspected risk factors. However, all these studies lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs.

Biologic Plausibility

The diagnosis of NHL encompasses a wide variety of lymphoma subtypes. In humans, about 85% are of B-cell origin and 15% of T-cell origin. In commonly used laboratory mice, the lifetime incidence of spontaneous B-cell lymphomas is about 30% in females and about 10% in males. Although researchers seldom note the subtypes of B-cell lymphomas observed, lymphoblastic, lymphocytic, follicular, and plasma-cell lymphomas are seen in mice and are similar to the types of NHL seen in humans. Laboratory rats, on the other hand, are less prone to develop lymphomas, although Fisher 344 rats do have an increased incidence of spontaneous mononuclear-cell leukemia of non-specific origin. The lifetime incidence of leukemia is about 50% in male rats and about 20% in female rats. Neither mice nor rats develop T-cell lymphomas spontaneously at a predictable incidence, but T cell–derived tumors can be induced by exposure to some carcinogens.

Several long-term feeding studies of various strains of mice and rats have been conducted over the past 30 years to determine the effects of TCDD on cancer incidence. Few of them have shown effects of TCDD on lymphoma or leukemia incidence. NTP (1982a) reported no increase in the overall incidence of lymphoma in female B6C3F1 mice exposed to TCDD at 0.04, 0.2, or

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2.0 µg/kg per week for 104 weeks, but it did find that histiocytic lymphomas (now considered to be equivalent to large B-cell lymphomas) were more common in the high-dose group. No effects on lymphoma incidence were seen in Osborne–Mendel rats treated with TCDD at 0.01, 0.05, or 0.5 µg/kg per week. Sprague Dawley rats treated with TCDD at 0.003, 0.010, 0.022, 0.046, or 0.100 µg/kg per day showed no change in the incidence of malignant lymphomas. Nor has long-term exposure to phenoxy herbicides or cacodylic acid resulted in an increased incidence of lymphomas in laboratory animals. Thus, few laboratory animal data support the biologic plausibility of the promotion of NHL by TCDD or the other COIs, but it should be noted that the standard rodent models are not particularly sensitive for the detection of chemicals that cause lymphohematopoietic cancers.

In contrast, more recent studies at the cellular level indicate that activation of AHR by TCDD inhibits apoptosis, a mechanism of cell death that controls the growth of cancer cells. Vogel et al. (2007) studied human cancer cells in tissue culture and showed that the addition of TCDD inhibited apoptosis in histiocytic-lymphoma cells, Burkitt-lymphoma cells, and NHL cell lines. The reduction in apoptosis was associated with an increase in the expression of *Cox-2*, *C/EBP* β, and *Bcl-xL* mRNA in the cells. Those genes code for proteins that protect cells from apoptosis. The effects of TCDD on apoptosis were blocked when an AHR antagonist or a Cox-2 inhibitor was added to the culture; this demonstrated the underlying AHR-dependent mechanism of the effects. More important, when C57Bl/10J mice were given multiple doses of TCDD over a period of 140 days, premalignant lymphoproliferation of B cells was induced before the appearance of any spontaneous lymphomas in the control mice. When the B cells were examined, they were found to manifest changes in gene expression similar to those induced by TCDD in the human cell lines, which provided support for this mechanism of lymphoma promotion by TCDD. Work by Phadnis-Moghe et al. (2015) found that TCDD exposure in human B-cell culture impairs B-cell lymphoma-6 activity, which may contribute to the development of NHL.

It is well established that AHR activation by TCDD in human breast and endocervical cell lines induces sustained high concentrations of the IL-6 cytokine, which has tumor-promoting effects in numerous tissues (Hollingshead et al., 2008). IL-6 plays a role in B-cell maturation and induces a transcriptional inflammatory response. It is known to be increased in B-cell neoplasms, including multiple myeloma and various lymphomas, and especially diffuse large B-cell lymphomas (Hussein et al., 2002; Kato et al., 1998; Kovacs, 2006).

An alternative link that could help explain the association between TCDD and NHL has been explored in human studies. Chromosomal rearrangements, with the consequent dysregulation of the expression of various genes, are prevalent in B-cell lymphomas, and the t(14;18) reciprocal translocation, which juxtaposes the BCL2 with the locus of the immunoglobin heavy chain, is found in tumor cells in most cases of follicular lymphoma. Roulland et al. (2004)

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investigated the prevalence of the t(14;18) translocation, which is characteristic of most cases of follicular lymphoma, in 53 never-smoking and pesticide-using men in a cohort of French farmers whose pesticide exposures and confounding information had previously been well characterized; blood samples had been gathered from 21 of them during periods of high pesticide use, and samples from the other 32 had been collected during a period of low pesticide use. The authors found a higher prevalence of cells carrying the translocation in the farmers whose blood had been drawn during a period of high pesticide use than in those whose blood had been drawn during a low-use period. Baccarelli et al. (2006) reported an increase in t(14;18) chromosomal translocation in lymphocytes from humans who were exposed to TCDD in the Seveso accident. In most cases of follicular lymphoma, tumor cells carry the t(14;18) chromosomal translocation, and there is evidence that an increased frequency of lymphocytes from the peripheral blood carrying this tumor marker may be a necessary but not sufficient step toward the development of follicular lymphoma (Roulland et al., 2006).

More recently, Saberi Hosnijeh et al. (2011, 2012a,b, 2013a,b) have published a series of papers examining factors associated with immune regulation and possibly related to B-cell neoplasms and serum TCDD levels in Dutch production workers from a subcohort of the IARC study sample. The mortality status of the entire subcohort was updated, and blood samples were gathered in 2007–2008 from a small number of survivors—45 who had TCDD exposure in factory A, 39 whose jobs in factory A did not expose them to TCDD, and 69 in factory B that produced phenoxy herbicides not subject to TCDD contamination (Boers et al., 2010). Boers et al. (2012) modeled the resulting contemporary TCDD serum levels to back-extrapolated TCDD concentrations at the end of employment for each worker. When examining immunoglobulin (IgG, IgA, IgM, IgD, and IgE) and complement (C3 and C4) concentrations measures of humoral immunity, Saberi Hosnijeh et al. (2011) found a consistent pattern only for C4, which was negatively associated with both measured current and estimated maximum TCDD serum concentrations. Limiting the analyses to workers from factory A and examining serum concentrations of 16 cytokines, 10 chemokines, and 6 growth factors, Saberi Hosnijeh et al. (2012a) found that most analytes were negatively associated with the current and estimated past maximum TCDD levels. Saberi Hosnijeh et al. (2012b) found that for both cell counts and lymphocytes, the results were similar between high- and low-exposed workers from factory A, except for a non-dose-dependent increase in the CD4/CD8 ratio among the high-exposed workers. Most lymphocyte subsets, in particular the B-cell compartment, showed decreases with higher levels of both current and estimated maximum levels of TCDD. Saberi Hosnijeh et al. (2013a) addressed plasma levels of CD27, CD30, and IL-1RA, which are proteins that regulate immune function and are thought to be involved in lymphopoeitic neoplasms, and they found a tendency toward decreased levels with increasing TCDD concentrations, which would be consistent with immune suppression. Similarly, Saberi Hosnijeh et al. (2013b) investigated

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the possibility of a relationship between TCDD levels and serum metabolites, but they found no notable patterns. Overall, this set of findings in a group of workers with an elevated incidence of NHL at its most recent mortality update provides some insight into the biological processes, particularly immunological ones, that TCDD might stimulate on the path to this malignancy.

Synthesis

The first VAO committee concluded that there was sufficient evidence of an association between exposure to at least one of the COIs and NHL. The evidence was drawn from occupational and other studies in which subjects were exposed to a variety of herbicides and herbicide components. Although the new studies of NHL incidence and mortality that were reviewed in this update (Coggon et al., 2015; Collins et al., 2016; Czarnota et al., 2015) mostly found increased, but not statistically significant, risks of NHL after exposure to at least one of the COIs, based on the results of studies previously reviewed, the committee maintains the conclusion of sufficient evidence of an association.

Individual findings on CLL are fairly few compared with the considerable number of studies supporting an association between exposure to the COIs and NHL. Two new studies of CLL in U.S. Vietnam veterans were reviewed. Baumann Kreuziger et al. (2014) found that veterans who reported having been exposed to Agent Orange had a statistically significantly lower age of presentation and time to first treatment, but the number of therapies and the rate of transformation were not different between exposed and unexposed patients. In the second study, Mescher et al. (2018) used data from the National VA Tumor Registry to identify cases of CLL. Similar to the findings of Baumann Kreuziger et al., veterans presumed to have been exposed to Agent Orange were diagnosed at a younger age and had shorter time to initial treatment, but in this analysis those exposed had significantly longer overall survival. Results of some high-quality studies show that exposure to 2,4-D and 2,4,5-T appears to be associated with CLL; these studies include the incidence study of Australian veterans (ADVA, 2005a), the case-control study by Hertzman et al. (1997) of British Columbia sawmill workers who were exposed to chlorophenates, the Danish-gardener study (Hansen et al., 1992), and the population-based case-control study in two U.S. states by Brown et al. (1990) that showed increased risks to be associated with any herbicide use and, specifically, with the use of 2,4,5-T for at least 20 years before the interview. Other studies that showed positive associations have lacked exposure-specificity in the the populations studied.

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Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is sufficient evidence of an association between exposure to at least one of the COIs and NHL.

Plasma Cell Dyscrasias

Plasma cell dyscrasias are a heterogeneous group of disorders characterized by the presence of monoclonal immunoglobulins in the serum, which reflects a monoclonal proliferation of lymphoplasmacytic cells in the bone marrow (Wahed and Dasgupta, 2015). Plasma cell neoplasms are lymphoid neoplasms of terminally differentiated B cells, all of which exhibit the expansion of a single clone of Ig-secreting plasma cells. Included in this group are MGUS, multiple myeloma, Waldenstrom's macroglobulinemia, immunoglobulin deposition diseases (such as AL amyloidosis and primary amyloidosis), plasmacytoma, and plasma cell leukemia.

Monocolonal Gammopathy of Undetermined Significance

MGUS is a precursor condition of multiple myeloma. However, only an estimated 1% of MGUS cases progress to multiple myeloma each year (Kyle et al., 2018; Mateos and Landgren, 2016). It is a clinically silent condition, meaning that there are no apparent signs or symptoms or other clinical manifestations, although MGUS has been associated with osteoporosis, hip fractures, and peripheral neuropathy (Bida et al., 2009). MGUS is defined by the presence of a monoclonal antibody, antibody heavy chain, or antibody light chain in the blood or urine of a person lacking the symptoms or signs of a more serious plasma cell dyscrasia. MGUS is categorized into subtypes based on the identity and levels of the myeloma proteins detected as well as the prognosis for progressive disease. IgG and IgA MGUS are precursor conditions of multiple myeloma, whereas IgM MGUS is a precursor of Waldenstrom's macroglobulinemia and other lymphoproliferative disorders (van de Donk et al., 2014). The condition is typically discovered as an incidental finding when a protein electrophoresis test is performed for reasons unrelated to plasma cell dyscrasias.

A prospective longitudinal study of 1,384 (predominantly white) patients who were residing in southeastern Minnesota and who were diagnosed with MGUS at the Mayo Clinic from 1960 through 1994 were followed for progression to multiple myeloma or another plasma-cell or lymphoid disorder. The patients were stratified into IgM and non-IgM MGUS to determine their risk factors for progression. The presence of one of two factors (an abnormal serum free light-chain ratio and a high serum M protein level [$\geq$ 1.5 g per deciliter]) was associated with a higher risk of progression in both groups; IgM MGUS patients

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had an even higher risk than the non-IgM MGUS patients. After an adjustment for competing causes of death, the risk of progression was 10% at 10 years, 18% at 20 years, 28% at 30 years, 36% at 35 years, and 36% at 40 years (Kyle et al., 2018). Using the same population in another analysis, the prevalence of MGUS in people 50 years and older was found to be 3.2% and 5.5% in people over 70 years (Kyle et al., 2007).

A systematic review and meta-analysis of MGUS conducted in 2010 found that the prevalence of MGUS ranged from 0.05% to 6.1% in 11 different studies. The prevalence of MGUS increases with age and is affected by race (higher among blacks than whites and Japanese), sex (higher in men than women), family history, immunosuppression, and pesticide exposure (Wadhera and Rajkumar, 2010).

The risk factors for the progression of clinical MGUS to multiple myeloma that have been reported are the size and type of serum M protein and the presence of an abnormal serum free light chain ratio (Rajkumar et al., 2005), immune status (with higher prevalence among immunosuppressed and immunocompromised patients), hereditary or familial factors (Landgren et al., 2006; Vachon et al., 2009), and some occupational and environmental factors such as exposure to certain pesticides and fertilizers in the AHS (Landgren et al., 2009), but those associations have been inconsistent. Heavy tobacco smoking has also been associated with the prevalence of MGUS (Pasqualetti et al., 1997).

Update of the Epidemiologic Literature MGUS was not considered as a separate outcome in any of the prior VAO updates primarily because most relevant studies focused on multiple myeloma and did not include MGUS. The present committee determined that because it is a precursor condition of multiple myeloma, it would be considered as a separate outcome with respect to exposure to the COIs. It was felt that an increased incidence of MGUS, while itself clinically silent, could be a harbinger of an increase in the incidence of multiple myeloma; however, there are not yet any data to support this inference. One published study that specifically examined the prevalence of MGUS in Vietnam veterans who were responsible for spraying herbicides was identified and reviewed; no other studies of MGUS and the COIs were identified. Table 27, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to plasma cell dyscrasias and includes the studies of MGUS.

In a new analysis using data and biospecimens collected from the AFHS, Landgren et al. (2015) examined the association between serum TCDD levels and the presence of MGUS. Data and biospecimens were collected prospectively from individuals to whom structured questionnaires and physical exams were given at set times over 20 years, with the final exam conducted in 2002. The study included 479 Ranch Hand veterans (who conducted aerial spray missions of the herbicides from 1962 to 1971) and 479 controls (comparison veterans who were also in the Air Force and had similar job duties and were deployed to

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Southeast Asia during the same period) who participated in the 2002 follow-up examination and had given a serum specimen and were at least 50 years old at the 2002 follow-up. Individuals with a history of multiple myeloma, Waldenstrom macroglobulinemia, solitary plasmacytoma, or amyloidosis were excluded. All serum specimens were tested without knowledge of the exposure status. TCDD concentrations were either measured in the 1987 exam or reconstructed from the measurement in the nearest subsequent follow-up (1992, 1997, 2002 follow-ups). Logistic regression was used for analyses, and models were adjusted for age, race, BMI at the 2002 examination, and changes in BMI between 2002 and the time of blood draw for the TCDD measurement; additional variables of military occupation, smoking history, drinking history, and history of radiation therapy or chemotherapy for cancer treatments were also considered. The Ranch Hand veterans and comparison veterans had similar demographic and lifestyle characteristics and medical histories. The crude prevalence of overall MGUS was 7.1% in Ranch Hand veterans and 3.1% in comparison veterans, which is equivalent to a 2.4-fold increased risk for MGUS in Ranch Hand veterans versus the comparison veterans, after adjustment (OR = 2.37, 95% CI 1.27–4.44, p = 0.007). The risk of MGUS was significantly increased in veterans younger than 70 years (OR = 3.4, 95% CI 1.46–8.13, p = 0.004), whereas no significant increase in the risk was seen in those 70 years or older (OR = 1.4, 95% CI 0.55–3.63, p = 0.63), although both estimates were imprecise. When compared with the veterans in the lowest TCDD level ($\leq$ 3.65 ppt), the crude ORs for having MGUS were statistically significant for veterans with TCDD levels of 5.81–10.92 ppt and more than 10.92 ppt, but an adjustment eliminated the significant effect of TCDD at the highest level. In both Ranch Hand veterans and comparison veterans, the prevalence of MGUS increased with age. This study is the first to correlate objective measurement of levels of TCDD exposure with MGUS. This study was well designed and is strengthened by its use of objective measurements of serum TCDD levels in both exposure groups, long-term follow-up with prospective data and biospecimen collection, and the use of standard laboratory analyses. Although the serum samples taken to measure TCDD levels were collected at least 15 years after military exposure and it was not possible to measure exposure to 2,4-D or 2,4,5-T, TCDD levels may be used as a surrogate of exposure for the Ranch Hands and comparison veterans. However, the findings strongly support an association between TCDD exposure and MGUS, and therefore, multiple myeloma.

Multiple Myeloma

Multiple myeloma (ICD-9 203.0; ICD-10 C90.0) is characterized by a proliferation of bone marrow cells that results in an excess of neoplastic plasma cells and in the production of excess immunoglobulin protein. Multiple myeloma is sometimes grouped with other immunoproliferative neoplasms (ICD-9 203.8;

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ICD-10 C90.2). The American Cancer Society estimated that 16,400 men and 14,370 women would receive diagnoses of multiple myeloma in the United States in 2018 and that 6,830 men and 5,940 women would die from it (ACS, 2018e). The incidence of multiple myeloma is highly age-dependent and is relatively low in people under 40 years old. The incidence is slightly higher in men than in women; for all races the incidence rate for 2009–2013 was 8.0 per 100,000, and for women it was 5.2 per 100,000. The difference becomes more pronounced with age. In the age groups that include most Vietnam veterans, the age-adjusted modeled incidence rate of myeloma for men 50–64 years old of all races combined was 13.1 per 100,000 in 2014, increasing to 39.0 for 65- to 74-year-olds and 61.1 for men over 75 years.[21]

An increased incidence of multiple myeloma has been observed in several occupational groups, including farmers and other agricultural workers and those with workplace exposure to paint strippers, petroleum, and certain metals, minerals, and chemical substances (Sergentanis et al., 2015). People who have high exposure to ionizing radiation and those who have other plasma-cell diseases, such as MGUS or solitary plasmacytoma, are also at greater risk (Sergentanis et al., 2015).

Conclusions from *VAO* and Previous Updates The committee responsible for *VAO* concluded that there was limited or suggestive evidence of an association between exposure to the COIs and multiple myeloma. Few studies of multiple myeloma have been conducted among Vietnam veterans, and most of them have reported no cases of or decreased risks of multiple myeloma (Akhtar et al., 2004; Boyle et al., 1987; Breslin et al., 1988; Cypel and Kang, 2008; Goun and Kuller, 1986; Wolfe et al., 1990). Follow-up analyses of the New Zealand cohort of veterans who served in Vietnam found no statistically significant increase in the risk of deaths from or incidence of multiple myeloma when compared to the standardized general New Zealand population (McBride et al., 2013). Likewise, in the Korean cohort of veterans who served in Vietnam, there was no statistically significant increase in multiple myeloma in the internal comparison of the high- and low-exposure groups based on EOI scores (Yi and Ohrr, 2014). Similarly for multiple myeloma mortality, Yi et al. (2014b) reported a decreased risk for the high- versus low-exposure groups and with the individual log-transformed EOI scores.

However, several occupational cohort studies with well-characterized exposures to one or more of the COIs found a statistically significant increased incidence or mortality of myeloma among agricultural workers and farm workers (Blair et al., 1993; Boffetta et al., 1989; Brown et al., 1993; Burmeister et al., 1983; Eriksson and Karlsson, 1992; La Vecchia et al., 1989; Morris et al., 1986).

[21] As calculated on the site https://seer.cancer.gov/faststats/selections.php?#Output using the SEER 13 dataset and by choosing age-adjusted rates, myeloma, all races, age groups 50–64, 65–74, and ≥ 75 years, and male sex.

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Additional studies reviewed since *VAO* have reported increased risks of incidence or mortality, but in general the findings have not been statistically significant. Most recently, a follow-up analysis of the Cross-Canada Study of Pesticides and Health was reviewed by the Update 2014 committee. The association between lifetime use of multiple pesticides and multiple myeloma risk was examined among men 19 years and older who worked in agriculture and had a first diagnosis of STS, NHL, multiple myeloma, or HL during the original study period of 1991–1994 (Kachuri et al., 2013). Pesticides were grouped by type, chemical class, and their carcinogenic potential. The investigators recruited 342 cases and 1,357 controls. Odds ratios were calculated and adjusted for age, residence, medical history, and smoking. A statistically significant increased risk of multiple myeloma was observed with exposure to Mecoprop but not with exposure to 2,4-D. An evaluation of days per year of mixing or applying phenoxy herbicides was not statistically significant for any category ($\leq$ 2 days per year, 2–5 days, and > 5 days).

Update of the Epidemiologic Literature No new studies of multiple myeloma in Vietnam veterans or environmental or case-control studies of exposure to the COIs and multiple myeloma have been published since *Update 2014*. Reviews of the relevant studies are presented in the earlier reports. Tables 26 and 27, which can be found at www.nap.edu/catalog/25137, summarize the results of studies related to CLL and plasma cell dyscrasias and include the studies of multiple myeloma.

Occupational Studies In an extension of the follow-up of UK phenoxy herbicide manufacturers and sprayers to examine the carcinogenicity of phenoxy herbicides and their association primarily with HL, STS, and CLL, Coggon et al. (2015) also reported mortality from other types of cancer, including multiple myeloma. A total of 18 deaths from multiple myeloma were reported, but although risk estimates were slightly elevated, none were statistically significant for any of the groups of workers: all workers (n = 18; SMR = 1.04, 95% CI 0.62–1.64), workers exposed to herbicide levels above background (n = 15; SMR = 1.18, 95% CI 0.66–1.94), or workers exposed for more than 1 year at levels above background (n = 6; SMR = 1.01, 95% CI 0.37–1.86).

Other Identified Studies One other study that reported mortality outcomes from multiple myeloma was identified, but it lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs (Ruder et al., 2014).

Biologic Plausibility No animal studies have reported an association between exposure to the COIs and multiple myeloma. AHR activation by TCDD in human

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breast and endocervical cell lines induces sustained high concentrations of IL-6, which has tumor-promoting effects in numerous tissues (Hollingshead et al., 2008). IL-6 plays a role in B-cell maturation and induces a transcriptional inflammatory response. It is known to be increased in B-cell neoplasms, including multiple myeloma and various lymphomas (Hussein et al., 2002; Kovacs, 2006).

In comparing the frequency of specific variants of several metabolic genes between multiple myeloma cases and controls, Gold et al. (2009) found some indication of differences, particularly in *CYP1B1* and *AHR* alleles, that might reflect an increased susceptibility to multiple myeloma after exposure to particular chemicals. A biochemical link to the COIs, however, is far from being established.

Synthesis Previous VAO reports found limited or suggestive evidence of an association between exposure to at least one of the COIs and multiple myeloma. The evidence of an association between the COIs and lymphomas (NHL, HL, and CLL/HCL) has been classified as sufficient. Most of these cancers also arise from B cells, so the committee hypothesized that it would be etiologically plausible for the association with multiple myeloma to belong with the lymphomas in the sufficient category. Although many studies of exposure to pesticides in general and multiple myeloma found strong or at least positive associations, a review of studies that addressed an association between exposure to the specific COIs and multiple myeloma found that the results were considerably weaker than those for the other B-cell neoplasms; in particular, the results did not justify advancing multiple myeloma out of the limited or suggestive category. No animal studies have reported an association between exposure to the COIs and multiple myeloma. However, given the close relationship between myeloma and other lymphoproliferative neoplasms, biologic and mechanistic studies implying a connection between the COIs and NHL likely link to similar pathways in plasma cell dyscrasias. Two well-designed studies of well-characterized cohorts were reviewed in the current update. In an analysis using data and preserved serum samples from the AFHS, Landgren et al. (2015) found a 2.4-fold increased risk of MGUS in Ranch Hand veterans compared with a matched group of veterans who did not participate in aerial herbicide spraying missions. In both Ranch Hand veterans and comparison veterans, the prevalence of MGUS increased with age. This study is the first to provide objective evidence of an association between higher levels of TCDD exposure and MGUS, and therefore, possibly multiple myeloma. In the extended follow-up of UK men who worked manufacturing or spraying phenoxy acids in the United Kingdom, Coggon et al. (2015) reported slightly increased, but not statistically significant, risks of multiple myeloma, and the increased risk was lowest in the most highly exposed group. No toxicologic studies on exposure to the COIs and MGUS or multiple myeloma have been identified.

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Amyloid Light Chain Amyloidosis

AL amyloidosis is a rare condition that is a complication of multiple myeloma. The committee responsible for *Update 2006* moved the discussion of AL amyloidosis from the chapter on miscellaneous non-neoplastic health conditions to the cancer chapter to put it closer to related neoplastic conditions, such as multiple myeloma and some types of B-cell lymphomas. The conditions share several biologic features, notably the clonal hyperproliferation of B cell–derived plasma cells and the production of abnormal amounts of immunoglobulins.

The primary feature of amyloidosis (ICD-9 277.3; ICD-10 E85) is the accumulation and deposition in various tissues of insoluble proteins, or amyloids. Amyloid protein accumulates in the extracellular spaces of various tissues. The pattern of organ involvement depends on the nature of the protein; some amyloid proteins are more fibrillogenic than others. Amyloidosis is classified according to the biochemical properties of the fibril-forming protein. Excessive amyloid protein can have modest clinical consequences, or it can produce severe, rapidly progressive multiple-organ-system dysfunction. The Amyloidosis Foundation estimates that approximately 4,500 new cases are diagnosed each year (Amyloidosis Foundation, 2018). It usually affects people from ages 50 to 80 years and occurs more often in males than in females.

AL amyloidosis is the most common form of systemic amyloidosis; the A stands for amyloid, and the L indicates that the amyloid protein is derived from immunoglobin light chains. AL amyloidosis results from the overproduction of immunoglobulin light-chain protein from a monoclonal population of plasma cells; some of these light chains can form a beta-pleated sheet, engendering protein deposition in tissue. Clinical findings can include excessive AL protein or immunoglobulin fragments in the urine or serum, renal failure with nephrotic syndrome, liver failure with hepatomegaly, heart failure with cardiomegaly, marcroglossia, carpal tunnel syndrome, and peripheral neuropathy. Bone marrow biopsies commonly show an increased density of plasma cells, which suggests a premalignant state. Historically, bone marrow biopsies emphasized routine histochemical analysis, but modern immunocytochemistry and flow cytometry now commonly identify monoclonal populations of plasma cells with molecular techniques. AL amyloidosis can progress rapidly and is often far advanced by the time it is diagnosed (Buxbaum, 2004).

Conclusions from *VAO* and Previous Updates The committees responsible for *Update 2000*, *Update 2002*, and *Update 2004* concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and AL amyloidosis. Although there are few epidemiologic data specifically on AL amyloidosis, the committee responsible for *Update 2006* changed the categorization to limited or suggestive evidence

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of an association on the basis of commonalities in its cellular lineage with multiple myeloma and B-cell lymphomas. Later committees have not changed that categorization.

Epidemiologic results for amyloidosis were reported for the first time in Vietnam veterans in the publication from the Korean Veterans Health Study (Yi et al., 2014a) on the prevalence of diseases as confirmed by insurance records. From the internal comparison of veterans in the category with high EOI scores (nine cases) to those in the low-potential-exposure group (six cases) with adjustments for age, rank, smoking, drinking, physical activity, domestic herbicide use, education, income, and BMI, a statistically significantly elevated, but imprecise, risk of amyloidosis (OR = 3.02, 95% CI 1.02–8.93) was found. When regression with the same adjustments was performed on the logarithms of the individual EOI scores for the entire set of veterans, a statistically significant increased risk was again found but it was lower in magnitude (OR = 1.32, 95% CI 1.02–1.71).

Update of the Epidemiologic Literature No new studies of exposure to the COIs and AL amyloidosis (human or animal) have been identified since those reviewed in *Update 2014*. Reviews of the relevant studies are presented in the earlier reports. Table 27—contained in the supplementary tables available at www.nap.edu/catalog/25137—summarizes the results of studies related to plasma cell dyscrasias and includes the studies of amyloidosis.

Biologic Plausibility A 1979 study reported the dose-dependent development of a "generalized lethal amyloidosis" in Swiss mice that were treated with TCDD for 1 year (Toth et al., 1979). That finding has not been validated in 2-year carcinogenicity studies of TCDD in mice or rats, but the use of different strains may explain the discrepancies. Thus, few animal data support an association between TCDD exposure and AL amyloidosis in humans, and no animal data support an association between the other COIs and AL amyloidosis.

It is known, however, that AL amyloidosis is associated with B-cell diseases, and 15–20% of cases of AL amyloidosis occur with multiple myeloma. Other diagnoses associated with AL amyloidosis include B-cell lymphomas (Cohen et al., 2004), MGUS, and agammaglobulinemia (Rajkumar et al., 2006).

Synthesis AL amyloidosis is very rare, and previous VAO committees have thought that it was unlikely that population-based epidemiology will ever provide substantial direct evidence regarding its causation. The assignment of this condition to the "limited or suggestive" category of association has been based on the biologic and pathophysiologic features linking AL amyloidosis, multiple myeloma, and some types of B-cell lymphomas—especially the clonal hyperproliferation of plasma cells and abnormal immunoglobulin production—thus indicating that AL amyloidosis is pathophysiologically related to these conditions. No new epidemiologic or mechanistic studies of exposure to the COIs and AL

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amyloidosis have been identified since *Update 2014*, and therefore the committee maintains the conclusion of limited or suggestive evidence of an association.

Conclusion on Plasma Cell Dyscrasia

Based on new information from an analysis of the AFHS that examined MGUS and used objective measures of dioxin exposure in a well-characterized exposed group of Vietnam veterans, the committee concludes that there is sufficient evidence of an association between exposure to at least one of the COIs and MGUS. However, the committee notes that the clinical and health implications of a diagnosis of MGUS are minimal and that only a small percentage of people with MGUS progress to multiple myeloma. Although an increase in MGUS may be inferred to predict an increased incidence of multiple myeloma, there is no scientific evidence at this time to confirm that the elevated rates of MGUS seen in the exposed population will translate to higher rates of multiple myeloma. Therefore, based on the evidence reviewed here and in previous VAO reports, the committee concludes that there remains limited or suggestive evidence of an association between exposure to at least one of the COIs and multiple myeloma and AL amyloidosis. The close association of these diagnoses to a previous diagnosis of MGUS strengthens this association; however, further study would be needed to establish definitively that the increased prevalence of MGUS will translate to higher levels of these clinically significant plasma cell neoplasms.

Leukemias

Leukemias (ICD-9 202.4, 203.1, 204.0–204.9, 205.0–205.9, 206.0–206.9, 207.0–207.2, 207.8, 208.0–208.9; ICD-10 C90.1–C95.9) have traditionally been divided into four primary types: acute and chronic lymphocytic leukemias and acute and chronic myeloid leukemias. There are numerous subtypes of acute myeloid leukemia (ICD-9 205; ICD-10 C92), which is also called acute myelogenous leukemia, granulocytic leukemia, or acute non-lymphocytic leukemia.

NCI estimated that in the United States in 2018, 60,300 people would receive a new diagnosis of and 24,370 men and women would die from some form of leukemia. Collectively, leukemias were expected to account for 3.7% of all new diagnoses of malignancies and 4.1% of deaths from malignancy in 2017 (NCI, n.d.bb). Grouping all different forms of leukemias into a single group is not informative because the different forms have different patterns of incidence and different risk factors.

Myeloid Leukemias

In adults, the majority of acute leukemias are AML (ICD-9 205.0, 207.0, 207.2; ICD-10 C92.0, 92.4–92.5, 94.0, 94.2). NCI estimated that 19,520 people

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would receive a new diagnosis of AML in the United States in 2018 and that 10,670 men and women would die from it. Overall, AML is slightly more common in men than in women of the same race and incidence is highest in whites and lowest in American Indians/Alaska Natives (NCI, n.d.cc). Risk factors associated with AML include high doses of ionizing radiation, occupational exposure to benzene, and exposure to some medications used in cancer chemotherapy (such as melphalan) (Deschler and Lubbert, 2006; IARC, 2016). Several bone marrow failure syndromes (severe congenital neutropenia, Fanconi anemia, Schwachman-Diamond syndrome, and dyskeratosis congenital) and Down syndrome are associated with an increased risk of AML, and tobacco use is thought to account for about 20% of AML cases.

Vietnam veterans have expressed concern about whether myelodysplastic syndromes, which can transform to AML, are associated with Agent Orange exposure. However, no results on those conditions in conjunction with the COIs have been found in VAO literature searches. Epidemiologic research on those hematologic disorders has been undertaken fairly recently; for instance, the LATIN case-control study (Maluf et al., 2009) has undertaken an investigation of aplastic anemia in South America, but the reported exposures have been only as specific as "herbicides" and "agricultural pesticides."

The incidence of CML increases steadily with age in people older than 30 years. NCI estimated that 8,430 people would receive a new diagnosis of CML in the United States in 2018 and that 1,090 men and women would die from it. Its lifetime incidence is roughly equal in whites and blacks and is slightly higher in men than in women of the same race and ethnicity (NCI, n.d.dd). CML accounts for about one-third of cases of leukemias in people in the age groups that include most Vietnam veterans (60–80 years). It is associated with an acquired chromosomal translocation known as the Philadelphia chromosome, for which exposure to high doses of ionizing radiation is a known risk factor.

Lymphoid Leukemias

ALL is a disease of young children (peak incidence at the age of 2–5 years) and of people over 70 years old. NCI estimated that 5,960 people would receive a new diagnosis of ALL in the United States in 2018 and that 1,470 men and women would die from it (NCI, n.d.ee). The lifetime incidence of ALL is slightly higher in whites than in blacks and about the same for men and women. Exposure to high doses of ionizing radiation is a known risk factor for ALL, but there is little consistent evidence on other factors.

CLL is more appropriately classified as a form of B-cell NHL given its immunohistochemistry, B-cell origin, and progression to an acute, aggressive form of NHL. Therefore, the committee considers it in the section above on NHL, as classified in the WHO system.

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Conclusions from *VAO* and Previous Updates

The committee responsible for VAO concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and all types of leukemias. Additional information available to the committees responsible for *Update 1996* through *Update 2010* did not change that conclusion. The committee responsible for *Update 2002*, however, considered CLL separately and judged that there was sufficient evidence of an association with the herbicides used in Vietnam and CLL alone, and *Update 2008* noted that HCL is closely related to CLL.

The committee responsible for *Update 2006* considered AML individually but did not find evidence to suggest that its occurrence is associated with exposure to the COIs, and there is still not sufficient evidence to support such an association, so AML has been retained with other non-CLL leukemias in the category of inadequate or insufficient evidence.

The *Update 2014* committee assessed two cohorts of veterans who served in Vietnam. In the New Zealand cohort, McBride et al. (2013) reported four leukemia deaths, which was not statistically different from the general standardized population. They also reported the incidence of 21 leukemias overall, which resulted in a statistically significantly elevated standaridized incidence ratio. Results were stratified by incident non-lymphoid and lymphoid leukemias, but only the lymphoid leukemia standardized incidence ratio was statistically significant. From the Korean Health Study, Yi et al. (2014b) reported 107 leukemia deaths, but neither the low-exposure nor the or high-exposure hazard ratios were statistically significant. That included 5 ALL, 46 AML, and 15 CML deaths. The small number of low-exposure deaths makes the reported estimates quite unstable. When examined using EOI scores, the adjusted hazard ratios for myeloid leukemia in the low-exposure and high-exposure groups were not statistically significant (Yi and Ohrr, 2014).

Update of the Epidemiologic Literature

No studies of leukemia of any type in Vietnam veterans have been published since *Update 2014*. Likewise, no environmental or case-control studies of the COIs and leukemia were identified. Reviews of the relevant studies are presented in the earlier reports. Table 28, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to leukemia.

Occupational Studies Among the Dow Midland, Michigan, worker cohort, Collins et al. (2016) reported categories of leukemia and leukemia (C91–C95), total lymphoid leukemia (C91–C95), total myeloid leukemia (C92), acute nonlymphatic leukemia (92.0, 93.0, 94.0), and all other leukemia (C93–C95). Compared with the standardized U.S. population, an increased risk of mortality for

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all leukemias was found for the TCP workers (n = 17; SMR = 1.78, 95% CI 1.04–2.85) but not for the PCP workers (n = 3; SMR = 0.67, 95% CI 0.14–1.96). Likewise, an increased risk of death was seen among TCP workers for total myeloid leukemia (C92) (n = 10; SMR = 2.42, 95% CI 1.16–4.46) and acute non-lymphatic leukemia (C92.0, 93.0, 94.0) (n = 9; SMR = 2.88, 95% CI 1.32–5.47), but no difference was found for PCP workers, for which there were only two reported deaths from each of those leukemias. No differences in mortality for total lymphoid leukemia was found for either the TCP workers (n = 5; SMR = 1.99, 95% CI 0.64–4.64) or the PCP workers, for which there was only one death. Only two deaths among TCP workers and none among the PCP workers were found for all other leukemias.

Coggon et al. (2015) extended the follow-up period of a large IARC-sponsored study and examined the carcinogenicity of phenoxy herbicides and their association primarily with HL, STS, and CLL, but deaths from other types of cancer were also reported. A total of 40 leukemia deaths were reported. Although risk-of-death estimates from leukemia were slightly elevated for all three groups of workers, none reached statistical significance: all workers (SMR = 1.27, 95% CI 0.91–1.73), workers exposed to herbicide levels above background (n = 30; SMR = 1.29, 95% CI 0.87–1.84), and workers exposed to phenoxy acids above background for 1 year or longer (n = 11; SMR = 1.04, 95% CI 0.52–1.86). Mortality from myeloid leukemia specifically (n = 22) was also not statistically significant for any of the groups of workers: all workers (SMR = 1.21, 95% CI 0.76–1.84), workers exposed to herbicide levels above background (n = 15; SMR = 1.11, 95% CI 0.62–1.83), or workers exposed for more than 1 year at levels above background (n = 6; SMR = 0.98, 95% CI 0.36–2.13).

Other Identified Studies Several other studies (occupational, environmental, and case-control designs) were identified that examined leukemia outcomes, but all lacked sufficient exposure specificity (e.g., mixtures to several chemicals with no concentrations measured, general herbicide exposure, or exposure determination based on broad categories of occupation with no further details collected) to be included further as contributing to the evidence base of the potential effect of the COIs (Akahane et al., 2017; Levine et al., 2016; Maryam et al., 2015; Poynter et al., 2017; Punjindasup et al., 2015; Ruder et al., 2014).

Biologic Plausibility

Leukemia is a relatively rare spontaneous neoplasm in mice, but it is less rare in some strains of rats. A small study reported that 5 of 10 male rats fed TCDD at 1 ng/kg per week for 78 weeks showed an increased incidence of various cancers, one of which was lymphocytic leukemia (Van Miller et al., 1977). Later studies of TCDD's carcinogenicity have not shown an increased incidence of lymphocytic leukemia in mice or rats.

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Two studies that used cells in tissue culture suggested that TCDD exposure does not promote leukemia. The proliferation of cultured human bone marrow stem cells (the source of leukemic cells) was not influenced by the addition of TCDD to the culture medium (van Grevenynghe et al., 2005). Likewise, Mulero-Navarro et al. (2006) reported that the AHR promoter is silenced in ALL—an effect that could lead to a reduced expression of the receptor, which binds TCDD and mediates its toxicity. No reports of animal studies have noted an increased incidence of leukemia after exposure to the phenoxy herbicides or other COIs. AHR plays a role in hematopoetic stem cell expansion as well as in erythroid and megakaryocytic differentiation (B. W. Smith et al., 2013). In this context, information in a letter to the editor of the *American Journal of Hematology* from Nguyen-Khac et al. (2014) is interesting. The researchers described a chromosomal translocation found in a human acute leukemia that recombines the *TEL* gene with the *ARNT* (Arylhydrocarbon Receptor Nuclear Translocator) gene, producing a fusion gene product. This recent functional work strongly suggests that the translocation impairs the normal functions of ARNT, potentially contributing to leukemogenesis.

Synthesis

Several studies of the incidence of or mortality from leukemia have been reviewed in the VAO series. Among U.S. Vietnam veterans, findings have been null, and risk estimates have been less than 1.0; among international cohorts of Vietnam veterans (Australia, New Zealand, and Korea), risk estimates have been mixed in direction, but most continue not to be statistically significant. No new studies of leukemia in Vietnam veterans were identified for the current update. Two mortality updates of occupational cohorts were reviewed that examined all leukemia and subtypes. Collins et al. (2016) extended the follow-up period of workers who were exposed to dioxins during the manufacturing process of PCP and TCP in Midland, Michigan, and found a statistically significant elevated risk of mortality from all leukemias for the TCP workers, but not for PCP workers compared with the standardized U.S. population. Likewise, a statistically significant increased risk of death compared with the standardized U.S. population was seen among TCP workers for total myeloid leukemia and acute non-lymphatic leukemia, but no difference was found for PCP workers for which there were only two reported deaths from each of those leukemias. A small number of deaths from total lymphoid leukemias and all other leukaemia were reported for both TCP and PCP workers, and neither estimate was statistically significant. In the follow-up study of UK workers who manufactured or sprayed phenoxy herbicides, SMR estimates from all leukemia and myeloid leukemia specifically were slightly elevated for all three groups of workers, but none reached statistical significance (Coggon et al., 2015). Given the new mixed findings from the well-characterized occupational cohorts and the lack of reported findings of increased incidence

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of leukemia after exposure to the phenoxy herbicides or other COIs in animal models, the committee maintains the conclusion of inadequate or insufficient evidence of an association between exposure to the COIs and leukemias in general.

Conclusion

Based on the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and leukemias in general. An exception is the specific leukemia subtypes of chronic B-cell hematoproliferative diseases, including CLL and HCL, which are more appropriately grouped with lymphomas.

Other Myeloid Diseases

Myelodysplastic syndromes (MDSs) are a collection of clonal diseases (ICD-9 238.7; ICD-10 D46) that involve dysplastic maturation and cytopenias. The malignant potential of MDS is variable, but with time, many patients will progress to AML. MDS was not required to be reported to cancer registries until 2001. U.S. incidence was estimated at 4.9 per 100,000 people per year for 2007–2011, but this is thought to be an underestimate due to underreporting and underdiagnosis (Cogle, 2015). NCI has estimated the incidence rate of MDS to be even higher in older age groups: 13.5 per 100,000 in those aged 65–69 years to 63.6 per 100,000 in those aged 85 years and older. The median age of diagnosis is 70 years (Montalban-Bravo et al., 2016). In addition to increasing incidence with older age, MDS is most prevalent in individuals who are white and male. It has been conservatively estimated that 10,000 new cases of MDS occur in the United States annually and that more than 60,000 individuals in the United States are currently living with MDS (Ma, 2012).

Various factors determine the prognosis for MDS, and several scoring systems are used. Most involve the number of cytopenias, dependence on transfusion, cytogenetic abnormalities, and the number of blasts in the marrow. For low-risk disease, the median survival is about 7 years; for high risk, it is less than 1 year. MDS does not always progress to AML, and the incidence of progression varies with the risk category. Of cases with high-risk MDS, around 25–35% progress to AML. More people die from complications of infection or bleeding related to cytopenias than through transformation to AML.

Myeloproliferative neoplasms (MPNs) (ICD-9 205.1, 238.4, 289.89, 289.9; ICD-10 D47.1) are clonal diseases of the myeloid lineage associated with hyperproliferation and elevated blood counts, usually associated with constitutive tyrosine kinase activation. These syndromes include polycythemia vera, essential thrombocytosis, myelofibrosis, and CML. The non-CML MPNs have a closely-related pathogenesis. Nearly all of patients with polycythemia vera have a point

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mutation in the *JAK2* gene (*JAK2V617F*), which is also present in about half of patients with essential thrombocytosis and myelofibrosis. The majority of *JAK2*-negative essential thrombocytosis and myelofibrosis are associated with mutations in the calreticulin gene.

The non-CML myeloproliferative neoplasms are characterized by abnormal peripheral blood counts with increased reticulin fibrosis in the bone marrow; the degree to which the abnormalities relate to red cells (polycythemia vera), platelets (essential thrombocytosis), and fibrosis (myelofibrosis) determines the phenotype. All of these closely related illnesses are associated with increased clotting and bleeding risk, and all have the potential to progress to marrow failure (secondary to myelofibrosis) and AML.

Aplastic anemia (ICD-9 284; ICD-10 D60–D61) is a bone marrow failure syndrome associated with the primary loss of stem cells or the immunologic suppression of stem-cell proliferation and maturation leading to pancytopenia. Exposures to radiation, a number of drugs, and some industrial chemicals (such as benzene) are recognized as risk factors for this condition, but it may also arise from an autoimmune disease.

Conclusions from *VAO* and Previous Updates

MDS was not reviewed as a separate category until *Update 2014*, which reviewed only one paper that included MDS as an outcome. Yi and Ohrr (2014) assessed cancer incidence among Korean veterans who had served in Vietnam between 1964 and 1973 and reported a non-significant increased risk of MDS in the internal comparison of the high- and low-exposure groups, based on the EOI scores.

Update of the Epidemiologic Literature

Although VA charged the committee with specifically considering and reviewing the evidence of exposure to the COIs and MPNs, no published studies were identified (in humans or in animals or cell lines). There have been no published data on the relationship between the COIs and MPNs; therefore no evaluation of the literature could be performed.

Biologic Plausibility

K. P. Singh et al. (2014) explored the relationship of the absence of the AHR locus and changes in hematopoetic stem cells associated with aging. They followed *Ahr*-null mice, showing that they have diminished survival, splenomegaly, leukocytosis, and anemia. The hematopoetic stem cells showed diminished self-renewal capacity, with somatic changes compatible with a profile of accelerated aging and hematopoetic stem cell exhaustion.

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Synthesis

There are minimal data to assess the role that specific COIs may play in the occurrence of the various nonmalignant bone marrow–derived diseases. Based on the one null study of Korean veterans who served in Vietnam that was reviewed in *Update 2014* and the paucity of new studies, the committee maintains the conclusion of inadequate or insufficient evidence of exposure to any of the COIs and other myeloid diseases including MPNs.

Conclusion

Based on the available information, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and other myeloid diseases including myeloproliferative neoplasms.

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8

Reproductive Health Effects and Effects on Descendants

Chapter Overview

*Based on new evidence and a review of prior studies, the current committee did not find any new associations between outcomes related to the reproductive health of veterans or effects on their descendants and exposure to the chemicals of interest (COIs; 2,4-dichlorophenoxyacetic acid [2,4-D], 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], picloram, dimethylarsinic acid [DMA or cacodylic acid], 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]).*

Thus, the findings on these outcomes can be summarized as follows:

- *None of the outcomes met the committee's criteria for determining that there was sufficient evidence of an association with exposure to the COIs.*
- *None of the outcomes met the committee's criteria for determining that there was limited or suggestive evidence of an association with exposure to the COIs.*
- *There is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and endometriosis; decreased sperm counts or sperm quality, subfertility, or infertility; spontaneous abortion, stillbirth, neonatal death, or infant death; and low birth weight or preterm delivery, birth defects, childhood cancers, or other disease in their children as they mature or in later generations.*
- *There is limited or suggestive evidence of no association between paternal exposure to TCDD and spontaneous abortion.*

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This chapter summarizes the scientific literature published since *Veterans and Agent Orange: Update 2014*, hereafter referred to as *Update 2014* (NASEM, 2016a), on the association between exposure to herbicides and adverse effects on the reproductive health of male and female Vietnam veterans and the health of their children and later generations. The literature considered in this chapter includes studies of a broad spectrum of reproduction-related effects in veterans and in other populations exposed occupationally or environmentally to the herbicides sprayed in Vietnam or to TCDD. Because some polychlorinated biphenyls (PCBs), some polychlorinated dibenzofurans (PCDFs or furans), and some polychlorinated dibenzodioxins (PCDDs) other than TCDD have dioxin-like biologic activity, studies of populations exposed to these chemicals were reviewed if their results were presented in terms of TCDD toxic equivalents (TEQs). As noted in Chapter 3, studies that report TEQs based only on mono-ortho PCBs (which are PCBs 105, 114, 118, 123, 156, 157, 167, and 189) are considered even though their TEQs are several orders of magnitude lower than those of the non-ortho PCBs (77, 81, 126, and 169), based on the revised World Health Organization (WHO) toxicity equivalency factor (TEF) scheme of 2005 (La Rocca et al., 2008; van den Berg et al., 2006). This is because the lower TEQs of the mono-ortho PCBs may be counterbalanced by their abundance, which is generally many orders of magnitude higher than the abundance of the non-ortho PCBs (H.-Y. Park et al., 2010).

The adverse outcomes evaluated in this chapter are male reproductive health effects such as alterations in sperm quality, semen, sex ratio, or hormonal levels; female reproductive health effects, including endometriosis and outcomes related to alterations in hormonal levels such as polycystic ovary syndrome and gestational diabetes; increased fetal loss (spontaneous abortion and stillbirth); neonatal and infant mortality; adverse gestational outcomes of low birth weight and preterm delivery; and the possibility of adverse health outcomes (birth defects, cancer; and changes in growth and physical parameters and in immune, allergic, motor development, cognitive, behavioral and socio-emotional outcomes) at any time during the lives of all progeny of Vietnam veterans. The committee responsible for *Updates 2012* and *2014* separated those outcomes most directly related to reproductive health and to the health of progeny into separate chapters. This report combines them because the committee believes that reproduction-related effects are best understood as a continuum.

Because the vast majority of Vietnam veterans are men, the primary focus of the *Veterans and Agent Orange* (*VAO*) series has been on potential adverse effects of herbicide exposure on men, and the etiologic importance of the exposed party's sex does not play the same dominant role in non-reproductive outcomes that it does in reproductive outcomes. However, an estimated 7,500 women are thought to have served in Vietnam (VA, 2017a), so findings relevant to female reproductive health, such as those concerning endometriosis, are also included in the chapter. Whenever the information was available, an attempt has

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been made to evaluate the effects of exposure on males and females separately. It should be kept in mind, though, that the amount of research providing reliable information on the consequences of paternal exposure is extremely sparse for the COIs in the VAO report series and also for the full array of environmental agents that may pose threats to the health of future generations.

In addition, for published epidemiologic or experimental results to be fully relevant to the evaluation of the plausibility of reproductive effects in Vietnam veterans, whether female or male, the veteran's exposure needs to have occurred before the conception of the child. With the exception of female veterans who became pregnant while serving in Vietnam, pregnancies that might have been affected occurred after deployment, when primary exposure had ceased but fetal exposure via dioxin stored in maternal tissue was possible. In the case of pregnancies of women who have previously been substantially exposed to the lipophilic dioxins, the direct exposure of the fetus throughout gestation is possible through the mobilization of toxicants from the mother's adipose tissue. In contrast, adverse effects on offspring mediated by male veterans would be via alterations in the sperm genome and associated ribonucleic acids (RNAs) or semen that would have been transmitted after exposure and deployment.

The categories of association and the approach to categorizing the health outcomes are discussed in Chapter 3. To reduce repetition throughout the report, Chapter 5 characterized study populations and presents design information related to new publications that report findings or that revisit study populations considered in earlier updates.

BIOLOGIC PLAUSIBILITY OF REPRODUCTIVE HEALTH EFFECTS

There have been few studies of the effects on reproductive outcomes of exposure to the four herbicides in question, particularly picloram and cacodylic acid, and the available studies generally have shown toxicity only at very high doses. Much of the following discussion thus concerns TCDD, which, other than in controlled experimental circumstances, usually occurs in a mixture of dioxins (dioxin congeners in addition to TCDD).

TCDD is stored in fat tissue and has a long biologic half-life, so internal exposure at generally constant concentrations may continue after an episodic, high-level exposure to an external source is discontinued. If a person had a high exposure, then high amounts of dioxins may still be stored in fat tissue and be mobilized, particularly at times of weight loss. That would not be expected to be the case for nonlipophilic chemicals, such as cacodylic acid.

Dioxin exposure has the potential to disrupt male reproductive function by altering the expression of genes that are pertinent to spermatogenesis and by altering steroidogenesis (Wong and Cheng, 2011); it has the potential to disrupt female reproductive function by altering the expression of genes relevant to

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ovarian follicle growth and maturation, uterine function, placental development, and fetal morphogenesis and growth (Bruner-Tran et al., 2017).

A father's direct contribution to a pregnancy is limited to the contents of the sperm that fertilizes an egg; those contents had long been thought to consist of greatly condensed, transcriptionally inert deoxyribonucleic acid (DNA) constituting half the paternal genome (a haploid set of chromosomes). Consequently, it was once believed that paternally derived damage to the embryo or offspring could only result from changes in sperm DNA, and dioxins have not been shown to mutate the DNA sequence. However, as discussed in greater detail below, TCDD can have epigenetic effects that modify the expression of a cell's genetic material, and those modifications persist in the daughter cells following cell division, whether the division involves an individual's own somatic tissues or the production of his (or her) gametes. This provides an alternative pathway to creating permanent (heritable) changes in gene expression—a pathway that does not involve altering the DNA sequence. Epigenetic changes include chemical modifications made to DNA (usually involving methylation) or to other cellular components such as histones and RNAs (Jirtle and Skinner, 2007). As a sperm matures, most of its histones are replaced by protamines, which renders it transcriptionally quiescent and permits extensive DNA compaction. The core histones that are retained in human sperm carry epigenetic modifications to maintain open nucleosomes, which permits the transcription of genes that are important during embryo development (Casas and Vavouri, 2014). Sperm also carry a considerable collection of RNA fragments (Kramer and Krawetz, 1997; Krawetz et al., 2011), including ribosomal RNAs, messenger RNAs, and small noncoding RNAs (Casas and Vavouri, 2014; Lane et al., 2014). Small RNAs have been found to play critical roles in fertilization (Amanai et al., 2006), early embryonic development (Hamatani, 2012; Suh and Blelloch, 2011), and epigenetic modifications (Gapp et al., 2014; Kawano et al., 2012). Therefore, male infertility or fetal loss associated with exposure to the COIs might be mediated by epigenetic modifications to components of sperm other than the DNA (Krawetz, 2005; Vecoli et al., 2016).

A mother's contribution to a pregnancy is obviously more extensive, and damage to an embryo or offspring can result from epigenetic changes in the egg DNA or from the direct effects of exposure on placenta formation and on the fetus during gestation. The mobilization of dioxin during pregnancy may be increased because the body is drawing on fat stores to supply nutrients to the developing fetus. TCDD has been measured in human circulating maternal blood, cord blood, and placenta. Thus, dioxin in the mother's bloodstream could cross the placenta and expose the developing embryo and fetus. Data indicate that dioxin can accumulate in placental tissue and that dioxin can transfer from the placenta to the developing fetus (Mose et al., 2012).

On the basis of laboratory animal studies, it is known that TCDD can affect reproduction, so a connection between TCDD exposure and human reproductive and gestational effects is biologically plausible. However, making definitive

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conclusions based on animal studies about the potential for TCDD to cause reproductive and gestational toxicity in humans is complicated by differences in sensitivity and susceptibility among different species, including strain-specific differences; by differences in the route, dose, duration, and timing of exposure between experimental protocols and real-world exposure; and by substantial differences between laboratory animals and humans in the toxicokinetics of TCDD. Experiments with 2,4-D and 2,4,5-T indicate that these chemicals have subcellular effects that could constitute a biologically plausible mechanism for reproductive and gestational effects. However, the preponderance of evidence from animal studies indicates that these chemicals do not have reproductive effects. There is insufficient information on picloram and cacodylic acid to assess the biologic plausibility of their potential reproductive or gestational effects.

The sections on the biologic plausibility of the specific outcomes considered in this chapter present more detailed toxicologic findings that are of particular relevance to the outcomes discussed.

MALE REPRODUCTIVE HEALTH

Male reproductive function is under the control of a variety of components whose proper coordination is important for normal fertility. Several of these components and some health outcomes related to male fertility, including reproductive hormones and sperm characteristics, can be studied as indicators of fertility. The reproductive neuroendocrine axis involves the hypothalamus, the anterior pituitary gland, and the testis. Gonadotropin-releasing hormone is secreted from the hypothalamus in a pulsatile fashion and acts on the anterior pituitary gland, leading to the release of both follicle-stimulating hormone and luteinizing hormone. Both are secreted into the circulatory system in episodic bursts by the anterior pituitary gland and are necessary for normal spermatogenesis. In the testis, luteinizing hormone interacts with receptors on Leydig cells, where it stimulates increased testosterone synthesis. Follicle-stimulating hormone and the testosterone from the Leydig cells interact with Sertoli cells in the seminiferous tubule epithelium to regulate spermatogenesis. A more detailed review of the male reproductive hormones can be found elsewhere (Strauss and Barbieri, 2013). Several agents, such as lead and dibromochloropropane, affect the neuroendocrine system and spermatogenesis (for reviews, see Schrader and Marlow, 2014; Sengupta, 2013). Reviews on the effects of various environmental toxicants, including TCDD, on testicular steroidogenesis and spermatogenesis provide insights into the potential underlying mechanisms, including reducing testosterone production in Leydig cells and inhibiting the formation of cyclic adenosine monophosphate (Mathur and D'Cruz, 2011; Svechnikov et al., 2010).

The committee responsible for the original VAO report (IOM, 1994) concluded that there was inadequate or insufficient evidence of an association between exposure to 2,4-D, 2,4,5-T, picloram, cacodylic acid, or dioxin and

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alterations in sperm characteristics or other male reproductive health parameters, finding, generally, that existing studies reported inconsistent or non-significant results. Additional information available to the committees responsible for subsequent updates did not change these conclusions. Reviews of the relevant studies are presented in the earlier reports. Table 29, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to male reproductive health outcomes.

Update of the Epidemiologic Literature

Environmental Studies

Mumford et al. (2015) examined the relationship between exposure to a number of persistent organic pollutants (POPs) and semen quality as part of the Longitudinal Investigation of Fertility and the Environment (LIFE) Study of environmental influences on human fecundity and fertility. Participants were 501 male partners of couples discontinuing contraception for the purposes of becoming pregnant, who were recruited in Michigan and Texas during 2005–2009. Upon enrollment, in-person interviews were conducted with each male partner to ascertain health, demographic, and reproductive histories. All data and biospecimens were collected in the home, and baseline interviews were followed by a standardized anthropometric assessment for the determination of BMI conducted by research nurses, and the research nurse also obtained non-fasting blood (10 mL) for quantification of serum chemicals and lipids. The quantification of POPs in serum included 1 polybrominated biphenyl (PBB 153); 9 organochlorine pesticides, and 10 polybrominated diphenyl ethers. PCBs with TEFs include 105, 114, 118, 156, 157, 167, and 189. PCDFs and the dioxin-like PCBs 77, 81, 123, 126, and 169 were not measured, leading to an underestimation of the TEQ. A baseline semen sample was obtained, followed by a second sample approximately 1 month afterwards, irrespective of the couples' pregnancy status. A total of 35 semen parameters were measured, including five reflecting general characteristics (volume, straw distance [a motility marker], sperm concentration, total sperm count, and percent hypo-osmotic swollen [a marker of sperm quality]), 8 motility measures, 12 morphometry measures, 8 morphology measures, and 2 sperm chromatin stability assay measures. The models were adjusted for age, BMI, cotinine (a marker of tobacco smoke exposure), research site, total serum lipids, fish consumption, abstinence time, and sample age. A total of 468 men had measured chemical concentrations and semen quality and were included in the analysis. The levels of PBBs and some other chemicals were lower in this cohort than those observed in the U.S. National Health and Nutrition Examination Survey (NHANES) although the exposures were comparable. When males with chemical concentrations in the fourth quartile were compared with those in the first quartile, significant associations (at the 0.05 level) were found for several individual POPs and semen quality parameters. Although the majority of the comparisons were null, the researchers did observe associations between each chemical

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class and each type of semen quality parameter, with results indicating both positive and negative associations with semen quality. At the 0.05 level of significance, among the PCBs with a TEF, only the 4th quartile of PCB 156 showed a positive association with a sperm morphology marker indicative of reduced semen quality. At the 0.01 level of statistical significance, PCBs 157 and 189 were associated with markers of improved semen quality. This report was based on a relatively large study cohort, and the results were adjusted for BMI and other potential confounders. Without a direct measurement of TCDD, though, the report is of modest utility.

Den Hond et al. (2015) measured biomarkers of exposure in 163 men recruited through academic fertility clinics in Belgium. All were under 50 years of age, with a body mass index (BMI) ≤ 35 and no known congenital, genetic, or acquired cause of infertility. Blood, urine, and two serially collected semen samples were obtained along with patient-provided information on smoking, food intake, physical activity, socioeconomic status, health status, and living conditions. Exposure characterization consisted of an evaluation of the serum for levels of endocrine-disrupting chemicals, including dioxins and dioxin-like PCBs. Men who had total motility counts of less than 20 million were classified as subfertile. The investigators found that elevated levels of PCDDs and PCDFs were associated with a non-significant increase in the risk of subfertility (1.59; 95% CI 0.96–2.65; $p = 0.07$), and elevated levels of dioxin-like PCBs were associated with a non-significant decrease in that risk (0.45; 95% CI 0.17–1.22; $p = 0.12$) after adjustment for confounders. This study was limited by its very small sample size and by a failure to use all of the semen quality markers available. It also had a confusing sampling frame with cases and controls sampled first based on an unsuccessful conception within 12 months status, and then further divided by total motile count. The generalizability of results from fertility clinic patients to Vietnam veterans is also uncertain. The report is thus of limited utility for the committee.

Galimova et al. (2015) measured PCDD/F levels in the semen of 168 infertile and 49 fertile men in Ufa, Russia, a city close to a manufacturing plant that produced, among other herbicides, 2,4,5-T during the 1960s through the 1980s. All subjects were patients of reproductive health clinics. Testing found PCDD/F TEQ levels to be 2.2–2.3 times higher in the ejaculate of infertile men than in that of fertile men. The highest concentration of the 2,3,7,8-TCDD congener was found in the ejaculate of men with abnormal sperm. 2,3,7,8-TCDD did not make a material contribution to the total level of dioxin load—its share was 12% of the equivalent dose, whereas the major part of toxicity as measured by the TEQ was determined by the presence of PCDFs. Other congeners also showed consistently higher levels among the men in the infertile groups. The paper lacked many details on the recruitment of the men, the number of men was small, and no analysis of the impact of adjustment for other factors was presented.

Mínguez-Alarcón et al. (2017) examined a group of young Russian men to determine whether peripubertal serum organochlorine concentrations affect

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semen parameters. The analysis was based on the Russian Children's Study, which is an ongoing prospective study of 516 males. Boys were enrolled at age 8–9 years and underwent a physical exam, blood sampling and, together with the mother or guardian, completed a questionnaire. Annual follow-ups were conducted. Because of loss to follow-up and other reasons, the analysis is based on 133 males (18–19 years) who had serum organochlorine concentration data collected and who provided one or two semen samples. One hundred twenty-three men provided two semen samples a week apart, and 10 provided one sample. Semen analysis included sperm motility, semen volume, and sperm concentration. Sera samples were used to measure 7 PCDDs, 10 PCDFs, 4 coplanar PCBs, 6 mono-ortho-substituted PCBs, and 31 other PCBs (non-dioxin-like PCBs). The measured TCDD concentrations (pg TEQ/g lipid) were: minimum, 0.35; 25th percentile, 1.77; 50th percentile, 2.9; 75th percentile, 4.2; and maximum, 12.1. A general pattern of decreased semen quality (concentration, count, motile sperm) with increasing TCDD was found. In adjusted models, men in the highest quartile of serum TCDD TEQs had, on average, a lower sperm concentration, lower sperm count, and lower total motile sperm count than those in the lowest quartile. There were no significant associations for the summed concentration of PCDD, PCDFs, coplanar PCBs or ∑CBs. This report is based on a well-designed study, including a prospective follow-up and adjustment for multiple potential confounders. The study was not able to isolate possible in utero exposure and postnatal exposure. Moreover, its utility is limited by the fact that subjects were exposed to dioxins in a different period of their life (infancy, childhood, and adolescence) than the Vietnam veterans, and the generalizability of the results is open to question.

Case-Control Studies

Paul and colleagues (2017) conducted a case-control study of the association between serum and semen levels of dioxin-like PCBs and post-testicular sperm maturation. The study group comprised 56 adult (aged 30–55 years) males from subfertile couples who were being evaluated for infertility at an in vitro fertilization clinic in Alicante, Spain, from May 2012 to June 2014. Cases (n = 24) were men whose semen quality was considered low based on having at least an alteration in at least one semen quality parameter as compared with baseline values. Controls (n = 26) were men with normal semen quality (all parameters above WHO 2010 cutpoints). Participants underwent a complete clinical examination; completed a questionnaire soliciting socioeconomic information, medical history, tobacco and alcohol consumption, and likely exposure to environmental chemicals; and gave blood and semen samples. The semen parameters that were measured included sperm concentration, volume, percentage of motile sperm, and percentage of sperm that were morphologically normal. The levels of 12 non-ortho (77, 81, 126, 169) and mono-ortho (105, 114, 118, 123, 156, 157, 167, 189) dioxin-like PCBs were measured in the serum samples. The authors also examined total TEQs as the sum of the TEQs obtained from the dioxin-like

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PCBs. The mean levels (expressed at WHO-TEQ/g lipid) of the total dioxin-like PCBs and the non-ortho PCBs were higher in the low-semen-quality case group (22.32 ± 21.33 pg WHO-TEQ/g lipid and 22.52 ± 21.2 pg WHO-TEQ/g lipid, respectively) than in the control group (14.00 ± 10.82 pg WHO-TEQ/g lipid and 13.85 ± 10.69 pg WHO-TEQ/g lipid, respectively), although the differences were not statistically significant. The levels of mono-ortho PCBs were statistically significantly higher in low-semen-quality cases than in controls. When levels were expressed as pg/g lipid, all comparisons showed higher levels in the case group, which were statistically significant. Several specific PCBs had higher levels among cases than among controls, including PCB 126, but for only one (PCB 105) was the difference statistically significant ($p = 0.031$). Significant decreased correlations were found between PCB 126 and viability ($r = -0.645$; $p = 0.013$) and between PCB 77 ($r = 0.671$; $p = 0.009$) and PCB 81 ($r = 0.552$; $p = 0.041$) and sperm morphology among the cases. Other PCBs showed a positive correlation with specific sperm parameters, while parameters such as sperm count and concentration showed no correlation with total PCB levels. The study results suggest an association between exposure to dioxin-like PCBs and semen quality, but it is limited by the small number of participants, and its generalizability is uncertain.

Other Identified Studies

Cremonese and colleagues (2017) conducted a cross-sectional study of 99 rural and 36 urban men aged 18–23 years living in southern Brazil. Occupational exposure to herbicides (not otherwise specified) and other agricultural chemicals was assessed via a structured questionnaire. Information was also gathered on demographics, occupation, and other factors. Whole blood and semen samples were collected. The investigators found a statistically significant dose–response relationship between higher reported herbicide use and both poorer sperm morphology ($p < 0.001$) and reduced levels of luteinizing hormone ($p = 0.002$). The study's strengths include the use of trained researchers to collect data on randomly sampled subjects who were selected to be representative of the underlying population, while its primary weakness is the small size of that cohort and the lack of specificity of the exposures.

Among the newly reviewed studies, Tan et al. (2016) found that the introduction of 2,4-D to in vitro samples of ejaculated human sperm did not affect their viability, capacitation, or spontaneous acrosome reactions, but it inhibited total motility, progressive motility, the ability to penetrate viscous media, and progesterone-induced capacitation and acrosome reaction rates in a dose-dependent manner. Sun and colleagues (2016) measured levels of prostate-specific antigen, dioxins, and steroid hormones in the serum of 97 men who had resided in a dioxin "hot spot" in Vietnam near a former U.S. airbase. Eighty-five men from a non-sprayed region in the north of the country served as controls. The investigators collected information on the subjects' health status, residence history, smoking

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habit, alcohol consumption, and occupation via a questionnaire. While dioxins, furans, and non-ortho PCBs levels were significantly higher in men in the hot spot region, prostate-specific antigen concentrations did not differ significantly between the groups. Levels of testosterone ($p = 0.003$) and estradiol ($p = 0.024$) were significantly higher in hot spot subjects and those of dehydroepiandrosterone ($p = 0.047$) significantly lower, but there were no significant differences for the other steroid hormones (androstenedione, cortisol, cortisone, dihydrotestosterone, estrone, and progesterone) measured. The study's primary weaknesses are its cross-sectional design, and its lack of control for possible confounders, and the fact that prostate-specific antigen level is not a health outcome.

Biological Plausibility

Although a study reported that doses of 2,4-D greater than 50 mg/kg/day produce acute testicular toxicity in male rats (Joshi et al., 2012), there is little evidence that lower doses of either 2,4-D or 2,4,5-T (when free of TCDD contamination) given chronically have substantial effects on either the reproductive organs or fertility (Charles et al., 2001; Munro et al., 1992). The no-observed-adverse-effect level for 2,4-D is recognized as 15 mg/kg/day (Gervais et al., 2008). In contrast, many diverse laboratory studies have provided evidence that TCDD can affect reproductive-organ function and reduce fertility in both males and females.

The administration of TCDD to male animals elicits reproductive toxicity by affecting testicular, epididymal, prostate, and seminal vesicle weight and function and by decreasing the rate of sperm production (Foster et al., 2010; Rider et al., 2010; Schneider et al., 2014). The mechanisms underlying those effects are not known, but the primary hypotheses are that the changes are mediated through the dysregulation of testicular steroidogenesis, altered Sertoli cell function, and increased oxidative stress. The exposure of cultured testicular Leydig cells to 25 nM TCDD markedly alters gene expression (Naville et al., 2011), and the exposure of cultured Sertoli cells to 5 nM TCDD decreases viability and increases markers of oxidative stress (Aly and Khafagy, 2011). The exposure of adult rats or mice to TCDD (2–7 µg/kg/week for 45–60 days) reduces testicular and reproductive function, and these effects can be attenuated by co-treatment with various antioxidants (Beytur et al., 2012; Ciftci et al., 2012; Sönmez et al., 2011; H. P. Yin et al., 2012). The results of those studies are supported by the transgenic mouse model that harbors a constitutively active aryl hydrocarbon receptor (Ahr) in which testicular and ventral prostate weights and sperm number are reduced (Brunnberg et al., 2011).

The discussion of the influence of paternal exposures on outcomes in offspring later in the chapter contains additional information on the biologic plausibility of COI exposure affecting male reproductive health.

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Synthesis

Reproduction is a sensitive toxic endpoint of TCDD and dioxin-like chemicals in rodents, and there are several species and strains of animals for which the fetus is more sensitive than the adult rodent to the adverse effects of TCDD. The sensitivity of these endpoints in humans, however, is less apparent. Although AHR plays an important role in normal sperm development (D. A. Hansen et al., 2014), there remains little evidence that exposure to dioxin is associated with a reduction in sperm quality or a reduction in fertility. Some of the studies reviewed in this update found an association between dioxin or dioxin-like PCB exposure and one or more parameters associated with male reproductive health, primarily poor sperm morphology (Mínguez-Alarcón et al., 2017; Paul et al., 2017). However, others reported only non-significant associations or none at all. The various weaknesses in these studies' methodologies that were noted by the committee, including small sample sizes and difficulty in generalizing the results to the exposure experience of Vietnam veterans, greatly limits their usefulness.

Conclusion

On the basis of its evaluation of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence of an association between exposure to the COIs and alterations in semen quality or other male reproductive health markers.

FEMALE REPRODUCTIVE HEALTH

Studies of the relationship between chemicals and fertility are less common in women than in men. Some chemicals may disrupt the female hormonal balance necessary for proper functioning. Normal menstrual-cycle functioning is also important in the risk of hormonally related diseases, such as osteopenia, breast cancer, and cardiovascular disease. Generally speaking, chemicals can have multiple effects on the female system, including the modulation of hormone concentrations that results in uterine-cycle or ovarian-cycle irregularities, changes in menarche and menopause, and the impairment of fertility (Bretveld et al., 2006a,b). Past and current literature reviews have found studies relevant to the committee's Statement of Task addressing endometriosis, hormonal levels, polycystic ovary syndrome, and gestational diabetes.

The committee responsible for the original *VAO* report (IOM, 1994) concluded that there was inadequate or insufficient evidence of an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and alterations in female reproductive health outcomes. Additional information available to the committees responsible for subsequent updates did not change the conclusion that exposure to the COIs had not been found to be associated with these outcomes.

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Reviews of the relevant studies are presented in the earlier reports. Tables 30 and 31, which can be found at www.nap.edu/catalog/25137, summarize the results of studies related to endometriosis and other female reproductive health outcomes reviewed in the VAO series.

Endometriosis

Endometriosis (*International Classification of Diseases*, 9th revision [ICD-9] 617; ICD-10 N80.8) affects more than 5 million women in the United States and Canada at any given time (NICHD, 2017). The endometrium, the tissue that lines the inside of the uterus, is built up and shed each month during menstruation. In endometriosis, endometrial cells are found outside the uterus—usually in other parts of the reproductive system, in the abdomen or pelvis, or on surfaces near the reproductive organs. The ectopic tissue develops into growths or lesions that continue to respond to hormonal changes in the body and break down and bleed each month in concert with the menstrual cycle. Unlike blood released during normal shedding of the endometrium, blood released from degenerating ectopic endometrium has no way to leave the body. The blood sets up an inflammatory reaction causing pain, adhesions (scars), infertility, intestinal problems, or hematuria (blood in urine).

There are several theories of the etiology of endometriosis, including one that posits a genetic contribution, but the cause remains unknown. Estrogen dependence and immune modulation are established features of endometriosis, but they do not adequately explain its cause. It has been proposed that endometrium is distributed through the body via blood or the lymphatic system; that menstrual tissue backs up into the fallopian tubes, implants in the abdomen, and grows; and that all women experience some form of tissue backup during menstruation but only those who have immune-system or hormonal problems experience the tissue growth associated with endometriosis. Despite numerous symptoms that can indicate endometriosis, definitive diagnosis is possible only through laparoscopy or a more invasive surgical technique. Several treatments for endometriosis are available, but there is no cure.

Endometriosis was first reviewed in *Update 2002*, which identified two relevant environmental studies. Additional studies considered in later updates did not change the conclusion that the evidence is inadequate or insufficient to support an association with herbicide or dioxin exposure. Table 30, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to endometriosis.

Update of the Epidemiologic Literature

No Vietnam-veteran, occupational, or environmental studies of exposure to the COIs and endometriosis have been published since *Update 2014*. The new

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case-control studies regarding this outcome that met the committee's criteria for review are summarized below.

Case-Control Studies Martínez-Zamora et al. (2015) used a case-control design to evaluate levels of dioxin-like chemicals in the adipose tissue in 30 women diagnosed with deep infiltrating endometriosis versus women in a control group who were undergoing laparoscopic surgery due to benign adnexal gynecological diseases other than endometriosis. All subjects were recruited from a university hospital located in Catalonia, Spain. Seventeen dioxins (7 PCDDs and 10 PCDFs) and 12 PCBs were analyzed. The investigators found that the TEQs and concentrations of both dioxins and PCBs were significantly higher in patients with deep infiltrating endometriosis than in the control group ($p = 0.05$), due primarily to significantly higher values of TCDD (odds ratio [OR] = 1.41, 95% confidence interval [CI] 1.12–2.10; $p < 0.01$) and 1,2,3,7,8-PeCDD (OR = 1.82, 95% CI 1.36–7.14, $p < 0.01$), which the authors observed was suggestive of a potential role for dioxin-like chemicals in the pathogenesis of deep infiltrating endometriosis. A strength of the study was the measurement of dioxins and PCBs in adipose tissue and the calculation of TEQs; its limitations included the small sample size (30 cases; 30 controls) and the selection bias induced by the recruitment of hospital surgery patients as subjects, which limits the generalizability of the results to other populations like female Vietnam veterans.

Ploteau et al. (2017) examined whether deep infiltrating endometriosis with or without concurrent ovarian endometrioma was associated with levels of POPs measured in the adipose tissue of cases and controls undergoing surgery in a clinic in the Pays de la Loire region of France during 2013–2015. Cases (n = 55) were 18 to 45 years old with a surgical diagnosis of deep infiltrating endometriosis; controls (n = 44) were women of similar age and BMI who had presented for other benign gynecological conditions. All subjects were interviewed for information on their health, physical state, and other factors thought to be associated with POP exposure. Serum was collected the day before the surgical procedure that led to their participation in the study; parietal and omental fat samples were obtained during the procedure. Biospecimens were tested for a number of POPs, including dioxins and dioxin-like PCBs. The investigators found statistically significant associations between deep infiltrating endometriosis and adipose tissue levels of four mono-ortho dioxin-like PCBs: 105 (adjusted odds ratio [aOR] = 2.09, 95% CI 1.24–3.77), 114 (aOR = 1.89, 95% CI 1.04–3.69]), 118 (aOR = 2.30, 95% CI 1.31–4.36), and 123 (aOR = 2.47, 95% CI 1.42–4.66) versus controls. The statistical significance held up when subjects with deep infiltrating endometriosis with ovarian endometrioma were examined. No such association was observed for the dioxin-like PCB 169. The authors noted that the associations were stronger for the subjects with deep infiltrating endometriosis with ovarian endometrioma but that the small number of such cases complicated the interpretation of the results. They did not calculate a TEQ, limiting the usefulness of their findings.

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Other Identified Studies A 2017 review article by Parazzini et al. concluded that there were few studies on endometriosis and exposure to dioxin and that the available literature had inconsistent findings. Soave and colleague's 2015 review contains information on several endometrial studies referenced in *Update 2014*, plus the Martínez-Zamora paper reviewed above.

Biological Plausibility

As observed in *Update 2014*, laboratory studies that used animal models and examined gene-expression changes associated with human endometriosis provide evidence of the biologic plausibility of a link between TCDD exposure and endometriosis. Genetic polymorphisms in the AHR signaling complex have been associated with a susceptibility to advanced endometriosis in humans (D. Li et al., 2013; C. H. Wu et al., 2012), although another study found no association in Japanese women (Matsuzaka et al., 2012). The first suggestion that TCDD exposure may be linked to endometriosis came as a secondary finding of a study that exposed female rhesus monkeys (*Macaca mulatta*) chronically to low concentrations of dietary TCDD for 4 years (Bowman et al., 1989). Ten and 13 years after the exposure ended, the investigators documented an increased incidence of endometriosis in the monkeys that correlated with the TCDD exposure concentration (Rier et al., 1993, 2001). The sample was too small to yield a definitive conclusion that TCDD was a causal agent of endometriosis, but this study led to additional studies of the ability of TCDD to promote the growth of pre-existing endometriotic lesions (Bruner-Tran et al., 1999; Johnson et al. 1997; J. Z. Yang et al., 2000).

There are a number of mechanisms by which TCDD may promote endometrial lesions, which provide additional support for the biologic plausibility of a link between TCDD and endometriosis. Human endometrial tissue and cultured human endometrial epithelial cells both express AHR; its dimerization partner, the aryl hydrocarbon nuclear translocator (Khorram et al., 2002); and three AHR target genes—*CYP1A1*, *1A2*, and *1B1* (Bulun et al., 2000; Willing et al., 2011). These findings suggest that endometrial tissue is responsive to TCDD. M. N. Singh et al. (2008) showed that *CYP1A1* expression is greater in ectopic endometrial tissue than in eutopic uterine tissue in the absence of TCDD exposure, which suggests that *CYP1A1* may play a role in the etiology of the disease or that AHR and its signaling pathway have been activated by an endogenous ligand other than TCDD. Other mechanisms by which TCDD may promote endometriosis include altering the ratio of progesterone receptor A to progesterone receptor B and blocking the ability of progesterone to suppress matrix metalloproteinase expression—actions that promote endometrial-tissue invasion and that are observed in women who have endometriosis (Igarashi et al., 2005).

TCDD also induces changes in gene expression that mirror those observed in endometrial lesions. In addition to the induction of *CYP1A1* noted above,

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TCDD can induce expression of histamine-releasing factor, which is increased in endometrial lesions and accelerates their growth (Oikawa et al., 2002, 2003). TCDD disrupts cannabinoid signaling in endometrial stromal cells by inhibiting the progesterone-induced expression of cannabinoid receptor type 1, which is also observed in women with endometriosis (Resuehr et al., 2012). TCDD also stimulates the expression of RANTES (regulated on activation, normal T-cell–expressed, and secreted protein) in endometrial stromal cells, and RANTES concentration and bioactivity are increased in women who have endometriosis (Zhao et al., 2002). The two CC-motif chemokines (chemotactic cytokines), RANTES and macrophage-inflammatory protein-1α (MIP-1α), have been identified as potential contributors to the pathogenesis and progression of endometriosis. Previous studies have shown that the combination of 17β-estradiol and TCDD increases the secretion of RANTES and MIP-1α in endometrial stromal cells (Yu et al., 2008), and a more recent study showed that the same combination suppresses the expression of tetraspanin CD82, a tumor-metastasis suppressor, and thus promotes the invasion of endometrial stromal cells (M. Q. Li, 2011). Those results support the idea that TCDD in combination with estradiol may contribute to the development of endometriosis by increasing the invasiveness of endometrial cells. Despite that evidence, chronic exposure of rats to TCDD, non-dioxin-like PCB 153, dioxin-like PCB 118 or PCB 126, or 2,3,4,7,8-PeCDF (the furan congener with the highest TEF), either individually or in various combinations, fails to alter endometrial histology in a consistent manner (Yoshizawa et al., 2009). The differences between rodent and human endometrium could account for the lack of observed effects in rats.

In summary, experimental studies, particularly ones that used human eutopic and ectopic endometrial tissue, provide evidence of the biologic plausibility of a link between TCDD exposure and endometriosis.

Synthesis

The human studies linking dioxin exposure with endometriosis are few and inconsistent; information related to exposures to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid is lacking. Although animal studies support the biologic plausibility of an association, contemporary human exposures may be too low to show an association should one exist.

Conclusion

On the basis of the evidence reviewed here, in *VAO*, and in the previous VAO updates, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and endometriosis.

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Other Female Reproductive Health Outcomes

Update of the Epidemiologic Literature

Polycystic Ovary Syndrome (PCOS) PCOS is a hormonal disorder characterized by overproduction of the androgen testosterone. Three potentially relevant studies published since *Update 2014* addressed this condition. Vagi et al. (2014) used a case-control pilot study design with Cedars Sinai Medical Center, Los Angeles, California, patients. Serum PCBs—including the dioxin-like PCBs 105, 118, and 156—organochlorine pesticides, and other compounds were measured in the urine of 52 cases and 50 controls. None of the dioxin-like PCBs was significantly elevated in cases compared to controls.

Q. Yang et al. (2015) used a case control design to evaluate serum PCBs, pesticides, and polycyclic aromatic hydrocarbons in 50 cases and 30 control ethnic Han females recruited from the Peking University Third Hospital in northern China. Exposure to dioxin-like PCBs was associated with an OR of 4.89 (95% CI 1.81–13.2), which remained significant after adjusting for confounders including education and occupation (aOR = 5.52; 95% CI 1.51–20.2). Study limitations include a small sample size and metabolic differences between the cases and control that could affect the absorption, distribution, metabolism, and excretion of COIs.

J. Zhang et al. (2014) used a cross sectional stratified case control design to examine PCOS in females aged 12–44 years in Chengdu, China; 169 cases and 338 controls were analyzed. Questionnaire responses were used to characterize exposure. No exposures to COIs were measured, although they can be inferred for two categories characterizing a history of contact with polybrominated biphenyls: "pesticide," which yielded an odds ratio of 1.76 (95% CI 1.09–2.87) and "eating fruit with pericarp" 5.00 (95% CI 2.67–9.48). However, the considerable uncertainty associated with this inference greatly limits the usefulness of the study results to the committee.

Gestational Diabetes Mellitus Vafeiadi et al. (2017) used a cross-sectional/semi-prospective design to evaluate PCBs and other compounds in first-trimester maternal serum (dioxin-like PCBs 118 and 156 summed) with relation to gestational diabetes mellitus measured at 24–28 weeks in 939 mother–infant pairs from the Rhea pregnancy cohort in Crete, Greece. Sixty-eight (7%) mothers presented with gestational diabetes mellitus. The adjusted odds ratio of gestational diabetes mellitus in women in the "medium" (middle) tertile of dioxin-like PCBs was 5.63 (95% CI 1.81–17.51) and for the high tertile 4.71 (95% CI 1.38–16-01). The study controlled for maternal fish consumption, but the authors could not rule out undiagnosed type 1 or type 2 diabetes or other unmeasured lifestyle factors as confounders, limiting the usefulness of the results.

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Alteration in Hormonal Levels Anh et al. (2017) examined the relationship between adrenal hormone disruption in lactating mothers and their children in two locations of Vietnam, one of which was known to be contaminated with dioxin. Thirty-seven mother–child pairs were recruited from the area near the former U.S. air base at Bien Hoa, which stored tactical herbicides during the Vietnam War and where at least four accidental releases of Agent Orange took place; 47 control pairs from a rural community in northern Vietnam—where no U.S. spraying took place—were also examined. Breast milk and blood samples were collected from all mothers 4–16 weeks after the birth of their first child. Data on subjects' social characteristics, diseases (including hormonal therapy), and body measurements were obtained via questionnaire. The researchers found a statistically significant difference ($p < 0.001$) between serum androstenedione[1] levels in mothers who resided in the dioxin contaminated region ($n = 37$; 1.91 ng/mol ± 1.00) versus the non-contaminated region ($n = 47$; 0.61 ng/mol ± 0.27). They also noted an association between maternal levels of several dioxin congeners and subsequent salivary dehydroepiandrosterone levels in children (total TEQ $\beta = 0.42$; $p < 0.001$). These results suggested to them that dioxin disrupts adrenal androgens in mothers and breastfeeding children through the same mechanism. However, since the children were exposed both before and after birth, this study is of limited relevance to the committee.

A cross-sectional study by Lignell and colleagues (2016) evaluated PBDE, PCB, and dioxin exposures in relation to thyroid hormone status early in pregnancy in randomly selected first-time mothers (1996–1999) from Uppsala County, Sweden. Maternal body burden of PCDD/Fs was inversely associated with first-trimester T3 thyroid hormone levels. In fully adjusted models, no PCBs were associated with first-trimester thyroid hormone levels; however, in crude analysis di-ortho and mon-ortho PCB TEQ concentrations were negatively associated with serum lipids in the third trimester and T3 in the first trimester. This result was deemed noteworthy because maternal thyroid hormone status influences fetal development in early pregnancy. However, reverse causality cannot be discounted in this study as maternal T3 could influence the absorption, distribution, metabolism, and excretion of toxicants.

Biological Plausibility

Many studies have examined the effects of TCDD on the female reproductive system. The two primary mechanisms that are believed to contribute to abnormal follicle development and decreased numbers of ova after TCDD exposure are the "cross-talk" of the AHR with the estrogen receptor and the dysregulation of the hypothalamic–pituitary–gonadal axis (Pocar et al., 2005; Safe and Wormke,

[1]Androstenedione and dehydroepiandrosterone are so-called "pro-hormones" that are precursors in the production of testosterone and estrogen.

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2003). Oocytes are directly responsive to TCDD, so TCDD's effects on hormone concentrations, hormone-receptor signaling, and ovarian responsiveness to hormones all probably contribute to TCDD-induced female reproductive toxicity. The data of Jung et al. (2010) in rats show that a single high-dose gavage treatment of 32 µg/kg TCDD reduces the proliferation of granulosa cells and thus attenuates cell-cycle progression and potentially contributes to the reduction in ovulation rates observed in other studies. In contrast, Karman et al. (2012) found that 1 nM TCDD exposure in vitro did not reduce the rates of growth of murine antral follicles, but did reduce the secretion of progesterone and estradiol by the follicles. The concentrations of those hormones could be restored by the addition of the precursor pregnenolone, which suggests that TCDD acts upstream of pregnenolone formation. Baldridge et al. (2015) have also shown that rat granulosa cells are highly sensitive to low-level (femtomolar) TCDD, resulting in disrupted steroid hormone secretion. A similar result was demonstrated by Jablonska et al. (2014) in porcine granulosa cells. This would be consistent with previous observations in zebrafish that 10, 40, and 100 parts per billion TCDD in food depressed estradiol biosynthesis (Heiden et al., 2008).

Dioxin's effects on early embryo development and on placenta formation are well documented (S. C. Chen et al., 2010; Ishimura et al., 2009; Tsang et al., 2012). Petroff et al. (2011) used a rat in vitro fertilization model to demonstrate that 100 nM TCDD perturbs chromatin and cytoskeletal remodeling at the earliest stages of embryo development, but these changes failed to result in any apparent morphologic changes at later stages of development. The long-term potential effects of these early changes on pregnancy outcome are unknown. It has previously been shown that TCDD may have direct effects on human trophoblast formation at 0.2–2.0 nM in vitro and thus may have the capacity to influence the developing fetus (S. C. Chen et al., 2010). That idea is supported by a study showing the ability of 5 nM TCDD to activate the AHR signaling pathway in both rat and human placental trophoblasts (Stejskalova et al., 2011). Finally, a study has demonstrated that TCDD at 0.1, 1.0, and 10.0 nM reduces in a dose-dependent fashion the ability of trophoblastic spheroids (which constitute an embryo surrogate) to attach to endometrial epithelial cells (Tsang et al., 2012). The more recent literature continues to support the biologic plausibility of TCDD having effects on male and female reproduction.

Synthesis

Eskenazi et al. (2010)—reviewed in *Update 2010*—published the only study to date that has examined dioxin exposure in women with respect to time-to-pregnancy (number of contraceptive-free months before pregnancy) and infertility (more than 12 contraceptive-free months to pregnancy). Dose–response relationships between TCDD serum levels in women who were less than 40 years of age at the time of the Seveso accident and both time-to-pregnancy and

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infertility were observed, which is consistent with published observations in the rat model. Epidemiologic studies have not provided sufficient data to interpret the effects of dioxin specifically on menstrual-cycle function in humans.

The studies reviewed for this update yielded inconsistent results regarding any association between exposure to the COIs and the outcomes studied, and they exhibited weaknesses such as the failure to measure dioxin levels or calculate TEQs that limit their applicability to the evaluation of outcomes for Vietnam veterans.

Conclusion

On the basis of its evaluation of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence of an association between exposure to the COIs and disturbances in hormonal levels in females and diseases that may be associated with such disturbances.

GESTATION AND NEONATAL EFFECTS IN OFFSPRING

The gestation and neonatal periods are uniquely vulnerable times in life. Data on health outcomes either occurring or first identified during these periods have been collected on the offspring of those exposed to the COIs. Information concerning spontaneous abortion, stillbirth, neonatal death, and infant death; sex ratio; birth weight and preterm delivery; and birth defects are discussed below.

General Biologic Plausibility

Influence of Paternal Exposure

James (2006) has interpreted the perturbation of sex ratios by dioxins and other agents as being an indicator of parental endocrine disruption and, indeed, a population-level finding of a paternally mediated effect would be a strong indicator that dioxin exposure can interfere with the male reproductive process. If this observation were demonstrated to be true, then it would be concordant with a reduction in testosterone in exposed men (Egeland et al., 1994). Another pathway to an altered sex ratio might involve male embryos experiencing more lethality from the induction of mutations due to their unmatched X chromosome. TCDD has not been thought to have genotoxic effects, but sex-specific adverse consequences of the modified imprinting of gametes might be a possible mechanism leading to the observation of altered sex ratios at birth. To date, however, the proportion of sons among the children of fathers exposed to dioxin-like chemicals does not present a clear pattern of reduction.

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The idea that the exposure of either parent to a toxicant before conception could result in an adverse outcome in offspring is not new and remains a topic of much interest (Schmidt, 2013). Epidemiologic studies have reported occasional findings of paternally transmitted adverse outcomes associated with paternal exposures to certain agents, but none has been replicated convincingly. Even in instances in which an agent is recognized as mutagenic or potentially carcinogenic for exposed men, adverse consequences have not been demonstrated in their children. For example, the hypothesis was extensively investigated in the early 1990s in relation to fathers' exposure to ionizing radiation before conception and an increase in leukemias in their offspring. The initial study (Gardner et al., 1990) was conducted in men who worked at the Sellafield nuclear facility in West Cumbria, United Kingdom. It was presumed that the men were exposed to radiation as a result of working at Sellafield. An association was found between radiation exposures to fathers before their children's conception and an increase in leukemias among those children. However, later studies failed to confirm that finding (Draper et al., 1997; Kinlen, 1993; Kinlen et al., 1993; Parker et al., 1993; Urquhart et al., 1991). Similarly, a rigorous follow-up of children of atomic-bomb survivors has not demonstrated increased risks of cancer or birth defects (Fujiwara et al., 2008; Izumi et al., 2003; Schull, 2003), and other studies of birth defects and cancer in the children of male cancer survivors after chemotherapy or radiation treatment have found little support for paternal transmission (Chow et al., 2009; Dohle, 2010; Howell and Shalet, 2005; Madanat-Harjuoja et al., 2010), although sperm and fertility clearly are adversely effected (Green et al., 2010).

An additional problem when trying to determine whether adult male exposure of any type (including to the COIs) can lead to pathological effects in descendants is that almost all experimental exposure studies designed to identify male transmission have been limited to developmental exposures in rodents (Guerrero-Bosagna and Skinner, 2014; Paoloni-Giacobino, 2014). An early experiment examining male mice treated with simulated Agent Orange mixtures prior to breeding with unexposed females failed to find an increase in a variety of different birth defects in progeny compared with the progeny of untreated males (Lamb et al., 1981).

Prior VAO committees have been unable to identify epidemiologic evidence that convincingly demonstrated paternal exposure to any particular chemical before conception that resulting in cancers or birth defects in offspring. However, few data exist to address the hypothesis of paternal exposure and adverse effects in human offspring in which the exposure occurred before conception only to the father, and what little information exists comes from the radiation effects literature. Thus, it is difficult to assert conclusively that the available epidemiologic evidence either supports or does not support paternal transmission; considerable uncertainty remains on many fronts and would presumably vary by agent and mode of exposure. Several systematic reviews of the topic have been conducted

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(Chia and Shi, 2002; Weselak et al., 2007, 2008; Wigle et al., 2007, 2008), and none have established firm relationships between specific agents and particular effects in offspring. Paternal occupation (characterized by exposure to chemicals via job title or job–exposure matrices) has been linked to an increased risk of selected birth defects (Desrosiers et al., 2012; Fear et al., 2007; Shaw et al., 2002) and neuroblastoma (De Roos et al., 2001a,b). Moreover, increased risks of childhood brain cancer have been reported in relation to paternal exposure to selected pesticides, particularly herbicides and fungicides (van Wijngaarden et al., 2003), although the authors noted considerable uncertainty in the robustness of the findings. Therefore, the hypothesis that paternal preconception exposure to toxic agents may result in harm to a man's children remains unresolved in significant part because of the sparseness of epidemiologic research on the subject.

Paternal Preconceptual Exposure There is no evidence that dioxins can mutate DNA sequences (see Chapter 4). Genetic changes in sperm genes due to preconception exposures to TCDD—as has been shown, for example, in connection with irradiation or the anticancer drug cyclophosphamide (Codrington et al., 2004)—are thus unlikely. The potential does exist, however, for TCDD to alter the sperm cells of adults before fertilization through epigenetic pathways. The sperm epigenome is distinctive from that of the egg (oocyte) or somatic cells (all other non-gamete cells in the body). The mature sperm cell has less global methylation than somatic cells, particularly at gene promoters, and has unique DNA methylation marks (particularly on paternally imprinted genes) that put the sperm genomes in a pluripotent-like state before fertilization (Hales et al., 2011). However, rapid demethylation of most of the remainder of the paternal genome occurs shortly after fertilization (Dean, 2014), suggesting that additional changes are required for the nascent embryo to become truly pluripotent. Chemical alterations of DNA methylation foci of adult sperm have the potential to contribute to permanent effects in offspring, as has been suggested for male transmittance in fetal alcohol syndrome (Jenkins and Carrell, 2012a). During spermatogenesis in the adult, most sperm histones are replaced by protamines, which render the sperm transcriptionally quiescent and permit extensive DNA compaction. However, some core histones are retained in human sperm with appropriate epigenetic modifications in order to maintain open nucleosomes at sites that are important during embryo development (Casas and Vavouri, 2014), so their perturbation by exogenous chemicals remains a possibility. This is particularly important because although genome-wide DNA demethylation occurs in paternal DNA after fertilization (Dean, 2014) and should erase most sites that have been reprogrammed by chemicals, histone modification patterns are retained and thus may transmit chemical-induced alterations across generations (Puri et al., 2010).

Despite the exclusion of almost all cytoplasm, mature sperm, as noted above, have been found to carry a diverse spectrum of RNAs, including messenger RNAs, ribosomal RNAs, and small noncoding RNAs, which may affect the developing

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embryo (Casas and Vavouri, 2014; Hamatani, 2012; Kawano et al., 2012; Krawetz, 2005; Krawetz et al., 2011; Lane et al., 2014; Suh and Blelloch, 2011). For example, small RNAs of paternal origin may direct epigenetic modifications during embryo development and lead to changes in phenotype later in life (Hales et al., 2011). When newborn male mice were stressed by unpredictable separation from their mothers, messenger RNAs in their sperm have been shown to transmit the effects of this early trauma for two generations (Gapp et al., 2014). Heavy metals interact with sperm's nuclear proteins, and this mechanism is suspected to be a basis of the paternally mediated effects of lead (Quintanilla-Vega et al., 2000). Disturbances in the establishment of the epigenetic marks in mature sperm may change cell fate in the early embryo and have effects throughout development and postnatal life (Jenkins and Carrell, 2012b).

Direct evidence of dioxin-mediated changes in the epigenome of mature sperm is not available. However, dioxins have been shown to modify DNA methylation in somatic cells (Hou et al., 2012), so an epigenetic pathway is plausible.

Paternal Postconceptual Exposure Contaminants such as TCDD that are present in the tissues and blood of exposed males can be transported as parent compounds or as metabolites into seminal fluid, the noncellular component of the ejaculate. Typically, the concentrations of contaminants in seminal fluid are lower than those in serum, but no direct assessments of the ratios of serum to seminal fluid in TCDD have yet been reported. Seminal-fluid contaminants can be transmitted to a female during sexual intercourse and be absorbed through the vaginal wall; if the concentrations are high, then they could potentially affect a current pregnancy (Chapin et al., 2004; Klemmt and Scialli, 2005). TCDD and other POPs have been identified and quantified in the seminal plasma of exposed men, including Vietnam veterans (Schecter et al., 1996; Schlebusch et al., 1989; Stachel et al., 1989); thus, this transmission route is theoretically possible. In the Schecter et al. (1996) study, serum TCDD was measured in 50 Vietnam veterans from Michigan who had a confirmed or self-reported potential for herbicide exposure and had blood drawn an average of 26 years after the possible exposure. Of those, six had TCDD levels greater than 20 parts per trillion (ppt) on a lipid-adjusted basis, which supports the idea that some veterans had high initial exposures. A subgroup of 17 men contributed semen at the time of blood draw, and dioxin congeners were analyzed in three randomly pooled samples—a process necessary to provide sufficient volume for chemical analysis. Although the measured concentrations were very low, the results documented the existence of dioxins and dibenzofurans in the seminal plasma of the veterans long after the possible herbicide exposure to TCDD-contaminated herbicides. Because the results on serum and semen concentrations could not be linked to individual veterans and because it is unknown whether any of the individuals who had high serum dioxin concentrations after 26 years contributed semen for the seminal-fluid measurements, the value of this information is

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minimal. Seminal-fluid concentrations of TCDD and related chemicals closer to the period of exposure in Vietnam have not been determined, so it is not possible to assess the clinical consequences of this exposure route for female partners and gestating offspring. Banked Ranch Hand specimens, however, might provide a valuable resource for comparing TCDD concentrations in serum and seminal fluid. A 2015 Institute of Medicine report describes the available data and biospecimens from the Air Force Health Study (AFHS) and the potential for future analyses (IOM, 2015).

Despite the potential for a seminal fluid route of exposure, the critical question of dose sufficiency remains unanswered. That is, could absorbed TCDD concentrations be high enough to transmit adverse effects to the fetus? To answer that question, one must take into account several factors. First, the volume of seminal plasma is relatively low (1–5 mL) and, because of leakage, only a fraction of seminal constituents is absorbed across the vaginal wall. Moreover, the dilution of absorbed chemicals in the female bloodstream before transmission across the placenta is estimated at 3 orders of magnitude or more (Klemmt and Scialli, 2005), which reduces a serum concentration of 20 ppt to a scale of parts per quadrillion (10^{-15}). Although no studies have been undertaken to address the issue directly, the dilution factor makes it extremely unlikely that adverse fetal and offspring outcomes would occur as a consequence of seminal plasma exposures to TCDD during pregnancy. One caveat to this conclusion, however, is that seminal fluid is now known to play an important role in the metabolic phenotype of offspring because it stimulates embryotrophic factors (Bromfield, 2014; Bromfield et al., 2014). Whether TCDD contamination of the seminal fluid can affect this function is not known and should be tested.

Influence of Maternal Exposure

Maternal exposures can affect a pregnancy and the resulting offspring far more extensively than can paternal exposures. Because of the long half-life of TCDD and its bioaccumulation in adipose tissues, women exposed to herbicides in Vietnam would have the potential to expose their offspring to TCDD directly during later pregnancies. Thus, damage to the resulting offspring or future generations could result from epigenetic changes in an egg before conception or from the direct effects of exposure on the fetus during gestation and on the neonate during lactation. Dioxin in the mother's bloodstream can cross the placenta and expose the developing embryo and fetus.

Furthermore, the mobilization of dioxin during pregnancy or lactation may be increased because the body is drawing on fat stores to supply nutrients to the developing fetus or nursing infant. TCDD has been measured in circulating human maternal blood, cord blood, placenta, and breast milk (G. Suzuki et al., 2005), and it is estimated that an infant breastfed for 1 year accumulates a dose of TCDD that is six times as high as an infant who is not breastfed (Lorber and Phillips, 2002).

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The offspring effects of maternal exposures may not be manifested immediately and could be a result of a dioxin-mediated reprogramming of developing organs and lead to a disease onset later in life. As noted elsewhere, placental structure and function are believed to play a major role in fetal growth, and TCDD has been shown to alter placental vascular remodeling (Y. Wu et al., 2013, 2014).

As mentioned in conjunction with the role of the placenta in fetal development, the developmental basis of adult disease (Barker et al., 2012) is being actively researched through the investigation of maternal nutritional exposures, stress, and alcohol exposure, and more recent studies have examined exposures to TCDD and other environmental toxicants. The molecular basis of the later-life effects is believed to be primarily epigenetic. Maladies that may be manifested later in life include neurologic and reproductive disorders, thyroid changes, diabetes, obesity, and adult-onset cancers. Furthermore, germ cells (eggs and spermatogonia) in offspring pass through critical developmental stages during fetal life (D. A. Hansen et al., 2014), and emerging evidence demonstrates that fetal exposures are capable of altering the germ cells epigenetically, resulting in a transmission of adverse effects to future generations (intergenerational and transgenerational inheritance) (D. A. Hansen et al., 2014).

Spontaneous Abortion, Stillbirth, Neonatal Death, and Infant Death

Spontaneous abortion is the expulsion of a nonviable fetus—generally before 20 weeks of gestation—that is not induced by physical or pharmacologic means. The background risk of recognized spontaneous abortion is generally 11–22% (Avalos et al., 2012), but it is established that many more pregnancies terminate before women become aware of them (Wilcox, 2010). Such terminations are known as subclinical pregnancy losses and generally are not included in studies of spontaneous abortion. The estimates of the risk of recognized spontaneous abortion vary with the design and method of analysis. Studies have included cohorts of women asked retrospectively about pregnancy history, cohorts of pregnant women (usually those receiving prenatal care), and cohorts of women who are monitored for future pregnancies. The value of retrospective reports can be limited by the differential recall of details (exposure history, for example) specific to pregnancies that occurred long before the interview. Studies that enroll women who present for prenatal care require the use of life tables and specialized statistical techniques to account for differences in the times at which women seek medical care during pregnancy. The enrollment of women before pregnancy provides the theoretically most valid estimate of risk, but it can attract non-representative study groups because the study protocols are demanding for the women.

Countries and U.S. states have different legal definitions of the age of fetal viability and apply these terms differently. The American College of Obstetricians and Gynecologists defines “stillbirth” as the delivery of a fetus that shows no signs of life (that is, an absence of breathing and heartbeat; pulsations in umbilical cord

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are absent; no voluntary movement of muscle) at 20 weeks or greater of gestation (if the gestational age is known) or at a weight greater than or equal to 350 g if the gestational age is not known (Da Silva et al., 2016). "Neonatal death" refers to the death of a live-born infant within 28 days of birth (WHO, 2006) and "postnatal death" refers to a death that occurs before the first birthday (Andrews et al., 2008).

The causes of stillbirth and early neonatal death overlap considerably, so they are commonly analyzed together in a category referred to as "perinatal mortality" (Andrews et al., 2008). Stillbirths make up less than 1% of all births (CDC, 2000). The most common causes of mortality during the neonatal period are low birth weight (< 2.5 kg at birth), preterm delivery, congenital malformations, pregnancy or delivery complications, and placenta or cord conditions. The most common causes of postnatal death in infants is SIDS (sudden infant death syndrome) (Andrews et al., 2008).

The committee responsible for the original VAO report concluded that there was inadequate or insufficient evidence of an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and spontaneous abortion or perinatal death. Additional information available to the committees responsible for *Update 1996*, *Update 1998*, and *Update 2000* did not change that conclusion.

The committee responsible for *Update 2002*, however, found that there was enough evidence available to conclude that there was "limited or suggestive evidence of *no* association" between paternal exposure to TCDD and the risk of spontaneous abortion. That conclusion was based primarily on a National Institute for Occupational Safety and Health study (Schnorr et al., 2001) that investigated a large number of pregnancies fathered by workers whose serum TCDD concentrations were extrapolated back to the time of conception; no association was observed up to the highest-exposure group (1,120 ppt or higher). Indications of a positive association were seen in studies of Vietnam veterans (CDC, 1989c; Field and Kerr, 1988; S. D. Stellman et al., 1988b), but the committee responsible for *Update 2002* asserted that they might be due to an exposure to phenoxy herbicides rather than to TCDD and concluded that there was insufficient information to determine whether there is an association between maternal exposure to TCDD and the risk of spontaneous abortion or between maternal or paternal exposure to 2,4-D, 2,4,5-T, picloram, or cacodylic acid and the risk of spontaneous abortion.

The additional information (none of which concerned paternal exposure) reviewed by the committees responsible for the *Update 2004* through *Update 2014* reports did not change these conclusions.

The relevant studies concerning perinatal death are reviewed in the earlier reports. Table 32, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to spontaneous abortion.

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Update of the Epidemiologic Literature

No Vietnam-veteran, occupational, environmental or case-control studies of exposure to the COIs and spontaneous abortion or perinatal death have been published since *Update 2014*.

Biological Plausibility

Laboratory animal studies have demonstrated that TCDD exposure during pregnancy can alter the concentrations of circulating steroid hormones and disrupt placental development and function and thus contribute to a reduction in the survival of implanted embryos and to fetal death (L. Huang et al., 2011; Ishimura et al., 2009; J. Wang et al., 2011; Y. Wu et al., 2013, 2014). There is no evidence of a relationship between paternal or maternal exposure to TCDD and spontaneous abortion. Exposure to 2,4-D or 2,4,5-T causes fetal toxicity and death after maternal exposure in experimental animals. However, that effect occurs only at high doses and in the presence of maternal toxicity. No fetal toxicity or death has been reported to occur after paternal exposure to 2,4-D.

Animal studies of maternal TCDD exposure during pregnancy have demonstrated the induction of fetal death; neonatal death, however, is only rarely observed and is usually the result of TCDD-induced cleft palate, which leads to an inability to nurse. A single study by Y. Wu et al. (2016) suggests that AHR may be involved in unexplained recurrent spontaneous abortion without consideration of TCDD or other ligands. Further study in this area is warranted. Studies addressing the potential for perinatal death as a result of paternal exposure to TCDD or herbicides are at this point inadequate to support drawing any conclusions.

Synthesis

No studies concerning the COIs and spontaneous abortion, stillbirth, neonatal death, or infant death have been published since *Update 2014*, and available toxicologic studies do not provide clear evidence for the biologic plausibility of an association with paternal exposures.

Conclusion

On the basis of the evidence reviewed to date, the committee concludes that there is limited or suggestive evidence that paternal exposure to TCDD is *not* associated with risk of spontaneous abortion and that insufficient information is available to determine whether there is an association between maternal exposure to TCDD or either maternal or paternal exposure to 2,4-D, 2,4,5-T, picloram, or cacodylic acid and the risk of spontaneous abortion. The committee concludes that there is inadequate or insufficient evidence to determine whether there is an

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association between exposure to the COIs and stillbirth, neonatal death, or infant death.

Sex Ratio

Although it would not constitute an adverse health outcome in an individual veteran, perturbations in the sex ratio of children born to an exposed population would suggest that the exposure had an impact on the reproductive process. Previous reports in the VAO series have reviewed the literature on this topic. *Update 2014* contains a summary of this work, which is also cataloged in Table 33, which may be found in the supplementary tables available at www.nap.edu/catalog/25137. In brief, studies have found an alteration—most often, a reduction—in the expected proportion of male infants at birth, but results are inconsistent and only some reach statistical significance.

Update of the Epidemiologic Literature

One occupational cohort study on TCDD exposure and sex ratio has been published since *Update 2014* was released. 't Mannetje et al. (2017) conducted a retrospective study of workers employed for at least 1 month between 1969 and 1984 at a phenoxy herbicide production plant in New Zealand. In 2007–2008, a sample of 430 workers out of 631 known to be alive under the age of 80 years and living in New Zealand was randomly selected. Of these, 244 responded, and 212 had a biological child. Blood samples were collected and a survey was conducted regarding biological children, their birthdates, sex, and medical conditions. Logistic regression was conducted with the outcome of male versus female sex, with adjustment for the parent's age at the time of birth, BMI at the time of blood draw, and smoking status. Both female and male employees were included, and analyses were conducted separately; however, the number of female employees was small. Exposures were estimated for the time of the child's birth by back-calculation using first-order toxicokinetics and a half-life of 7.6 years. Results showed reduced odds of a male birth when comparing high- versus low-TCDD exposure. For a father's exposure in 2007–2008, reduced odds of a male birth (OR = 0.46, 95% CI 0.29–0.73) were observed for TCDD serum concentration $\geq$ 4 versus < 4 pg/g lipid (OR = 0.49, 95% CI 0.30–0.79). Similarly, for a father's exposure at time of the child's birth, reduced odds of a male birth were observed, comparing TCDD concentration $\geq$ 20 versus < 20 pg/g lipid (OR = 0.49, 95% CI 0.30–0.79). Additionally, a significant dose–response was observed, both for the TCDD as measured in 2007–2008 (p trend = 0.01) and for exposure estimated at time of the child's birth (p trend = 0.007). In both of these analyses, the upper two exposure categories showed significant ORs of approximately half the probability of a male child compared with fathers whose exposure to TCDD was in the low-dose group. The study found that paternal serum TCDD concentrations in

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excess of an estimated 20 pg/g lipid at time of conception were associated with a reduced sex ratio. No significant association was observed for the mother's exposure to TCDD, although the ORs were elevated, not reduced. These findings are consistent with a male-mediated reduction in sex ratio, which had been observed in some prior studies (del Rio Gomez et al., 2002; Hertz-Picciotto et al., 2008; Mocarelli et al., 1996, 2000). The lack of an association for maternal exposure is consistent with an endocrine-mediated outcome.

Biological Plausibility

In a previously unreviewed paper, Ishihara et al. (2007) found that male ICR mice administered TCDD orally with an initial loading dose of 2,000 ng TCDD/kg followed by a weekly maintenance dose of 400 ng/kg (T2,000/400) prior to mating produced a significantly lower proportion of male offspring than controls (controls: 53.1% ± 1.7; T2000/400: 46.2% ± 2.1) while there was no alteration in litter size. The authors speculated that TCDD might selectively reduce the fertility potential of Y-bearing gametes before conception but stipulated that no mechanism could be identified.

An investigation by You et al. (2018) treated mouse spermatozoa with different concentrations of TCDD in vitro (0.25, 25, 2,500 ng/mol) and then performed in vitro fertilization. The sex ratio of two-cell embryos was significantly decreased at all TCDD treatment groups compared with the control ($p < 0.05$). In addition, the sex ratio of blastocysts decreased significantly for the 25 and 2,500 ng/ml of TCDD treatment groups ($p < 0.01$), and embryo sex ratios were negatively correlated with live X sperm proportion (two-cell embryos, $r = –0.697$; blastocyst, –0.856; $p < 0.05$). The researchers concluded that TCDD may affect the fertility of Y spermatozoa more than X spermatozoa but asserted that further studies are needed to evaluate the source of the difference of fertilizing capability between X and Y spermatozoa exposed to TCDD.

Synthesis

Prior to this update, committees had not identified any experimental animal studies that specifically examined the effects of the COI on the sex ratios of offspring, nor had any alterations in sex ratio been reported in animal studies that examined the developmental effects of the COIs on offspring. However, the recent publication of You and colleagues' (2018) study and the previously unreviewed Ishihara et al. (2007) paper yielded information for their consideration. The committee finds that the papers' results are consistent with those observed in human studies but that the lack of an identified mechanism underlying the observations limits their usefulness. These data, along with the new 't Mannetje et al. (2017) retrospective study add incrementally to the body of knowledge on the topic but do not change any of the conclusions.

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Conclusion

On the basis of its evaluation of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence of an association between exposure to the COIs and altered sex ratio.

Birth Weight and Preterm Delivery

Birth weight and the length of the gestation period can have important effects on neonatal morbidity and mortality and on health over the life span. Typically, low birth weight (LBW) is defined as a birth weight under 2,500 g (~5.5 lbs.) (UNICEF, 2004). In the absence of congenital malformations or chromosomal anomalies, LBW is the consequence of either preterm delivery or an intrauterine growth restriction. Preterm delivery is delivery at less than 259 days or 37 weeks of gestation from the date of the first day of the last menstrual period (Jones and Lopez, 2013). Intrauterine growth restriction is functionally defined in terms of LBW relative to gestational age when compared to local or national fetal-growth graphs (Romo et al., 2009). LBW occurs in about 7% of live births. When no distinction is made between intrauterine growth restriction and preterm delivery, the factors most strongly associated with LBW are maternal tobacco use during pregnancy, multiple births, and race or ethnicity. Other potential risk factors are low socioeconomic status, malnutrition, maternal weight, birth order, maternal complications during pregnancy (such as severe pre-eclampsia or intrauterine infection), obstetric history, job stress, and cocaine or caffeine use during pregnancy (Alexander and Slay, 2002; Alexander et al., 2003; Ergaz et al., 2005; Jones and Lopez, 2013; Peltier, 2003). Established risk factors for preterm delivery include race (black), extremes of maternal age, low socioeconomic status, previous LBW or preterm delivery, multiple gestations, tobacco use, and low maternal pre-pregnancy weight or poor pregnancy weight gain (Rubens et al., 2014).

The importance and interpretation of risk factors for associations with birth weight are often unclear and a subject of controversy among researchers (Barker et al., 2012; Wilcox, 2010). Across populations, the frequency distribution of birth weight is Gaussian, with an extended lower tail, or "residual distribution," that includes preterm and LBW infants. The predominant, normal distribution corresponds largely to term births. In general, shifts in the predominant distribution do not tend to correspond to notable shifts in infant mortality (Wilcox, 2001). A number of factors may result in shifts in the predominant distribution; altitude, race or ethnicity, and maternal smoking are among the better studied, and these factors can produce either a larger or, sometimes, smaller percentage of LBW babies. However, populations that have a larger percentage of LBW infants do not always have higher infant mortality (Wilcox, 2001, 2010). While birth weight is tracked internationally as a public health indicator to identify opportunities for

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intervention and to understand country-specific infant mortality (UNICEF, 2004), strategies to increase birth weight have not been effective in reducing mortality.

The committee responsible for *VAO* concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and LBW or preterm delivery, and additional information available to the committees responsible for the subsequent updates did not change that conclusion. Reviews of the relevant studies are presented in the earlier reports. Tables 34 and 35, which can be found at www.nap.edu/catalog/25137, summarize the results of studies related to birth weight after paternal and maternal exposure to the COIs.

Update of the Epidemiologic Literature

No Vietnam-veteran, occupational, or case-control studies of exposure to the COIs and LBW or preterm delivery have been published since *Update 2014*. Four new environmental studies are summarized below.

A study of 234 couples by Robledo et al. (2015) examined how birth size varied with maternal and paternal exposures to 63 POPs from five major classes, including 36 PCBs, seven of which were dioxin-like (PCBs 105, 114, 118, 156, 157, 167, and 189). Exposure was measured in parental serum collected before conception. Differences in birth weight and other growth-related parameters were estimated using multiple linear regression per 1 standard deviation increase in natural log-transformed (ln-transformed) chemicals. The subjects were participants in the LIFE prospective cohort study,[2] which was conducted in Michigan and Texas between 2005 and 2009. Models were estimated separately for each parent and adjusted for maternal age, maternal prepregnancy BMI, and other confounders, and all models included an interaction term between infant sex and each chemical. The authors found that for every 1 standard deviation increase in ln-transformed concentration of paternal serum levels of PCB 167, the mean birth weight among female newborns (n = 117) was 97.49 g lower (95% CI [–187.45, –7.54]), and mean length (β = –0.57 cm; 95% CI [–1.12, –0.02]) and head circumference (β = –0.45 cm; 95% CI [–0.86, –0.03]) were smaller. No statistically significant outcomes were observed for paternal exposures and male newborns (n = 113). For maternal serum PCB 167 levels, male newborn birth weight was significantly lower (–129.24 g, 95% CI [–228.16, –30.31]) and head circumference smaller (–0.47 cm, 95% CI [–0.95, 0.00]), and there were no statistically significant outcomes for female newborns.

A birth cohort study of 484 children in Hokkaido, Japan, by Kobayashi et al. (2017)—the Hokkaido Study on Environment and Children's Health—measured total dioxin levels as the sum of 29 congeners in maternal blood samples taken

[2]Cited earlier in this chapter in the review of a paper by Mumford et al. (2015) in the section on male reproductive health outcomes.

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either during the third trimester or within 1 week after delivery. (In Japan, the largest source of dioxin exposure is dietary, and of the total dioxin intake in this population, the main source is seafood.) This new study expanded results from a previous study conducted in the same birth cohort (Konishi et al., 2009; reviewed in *Update 2010*) that reported a significant adverse effect on birth weight for total PCDDs TEQ levels (adjusted $\beta = -231.5$ g, 95% CI [−417.4, −45.6]) and total PCDFs TEQ levels (adjusted $\beta = -258.8$ g, 95% CI [−445.7, −71.8]). Sex-stratified analyses suggested that these reductions in birth weights were stronger in boys. Here, the investigators assessed polymorphisms in the women's genes encoding the aryl hydrocarbon receptor (AHR [G > A, Arg554Lys]), cytochrome P450 (*CYP1A1*) (T6235C), and glutathione S-transferase mu 1 (GSTM1; non-null/null), genes that code for three metabolizing enzymes that had previously been related to dioxin or birth weight.[3] Linear regression analyses that were adjusted for confounding factors found that pregnant women with a GSTM1 null genotype—a genotype previously established to be associated with a high inducibility of cytochrome P450 1A1 gene transcription—gave birth to infants with a 345 g (95% CI [−584, −105]) reduction in birth weight for each 10-fold increase in total dioxin TEQ. They also noted birth weight reductions for the children of GSTM1-null mothers for some of the individual congeners. These results suggest that PCDDs and PCDFs may accumulate in the placenta and, through interference with placental function, affect birth weight.

Miyashita and colleagues (2015b) investigated 70 PCB congeners, including dioxin-like PCBs, in the blood of a smaller subgroup of 367 woman–child pairs from the Hokkaido Study cohort cited above. Mothers completed self-administered questionnaires on demographic characteristics, socioeconomic status, personal habits, and food consumption; medical records were used to extract details for the delivery and characteristics of the infants. The authors found no associations between the concentrations of dioxin-like PCBs (or non-dioxin-like PCBs) and newborn anthropometric measurements of birth weight (small for gestational age), length, chest circumference, and head circumference in analyses with multiple linear regression models with or without adjustment for confounding factors. A reduction in birth weight and other measures of size was observed for dioxin-like PCBs, but these results were not statistically significant.

A study by Van Tung et al. (2016) of residents of a dioxin hot spot area in Vietnam[4] reported on 58 mother–infant pairs who were compared with 62 pairs from a region thought to be uncontaminated. Breast milk from lactating mothers with infants ages 7 and 16 weeks at the time of sampling was analyzed for

[3]Research indicates that dioxins bind AHR and induce *CYP1A1* expression (Harper et al., 2002) and absence of human glutathione S-transferase mu 1 (GSTM1; null genotype) is associated with increased induction of *CYP1A1* expression (Vaury et al., 1995).

[4]Males in this study cohort were reported on by Sun et al. (2016) in a paper addressed in the male reproductive outcomes discussion earlier in this chapter.

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dioxin congener levels. Samples from mothers living in the hot spot had fivefold higher levels of dioxin than breast milk from controls. Birth weight was inversely correlated with both 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF congener levels. The rate of newborns with a birth weight less than 2,500 g was threefold higher in the hot spot (12%) than in the control region (4%). However, at 8–9 or 12–14 weeks of age, no significant associations were observed between infant size and dioxin isomer levels.

Biological Plausibility

The available evidence from experimental animal studies indicates that TCDD exposure during pregnancy can reduce body weight at birth, but only at high doses. A study in human placental explants suggests that TCDD exposure may enhance placental inflammation and may increase the risk of preterm births associated with infection (Peltier et al., 2013). Laboratory studies of the potential male-mediated developmental toxicity of TCDD and herbicides resulting from the prior exposure of the fathers are inadequate to support conclusions. TCDD and herbicides are known to cross the placenta, which leads to the direct exposure of the fetus. Data from studies of experimental animals also suggest that the pre-implantation embryo and developing fetus are sensitive to the toxic effects of 2,4-D and TCDD after maternal exposure.

Synthesis

Studies reviewed in this update continue the pattern observed in earlier research of identifying either no or small decrements in birth weight and size parameters for children born to parents with the highest levels of dioxin exposure. As has been noted by previous committees, there are a number of challenges in conducting these types of epidemiologic studies in a rigorous way. First, the prenatal and immediate postpartum period is not a stable pharmacokinetic state; there are substantial changes in body volume and fat mobilization during that time. Biomarker measures during pregnancy may be substantially affected by weight change during pregnancy. Moreover, the extrapolation of a more recent biomarker measure back many years to a more relevant period is complicated by intervening pregnancy and breastfeeding events, which result in a substantial uncertainty in the index exposure level. Overall, although the committee notes that the animal literature does support an effect of TCDD exposure at high doses on birth weight, the epidemiologic literature is insufficiently robust to allow a final determination.

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Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and LBW or preterm delivery.

Birth Defects

A birth defect is an abnormality of structure, function, or metabolism, whether genetically determined or resulting from an environmental influence during embryonic or fetal life (Christianson et al., 2006). Other terms for birth defect, often used interchangeably, are "congenital anomaly" and "congenital malformation." Major birth defects, which occur in 2–3% of live births, are abnormalities present at birth that are severe enough to interfere with viability or physical well-being. Birth defects are detected in another 5% of babies through the first year of life. Genetic factors, exposure to some medications, exposure to environmental contaminants, occupational exposures, maternal infections, and lifestyle factors have been implicated in the etiology of birth defects (Christianson et al., 2006), although the causes of the vast majority of birth defects are unknown. Most etiologic research has focused on the effects of maternal and fetal exposures, but, as discussed in the beginning of this chapter, it is theoretically possible that epigenetic alterations in the paternal gamete caused by preconception exposures could result in paternally mediated effects. It should be noted that a substantial amount of epidemiologic research on suspect toxic agents has been conducted, but none of it has definitively established *paternal* preconception exposures as a contributing factor to the occurrence of birth defects (Chow et al., 2009; Desrosiers et al., 2012; Dohle, 2010; Schull, 2003).

The committee responsible for *VAO* concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to 2,4-D, 2,4,5-T or its contaminant TCDD, picloram, or cacodylic acid and birth defects in offspring. Additional information from the AFHS available to the committee responsible for *Update 1996* led it to conclude that there was limited or suggestive evidence of an association between at least one of the COIs and spina bifida in the children of veterans; there was no change in the conclusions regarding other birth defects.

The Update 2002 committee, which reviewed a study of female Vietnam veterans that reported significant increases in birth defects in their offspring (Kang et al., 2000a), did not find those results sufficient to modify prior conclusions. Nonetheless, Congress mandated that a number of birth defects in the children of female Vietnam veterans be assigned service-related status. Later committees have not encountered data that would merit changing the conclusion that the evidence

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is inadequate to support an association between exposure to the COIs and birth defects (aside from spina bifida) in the offspring of either male or female veterans.

The Update 2014 committee concluded that the new evidence it identified concerning the occurrence of birth defects in association with exposure to the COIs, in combination with existing evidence, was inadequate or insufficient to support an association for birth defects overall in the children of Vietnam veterans. In light of the fact that evidence anticipated by the committee responsible for *Update 1996* that would support an association between paternal exposure to the COIs and spina bifida had not materialized, that committee concluded that spina bifida should be moved from the category of limited or suggestive evidence of an association to the default category of inadequate or insufficient evidence of an association. An increased scrutiny of mechanisms by which paternal exposure might contribute to adverse effects in offspring has not definitively established the biologic plausibility of this phenomenon, whereas the understanding of how maternal exposures may disrupt fetal development has grown substantially. There were, however, no epidemiologic results supporting an association between maternal exposure to the COIs and spina bifida specifically, so spina bifida in association with exposure of either parent was moved to the inadequate or insufficient category of association. Chapter 10 of *Update 2014* contains a detailed discussion of the evidence and reasoning underlying this determination.

Tables 36 and 37, which can be found at www.nap.edu/catalog/25137, summarize the results of studies related to birth defects and specifically of neural-tube defects such as spina bifida.

Update of the Epidemiologic Literature

No Vietnam-veteran studies of exposure to the COIs and birth defects have been published since *Update 2014*. New occupational, environmental, and case-control studies addressing neural-tube defects, cryptorchidism or hypospadias, gastrointestinal tract defects, cardiovascular defects, and general or multiple congenital anomalies are addressed below. All are classified as "Other Identified Studies" (as delineated in Chapter 3) because the exposures they addressed were not described in sufficient detail to allow the committee to determine their relevance to the experience of Vietnam veterans.

Other Identified Studies–Occupational Studies Makelarski et al. (2014) report results from a case-control analysis of maternal periconceptional occupational exposure to pesticides and association with neural-tube defects in offspring. Cases and controls were derived from the U.S. National Birth Defects Prevention Study (NBDPS), a large national case-control study of risk factors for birth defects. For any insecticide and herbicide use the odds ratio for spina bifida was 2.1 (95% CI 1.0–4.1), based on 14 exposed. There was no dose–response gradient

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for cumulative exposure to insecticide and herbicide and spina bifida. The study's strengths include the national context of the study with a relatively large, well-defined case group and extensive covariate information. The limitations include a lack of specific herbicide exposure information, the use of the general job-exposure matrix for exposure assessment, and the fact that small sample sizes precluded analyses of exposure to herbicides only.

Using cases and controls from the NBDPS, Pettigrew and colleagues (2016) examined the joint effect of parental occupational exposure to pesticides on the risk of spina bifida in offspring (291 cases). The analysis included joint parental exposure to insecticides and herbicides and fungicides, which yielded an odds ratio of 0.8 (95% CI 0.4–1.5). There was only one case with joint parent herbicide-only exposure (the odds ratio was not estimated). Herbicide exposure adjusted for other pesticides had decreased odds of spina bifida (OR = 0.6, 95% CI 0.3–125.0). The study's strengths include the national context of the study with a well-defined case group and extensive covariate information. Its limitations include the lack of specific herbicide exposure information, the lack of paternal self-reported occupational history (it was reported by the mother), the use of the general job-exposure matrix for exposure assessment, and the very small sample sizes for herbicide exposure.

Rocheleau et al. (2015) examined the association between maternal occupational exposure to fungicides, insecticides, and herbicides and the risk of congenital heart defects among offspring. Cases and controls were drawn from the NBDPS, a multisite case-control study. Maternal occupational exposure to pesticides was analyzed for 3,318 cases and 2,979 controls using data for births in 1997–2002. Only 3 case mothers and 3 control mothers were determined to have had exposure to only herbicides. Analyses of estimated high exposure to herbicides and insecticides found associations with any simple, isolated congenital heart defects (OR = 1.90, 95% CI 1.05–3.44), pulmonary valve stenosis (OR = 3.64, 95% CI 1.31–10.15), right ventricular outflow tract obstruction (OR = 3.40, 95% CI = 1.41–8.16), and hypoplastic left heart syndrome without ventricular septal defect or anomalous pulmonary venous return (OR = 5.11; 95% CI 1.70–15.35). The study's strengths include its use of a large population-based case-control design with careful ascertainment and classification of congenital heart defects, adjustment for multiple potential confounders, and an expert-based assessment of potential pesticide exposure. The major study limitation was the lack of information on exposure to specific pesticides.

Jorgensen and colleagues (2014) used the national Danish registries to determine parental employment in farming or horticulture and to identify their male offspring born between 1980 and 2007. Boys were followed for a hospital diagnosis of cryptorchidism recorded in the Danish National Patient Registry. Hazard ratios (HRs) and 95% CIs were calculated using Cox regression to compare the occurrence of cryptorchidism among the male children of mothers and fathers working as horticultural workers and farmers with the male children of

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parents in other occupations. Adjustment was made for birth year, parental age, parity, and geographic region. A total of 618,081 boys born to actively employed mothers during 1980–2007 were identified, including 2,105 and 4,520 born to maternal horticultural workers and farmers, respectively. The adjusted HR for cryptorchidism showed an elevated risk among boys of maternal horticultural workers (HR = 1.20, 95% CI 0.95–1.52) and an increased risk among boys of maternal farmers (HR = 1.31, 95% CI 1.12–1.53) compared with boys of mothers in all other occupations. For fathers, a total of 708,283 boys were identified, and 18,648 were diagnosed with cryptorchidism during follow-up. Fathers working in horticulture or farming had 2,157 and 24,348 sons, respectively, of whom 72 (3.3 %) and 742 (3.0%), respectively, were diagnosed with cryptorchidism. The HRs for cryptorchidism were 1.20 (95% CI 0.96–1.51) for boys of paternal horticultural workers and 1.04 (95% CI 0.96–1.12) for boys of paternal farmers. The study's strengths included a large national registry-based cohort of parental occupational information and outcome information for offspring. However, data on specific chemical exposures for the parents were not available, limiting the usefulness of the study for the evaluation of risks from the COIs.

Other Identified Studies–Environmental Studies Markel and colleagues (2015) conducted an ecologic study of hypertrophic pyloric stenosis identified by the Indiana Birth Defects Registry from 2005–2009. Birth defect cases were linked with their birth certificate record to obtain demographic and other information. County-level pesticide use data from available from the U.S. Geological Survey annual agricultural pesticide use database. The county-level pesticide data were categorized into high, moderate, and low level of use. Data were also subdivided into fungicides, fumigants, insecticides, and herbicides. Herbicides comprised 91% of all pesticides used. Total pesticide levels in the county of residence correlated significantly with the incidence of hypertrophic pyloric stenosis ($r = 0.029$, $p = 0.004$), as did herbicide use ($r = 0.029$, $p = 0.005$). This analysis is limited by the ecologic nature of the exposure data and outcome data. Although statistically significant, the correlation values were rather small and while logistic regression was used, the report only presents p-values and no effect estimates. There was no information on specific herbicides, other than to note that glyphosate and atrazine, which are not COIs, were the most heavily used.

A study on the island of Guam by Noel and colleagues (2015) examined the relationship between village-level estimates of alleged Agent Orange exposure and infant mortality due to congenital anomalies. Total births by village (n = 19) and infant mortality due to congenital anomalies for each year from 1970 to 1989 were obtained from the Office of Vital Statistics in the Guam Department of Public Health and Public Services. Each birth and death was assigned to a village based on the usual residence of the mother. The 1980 U.S. Census was used to obtain data on the median age of the village females, village fertility

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ratio, population density, persons per household, single-mother households, and the number of married females. Agent Orange exposure data were obtained from a U.S. Air Force veteran who conducted ground-level Agent Orange spraying for vegetation control. The veteran provided village-level spray estimates based on his recollection; however, there was no reported independent verification of this information. Eleven villages were classified as high risk (cause-specific death rate was 2.96 per 1,000 live births) and eight as low risk (1.31 per 1,000 live births). Twelve villages were considered to have Agent Orange spraying (10 high risk and 2 low risk, $p = 0.006$). In the multivariable linear regression model, which included an adjustment for the median age of village females, the association between Agent Orange spraying area and infant mortality due to congenital anomalies was statistically significant (standardized regression coefficient $\beta = 2.02$; 95% CI 0.08–3.96; $p = 0.042$) with the model explaining approximately 51% of the variance. Models adjusting for other Census-based covariates yielded similar results. The study had several limitations, prominently the highly subjective nature of the exposure characterization. The assignment of potential village exposure to Agent Orange spraying was obtained from a single source and was not independently confirmed by records or biomarker data. Lacking congenital anomaly prevalence data, the study relied instead on mortality data, which may be affected by village-specific differences in infant survival due to differences in medical care and other factors.

Other Identified Studies–Case-Control Studies Shaw et al. (2014) examined cases and controls ascertained in the San Joaquin Valley in California. Cases were born from 1997 to 2006 with gastroschisis confirmed by clinical geneticists; those with single-gene conditions or chromosomal abnormalities or with identifiable syndromes were ineligible. Controls were non-malformed live-born infants randomly selected from birth hospitals in the study area. Data were collected from maternal telephone interviews using a standardized, computer-based questionnaire. Interviews were conducted with mothers of 72% of eligible cases ($n = 193$) and 69% of controls ($n = 974$). Mothers with pregestational diabetes were excluded from the analyses. Exposure assessment was performed for 461 individual chemicals and 62 physicochemical groupings that were applied at > 100 lb. in any of eight San Joaquin Valley counties in any year during the study period. An exposure-time window of 1 month before to 2 months after the maternal reported date of conception was assigned for each case or control mother. Exposure assignments were made for 156 cases and 785 controls whose mothers lived in the geocoded addresses more than 68 days during the window. To estimate pesticide applications, statewide pesticide use reporting records from the California Department of Pesticide Regulation describing agricultural pesticide applications occurring in the study period were obtained. Pesticide exposure was based on pounds of pesticides used during the relevant time window within a 500-meter radius of a case or control's geocoded address. Logistic regression was

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used to estimate odds ratios for pesticide exposure (yes/no), with adjustments for race/ethnicity, prepregnancy BMI, any use of folic acid–containing supplements, and smoking during the month before and the first 2 months of pregnancy. 2,4-D dimethylamine salt exposure had an adjusted odds ratio of 1.7 (95% CI 0.7–4.1) based on 8 exposed cases. The study's strengths include the population-based design, comprehensive case ascertainment and classification, and extensive covariate data. The exposure assessment was performed using pesticide application data, although misclassification is expected because of the lack of data on individual factors that may influence exposure. Random error cannot be excluded when considering the result for 2,4-D dimethylamine salt.

Using the same study design as Shaw et al. (2014) above, Carmichael and colleagues (2016) examined the association between pesticide exposure and the risk of five different types of birth defects ascertained in the San Joaquin Valley of California. The analysis included 367 cases with one of five types of birth defects and 785 controls without any identified malformations born in 1997–2006. The case groups (with at least 50 cases) included anotia/microtia, anorectal atresia/stenosis, transverse limb deficiency, craniosynostosis, and diaphragmatic hernia. Among the published results (the criteria for presentation were an odds ratio > 2.0 or < 0.5 or confidence intervals that excluded 1.0), the authors reported associations between dichlorophenoxy acid or ester exposure and transverse limb deficiency (7 exposed cases; OR = 2.5; CI 1.1–6.0) and anotia/microtia (12 exposed cases; OR = 3.4; CI 1.6–7.6). The study's strengths and limitations are similar to those of the Shaw et al. (2014) study, although the sample sizes for the defects in this study were relatively small and the estimate imprecise.

Rappazzo et al. (2016) conducted a case-control study using subjects drawn from a cohort of geocoded singleton live births (n = 335,729) in North Carolina between 2003 and 2005. Birth records were linked to the North Carolina Birth Defects Monitoring Program, an active birth defects surveillance system. Crop maps and pesticide application data were combined to estimate the quantities and types of pesticides applied during the time window of interest (1 month before pregnancy through the third trimester). Women who either had no crops within the buffer or who had no exposure during the relevant window of pregnancy were considered unexposed. The analysis included 6,358 cases and 298,548 controls. A total of 42 birth defects were ascertained, and 4,634 cases had a single (isolated) congenital anomaly. Logistic regression was used to estimate the odds ratio for exposure (yes/no) and each birth defect with adjustments made for race, education, marital status, maternal age, and maternal smoking. Elevated adjusted odds ratios were observed for atrial septal defects (OR = 1.70, 95% CI 1.34–2.14) and for patent ductus arteriosus (OR = 1.50, 95% CI 1.22–1.85) for the highest (≥ 90th percentile) exposure subjects. The study used a population-based design with birth defects surveillance and linked crop and pesticide records. Its limitations include the lack of individual-level pesticide exposure data for specific

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pesticides, potential residual confounding due to the use of birth certificate data and unmeasured factors, and the small number of cases for some comparisons.

Koskenniemi and colleagues (2015) based their case-control study of 44 cases of cryptorchidism and 38 controls on subjects who were operated on for inguinal or umbilical hernia or hydrocele at two hospitals: one in Turku, Finland (2002–2006), and the other in Copenhagen, Denmark (2004–2005). Twelve of the Finnish cases and two of the Danish were referred for surgical procedures from a prospective cohort study. A subcutaneous adipose tissue biopsy was taken during the operation, and samples were analyzed for 37 PCBs, 17 PCDD/Fs and 14 PBDEs. Covariate data were obtained through parental interview and from medical records. Associations between adjusted and unadjusted chemical concentrations and the risk of cryptorchidism were calculated using logistic regression. The analysis included an adjustment of the chemical concentrations by factors influencing postnatal exposure (age at operation and duration of breastfeeding) by linear regression, but no adjustment was made for factors related to chemical concentrations in the mother (e.g., BMI, maternal age, parity). In a sensitivity analysis, cases and controls were excluded if the mother had gestational diabetes. After adjustment for the country of origin, age at operation, and the duration of breastfeeding, total TEQ (OR = 3.2, 95% CI 1.3–9.1) and sum of PCDD/Fs (OR = 3.7, 95% CI 1.5–10.9) were associated with an increased risk of cryptorchidism. The sum of PCBs had an elevated, but non-significant, odds ratio (OR = 1.9, 95% CI 0.9–4.0). The sum of PBDEs was not associated with an elevated odds ratio of cryptorchidism (OR = 0.86, 95% CI 0.47–1.54). The effect estimates were not materially different after restricting to boys who were born full term, biopsied at less than 5 years of age, and whose mothers did not have gestational diabetes. The study provides some evidence of an association with dioxin-like chemicals (TCDD-specific results were not reported). However, the study was very small and some potential confounders were not adjusted. The study is of limited value to the committee's assessment.

Kalfa et al.'s (2015) case-control study examined 408 boys with isolated hypospadias and 302 controls identified at multiple institutions in the south of France between 2009 and 2014. Control boys were matched by ethnic origin and had no congenital malformation; no urological, genital, or nephrological condition; and no inguinal hernia or endocrine disease. The reasons for hospitalization for the controls included acute appendicitis, idiopathic intussusception, minor abdominal trauma, and pyloric stenosis. Information about maternal and paternal occupational and professional exposure to endocrine-disrupting chemicals was obtained using a standardized questionnaire and a job-exposure matrix. Environmental exposure was estimated by geocoding the residence postal code at the time of pregnancy and factoring in the types of surrounding hazards and their distance from that residence. The odds ratio for maternal exposure to herbicides (otherwise unspecified) was 1.00 (95% CI 0.007–13.97); the paper did not specify whether this imprecise estimate was adjusted. Environmental

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exposure to intensive agriculture (residence within a 3-km radius) was statistically significantly different between cases and controls, as was residence in an industrial area or proximity to an incinerator or a waste area. The committee did not consider this study to be informative because results for specific COIs were not presented and there are concerns that the analysis and exposure information is based on questionnaire and a determination by simple distance to source for the environmental exposures.

A paper by Ueker and colleagues (2016) describes the results of a hospital-based case-control study conducted in Cuiabá, Mato Grosso, Brazil in 2011. The cases were children aged less than 5 years with a diagnosis of a congenital malformation in medical records who were identified at four referral institutions. The controls were children of those ages diagnosed with other conditions (respiratory diseases, infectious diseases, disorders of the perinatal period, and endocrine and metabolic diseases) at the same institutions. Controls were pair-matched with cases on sex. Information on parental exposure to pesticides (unspecified, except to note that glyphosate is the most commonly used) and other factors was collected by a maternal interview. The analysis group consisted of 137 cases and 274 controls. In the unadjusted analysis, several maternal pesticide exposure variables had an elevated odds ratio for birth defects, including living close to crop spraying with pesticides (OR = 1.61, 95% CI 0.88–3.03) and pesticide use at work (OR = 1.52, 95% CI 0.65–3.53). Paternal application of pesticides was also associated with an elevated odds ratio for birth defects (OR = 2.75, 95% CI 1.05–7.19). This study has several important limitations, including the lumping of all different birth defects into a single case group, a small sample, self-reported pesticide exposure (including maternal report of paternal exposures), no information on specific pesticides, and no information on case and control response rates.

Biological Plausibility

As was noted in *Update 2014*, 2,4-D has been previously shown to be a teratogen, although at exposure levels that exceed maternal renal clearance and thus are not relevant to herbicide exposure in Vietnam. A 2011 study showed that late in utero and early postnatal 2,4-D exposure can result in nephrotoxicity in offspring at one-sixth of the LD_{50}[5] (Troudi et al., 2011). Other herbicides of interest can induce fetal malformations but typically only at high doses that are toxic to pregnant women. TCDD is a potent teratogen in all laboratory species that have been studied, although the patterns of birth defects that are produced are often species-specific. However, specific mechanisms that link TCDD exposure to specific birth defects have not been fully elucidated.

[5]Lethal dose 50%: the dose required to kill half of the test population, usually expressed as a function of body weight.

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A variety of animal model studies, including in utero exposures, work with cultured cells, and studies with zebrafish embryos, have investigated the mechanisms underlying various TCDD-induced birth defects, including hydronephrosis, cleft palate, reproductive organ anomalies, neurogenesis, and perturbed heart, kidney, and lung development (Dong et al., 2010; Falahatpisheh et al., 2011; Jacobs et al., 2011; Lanham et al., 2012; Latchney et al., 2011; Neri et al., 2011; Tait et al., 2011; Yamada et al., 2014; Yoshioka et al., 2012; Yuan et al., 2012). Interestingly, AHR is required for TCDD-induced birth defects. In contrast, the induction of cytochrome P450 1A1 is not required (Dragin et al., 2006; Jang et al., 2007; Mimura et al., 1997). When pregnant *Ahr*-null mice are exposed to TCDD, the fetuses do not exhibit any of the typical developmental malformations associated with TCDD exposure, but fetuses of TCDD-exposed pregnant *CYP1A1*-null mice do. In addition, an Ahr antagonist can attenuate TCDD-induced birth defects in mice. Thus, the activation of Ahr by TCDD during development appears to be a key first step in mediating TCDD's developmental toxicity, but this step does not depend on *CYP1A1* activity. Although structural differences in AHR have been identified among species, it functions similarly in animals and humans. Therefore, a common mechanism mediated by AHR in which tissue growth and differentiation processes are affected probably underlies the developmental toxicity of TCDD in humans and animals.

Antioxidant treatment provides protection against some TCDD-induced teratogenicity, which suggests that reactive oxygen species might be involved in the pathways that lead to these structural changes (Jang et al., 2008). A few studies indicate that the stem cells and organ-specific progenitor cells may be direct targets and that maternal TCDD exposures interfere with proliferation and cell differentiation through AHR and result in defects in organ morphogenesis (Latchney et al., 2011; Neri et al., 2011). Few laboratory studies of potential male-mediated developmental toxicity (and specifically birth defects) attributable to exposure to TCDD and herbicides have been conducted. As noted, the feeding of simulated Agent Orange mixtures to male mice produced no adverse effects in offspring (Lamb et al., 1981).

In sum, studies with maternal exposure in animal models suggest that a role for TCDD and related chemicals in causing birth defects is plausible and also that AHR plays a causal role. However, translating these results to human populations has been difficult.

Synthesis

Given the long-standing concern of Vietnam veterans about the potential of the COIs to adversely affect the health of their children, birth defects have been among the outcomes considered by VAO committees since the first comprehensive review was published in 1994. Embryonic and fetal development in some species including rodents are sensitive to the toxic effects of exposure to TCDD and dioxin-like chemicals, and there are several species and strains of animals for

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which the fetus is more sensitive than the adult to the adverse effects of TCDD. Human data are generally lacking, however, and the sensitivity to developmental disruption in humans is less apparent, in part because contemporary studies of environmental dioxin exposure and birth defects have involved extremely low exposures. As noted, recent human population-based studies attempting to link TCDD or the other COI exposures to birth defects have provided mixed results.

Moreover, the study of birth defects in any population is complicated by the relatively rarity of specific birth defects. Attributing any one defect to a specific exposure is often difficult, and it is extremely difficult to conduct rigorous epidemiology studies assessing risks to children arising from their parents' exposure, particularly when a distinction between maternal and paternal contributions is sought. These challenges are highlighted in the studies considered by the committee, which exhibit sometimes significant weaknesses that limit their usefulness—particularly in assessing the effects resulting from the exposures experienced by Vietnam veterans. The new epidemiologic studies considered by the committee were mostly not informative because of a small sample or the absence of exposure data on COIs, or both. There were two relatively rigorous epidemiologic studies that suggested an association between two different COIs (2,4-D dimethylamine salt; dichlorophenoxy acid) and an increased risk of two unrelated birth defects. However, the recent studies did not change the previous conclusion of inadequate or insufficient evidence to support an association for birth defects overall in the children of Vietnam veterans.

Conclusion

The committee's review of newly published studies in combination with existing information leads it conclude that evidence remains inadequate or insufficient to determine whether there is an association between exposure to the COIs and birth defects in the children of Vietnam veterans.

EFFECTS OCCURRING LATER IN OFFSPRING'S LIVES

Information is available concerning parental exposure to the COIs and several outcomes occurring later in the lives of their offspring. Studies investigating cancers; growth and physical parameters; motor development, cognitive, behavioral, and socio-emotional outcomes; immune and allergic outcomes; and reproductive health are reviewed below.

Cancer in Offspring

The American Cancer Society estimates that 10,590 children under 15 years old will receive a new diagnosis of cancer in the United States in 2018 (ACS, 2018f). The treatment and supportive care of children who have cancer continue to improve. The 5-year survival rate for children who receive a cancer diagnosis

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has increased from less than 60% in the 1970s to more than 80% in 2013, the most recent year for which data are available. Despite advances, cancers remain the second leading cause of death in children under 15 years old (after accidents); 1,180 deaths are projected for 2018 (ACS, 2018f).

Leukemias are the most common cancer in children, accounting for about 29% of all childhood cancer cases. The second-most common group of cancers in children is cancer of the brain and tumors of other parts of the central nervous system (CNS) (26%), followed by neuroblastomas (6%) and Wilms tumor (also called nephroblastoma; 5%) (ACS, 2018f). Other cancers in children include lymphomas, bone cancers, soft-tissue sarcomas, renal cancers, eye cancers, and adrenal cancers. In contrast with adult cancers, relatively little is known about the etiology of most childhood cancers, especially about potential environmental risk factors and the effects of parental exposures.

The committee responsible for *VAO* concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid and childhood cancers. The additional information available to the committees responsible for *Update 1996* and *Update 1998* did not change that conclusion. The committee responsible for *Update 2000* reviewed the material in earlier VAO reports and in newly available published literature and concluded that there was limited or suggestive evidence of an association between exposure to at least one of the COIs and acute myeloid leukemia (AML).[6] However, after the release of *Update 2000*, investigators involved in one study discovered an error in their published data. The *Update 2000* committee reconvened to evaluate the previously reviewed and new literature regarding AML, and it produced *Acute Myelogenous Leukemia* (IOM, 2002). It reclassified AML from "limited/suggestive evidence of an association" to "inadequate evidence to determine whether an association exists."

The committees responsible for *Update 2002*, *Update 2004*, *Update 2006*, *Update 2008*, *Update 2010*, *Update 2012*, and *Update 2014* reviewed the material in earlier VAO reports and in newly available published literature and agreed that there remained inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and childhood cancers.

Table 38, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to cancers in children.

[6]AML (ICD-9 205; ICD-10 C92.A0) is also referred to as acute myelogenous leukemia, acute myeloblastic leukemia, and acute nonlymphocytic leukemia. For consistency, this report uses "acute myeloid leukemia," or AML, regardless of the usage in the source materials.

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Update of Epidemiologic Literature

New Vietnam-veteran, occupational, environmental, and case-control studies addressing childhood leukemias, central nervous system tumors, rhabdomyosarcoma, and retinoblastoma are addressed below.

Vietnam-Veteran Studies Grufferman and colleagues (2014) evaluated the role of parental military service in Vietnam and service-related exposures in the risk of rhabdomyosarcoma[7] occurring in offspring. Cases of rhabdomyosarcoma diagnosed under the age of 20 years were identified from the Intergroup Rhabdomyosarcoma Study Group clinical trial, which included hospitals in 46 U.S. states and the District of Columbia from 1982 to 1988. Controls were identified by telephone random-digit dialing and were matched to cases on race, sex, and age. Case and control families were interviewed by telephone. The interview included questions about childhood environmental exposures, parental occupational exposures, family demographic characteristics, parental lifestyle and behavioral characteristics, and medical history. Questions related to military service focused on the Vietnam War period. Each parent was asked if he or she had ever served in the armed forces before the date of the index child's diagnosis and, if so, in which years the service occurred. The parent was also asked if he or she was in contact with nuclear, chemical, and biological weaponry, radiation, radar or microwaves, or Agent Orange. Among the 440 cases that were eligible, 351 completed interviews, and, of those, 319 eligible cases had available information on parental occupation. Subjects were matched on age, sex, and race, and analyses were adjusted for family income, maternal education and recreational drug use, length of pregnancy, and maternal spotting/bleeding/cramping during pregnancy. The study reported that 3.4% of case mothers and 1.4% of control mothers reported a history of military service. Maternal history of military service was associated with an elevated adjusted odds for rhabdomyosarcoma (OR = 2.75, 95% CI 0.71–10.62). There were too few cases to refine this analysis by time period of service. A history of paternal military service was not associated with rhabdomyosarcoma (OR = 0.85, 95% CI 0.58–1.25); restricting the time of paternal military service to 1962–1970 yielded an adjusted odds ratio of 0.78 (95% CI 0.50–1.21), based on 53 cases (18.5%) and 64 controls (22.3%). Only 9 cases (1.7%) and 5 controls (3.1%) reported potential Agent Orange exposure during Vietnam War service. The adjusted odds ratio was elevated for this exposure, but the estimate was not statistically significant (OR = 1.72, 95% CI 0.55–5.41). This report was based on a nationally ascertained case groups and included a parental interview asking questions about a wide array of potential risk factors and confounders, with specific questions on military

[7]Rhabdomyosarcoma is a cancer of the muscle tissue. It is the most common form of soft-tissue sarcoma in children. See https://www.cancer.gov/types/soft-tissue-sarcoma/patient/rhabdomyosarcoma-treatment-pdq (accessed October 28, 2018).

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service and Agent Orange exposure. Concerns include the use of a control group based on telephone random-digit dialing and the recall of potential Agent Orange exposure. The analysis of paternal Agent Orange exposure was based on a very small number of exposed cases, and the confidence intervals associated with the odds ratios were correspondingly broad. This report found no association between paternal military service in Vietnam or Agent Orange exposure and an increased risk of rhabdomyosarcoma in offspring.

Other Identified Studies–Occupational Study Febvey et al. (2016) pooled three population-based case-control studies of childhood CNS tumors conducted from France, Germany, and the United Kingdom. Cases were children less than 15 years of age with CNS tumors; controls were matched by gender and age. Interview data (telephone or in-person) on parental occupational histories were collected. Parental occupational data were harmonized, and an existing general population job-exposure matrix was used to assign pesticide exposure into no-, low-, or high-exposure categories for insecticides, herbicides, fungicides, and all pesticides combined. The analysis included 1,361 cases and 5,498 controls. Only 0.9% of mothers and 4.2% of fathers were found to have occupational exposure to pesticides during the studied pregnancy. No association was observed between CNS tumors and maternal pesticide exposure (pooled OR = 0.76, 95% CI 0.41–1.41) and statistically significantly decreased odds were seen with paternal occupational pesticide exposure (OR = 0.71, 95% CI 0.53–0.95). The study included a relatively large number of subjects and used a common job-exposure matrix to assign potential pesticide exposure across studies. However, the analysis was limited by the low prevalence of parental exposure, precluding examination of dose categories and a breakdown of pesticide categories. No information was available on specific pesticides.

Other Identified Studies–Environmental Study Bailey and colleagues (2015) used pooled individual-level data from 12 studies that participated in the Childhood Leukemia International Consortium to examine the association between home pesticide exposure and the risk of childhood leukemia. The analysis included data from 12 consortium studies conducted in North America, Europe, and Australasia over a 30-year period. Self-reported home pesticide exposure data were harmonized across studies. The time periods of potential exposure included the period prior to conception, during pregnancy, and after the child's birth. Exposure could be to the mother, father, or child. A variety of factors were examined as potential confounders. The analyses were stratified by leukemia subgroups, including acute lymphoblastic leukemia (ALL) and AML, and cytogenetic data. Weakly elevated odds ratios for ALL were found for home herbicide use (otherwise unspecified) during the three time periods of interest: for example, the odds ratio for reported use within 1–3 months before conception was 1.23 (95% CI 1.04–1.45), based on 5 studies. Home herbicide use during pregnancy

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was associated with decreased odds of AML (OR = 0.84; 95% CI 0.56–1.26). These were the only two analyses with reported results for the herbicide exposure group. The study's strengths include a large pooled resource from multiple international studies. Limitations include self-reported home pesticide exposure and the inability to isolate effects of specific herbicides.

Other Identified Studies–Case-Control Studies Gunier et al. (2017) analyzed data from the California Childhood Leukemia Study, a population-based case control study conducted in 35 counties from 1995 to 2008. Cases were ascertained within 72 hours after diagnosis at Northern and Central California hospitals. Controls were randomly selected from California birth certificate files and individually matched to cases on the child's age, sex, race, and Latino ethnicity, and on maternal race. In-person interviews collected information on occupational history for each parent. The interview also collected information on whether the parent worked regularly with pesticides, insecticides, fungicides, or herbicides, otherwise unspecified, and whether the parent worked in agricultural occupations. Detailed occupational information was collected using specific job module interview questions for occupations with potential pesticide exposure, including farm or ranch worker; gardener, landscaper, nursery worker, or groundskeeper; agricultural packer; and pesticide applicator. The analysis was based on 669 children diagnosed with ALL and 1,021 controls. After adjustment for child's sex, age, ethnicity, mother's race, and household income an increased odds ratio of ALL was found for paternal occupational exposure to any pesticides (OR = 1.7, 95% CI 1.2–2.5). A statistically significant odds ratio was found for paternal pesticide exposure and ALL in children diagnosed before 5 years of age (OR = 2.3, 95% CI 1.3–4.1). Maternal pesticide exposure had a weakly elevated odds ratio for ALL (OR = 1.3, 95% CI 0.8–2.4). The study strengths include a population-based design and a comprehensive in-person interview-based collection and assessment of parental occupational exposures, including occupations associated with pesticide use. However, the study was not large enough to assess the effect of specific pesticides including the COIs.

Omidakhsh and colleagues (2017) conducted research based on data from a multi-center case-control study of retinoblastoma.[8] Cases included patients with sporadic retinoblastoma who were diagnosed or treated at the Children's Oncology Group institution or at the Wills Eye Institute in Philadelphia, Pennsylvania, between 2006 and 2011. A total of 282 cases (186 unilateral and 96 bilateral) were recruited. Controls were selected based on friends or non-biological relatives nominated by case families and with a child in the same age range as the index case child. Phone interviews were used to collect information on residential

[8]"Retinoblastoma is a rare childhood cancer in which malignant (cancer) cells form in the tissues of the retina. The retina is a thin layer of nerve tissue that lines the inside of the back of the eye and is sensitive to light." See https://www.cancer.gov/types/retinoblastoma (accessed October 28, 2018).

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pesticide use before conception and during pregnancy, including the use of professional lawn or landscape services; the use of pest control professionals or exterminators for their home; in-home use of insect or rodent killers; use of indoor foggers; home or garden use of herbicides, mold removal products, antifungals, or weed killers; insect repellant; head lice treatment on the children; and, for pets, the use of flea collars or flea or tick shampoos. When parents indicated that they had applied a product themselves in the home or garden, they were asked to identify the name of the product. The investigators' analysis was based parents of 99 unilateral and 56 bilateral case-control pairs. Odds ratios were adjusted for mother's race, mother's age, mother's work status, and whether the father was living at home at the start of the pregnancy. Parental home use of a weed killer (OR = 2.3; 95% CI 0.9–5.4) was associated with an elevated odds ratio for unilateral retinoblastoma. The use of weed control products on the garden or lawn had an adjusted odds ratio of 2.3 (95% CI 0.9–5.4). Associations between maternal residential pesticide exposure in the month before or during pregnancy and bilateral retinoblastoma were very imprecise. For example, the use of professional lawn or landscape services had an odds ratio of 3.4 (95% CI 0.6–18.0), while the use of weed control products on a garden or lawn had an adjusted odds ratio of 1.0 (95% CI 0.3–3.3). The patterns of association were similar for different exposure factors, including the types of products, locations, timing, and frequency of pesticide use. When asked about the specific pesticide product used, about half of participants responded with a product name for which ingredients could be identified. Reported products used for weed control included 2,4-D, dicamba, glyphosate, and 2-methyl-4-chlorophenoxyacetic acid (MCPA). The study was based on national case and control groups and a detailed interview regarding residential pesticide use and other factors. In addition, cases could be divided into unilateral and bilateral subgroups. Limitations included the use of friend controls that may introduce selection bias based on shared geography and other factors, the relatively small number of cases, and the inability to analyze by specific herbicides that may have been used, including those of particular interest (2,4-D and dicamba).

Biologic Plausibility

Laboratory animal studies have established that TCDD can affect development, so a connection between TCDD exposure and effects on offspring, including developmental disruption and disease onset in later life, is biologically plausible. It has been established in several animal studies that TCDD at high doses is a potent teratogen. Studies with rodent models have demonstrated male, female, and sex-independent effects in the immediate offspring of females exposed during pregnancy. These include epigenetic modification of imprinted genes (Somm et al., 2013), increased DNA methylation of the *BRCA1*

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tumor suppressor gene in mammary tissue (Papoutsis et al., 2013), altered uterine response to estradiol (K. A. Burns et al., 2013), the dysregulation of lipid metabolism in the presence of a high-caloric diet (Sugai et al., 2014), aberrant emotional behaviors (A. T. Nguyen et al., 2013), a reduced capacity for lymphocyte differentiation (Ahrenhoerster et al., 2014), testicular inflammation (Bruner-Tran et al., 2014), and a variety of adult diseases, including kidney, prostate, ovarian primordial follicle loss, and polycystic ovarian disease (Manikkam et al., 2012a). Transgenerational inheritance to the F3 generation was shown for the last two studies. However, definitive conclusions based on animal studies about the potential for TCDD to cause later-life effects in human offspring are complicated by differences in sensitivity and susceptibility among individual animals, strains, and species; by differences in the route, dose, duration, and timing of exposure in experimental protocols and real-world exposure; and by differences in the toxicokinetics of TCDD between laboratory animals and humans. Experiments with 2,4-D and 2,4,5-T indicate that they have subcellular effects that could constitute a biologically plausible mechanism for developmental effects, but only at very high doses. There is insufficient information on picloram and cacodylic acid to assess the biologic plausibility of their developmental or delayed effects in offspring.

Paternal or maternal exposure to xenobiotics potentially could increase the susceptibility of offspring to cancer through multiple mechanisms. Susceptibility could be increased by causing a tumor-promoting mutation in germ cells that would be present in all of the somatic cells of the child. This de novo mutation could then be passed on to subsequent generations via Mendelian inheritance, assuming that the child survived to reproduce. However, as discussed earlier in this chapter and in earlier chapters, TCDD and other COIs are not genotoxic (i.e., they do not cause mutations), which makes the mutation-induction and inheritance scenario unlikely. Alternatively, a maternally mediated increase in susceptibility to childhood cancers could result from the direct exposure of a fetus in utero or of the newborn via lactation to a xenobiotic that induces epigenetic alterations that increase cancer susceptibility.

As noted elsewhere in this chapter, there are several pre- and post-conception scenarios for how toxicant exposures could cause disease in first-generation offspring and perhaps in later generations based on epigenetic mechanisms (Vaiserman, 2014). Perhaps the most straightforward scenario is in utero exposure affecting the developing epigenome, predisposing the child to cancer. The best example of this happening is when otherwise very rare vaginal cancers arose in the daughters of women who took the estrogenic agent diethylstilbestrol to prevent miscarriage (Herbst et al., 1971). Thus, this scenario is plausible for humans. Although TCDD can have antiestrogenic effects, its toxicity via AHR likely involves transcriptional changes that could induce epigenetic mechanisms. With regard to cancers, if the affected gene or genes are involved in cancer pathways

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and epigenetic modifications stabilize the gene-expression changes, then the susceptibility to cancer could increase.

Prenatal TCDD exposure of rats is associated with altered mammary gland differentiation and an increase in the number of mammary adenocarcinomas (Brown et al., 1998). Perhaps related, prenatal TCDD exposure led to increased DNA methylation at the *BRCA1* (breast cancer) gene promoter in the female offspring of exposed pregnant rats (Papoutsis et al., 2013). The demonstration that early postnatal TCDD exposure does not increase mammary-cancer risk (Desaulniers et al., 2004) does not contradict the finding that TCDD-induced changes in utero mediate the increase in cancer susceptibility (Fenton et al., 2000, 2002), and it is consistent with the ultimate carcinogenic effect being greatest when epigenomic changes are the most dynamic. Thus, developmental epigenetic alterations may be involved in the prenatal effects. TCDD has been shown to suppress the expression of two tumor-suppressor genes, *p16Ink4a* and *p53*, via an epigenetic mechanism that appears to involve DNA methylation (Ray and Swanson, 2004). Similarly, it was reported that prenatal TCDD exposure increases the DNA methylation of two growth-related imprinted genes, *H19* and *Igf2*, in the developing fetus (Q. Wu et al., 2004).

No direct evidence from animal models shows that TCDD increases the risk of childhood cancers, such as acute leukemia and germ-cell tumors; although a 2014 study showed a reduced capacity of hematopoietic stem cells to undergo differentiation in offspring (Ahrenhoerster et al., 2014). Emerging research suggests that prenatal TCDD exposure can disrupt epigenetic imprinting patterns and alter organ differentiation and thus could contribute to an increased susceptibility to cancer later in life. Smith et al. (2005) showed that chromosomal rearrangements associated with childhood ALL are evident in the neonatal blood spots, which suggests that childhood leukemias begin before birth, perhaps due to maternal exposures to carcinogenic xenobiotics.

Synthesis

Newly identified studies of cancer in children of parents potentially exposed to the COIs included a case-control study in which parents were asked about military service and potential Agent Orange exposure. The study was small, and while an elevated odds ratio was found, the estimate was very imprecise and random error could not be ruled out. Other identified occupational and environmental studies had methodologic challenges and did not report results for specific COIs. In sum, the evidence is sparse and inconclusive.

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Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and childhood cancers.

Growth and Physical Parameters

Since *Update 2014*, seven studies identified as relevant by the committee have examined exposure to the COIs and the subsequent growth and development of children.

A case control study by Hsieh et al. (2017) explored whether polymorphisms and haplotypes of arsenic methyltransferase (*AS3MT*), glutathione-S-transferase omegas (*GSTOs*), and purine nucleoside phosphorylase (*PNP*) affect arsenic methylation capacity and developmental delay. From 2010 to 2014, 179 children with developmental delay and 88 children without delay were recruited from the Shin Kong Wu Ho-Su Memorial Teaching Hospital in Taiwan. Urinary arsenic species, including arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), and DMA^{V} were measured using a high-performance liquid chromatography-linked hydride generator and atomic absorption spectrometry. The polymorphisms of *AS3MT*, *GSTO*, and *PNP* were analyzed using the Sequenom MassARRAY platform with iPLEX Gold chemistry. Polymorphisms of AS3MT genes were found to affect susceptibility to developmental delay in children, but GSTO and PNP polymorphisms were not. Participants with the *AS3MT* rs3740392 A/G+G/G genotype, when compared with those with the *AS3MT* rs3740392 A/A genotype, had a significantly lower secondary methylation index. This may result in an increased odds ratio for developmental delay. Participants with the *AS3MT* high-risk haplotype had a significantly higher OR than those with *AS3MT* low-risk haplotypes (OR = 1.59; 95% CI 1.08–2.34). This study shows a joint dose–response effect of an *AS3MT* high-risk haplotype with inefficient arsenic methylation capacity on developmental delay, but it does not constitute persuasive evidence that the *AS3MT* genotype causes developmental delay by affecting arsenic methylation.

Three European birth cohorts (Belgian, Norwegian, Slovak) that assessed dioxin exposures in cord blood or breast milk were pooled by Iszatt et al. (2016) to investigate the influence of perinatal exposure to dioxins and dioxin-like chemicals on infant growth and BMI in childhood. The exposure was highest in the Belgian and lowest in the Norwegian cohort (the median and interquartile range of the pooled sample were 13 and 12.3 pg CALUX[9] TEQ/g lipid

[9]CALUX (Chemical-Activated LUciferase gene eXpression bioassay) is a test that may used to determine dioxin-like activity.

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respectively). Infant growth was defined as the cohort- and sex-specific change in weight-for-age z-score between birth and 24 months (n = 367). The investigators also calculated BMI at ~7 years of age in 251 children. Overweight was defined according to international standards for children equivalent to an adult BMI > 25 kg/m^2. In multivariate models based on generalized estimating equations, perinatal exposure to dioxins and dioxin-like chemicals appeared to be associated with increased growth between birth and 24 months (adjusted estimate for change in z-score: $\beta = 0.07$, 95% CI −0.01–0.14) and at age 7 years, and dioxin exposure was associated with a statistically significant increase in BMI in girls (adjusted estimate for BMI units $\beta = 0.49$, 95% CI 0.07–0.91) but not in boys ($\beta = -0.03$, 95% CI −0.55–0.49) (p-interaction = 0.044). Furthermore, only girls had an increased risk of being overweight at age 7 (54%, 95% CI −6% –151%). While these studies suggest that perinatal exposure to dioxin and dioxin-like chemicals may increase early infant growth and contribute to increased BMI in school age girls, the studies were still quite small in size, and additional studies of larger sizes are needed to re-examine these associations.

Mayhoub and colleagues (2014) used data from the MecoExpo study to investigate the relationship between parental exposures to pesticides (as reported by the mother) and neonatal parameters. The study included 993 mother–newborn pairs from the Picardy region of northern France. In this cohort, each mother completed a questionnaire that probed potential occupational, domestic, environmental, and dietary sources of parental exposure to pesticides during her pregnancy. Multivariate regression analyses found that maternal occupational exposure was associated with an elevated risk of low birth weight (OR = 4.2, 95% CI 1.2–15.4). Paternal occupational exposure to pesticides was associated with a lower-than-average gestational age at birth (−0.7 weeks, p = 0.0002) and an elevated risk of prematurity (OR = 3.7, 95% CI 1.4–9.7).

A study of 234 couples by Robledo et al. (2015)—also reviewed above in the section regarding low birth weight and preterm delivery—examined how birth size varied with maternal and paternal exposures to 63 POPs from five major classes, including 36 PCBs, seven of which were dioxin-like (PCBs 105, 114, 118, 156, 157, 167, and 189). Exposure was measured in parental serum collected before conception. Differences in length, head circumference, and ponderal index were estimated using multiple linear regression per 1 standard deviation increase in natural log-transformed (ln-transformed) chemicals. The subjects were participants in the LIFE prospective cohort study, which was conducted in Michigan and Texas between 2005 and 2009. Models were estimated separately for each parent and adjusted for maternal age, maternal pre-pregnancy BMI, and other confounders, and all models included an interaction term between infant sex and each chemical. Among girls (n = 117), mean length ($\beta = -0.57$ cm; 95% CI [−1.12, −0.02]) and head circumference ($\beta = -0.45$ cm; 95% CI [−0.86, −0.03]) were smaller in association, with a 1

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standard deviation increase in ln-transformed maternal serum concentrations of the dioxin-like PCB 167. No statistically significant differences in length, head circumference, and ponderal index were noted among boys (n = 113) relative to maternal or paternal exposure to the COIs.

Tai et al. (2016) enrolled 217 mother–infant pairs living in a dioxin-contaminated area in Vietnam and collected data on the birth cohort's physical growth during the first 3 years of life. Perinatal dioxin exposure of infants was estimated by the measurement of dioxin levels in the breast milk of the nursing mothers. Growth parameters—including weight, height, and head and abdominal circumferences—were measured at birth, 1 and 4 months, and 1 and 3 years of age. Multivariate mixed models were applied for analyzing repeated measures. All body size measures in boys were decreased in the high-exposure groups of TCDD (for example, head circumference age-adjusted z-score high-exposure: −0.87, 95% CI [−1.28, −0.46], versus low-exposure: −0.18, 95% CI [−0.29, −0.06]; $p < 0.01$). In girls, high TCDD exposure was associated with increased head circumference (age-adjusted z-score high-exposure: 0.18, 95% CI [−0.08, 0.44], versus low-exposure: −0.28, 95% CI [−0.42, −0.15]; $p < 0.01$). This study suggests that perinatal dioxin exposure affects the physical growth of infants and children in the first 3 years of life in a sex-specific manner.

S. Thomas and colleagues (2015) measured arsenic and chemical levels in blood or urine samples from the first and third trimesters in 1,835 pregnant women from across Canada to study the association with fetal growth. The investigators adjusted for maternal age, parity, pre-pregnancy BMI, and smoking and examined potential effect modification by single nucleotide polymorphisms (SNPs) in *GSTP1* and *GSTO1* genes. No association was found for arsenic and small-for-gestational-age as an outcome, but the researchers found an increased risk for small-for-gestational-age for the highest compared to the lowest tertile of exposure for the organic arsenic species arsenobetaine (> 2.25 µg/L, RR = 1.65, 95% CI 1.10–2.47) after adjusting for parity and smoking. A statistically significant interaction was observed in the relationship between DMA levels in urinary arsenic and small-for-gestational-age between strata of *GSTO1* A104A (p for interaction = 0.02). This study was deemed to be of limited usefulness to the committee because the relevance of the exposures to those experienced by Vietnam veterans is debatable.

Motor Development, Cognitive, Behavioral, and Socio-Emotional Outcomes

Since *Update 2014*, a number of studies identified as relevant by the committee have examined exposure to the COIs and cognitive or motor development outcomes in children.

Berghuis and colleagues (2014) reported on the results of a birth cohort study conducted in the Netherlands, with recruitment from 1998–2000. Maternal blood

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samples were collected in the second or third trimester and analyzed for 10 PCB congeners and 6 hydroxylated metabolites. Three mono-ortho PCBs (105, 118, and 156), but no coplanar PCBs were measured; the hydroxylated metabolite for PCBs 105 and 118 was measured. These prenatal exposures were analyzed in association with scores on the Touwen neurological examination administered at 3 months of age. Many empirical analyses were conducted, with results showing, in general, that prenatal PCB exposures were associated with more optimal development, both before and after adjustment for confounders of birth weight and child's age at assessment. None of the results assessing the association between prenatal exposure to mono-ortho PCBs and non-optimal development were statistically significant: PCB 105 (0.833 per 1 ng/g lipid weight, CI 0.550–1.262), 118 (0.932, CI 0.728–1.191), or 156 (0.910, CI 0.831–1.019) (both genders; after adjustment).

Braun and colleagues (2014) conducted an analysis of the effects of gestational exposure to endocrine-disrupting chemicals on 4- to 5-year-old children enrolled in the Health Outcomes and Measures of the Environment Study conducted in the Cincinnati, Ohio, metropolitan area. The study examined a total of 25 PCBs—including the dioxin-like PCBs 105, 118, 156, 157, 167, and 170—along with many phthalates, PBDEs, pesticides, and polyfluorinated compounds in relation to scores on the Social Responsiveness Scale (SRS). No dioxin-like PCB was found to be associated with SRS scores (data were presented in graphs with no accompanying numbers). The authors cautioned, though, that their modest sample size (n = 175) precluded them from dismissing possible effects from chemicals with null associations.

Caspersen et al. (2016) used data from the Norwegian Mother and Child Cohort Study, also known as MoBa, to estimate maternal dietary exposure to dioxins and PCBs using a validated food frequency questionnaire administered mid-pregnancy and a database of dioxin and PCB concentrations in foods. Exposure to dioxins and dioxin-like PCBs was characterized in terms of TEQs. Children's language skills at age 3 were assessed by parental report, including a grammar rating scale and questions about communication skills from the Ages and Stages Questionnaire (ASQ). The total sample analyzed was 44,092 children, and a thorough control for confounders included maternal age, education, parity, pre-pregnancy BMI, household income, bilingualism, maternal smoking, alcohol, and folate supplement use, dietary fatty acids intake, and dietary exposure to methylmercury during pregnancy. Statistically significant results included associations of either high maternal TEQ exposure from the diet or high intake (above the 97.5th percentile) of the non-dioxin-like PCB 153, with higher odds of incomplete grammar in boys and girls (aORs 1.1 to 1.3) and severe language delay (aOR = 1.6, 95% CI 1.1–2.4 for dioxin-like chemicals, and aOR = 1.9, 95% CI 1.1–3.4 for PCB 153). Higher exposure to dioxin-like chemicals was also associated with lower ASQ communication scores in girls (aOR = 1.4, 95% CI 1.1–1.9).

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Hui et al. (2016) recruited 161 mothers and their 11-year-old children living in Hong Kong to participate in a study of prenatal dioxin exposure and neurocognitive development that was a follow-up to earlier work. Neuropsychological assessments were performed by clinical psychology trainees or senior research assistants with graduate training in psychology who were blinded to the participating children's dioxin exposure. A registered clinical psychologist was responsible for ensuring assessment quality and interpreting the assessment results. All children first completed the cognitive assessments, which took approximately 3 hours, before other health assessments were performed. Most assessments were done in the research unit, but 15 were performed at home. Testing venues were required to be quiet and distraction free. No associations were found between any of the neurocognitive or behavioral tests administered with the TEQ levels from maternal breast milk obtained from their mothers 2 to 6 weeks postpartum, with p values for interaction ranging from 0.34 to 0.99 for children who were breastfed exclusively during infancy.

Kono and colleagues (2015) used a general population sample from an ongoing breast milk survey that has been conducted in several prefectures and cities in Japan since 1997, from which TEQs are calculated based on PCDD/Fs and coplanar PCBs. When the children were 6 to 13 years of age, parents were asked to complete the extended Japanese version of the Strengths and Difficulties Questionnaire (SDQ), which consists of 25 items on specific strengths and difficulties, with an overall rating of whether the child had behavioral or psychological problems. Four SDQ subscales were obtained—emotional symptoms, conduct problems, hyperactive/inattention, and peer problems—as well as a total difficulties score (TDS) based on the four subscale scores. Analyses were conducted stratified by age and sex of the child. Few covariates were considered in the multivariate analysis—maternal age, birth weight, any history of maternal cigarette smoking, and age at SDQ assessment—raising concerns about lack of control for the socioeconomic status of the family. None of several different metrics estimating the dioxin exposure showed any association with the TDS score in any of the analyses of boys, girls, or different age groups: in linear regression analyses, breast milk TEQ level was not significantly related to the TDS in boys (partial regression coefficient = 2.29; 95% CI [−7.60, 12.18]), or in girls, (partial regression coefficient = −1.04; 95% CI [−9.24, 7.15]) after adjustment for covariates. According to the authors, levels of dioxins in this population were comparable to other studies from the same time period that estimated dioxin exposures from breast milk in Japan, the United Kingdom, and Eastern Europe.

Nakajima et al. (2017) examined sex-specific differences in the effect of prenatal exposure to dioxin-like chemicals on neurodevelopment in children who were participants in the Sapporo cohort of the Hokkaido Study on Environment and Children's Health. Data included a blood draw from the mother in the second trimester of the pregnancy; a self-administered questionnaire that collected

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information on the mother's dietary habits, exposure to chemicals during daily life and at work site, home environment, smoking habit, and medical histories as well as the medical histories of the mother's partners; maternal and child medical records information; and a neurodevelopmental test (Bayley Scales of Infant Development, 2nd Edition) administered to the child at either 6 or 18 months. The exposure assessment included levels of a number of dioxin-like chemicals and TEQ values. One hundred ninety mother–infant pairs in the 6-month-old group and 122 mother-child pairs in the 18-month-old group were studied. The investigators reported that levels of 10 dioxin-like chemicals in male children were significantly negatively associated with the Psychomotor Developmental Index (PDI) scores at 6 months of age after adjustment for potential confounding variables (−0.21 point change in developmental score per dioxin level [common logarithm], adjusted; $p < 0.05$) but that the associations disappeared at 18 months of age. For female children, the level of only one mono-ortho PCB isomer (PCB 123) was significantly negatively associated with PDI at 6 months of age. However, in contrast to the males, levels of four dioxin-like chemicals (PCBs 114, 156, 157, and 189) in 18-month-old female children were significantly positively associated ($p < 0.05$) with Mental Developmental Index scores.

An English-language abstract of a paper published in Chinese by Ni et al. (2016) reports the results of a study involving 3,771 children whose mothers were recruited during pregnancy. Maternal occupational and life exposures were assessed in the period 6 months prior to pregnancy, and the BRIEF (Behavior Rating Inventory of Executive Function–Preschool version) was used to assess the preschool children's executive function. The investigators' findings included that maternal exposure to pesticides in the 6 months before the pregnancy were associated with Inhibitory Self-Control Index dysplasia (OR = 3.60, 95% CI 1.45–8.95), Flexibility Index dysplasia (OR = 6.72, 95% CI 2.50–18.07), and Global Executive Composite dysplasia (OR = 2.39, 95% CI 1.02–5.58) in the preschool children. No statistically significant associations between paternal pesticide exposures and adverse outcomes were reported. It is unclear from the abstract, however, whether any COI exposures were involved.

In a companion study to Pham et al. (2015), reviewed later in this section, Nishijo et al. (2014) examined 153 mother–infant pairs residing in a dioxin-contaminated area near a former U.S. base in Vietnam to evaluate potential associations between perinatal dioxin exposure and autism spectrum disorders in the children. Parents completed a full-length Autism Spectrum Rating Scale (ASRS) survey—which has not been validated in a Vietnam population but for which no comparable test was available—and researchers calculated several outcome and treatment scale scores from the results. These data were analyzed in conjunction with the Bayley-III neurodevelopmental battery test results described by Pham et al. The investigators found that high-TCDD-exposed (≥ 3.5 pg per g fat in mother's breast milk sampled 1 month after birth) male and female children showed significantly higher ASRS scores than less-TCDD-exposed children,

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without concomitant differences in their mental and psychomotor scores. In contrast, high TEQ level boys (≥ 17.9 pg-TEQ per g fat), had significantly lower neurodevelopmental scores than lesser-exposed boys without a comparable difference in their ASRS scores. These results suggest that perinatal TCDD exposure has an effect on autistic traits in childhood that is separate from neurotoxic effects of dioxin exposure in general.

A second follow-up to Pham et al. (2015) conducted by Nishijo et al. (2015) focused on urinary metabolite levels in 26 children who were part of their study cohort. The investigators found that urinary histidine is associated with dioxin exposure–induced neurodevelopmental deficits, suggesting that it might be a useful marker of dioxin-induced neurodevelopmental deficits. However, given the very small number of subjects and the very wide natural variability of urinary histidine (along with that of other urinary metabolites such as glycine and tryptophan), the results are not likely to be reliable and do not present data bearing on any direct evidence of a link between TCDD and clinical outcomes in descendants.

The Duisburg (Germany) birth cohort study by Nowack and colleagues (2015) recruited pregnant women from September 2000 to October 2002. Maternal blood samples were collected primarily in the third trimester (a few in the immediate postnatal period) and used for the quantitation of PCDD/Fs and dioxin-like PCBs in order to obtain a final TEQ for PCDD/Fs, PCBs, and PCDD/Fs+PCBs. These TEQ values were then examined in relation to child scores on the SRS, a standardized instrument suitable for administration to the general population that collects data from the parent on symptoms related to autism spectrum disorders. Parents rated the children on 65 items that were grouped into five subscales. In this study, the children were 9–12 years of age when the SRS was completed. Results showed that SRS Social Communication scores were significantly lower in male and female children exposed to higher TEQs from PCDD/Fs or from PCBs ($p < 0.05$). Autistic Mannerisms scores also correlated significantly with TEQs from PCDD/Fs or from PCDD/Fs+PCBs ($p < 0.05$). In sex-stratified analysis, the only significant association in boys was between TEQ from PCDD/Fs and the Social Motivation subscale ($p < 0.05$). In girls, however, significant associations were found for PCDD/Fs alone or combined with PCBs in relation to the total SRS score ($p < 0.05$) and the Social Communication subscale score ($p < 0.05$); and for all three TEQs (PCDD/Fs, PCBs, and PCDD/Fs+PCBs) in relation to the Social Cognition ($p < 0.05$) and Autistic Mannerisms ($p < 0.01$) subscales. The study's limitations were that postnatal exposures were not accounted for in the analysis of prenatal exposures and that the sample size for this follow-up was small ($n = 100$ for boys and girls combined). Also, other risk factors for autism spectrum disorder that have emerged were not measured: air pollution, maternal nutrition, maternal diabetes, and inter-pregnancy interval. However, the study's major strengths were the measurement of TEQs from the prenatal period and its control for potential

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confounders, such as maternal age, education, the presence of older siblings, the age of the child, alcohol and smoking during pregnancy, and nationality. Some factors were also associated with attrition, thereby reducing selection bias.

In a paper related to the Nowack et al. (2015) research described above, Neugebauer et al. (2015) examined attention performance and attention-related behavior among 117 school-aged children who were part of the Duisburg cohort. Increased prenatal PCDD/F and PCB concentrations were significantly ($p < 0.05$) associated with a higher number of omission errors in the subtest Divided Attention (47% and 42%; 95% CI 1.08–2.00 and 1.07–1.89, respectively). Orenstein and colleagues (2014) evaluated verbal memory, visual memory, and learning in 393 children born to mothers residing in New Bedford, Massachusetts, near a Superfund Site. They calculated TEQs based on measurements of the dioxin-like mono-ortho substituted PCBs 105, 118, 156, 167, and 189 in cord serum. Verbal memory, visual memory, and a learning index from the Wide-Range Assessment of Memory and Learning at 8 years of age were evaluated, with adjustments made for a broad array of confounders, including examiner, child's age at examination, sex, birth year, school grade, parental education, maternal age at birth, maternal birth place, household income, prenatal tobacco smoke exposure, prenatal alcohol exposure, prenatal omega-3 exposure, and maternal IQ. No significant associations were found.

A birth cohort studied by Pham et al. (2015) was established in Da Nang, Vietnam, in 2008–2009, at the site of a former U.S. air base, which is an area of documented high exposures to TCDD and other PCDD/Fs (Hatfield Consultants, 2009a,b). The study includes two districts in a surrounding area of 10 kilometers from the former air base. Exposure measurements were made in breast milk collected at approximately 1 month after delivery, and the chemical measured included 17 PCDD/Fs, including 2,3,7,8-TCDD. The Bayley Scales of Infant Development, 3rd edition (Bayley-III), was administered at age 12 months by an examiner who was supervised by a pediatric psychologist experienced with the Bayley-III. Adjustments were made for the gender, parity, gestational week, age (in days), and birth weight of the infants; the age, education, and drinking habit during pregnancy and residential location of the mothers; and the family income and smoking status of family members. Analyses found that the TEQs for three different metrics of exposure (all PCDD/Fs, TCDD, and the daily dioxin intake) showed virtually no associations with the overall cognitive score; the language composite, receptive language and expressive language subscales; and the motor composite, fine motor and gross motor subscales. The one exception was the daily dioxin intake (in pg TEQ/kg/day), which was significantly associated with improved overall cognitive scores in each of the three upper levels of exposure—mild (102.8; $p = 0.033$), moderate (102.1; $p = 0.042$), and high (102.4; $p = 0.041$), as compared with low exposures (97.7). No adaptive behavior skills showed any association with the exposures examined. However, based on parent report (not observation), scores on a social–emotional scale that assesses

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functional emotional skills and self-regulation were lower in the children with the highest category of prenatal overall TEQ and the highest category of the TCDD TEQ, as compared with the corresponding lowest-exposure categories ($p = 0.09$).

Tai et al. (2016)—whose results regarding physical growth in offspring are reviewed earlier in this chapter—enrolled 217 mother–infant pairs living in a dioxin-contaminated area in Vietnam and followed the neurodevelopment of the birth cohort longitudinally during the first 3 years of life. The perinatal dioxin exposure of infants was estimated by the measurement of dioxin levels in breast milk of the nursing mothers. The neurodevelopment of infants and children, including cognitive, language, and motor development, was determined at 4 months, 1 year, and 3 years of age. Multivariate mixed models were applied for analyzing repeated measures. In boys, composite motor (94.9, 95% CI 89.9–100, $p = 0.49$) and gross motor scores (9.1, 95% CI 7.9–10.2, $p = 0.36$) were significantly decreased with increasing exposure of 2,3,7,8-TCDD. The high PCDDs/PCDFs-TEQ group showed a significant decrease in expressive communication score compared with the low-exposure group, as measured by Bayley-III (8.4, 95% CI 7.8–8.8, $p = 0.30$). In girls, there was no decreased score in any neurodevelopment aspects in high-exposure groups. This study suggests that perinatal dioxin exposure affects the neurodevelopment of infants and children in the first 3 years of life in a sex-specific manner.

Tran et al. (2016) investigated the effects of early life exposure to dioxins in children living around Da Nang City, Vietnam, a location known to be contaminated with dioxin. Exposure was assessed via a breast milk sample from the nursing mother taken 1 month after birth. Demographic and confounding factors data were collected from the mother; physical measurements of the subjects—176 children—were taken at birth and 5 years of age. In boys, the total test (7.7 versus 9.7; $p = 0.018$) and balance (7.3 versus 9.3; $p = 0.013$) scores of the Movement Assessment Battery for Children-2 (Movement ABC-2) were significantly lower in the high-TEQ-PCDDs/Fs group compared with the moderate- and low-exposure groups. Nonverbal Index scores (77.0 versus 98.1; $p = 0.034$) and the pattern reasoning subscale of the Kaufman Assessment Battery for Children, 2nd Edition (KABC-II) (5.3 versus 7.1; $p = 0.041$), which measures planning ability, were also significantly lower in the high-TCDD-exposure group compared with the low-exposure group of boys. However, in girls no significant differences in Movement ABC-2 or KABC-II scores were found among the different TEQ-PCDDs/Fs and TCDD exposure groups. The five boys and one girl who were highly exposed to TEQ-PCDDs/Fs and TCDD were found to be at increased risk for difficulties in both motor coordination and cognitive function. Overall, these results suggest differing impacts of TEQ-PCDDs/Fs and TCDD exposure on motor coordination and higher cognitive ability, respectively. Moreover, high-TEQ-

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PCDDs/Fs exposure combined with high-TCDD exposure may increase autistic traits combined with developmental coordination disorder.

Immune and Allergic Outcomes

Since *Update 2014*, five studies identified as relevant by the committee have examined exposure to the COIs and immune or allergic outcomes in children.

P. H. Su et al. (2015) conducted a study of 56 8-year-old children from Taiwan. The subjects were stratified into high- and low-exposure groups based on maternal PCB and PCDD/F serum levels. No changes were found, although there were differences (t-test) in thyroid hormones, thyroxine binding globulin, IGFBP-3, and growth hormone.

El Majidi and colleagues (2014) used a procedure previously developed to standardize PCB biological concentration data between published studies to perform a systematic analysis of associations between PCB exposure and thyroid hormones (THs) (total and free T3 and T4) or thyroid-stimulating hormone (TSH) in pregnant women and newborns. The weight of evidence of a significant impact of PCB exposure on TSH and TH levels at the described biological levels in pregnant women and newborns (mean < 1,000 µg PCBΣEQ kg^{-1} lipids) appeared low, according to this analysis.

S. Hansen et al. (2014) examined the association between maternal serum concentrations of the dioxin-like PCB 118 and the risk of asthma in their offspring. Compared with subjects in the first tertile of maternal PCB 118 concentration, those in the third tertile had an adjusted HR of 1.90 (95% CI 1.12–3.23). Offspring were exposed in utero, and this is thus evidence of a developmental exposure leading to a later-life health impact.

A second study by S. Hansen et al. (2016) examined a cohort of 965 (421 in the follow-up) pregnant women and measured POPs, including PCBs. Exposures were separated into tertiles for the analysis, based on week-30 POP levels. A clinical evaluation of offspring took place at age 20. No association with asthma was found, but dioxin-like PCB exposure in the mother was associated with offspring airway obstruction (FEV1/FVC < 75%; OR = 2.96, 95% CI 1.14–7.70), with 9 cases versus 20 cases. This is an interesting finding in that this condition may be associated with chronic obstructive pulmonary disease (COPD) later in life.

Nicolle-Mir (2014)—in a paper published in French—examined the association between prenatal exposure to PCBs, hexachlorobenzene, and p-p′-DDE and the risk of asthma in children born to women included in a cohort of Danish births in 1988–1989 (the Danish Fetal Origins Cohort). The concentrations of POPs (congeners 118, 138, 153, 156, 170, and 180 for PCBs) were determined in a maternal serum sample taken at 30 weeks of pregnancy. Nine hundred sixty-five children in the cohort were contacted in 2008 to complete an online questionnaire covering their health status, lifestyle, and diet; 872 (90%) returned data that could

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be included in the analysis. Their government personal identification numbers were cross-referenced with the national prescriptions file, and subjects who had been treated for asthma between the ages of 6 and 20 years were classified as asthmatics. Those who had received a single prescription of beta-2 mimetics or inhaled corticosteroids were excluded. Three secondary criteria were also used: the diagnosis of asthma made by a doctor, the use of asthma treatments during the past 12 months (questions asked in the follow-up questionnaire), and a hospital diagnoses of asthma (outpatient or emergency, based on a central admissions registry). The cross-tabulation of these different sources showed a good match between the data from the national prescription file and the responses to the questionnaire. The prevalence of asthma was equal to 12.7% according to the file and to 13.6% according the questionnaire; 76% of subjects classified as asthmatics on the basis of the file report had an asthma diagnosis, and 72% of those who reported having been diagnosed with asthma were identified in the file. Serum concentrations of POPs were divided into tertiles, and the HR of asthma was calculated for the second and third tertiles with reference to the first. Analyses included those carried out with each individual congener and the sum of the dioxin-like congeners (PCBs 118 and 156). Models were adjusted to the child's sex and birth weight as well as to several maternal parameters: age at birth, pre-pregnancy BMI, parity, smoking, alcohol consumption, education level, plasma concentrations of triglycerides, and total cholesterol. The main analysis (asthma defined according to the national prescriptions file) showed an increase in the risk of asthma in the third tertile concentration of dioxin-like PCBs with reference to the first tertile (HR = 1.75, 95% CI 1.02–2.98), with significant dose–response trends. The association between dioxin-like PCBs and asthma is mainly due to the 118 congener (HR = 1.9, 95% CI 1.12–3.23). For PCB 156, PCBs with no dioxin activity, and the sum of the PCBs, the increases were not significant. The results of the secondary analyses were consistent: the strongest associations were observed with PCB 118 when the criterion "treatment of the asthma during the last 12 months" was used (HR in the last tertile respectively equal to 2.49 [95% CI 1.03–5.99] and 4.18 [95% CI 1.57–11.15]). The authors indicate, however, that the plasma levels of the dioxin-like PCBs and hexachlorobenzene were highly correlated (Spearman's R-value 0.53–0.99), with the mutual adjustments weakening the associations observed in the main analysis to HR = 1.47 (95% CI 0.75–2.86) for PCB 118. In addition, in the absence of information about breastfeeding, it is unclear whether these associations are attributable to prenatal or postnatal exposures or a combination of both.

Other Outcomes

No studies identified as relevant by the committee have examined exposure to the COIs and reproductive function in offspring since the publication of *Update*

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2014. However, since this category was included in the *Update 2014* report, it is noted here for completeness.

Li and colleagues (2015) conducted a study of the so-called "Yucheng children," descendants of mothers exposed to PCBs/PCDFS via a contaminated cooking oil incident in 1978–1979 in Taiwan, to evaluate the effect of this insult on their auditory function later in life. Exposed children were born June 1978–December 1998; control children were matched by age, sex, neighborhood, mother's age, and parents' educational level and occupation. Demographic data were collected via questionnaire, and pure-tone audiograms were obtained. Eighty-six Yucheng children and 97 controls participated in the study. Serum taken from the mothers during other studies had previously been screened for dioxins and PCBs. The study found that gestational exposure to 2,3,4,7,8-PeCDF was associated with an increase in hearing loss (increase in the pure tonal frequency threshold), although the frequency deficits were not the same in the right and left ears. Exposures to 1,2,3,4,7,8-HxCDF were associated with hearing loss in the left ear at 4,000 Hz. There was no effect of PCB exposure on hearing, although the PCB levels as measured by TEQ were rather low.

Biologic Plausibility

As noted in *Update 2014*, the results of studies in rodent models provide support for the idea that prenatal exposure to TCDD can result in adverse effects in offspring later in life, including immune disorders, behavioral disturbances, reproductive impairment, kidney disease, and cancers (Foster et al., 2010; Prescott, 2011; Puga, 2011; Takeda et al., 2012). Using two mouse models, investigators showed that prenatal TCDD (2.5–5.0 mg/kg) modified multiple immune signatures in the adult offspring that were indicative of adult-onset autoimmunity (Holladay et al., 2011). Adult-onset inflammatory disease and lupus-like autoimmunity were also observed in mice at 36 weeks of age after high-dose prenatal TCDD exposures (Mustafa et al., 2011). A single prenatal exposure of rats to TCDD (0.7 µg/kg of body weight) reduced brain developmental myelination and compromised remyelination potential in adults (Fernández et al., 2010), and in utero TCDD in mice altered neural progenitor differentiation (Mitsuhashi et al., 2010). However, another study suggested that, in contrast to wild-type murine neural progenitor cells (mNPCs), "human NPCs and AhR-deficient mNPCs were insensitive to AhR agonism or antagonism" (Gassmann et al., 2010, p. 1571). Maternal exposure to TCDD (0.2–0.4 µg/kg of body weight) in pregnant rats perturbed neuroendocrine function as measured by thyrotropin and growth hormone concentrations in exposed offspring through peripubertal postnatal day 30, which supports the idea of continued later-life thyroid hormone disturbances (Ahmed, 2011, 2014). These disturbances include the epigenetic modification of imprinted genes (Somm et al., 2013), increased DNA methylation of the *BRCA1*

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tumor suppressor gene in mammary tissue (Papoutsis et al., 2013), altered uterine response to estradiol (Burns et al., 2013), dysregulation of lipid metabolism in the presence of a high-caloric diet (Sugai et al., 2014), aberrant emotional behaviors (A. T. Nguyen et al., 2013), a reduced capacity for lymphocyte differentiation (Ahrenhoerster et al., 2014), testicular inflammation (Bruner-Tran et al., 2014), and a variety of adult diseases including kidney disease, prostate disease, ovarian primordial follicle loss, and polycystic ovarian disease (Manikkam et al., 2012a).

Other studies have focused on neurobehavioral outcomes following perinatal exposure, which is of relevance in understanding the possible consequences in the offspring of Vietnam veterans, particularly the offspring of female veterans. Haijima et al. (2010) found that gavage treatment of pregnant mice with 3 µg /kg TCDD on gestation day 12.5 (resulting in in-utero and lactational exposure of the offspring) impaired memory in the male offspring. Mitsui et al. (2006) reported that hippocampus-dependent learning could be impaired in male rats exposed to TCDD in utero and that the impairment could affect fear conditioning. Curran et al. (2011) assessed the effect of *CYP1A2* and Ahr genotypes on altered learning and memory in mice exposed to an environmentally relevant mixture of dioxin-like (coplanar) and non-dioxin-like PCBs in utero and during lactation. They observed the most significant deficits in response to PCB treatment in *Ahrb1 CYP1A2*(–/–) mice, including impaired novelobject recognition and increased failure rate in the Morris water maze test. Studies in week-old rodents have also detected molecular effects of TCDD in cerebellar granule cells and neuroblasts, which may be relevant to motor function and cognitive processes (Kim and Yang, 2005; Williamson et al., 2005). Stürtz et al. (2008) found alterations in how female rats that had been fed on postpartum days 1–7 with diets containing 15, 25, or 50 mg/kg 2,4-D interacted with their pups. The specific relevance of these findings to neurobehavioral effects in humans exposed as adults is unclear.

Another mode of epigenetic change is the modification of the spatial arrangement of chromosomes, which can influence gene expression and cell differentiation. Oikawa et al. (2008), for example, found that TCDD—through the AHR—modifies the positions of chromosomes in the interphase nuclei of human preadipocytes.

The studies discussed above suggest that TCDD has the potential to influence the epigenome and therefore could promote changes in offspring that lead to disease later in life, although the research addressing this issue in model systems is still at an early stage.

Synthesis

Epidemiologic studies designed to examine the effects of the COIs in more mature offspring have evaluated a variety of growth and development, neurologic, immunologic, reproductive, and endocrine system health outcomes. More studies are required before conclusions can be reached as to whether such outcomes in

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the offspring of exposed parents are replicable. In particular, it would be of interest to obtain information on neuropsychiatric conditions, such as attention-deficit hyperactivity disorder and other clinically defined neurodevelopmental outcomes in children who were exposed in utero.

Conclusion

There is inadequate or insufficient evidence to determine whether there is an association between the exposure of men and women to 2,4-D, 2,4,5-T, TCDD, picloram, or cacodylic acid before conception or during pregnancy and the adverse health outcomes in their children addressed in this section. Although the results of laboratory research support the plausibility of such clinical conditions, there are not yet sufficient human data to support an association between the COIs and such adverse outcomes in human offspring.

BIOLOGIC PLAUSIBILITY OF POSSIBLE EFFECTS IN SUBSEQUENT GENERATIONS

In response to a special request from the Department of Veterans Affairs, continuing inquiries from veterans and their families, and increasing attention in research efforts, the committee responsible for *Update 2010* explored the possibility of intergenerational or transgenerational effects resulting from exposure-related epigenetic changes in the parents or exposed fetuses that would lead to adverse health effects in later generations, such as grandchildren. Effects in persons exposed in utero are not considered transgenerational because the fetus was likely exposed directly. This exception includes the children of women exposed in Vietnam even if they are conceived after their tour of duty was over because TCDD remains in the body for a long time and is mobilized during pregnancy. Likewise, the children of men exposed to TCDD in Vietnam and born after the soldiers' tour of duty was over could possibly have health outcomes due in part to TCDD's effect on the sperm epigenome. In contrast, any adverse health effects in grandchildren associated with exposure would be considered to be transgenerational. Figure 8-1 illustrates the partitioning between intergenerational and transgenerational effects due to the exposure of a parent, delineating between those exposures that biologically persistent (like dioxin) and therefore dwell in the body long after exposure versus those that do not.

The Update 2010 committee did not identify any relevant scientific studies to review and concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to the COI and diseases in grandchildren or later descendants of Vietnam veterans. Committees responsible for *Updates 2012* and *2014* likewise failed to find any relevant human studies and affirmed the conclusion of inadequate or insufficient evidence.

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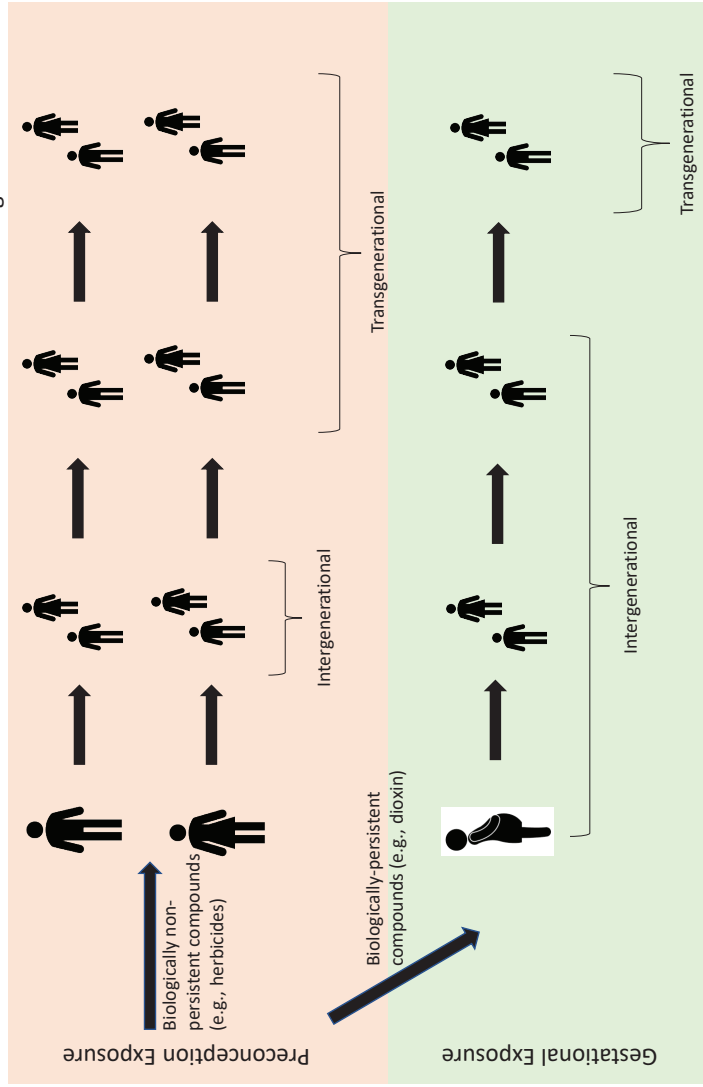

FIGURE 8-1 The partitioning between intergenerational and transgenerational effects due to the exposure of a parent.
SOURCE: Adapted from NASEM, 2018, Figure 3-2.

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Congress put forward and passed the Jeff Miller and Richard Blumenthal Veterans Health Care and Benefits Improvement Act in 2016, and it became Public Law 114-315. Among the law's provisions is Section 632, which called on the Secretary of Veterans Affairs to enter into a contract with the National Academies of Sciences, Engineering, and Medicine to conduct an assessment on scientific research relating to the descendants of individuals with toxic exposure. As part of its response to this directive, VA tasked this committee to "assess the current research available on possible generational health effects that may be the result of exposures to [the COIs]—including the biologic plausibility or potential for an exposure to lead to an increased risk of birth defects or other adverse conditions in the descendants of male Veterans."[10]

Epidemiological Studies

No relevant studies of potential transgenerational effects of exposure to the COIs in humans have been reported to date. The only transgenerational effect shown in humans has been from a comparison of food supplies in Sweden during the 1800s and health outcomes in the children and grandchildren of men who were prepubescent when food supplies were relatively high or low. These studies found an association between high food supply levels in grandfathers and decreased longevity and increased risk of cardiovascular disease and diabetes in grandsons that was paternally transmitted, although no mechanistic information was obtained (Kaati et al., 2002, 2007). Whether transgenerational effects can occur in humans from chemical exposures is unknown at this time.

Biologic Plausibility

Research on the biologic mechanisms that might underlie the potential effects of exposure to the COIs experienced by grandchildren and later generations is still sparse, although it has greatly expanded in the past few years. Epigenetic effects have been shown for male gametes in adult mice exposed to an endocrine-disrupting pesticide (methoxychlor) and fungicide (vinclozolin) (Paoloni-Giacobino, 2014). However, the chemically induced DNA methylation changes in sperm DNA were not transmitted from one mouse generation to the next for imprinted genes; they were, presumably, lost during the period of active demethylation that occurs shortly after fertilization. This observation suggests that transgenerational effects on imprinted genes in mice that might be paternally transmitted may not necessarily involve DNA methylation (Iqbal et al., 2015). Nonetheless, a 2013 study showed that odor fear-conditioning in the father could be paternally transmitted to the F2 generation (as well as the F1) and implicated

[10] *Gulf War and Health, Volume 11* (NASEM, 2018) addresses this mandate as it applies to exposures experienced during the conflicts in the Southwest Asia theater of military operations.

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reduced DNA methylation in the responsible odor receptor gene (Dias and Ressler, 2013). Thus, more research is required to understand better how transgenerational effects can be transmitted paternally once they have been demonstrated (Dias and Ressler, 2014).

A few animal studies have provided evidence of transmission of adverse effects to later generations. The mechanisms that could underlie later-life effects in offspring and effects in later generations (transgenerational inheritance) could involve epigenetic processes, as described earlier in this chapter. Research into dioxin's potential as an epigenetic agent is in its early stages, but a few studies have suggested that dioxin has such properties that are, in significant part, linked to the AHR. Direct evidence, however, is limited to maternal exposures of the developing embryo or fetus during in utero growth, and no reports exist showing paternal TCDD exposure and later-life effects in offspring or paternally mediated transgenerational effects in humans. Q. Wu et al. (2004) demonstrated that TCDD exposure of mouse embryos before implantation in unexposed females resulted in epigenetic changes, including increased DNA methylation and a reduced expression of imprinted genes, which implied that early embryonic exposure alone was sufficient to alter gene expression in the resulting offspring. The transmission of effects to later generations would involve epigenetic alterations in the developing germ cells of a fetus that was directly exposed to maternal TCDD in utero.

Results of a few studies support a transgenerational inheritance due to in utero exposure to TCDD. Exposing pregnant mice to TCDD (at 10 µg/kg) reduced fertility and increased premature birth in three later generations (Bruner-Tran and Osteen, 2011); effects were transmitted through both male and female offspring (Ding et al., 2011; McConaha et al., 2011). Exposing gestating female rats (F0) to dioxin (TCDD) at 100 ng/kg was shown to result in an earlier puberty in the offspring (F1) and in two later generations (F2 and F3) and to reduce ovarian follicle numbers in the females of the F3 generation; this implies transgenerational inheritance (Manikkam et al., 2012a). The F3 effects appear to be transmitted through the sperm that were initially exposed to maternal dioxin in utero. In a second paper by the same research team, additional diseases appeared later in life in the first generation (directly exposed offspring), including prostate disease in males and ovarian follicle loss and polycystic ovarian disease in females (Manikkam et al., 2012b). Further third-generation effects were noted, including kidney disease in males and polycystic ovarian disease in females, which imply transgenerational inheritance. The latter appear to be transmitted through the sperm originally exposed to maternal dioxin in utero inasmuch as sperm DNA methylation changes were observed at 50 chromosomal sites in generations F1–F3. Testicular inflammation from TCDD exposure has also been reported to manifest in multiple generations (Bruner-Tran et al., 2014).

The zebrafish has been used as a model to examine transgenerational effects from dioxin exposure, although different groups have reported different aspects of these effects since *Update 2012*. One group reported that exposing zebrafish at

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3 and 7 weeks old (during sexual development) to TCDD in water for 1 hour at 50 pg/ml increased female-to-male ratios and skeletal abnormalities and reduced fertility in the F1 and F2 descendants (equivalent to F2 and F3 in mammals) (Baker et al., 2014a,b). Another group studying DNA methylation changes in the offspring of mothers fed 20 µg/kg TCDD in their food reported no changes in global methylation in offspring when looking at the total levels of DNA methylation in the genome. However, gene-specific increases or decreases in promoter DNA methylation were observed with a tiling array assay for a limited number of genes in the F1 generation. *CYP1A1* transcription, a marker of TCDD exposure, was elevated in F1 offspring. Unfortunately, no F2 fish were generated from the TCDD exposure because the F1 fish died 1 to 2 weeks post hatching (Olsvik et al., 2014). Further work with this model will be helpful for providing targets for mammalian biologists as they continue to probe for transgenerational effects from TCDD and the other COIs.

Synthesis

No epidemiological information exists to evaluate whether paternal or maternal exposure to the COIs results in health effects in grandchildren or subsequent generations of descendants of Vietnam veterans. The animal literature contains evidence that environmental agents mediated by maternal exposure affect later generations through fetal and germline modifications, but in the case of adult male exposures before the conception of the next generation, there is insufficient evidence on which to draw a conclusion regarding transgenerational effects.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between paternal or maternal exposure to the COIs and health effects in grandchildren or later generations of descendants of Vietnam veterans.

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9

Neurologic Disorders

Chapter Overview

Based on new evidence and a review of prior studies, the current committee did not find any new associations between the relevant exposures and neurologic disorders. Current evidence supports the findings of earlier updates that:

- *There is limited or suggestive evidence of an association between the chemicals of interest (COIs) and Parkinson disease and diseases that present with Parkinson-like symptoms.*
- *There is inadequate or insufficient evidence to determine whether there is an association between the COIs and any of the other adverse neurologic outcomes.*

This chapter considers the possible effects of toxic exposure to the herbicides used during the Vietnam War and specific clinical conditions associated with the central nervous system (CNS) and the peripheral nervous system (PNS), primarily brain dysfunctions. The COIs are 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram (4-amino-3,5,6-trichloropicolinic acid), cacodylic acid (dimethyl arsenic acid [DMA]), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a contaminant of 2,4,5-T. As described in Chapter 3, studies of the effects of exposure to polychlorinated biphenyls (PCBs) and other dioxin-like chemicals were also considered informative if their results were reported in terms of TCDD toxic equivalents (TEQs) or concentrations of specific congeners of dioxin-like chemicals. Studies that report TEQs

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based only on mono-ortho PCBs (which are PCBs 105, 114, 118, 123, 156, 157, 167, and 189) are considered even though their TEQs are several orders of magnitude lower than those of the non-ortho PCBs (77, 81, 126, and 169), based on the revised World Health Organization (WHO) toxicity equivalency factor (TEF) scheme of 2005 (La Rocca et al., 2008; van den Berg et al., 2006). The lower TEQs of the mono-ortho PCBs, however, may be counterbalanced by their abundance, which is generally many orders of magnitude higher than the non-ortho PCBs (H.-Y. Park et al., 2010).

Examples of the diseases that result from the degeneration of specific brain areas are Parkinson disease (PD), Alzheimer disease (AD), spinocerebellar degeneration, and amyotrophic lateral sclerosis (ALS). These diseases may occur in the absence of any toxicant exposure, but all may be triggered by environmental factors, including toxicant exposure (Bronstein et al., 2009; Chin-Chan et al., 2015; de la Monte and Ming, 2014; H. Kang et al., 2014b; Tanner et al., 2014; M. D. Wang et al., 2014).

Disorders of the PNS are generally referred to as neuropathies. Neuropathies can be purely motor, presenting as deficits in strength, but most often they present with the involvement of both motor and sensory fibers. Neuropathies are often symmetric and start with symptoms related to dysfunction of fibers that travel the greatest distance to their target organ. For that reason, the symptoms of neuropathy often start in the digits and travel toward the torso. Many neuropathies also affect autonomic fibers and thus can result in changes in blood pressure and heart rate and in symptoms related to the control of digestion. Toxicant exposure can induce immediate (i.e., acute) damage to peripheral nerves, and previous updates found limited or suggestive evidence that dioxin exposure can cause such short-term effects. However, the overall focus of this chapter is on delayed adverse effects on both the PNS and the CNS.

The immediate effects of toxicants may involve all regions of the nervous system, whereas delayed effects are likely to be related to focal deficits. Diffuse damage to the CNS may cause alterations in thinking, consciousness, or attention, sometimes in combination with abnormalities of movement, while focal dysfunction can cause myriad syndromes, depending on which area of the brain is involved and the extent and severity of damage. For the purposes of this review, neurologic deficits associated with Vietnam service are distinguished from psychiatric and psychologic conditions—such as posttraumatic stress disorder (PTSD), depression, and anxiety—and from chronic fatigue syndrome.

In the original *Veterans and Agent Orange* (*VAO*) report (IOM, 1994), attention was focused on persistent neurobehavioral disorders. That focus was maintained through *Update 2002* (IOM, 2003c). A slight change in emphasis toward chronic neurodegenerative disorders was reflected in the name change of this chapter to "Neurologic Disorders" in *Update 2004* (IOM, 2005), which was carried forward in all subsequent updates. In this update, the chapter covers data pertinent to persistent neurologic disorders of all types.

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Many studies have addressed the possible contribution of various chemical exposures to neurologic disorders. However, for the purposes of the VAO committees, several of these studies have been limited by the use of nonspecific or mixed exposures and by a lack of adequate lag times between the exposure and the neurologic outcome of interest. Case identification of neurologic disorders is also an important consideration and is often difficult because there are few disorders for which there are specific diagnostic tests. Because the nervous system is not readily accessible for biopsy, pathologic confirmation is usually not feasible. However, identifiable neurologic disorders always result in objective abnormalities that are reflected in anatomic or functional tests or discovered via clinical examination.

This chapter reviews the association between exposure to the COIs and neurobehavioral disorders, neurodegenerative disorders, and chronic PNS disorders. The scientific evidence supporting the biologic plausibility of each category of disorders is also reviewed here. More complete discussions of the categories of association and of this committee's approach to categorizing health outcomes are presented in Chapter 3. For citations new to this update that revisit previously studied populations, the relevant details on the experimental design can be found in Chapter 5.

BIOLOGIC PLAUSIBILITY

Experimental data regarding the biologic plausibility of a connection between exposure to the COIs and various neurologic disorders continue to accrue. This section summarizes in a general way some of the information reviewed in the current update and, for completeness, includes pertinent information from prior updates.

Several studies have dealt with mechanisms of neurotoxicity that might be ascribed to the COIs, notably 2,4-D and TCDD. The molecular effects of the COIs are described in detail in Chapter 4. Some aspects of the biochemical activity of the COIs suggest pathways by which there could be effects on the neural systems. A number of studies suggest that the COIs, primarily 2,4-D, have neurologic effects, both neurochemical and behavioral, in animal models if exposure occurs during development or in cultured nerve cells (Konjuh et al., 2008; Pasandi et al., 2017; Rosso et al., 2000a,b; Stürtz et al., 2008); older references described the behavioral effects of a developmental exposure of rodents to a 2,4-D–2,4,5-T mixture (Mohammad and St. Omer, 1986; St. Omer and Mohammad, 1987). The exposure of zebrafish during development to the dioxin-like chemical PCB 126 leads to behavioral deficits in adults and substantial gene expression changes in the adult brain (Aluru et al., 2017). Juvenile TCDD exposure in zebrafish has been shown to lead to lesions in the olfactory neuroepithelium and to dramatic changes in the expression of genes important for neurological function (Q. Liu et al., 2014). Perinatal exposures to TCDD and to coplanar, dioxin-like PCBs

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have reportedly caused deficits in learning behavior in rats (Curran et al., 2011; Haijima et al., 2010; Hojo et al., 2008; Kakeyama et al. 2014). However, those studies should be interpreted with caution because the developing nervous system is different from the mature nervous system and may not be an appropriate model for the possible consequences of exposure to the COIs by adults, as was the case for Vietnam veterans.

Some studies further support suggestions that the concentration of reactive oxygen species could alter the functions of specific signaling cascades and be involved in neurodegeneration (Drechsel and Patel, 2008). Such studies do not specifically concern the COIs but they are potentially relevant to these chemicals inasmuch as TCDD and herbicides have been reported to elicit oxidative stress (Byers et al., 2006; Celik et al., 2006; J. Kumar et al., 2014c; D. Shen et al., 2005; Wan et al., 2014). TCDD has been shown to affect phosphokinase C biochemistry in nerve cells and so could affect the integrity and physiology of nerve cells (S. Y. Kim et al., 2007; H. G. Lee et al., 2007). TCDD has also been shown to affect signaling pathways that regulate nitric oxide synthesis in neural and glial cells, leading to neurotoxicity, senescence, and cell death (Duan et al., 2014; Jiang et al., 2014; Y. Li et al., 2013; Nie et al., 2015; Wan et al., 2014). Pellacani et al. (2014) found that PCB 126 reduced neuroblastoma cell viability. Zhao et al. (2016) exposed C6 glioma cell culture to TCDD and found dose- and time-dependent down-regulation of glutamate transporter-1 expression, which could contribute to neurotoxicity. Cytochrome P450 1A1, the aryl hydrocarbon receptor (AHR), and the AHR nuclear transporter occur in the brain, so TCDD may exert effects in the brain (P. Huang et al., 2000). In addition, earlier studies in hepatocytes indicated that 2,4-D affects aspects of mitochondrial energetics and mitochondrial calcium flux (Palmeira et al., 1994a,b, 1995a,b); if these effects occur in mitochondria of nervous-system cells, the energy balance and energy pathways of cells in the nervous system could be affected and disrupt nervous system function. This is supported by the work of Morales-Hernández et al. (2012), which showed that TCDD in cultured neuronal cells induces cell death by the disruption of intracellular calcium levels.

Laboratory-based studies have emphasized the importance of alterations in neurotransmitter systems as potential mechanisms underlying TCDD-induced neurobehavioral disorders (Jiang et al., 2014; H. Q. Xie et al., 2013). Neuronal cultures treated with 2,4-D exhibited decreased neurite extension associated with intracellular changes, including a decrease in microtubules, the inhibition of the polymerization of tubulin, disorganization of the Golgi apparatus, and the inhibition of ganglioside synthesis (Rosso et al., 2000a,b). Those mechanisms are important for maintaining the connections among nerve cells, which are necessary for neuronal function and are involved in axon regeneration and recovery from peripheral neuropathy. Early animal experiments have demonstrated that TCDD treatments affect the fundamental molecular events that underlie the neurotransmission initiated by calcium uptake (Hong et al., 1998). Mechanistic studies

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have demonstrated that 2,4,5-T can alter cellular metabolism and the cholinergic transmission necessary for neuromuscular transmission (Sastry et al., 1997).

TCDD treatment of rats at doses that do not cause general systemic illness or wasting produces electric changes in peripheral nerves that are associated with altered functions and pathologic findings that are characteristic of toxicant-induced axonal peripheral neuropathy (Grahmann et al., 1993; Grehl et al., 1993). In cultured cells, Jung et al. (2009) explored the mechanism by which TCDD inhibits neurite outgrowth and found it to be associated with a reduction in glutaminase.

As discussed in Chapter 4, extrapolating observations of cells in culture or in animal models to humans is complicated by differences in sensitivity and susceptibility among animals, strains, and species; by the lack of strong evidence of organ-specific effects occurring consistently across species; and by differences in the route, dose, duration, and timing of chemical exposures. Thus, although the toxicologic observations themselves cannot establish a conclusion that the COIs produced neurotoxic effects in humans, they establish biologic plausibility and point to potential mechanisms that might have come into play.

NERVOUS SYSTEM DISORDERS REPORTED OVERALL

The literature search for this update identified publications on populations with relevant exposures that examined overall mortality from any nervous system disorder (Collins et al., 2016) or hospitalization, again with all nervous system disorders combined, except epilepsy which was presented separately (Cox et al., 2015). However, while the results of grouping all nervous system disorders together are presented, they are not considered informative for assessing whether specific nervous system disorders may be due to an exposure to the COIs. Therefore, such results were not considered by the committee when weighing the evidence for specific conclusions.

Cox et al. (2015) used hospital discharge records from 1988 to 2009 to report the prevalent health conditions among a cohort of 2,783 male New Zealand Vietnam veterans, who served during 1964 to 1972 and were presumed to be exposed to dioxin. For participants 30 years of age or older, person-years of follow-up were calculated by 5-year age categories. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates were calculated for both the veteran cohort and the general population, and from these estimates a standardized hospitalization ratio (SHR) was calculated with 99% confidence limits as a means to address multiple tests performed for various outcomes. Results were presented for eight categories of mental and neurologic disorders and a ninth category for "other nervous disorders." Statistically significant increases in the hospitalization rates were observed for alcohol-related disorders (n = 89; SHR = 1.91, 99% confidence interval [CI] 1.39–2.43), which the authors note was often associated with PTSD, and other nervous disorders

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(n = 135; SHR = 1.32, 99% CI 1.02–1.61). "Other nervous disorder" was the largest single category among mental and neurologic disorders, but it was unclear which disorders were included in that category with the exception of those disorders listed under the general category of mental and neurological disorders; by a process of elimination, this category also likely included PD and ALS. However, hospitalization rates are not a good measure of PD and several other neurologic disorders because people with those conditions are not necessarily hospitalized for the symptoms. No difference in the hospitalization rates was seen for the category of "senility or organic mental illness" (n = 28; SHR = 0.95, 99% CI = 0.49–1.41); the authors do not list specific neurologic disorders of interest, and senility may have included both vascular and non-vascular or Alzheimer-like dementia. Overall, the results of this study for hospital admissions due to all causes combined showed a small increase in rates for Vietnam veterans compared with the population of New Zealand. However, exposure to the COIs was not validated through serum measurements, and the study did not control for smoking or ethnicity or other potentially important risk factors.

Collins et al. (2016) provides additional follow-up time to a retrospective analysis of a cohort of 2,192 workers (only 5 of whom were female) exposed to dioxins during trichlorophenol (TCP) and pentachlorophenol (PCP) production at a chemical manufacturing plant in Michigan. The U.S. population was used as the comparator to generate standardized mortality ratios. Work history records were used to determine the length of exposure. Serum samples to measure the levels of six types of dioxins were collected for 431 workers who had been exposed to TCP or PCP. Historic concentrations for each dioxin congener were calculated from the median concentrations of serum samples and the known half-lives associated with each congener. A job-exposure matrix was created for both the TCP and the PCP production facilities based on measured concentrations for workers in different jobs. A pharmacokinetic model was applied to job-specific concentrations and with the work history of each member of the study group to estimate each worker's time-dependent serum concentration profiles for each dioxin congener (i.e., TCDD as well as Hexa-CDD, Hepta-CDD, and Octa-CDD). Complete vital status follow-up through December 2011 was achieved for the cohort, and there were 1,198 decedents through the entire study period (1979–2011); 1,615 deaths were among TCP workers and 773 deaths occurred among PCP workers (196 workers were exposed to both TCP and PCP and are counted in each of those groups). Compared with the U.S. population, no difference in mortality from all diseases of the nervous system was found for all workers (n = 21; SMR = 0.74, 95% CI 0.46–1.13), and the SMRs were identical for TCP workers (n = 17; SMR = 0.79, 95% CI 0.46–1.26) and PCP workers (n = 7; SMR = 0.79, 95% CI 0.32–1.63).

A second occupational study reported on pesticide applicators from Argentina who completed a questionnaire to document self-reported use of pesticides and general symptoms or consultations (signs of irritation, fatigue/tiredness,

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headache, nervousness or depression, medical consultation, and hospitalization) (Butinof et al., 2015). Included among the pesticides were the herbicides 2,4-D, dicamba, and picloram—three of the COIs for Vietnam veterans. The percentage of completed questionnaires indicating the use of 2,4-D was 93.5%, with lower percentages for dicamba (69.4%) and picloram (46.0%). However, the survey respondents reported the use of 11 different pesticides on average, and effect estimates were based on grouped exposures or did not report any of the COIs separately, making this analysis of little utility for the purposes of the committee.

NEUROBEHAVIORAL, COGNITIVE, AND NEUROPSYCHIATRIC DISORDERS

This section summarizes the findings of *VAO* and previous updates on neurobehavioral disorders and incorporates information published since *Update 2014* into the evidence database.

Conclusions from *VAO* and Previous Updates

On the basis of the data available at the time, the committees responsible for *VAO* and all subsequent updates through *Update 2014* concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and neurobehavioral disorders. The data that informed that conclusion were mostly from the Air Force Health Study (AFHS, 1991a,b, 1995, 2000; Barrett et al., 2001, 2003). Urban et al. (2007) confirmed that acute neurologic symptoms experienced shortly after an acute exposure to TCDD could be sustained more than 30 years after the exposure, but this study did not address delayed effects. In general, many of the studies reviewed by previous VAO Updates were found to be methodologically flawed (Dahlgren et al., 2003; Pazderova-Vejlupkova et al., 1981; Pelclová et al., 2001, 2002) or uninformative (ADVA, 2005c; Decoufle et al., 1992; Kamel et al., 2007a; R. M. Park et al., 2005; Sapbamrer and Nata, 2014; Solomon et al., 2007; Visintainer et al., 1995). Difficulties in case identification and diagnosis, misclassification of exposures because of a lack of quantitative measures, subject ascertainment and selection bias, and uncontrolled confounding from many comorbid conditions are common weaknesses in the studies reviewed. The variability of the test results over time, the weak and inconsistent associations, and a lack of consistent dose–response relationships, also prevent those studies from supporting an association between the exposures of interest and neurobehavioral disorders.

More recent analyses using National Health and Nutrition Examination Survey (NHANES) data found no overall association between serum concentrations of PCBs with dioxin-like activity (mono-ortho PCBs 118 and 156 and non-ortho PCBs 126 and 169) and cognitive function scores in 708 adults aged 60–84 years, but a subgroup analysis demonstrated lower cognition with increasing serum

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concentrations of dioxin-like PCBs (mono-ortho and non-ortho substituted PCBs) (Bouchard et al., 2014). Also using NHANES data, Krieg (2013) performed a limited assessment of cognition in 700 adults, aged 20–59 years, and associated scores with 12 pesticide metabolites measured in the urine of subjects, including two chemicals found in the urine after 2,4-D exposure: unmetabolized 2,4-D and 2,4-dichlorophenol. No urinary marker of 2,4-D was associated with any deficit in any of the domains of neurobehavior that were tested. In contrast, increased urine levels of the trace metabolite 2,4-dichlorophenol were associated with an improved performance on the serial digit learning test ($p = 0.0002$).

Update of the Epidemiologic Literature

Since *Update 2014*, no new studies of Vietnam veterans or case-control studies that examined neurobehavioral, cognitive, or neuropsychiatric outcomes in relation to exposure to the COIs have been published.

Occupational Studies

In a study of workers exposed to PCBs from the Dortmund transformer and capacitor recycling plant in Germany, Fimm et al. (2017) examined a broad range of cognitive functions covering attention, executive processing, reasoning, memory, and motor performance. The cohort consisted of 237 individuals, half of whom were workers, and the other half were family members of the workers, employees of surrounding companies, and area residents. The mean age was 44 years, and 17% had completed high school, that is, 12–13 years of education, with the vast majority having completed 8–10 years and a few having completed less than 8 years of education. Subjects were excluded primarily for lack of German fluency, leading to 187 individuals with complete data on the neuropsychological battery. Blood plasma PCB levels were determined and analyzed as three mutually exclusive classes: low chlorinated (PCB 28, 52, 101), high chlorinated (PCB 138, 153, 180), and dioxin-like PCBs, which included mono- and non-ortho PCBs. However, fewer than 10% of the participants had detectable levels (limit of detection = 0.01 ug/L blood plasma) of the non-ortho (coplanar) PCBs (77, 81, 126, and 169), and hence these PCBs were excluded from the analysis, leaving the mono-ortho PCBs 105, 114, 118, 123, 156, 157, 167, and 189. Exposure was dichotomized into high and low, with the 95th percentile for each congener serving as the cutpoint. For each class (low, high, and dioxin-like), an individual was placed in the high category if his or her level of at least one congener in that class was elevated. With this definition, 72% of the original 237 people were classified as having high dioxin-like PCB exposure, though many of them also had high exposures to low- or high-chlorinated PCBs, or both. Forty of the participants had elevated levels for all of the dioxin-like PCB congeners. A structural equations model was fit to identify correlations among the various tests

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and PCB burdens. In multiple linear regression models, adjusting for education, dioxin-like PCBs showed no associations with any of the cognitive (vocabulary and other achievement, non-verbal reasoning), visuo-spatial processing, word fluency, flexibility, verbal and visual memory, nonverbal (geometric) learning, and several tests of attention and alertness. However, in the four sensorimotor tasks designed to assess fine motor coordination and the precision of arm/hand movement, dioxin-like PCBs were associated with poorer line tracking; specifically, a significant decrease ($\beta = -0.206$, $p = 0.002$) in performance on this test of fine motor control was associated with a 10-fold increase in the sum of 8 dioxin-like PCBs. The high-chlorinated PCBs were associated with poorer aiming, another fine motor task; in contrast, the low-chlorinated PCBs were associated with reduced scores on verbal tests.

Environmental Studies

Przybyla et al. (2017) conducted an analysis of the associations of whole-blood concentrations of four dioxin-like PCBs (118, 126, 156, and 169), six non-dioxin-like PCBs, and two metals (lead and cadmium) with cognitive impairment in adults aged 60–84 years as measured by the Digit Symbol Coding Test of the Wechsler Adult Intelligence Scale. The study sample consisted of 498 men and women who participated in the 1999–2000 and 2001–2002 cycles of NHANES and had not had a stroke. The final model included only those five neurotoxins (including PCB 118) that were significantly ($p \leq 0.05$) associated with cognitive functioning. Estimates were adjusted for poverty–income ratio, education, race, age, sex, and smoking status. Lower cognitive scores were found for older adults who had higher concentrations of PCB 146 ($\beta = -0.16$, 95% CI −0.29– –0.02; $p = 0.02$), whereas higher scores were observed for increased concentrations of PCB 153, both of these being non-dioxin-like PCBs. Lower cognitive scores were also found for older adults who had higher concentrations of PCB 118, but the association was not statistically significant ($\beta = -0.06$, 95% CI −0.20–0.08; $p = 0.41$). The results are difficult to interpret because PCBs are generally strongly correlated with one another (the substantial change in the beta coefficient comparing the fully adjusted model with the model with only the single PCB 153 provides evidence of such correlations in these data). Adjusting the model for highly correlated variables can introduce a large bias in a non-predictable direction. Thus, it is difficult to interpret the findings of this study, although it should be noted that the cross-sectional nature is not a weakness, given that the half-lives of these compounds are generally a decade or longer.

Ames et al. (2018) reported on neurocognitive and physical functioning in the Seveso Women's Health Study, which enrolled women who were between newborn and 40 years of age at the time of the explosion of the Seveso chemical plant in 1976 and who lived in the two closer exposure zones (A and B). The women were evaluated for their physical function in 1996 ($n = 154$) and with

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neurocognitive tests in 2008 (n = 459). All the women assessed for physical function were post-menarche at the time of the explosion and post-menopausal at the time of testing. Archived serum samples collected in 1976 that were stored at −20° C were used for an analysis of TCDD. Serum TCDD levels were reported in pg/g lipid or parts per trillion. The physical function tests were a 10-foot walking test of functional mobility, a coin-flipping test of manual dexterity, a grip strength test, and a reach down test of lower-body mobility. Working memory was assessed on the Wechsler Adult Intelligence Scale digit span and spatial span tests, each with both backward and forward tests. Multiple linear regression models were used to assess the dose–response relationship between TCDD concentration and the outcomes of interest, with a squared term to assess consistency with a linear relationship; they also conducted further sensitivity analyses using semiparametric methods to make fewer assumptions about functional forms. The potential confounding variables included educational attainment, smoking, alcohol consumption, age at interview, age at explosion, menarche status at explosion, menopause status (pre versus post) at interview (> 12 months without a menstrual cycle or surgical menopause), body mass index (BMI) category (referent: BMI < 25 kg/m^2, overweight: BMI ≥ 25 kg/m^2 and < 30 kg/m^2, and obese: BM ≥ 30 kg/m^2), and marital status. Directed acyclic graphs were used to inform covariate selection into the initial adjusted model, which was pared down following a change-in-estimate approach, retaining variables if their exclusion caused > 10% change in the TCDD coefficient. Results showed that serum TCDD was not associated with walking speed, upper-body mobility, or manual dexterity ($p > 0.05$ for these outcomes). An inverted U-shaped association was observed for grip strength, with poorer strength at both lowest and highest TCDD exposure levels, with significance for both the linear and quadratic terms in the non-dominant arm, and borderline significance in the dominant arm.[1] There was no association between TCDD and the Wechsler digit and spatial span tests ($p > 0.05$). Neither menopause status at assessment nor age at exposure modified the associations between TCDD and working memory, that is, the lack of association was observed in both the pre- and post-menopausal women and at all ages of exposure following the Seveso explosion.

[1] $p = 0.01$ for both linear (positive) and quadratic (negative) beta coefficients in the non-dominant arm; in the dominant arm, p-values were reported as < 0.05 for both the linear and quadratic terms in the table of the paper, but 0.07 and 0.08, respectively, in the figure; this discrepancy was due to use of the robust variance estimates for the figure but not the table (personal communication with the first author, Jennifer Ames).

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Other Identified Studies

One additional study in this area was identified by the committee, but it examined biologic markers of effect of neurotransmission pathways that do not relate to a diagnosable health outcome, and therefore it was given limited consideration. Putschogl et al. (2015) used health information and blood and urine samples collected from people who either worked at a transformer and capacitor recycling plant in Dortmund, Germany, or else who lived in the immediate area and might have been exposed to dioxin-like (PCBs 105, 114, 118, 156, 157, 167, and 189) and non-dioxin-like PCBs as a result of contamination of the area by the facility. The focus of the study was on determining associations with neurotransmitter metabolites for dopamine (homovanillin acid) and norepinephrine (vanillylmandelic acid) in urine as markers of targeted effects on these specific neurotransmission pathways. All 13 dioxin-like and non-dioxin-like PCB congeners examined were significantly associated with lower urinary metabolite levels of homovanillin acid after adjustment for creatinine. Moreover, highly chlorinated congeners were more strongly associated with increased concentration of homovanillin acid but significantly reduced concentration of vanillylmandelic acid, after adjustment for creatinine. These metabolites, however, are not specific to neuronal sources, as the dietary consumption of foods with high monoamine content (e.g., cheese, orange juice, and bananas) can also contribute to homovanillin. Only 12% of peripheral homovanillin acid derives from the brain, and vanillylmandelic acid is heavily influenced by hepatic function. Neither information on diet nor diagnoses of hypertension were collected, which may confound the association. Nevertheless, these measured metabolites are a poor indicator of CNS neurotransmission, and the problems with the overall design and analysis of the study (detailed in Chapter 5) seriously limit its contribution to the scientific evidence on effects of exposure to the COIs.

Biologic Plausibility

Some toxicologic studies have suggested a possible involvement of the COIs in the occurrence of neurobehavioral effects. Akahoshi et al. (2009) produced a mouse neuroblastoma cell line that overexpressed the Ahr, a TCDD-induced protein hypothesized to be important in the synthesis of dopamine, whose perturbation has been implicated in a number of neurobehavioral syndromes. An elevated expression of Ahr in these cells was associated with an increased production of neurotransmitters and augmented dopamine expression, but the implication of that finding is not clear. In vitro exposure of human CD34+ cells to TCDD has been associated with a modulation of gene expression of the GABAergic pathway, which may be associated with altered synaptic transmission, visual perception, and other neurologic conditions (Fracchiolla et al., 2011). Lensu et al. (2006) treated rats with 50 µg/kg TCDD or with leptin, a chemical with

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well-recognized effects on food consumption. When certain brain areas of the two treated animal groups were compared 24 hours later, the results were not consistent with a primary role for the hypothalamus brain region in TCDD-induced wasting syndrome.

Synthesis

There is not consistent epidemiologic evidence of an association between exposure to the COIs and neurobehavioral or cognitive disorders. One occupational study (Fimm et al., 2017) and two environmental studies (Ames et al., 2018; Przybyla et al., 2017) examined an array of neurocognitive tests, and none showed evidence of deficits in cognitive function or attention or alertness. An analysis of the Seveso Women's Health Study cohort found reductions in grip strength, one of four measures of motor function (Ames et al., 2018). More research on the COIs and these endpoints may be warranted, especially for adverse effects of the COIs on motor function.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and neurobehavioral (cognitive or neuropsychiatric) disorders.

NEURODEGENERATIVE DISEASES

This section summarizes the findings on neurodegenerative diseases—specifically PD, ALS, and AD—discussed in previous VAO reports. While multiple sclerosis would also be considered in this section, until *Update 2014* no published epidemiologic studies for it in relation to exposure to the COIs were identified. A publication by H. K. Kang et al. (2014a) found an elevated risk of death from multiple sclerosis among female Vietnam-era veterans; however, the estimate was imprecise due to the small number of cases. Among Korean veterans, Yi et al. (2014a) found no association between prevalent multiple sclerosis and herbicide exposure, but this very large study with substantial statistical power did identify associations for a number of neurological conditions (paroxysmal disorders, nerve/plexus disorders, and paralytic syndromes), which are also considerably more specific than the outcomes evaluated in previous VAO updates.

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Parkinson Disease and Parkinsonism

PD is a progressive neurodegenerative disorder that affects an estimated 6.3 million people worldwide (EBC, 2018), and it is the second-most common neurodegenerative disease (after AD). Its primary clinical manifestations are bradykinesia, resting tremor, cogwheel rigidity, and gait instability. These signs were first described as a single entity in 1817 by James Parkinson. Many non-motor manifestations of PD have been described, and they can precede the motor symptoms or present together with the motor symptoms of the disease. These include cognitive dysfunction that often progresses to frank dementia, sleep disturbances, hallucinations, psychosis, mood disorders, fatigue, and autonomic dysfunction affecting gastrointestinal, urinary, and heart function (Langston, 2006).

In the nearly two centuries since its initial description, much has been learned about some genetic predispositions and the pathophysiology of PD, but its etiology in most patients remains largely unknown, with environmental risk factors being largely understudied. The diagnosis of PD is based primarily on a clinical examination, although in recent years magnetic resonance imaging and functional brain imaging have become increasingly useful. Idiopathic PD at its onset may be difficult to distinguish from a variety of Parkinsonian syndromes, including drug-induced Parkinsonism, and neurodegenerative diseases, such as multiple systems atrophy, that present with Parkinsonian features but also develop additional brain abnormalities. Ultimately, a diagnosis of idiopathic PD can be confirmed with a postmortem pathology examination of brain tissue showing the characteristic loss of neurons from the substantia nigra and telltale protein aggregates known as Lewy body intracellular inclusions. Pathology findings in other forms of Parkinsonism show different patterns of brain injury and protein aggregation. Mortality and hospitalization records are systems with poor diagnostic standards for PD, and tend to under-report it. For example, on death records, PD is usually mentioned as a contributing cause instead of an underlying cause of death, and as such, leads to under-reporting. For PD, studies have shown that at best 60% of PD patients have PD mentioned on their death certificates (Benito-León et al., 2014; Désesquelles et al., 2014; Moscovich et al., 2017). Hospitalizations for PD do not occur until very late in the disease process, if at all, and thus even though the accuracy of hospital records in terms of diagnosis might be better than death certificates, they may miss the less severe cases. However, unless the exposure also contributes to differential under-reporting of PD on death certificates or admission to hospitals (and there is no obvious reason why this would be the case, unless exposed subjects are followed for disease more carefully by their health care system) one would expect disease misclassification to be non-differential. Therefore, as long as the specificity of the diagnosis is close to perfect (those who are listed as PD truly have the disease, which is what would be expected from death certificates and hospital records) the effect estimate would not be biased. Although the gold standard of diagnosis is pathology of the protein aggregates

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in the brain (Lewy-bodies), this standard is rarely, if ever, achieved in an epidemiologic investigation due to the low rate of autopsies or brain collection. On the other hand, the longer the disease durations, the more likely it is that the diagnosis is accurate (Adler et al., 2014; Wermuth et al., 2012, 2015). Diagnosis is more accurate for patients who develop PD when they are younger than 80 years, and the youngest onset patients are likely the most accurately diagnosed. Clinical accuracy also is much higher if patients are diagnosed in specialty clinics of tertiary care facilities (by movement disorder specialists).

Several studies have attempted to estimate the incidence and prevalence of PD, but methodological differences among the studies make direct comparisons of such estimates difficult. Overall prevalence estimates of PD range from 100 to 200 per 100,000 people (von Campenhausen et al., 2005), but a recent meta-analysis of 47 studies published between 1985 and 2014 estimated an overall PD prevalence of 315 (95% CI 113–873) per 100,000 people (Pringsheim et al., 2014). Restricting the analysis to the highest-quality studies, the estimated prevalence was 571 (95% CI 243–1,339) per 100,000 people, and the authors suggested that higher-quality studies may provide a more precise estimate of disease prevalence. Stratifying on age, the prevalence estimates clearly increase with increasing age: 41 per 100,000 in individuals 40–49 years; 107 per 100,000 in individuals 50–59 years; 428 per 100,000 in individuals 60–69 years; 1,087 per 100,000 in individuals 70–79 years; and 1,903 per 100,000 in individuals over age 80 years (Pringsheim et al., 2014). Meta-analyses and other data summaries suggest a slight male preponderance in the incidence and prevalence of PD, with PD starting earlier in men (Georgiev et al., 2017).

Similarly, the estimates of PD incidence in published studies vary considerably, possibly because of methodological differences in case ascertainment and in which diagnostic criteria were applied. In a 2017 review, Tysnes and Storstein (2017) estimated that the annual incidence of PD per 100,000 inhabitants ranges from less than 10 to more than 20. A meta-analysis by age group and sex found that males had a higher incidence of PD in all age groups and that the overall incidence rate of PD for people 40 years and older was 37.55 per 100,000 person-years (95% CI 26.20–53.83) for females compared with 61.21 (95% CI 43.57–85.99) in males (Hirsch et al., 2016). The incidence rate for both males and females increases with age. For females the incidence rates by age group were calculated as: 3.26 per 100,000 in individuals 40–49 years, 8.43 per 100,000 in individuals 50–59 years, 30.32 per 100,000 in individuals 60–69 years, 93.32 per 100,000 in individuals 70–79 years, and 103.48 per 100,000 in individuals over age 80 years. For males, the incidence rates by age group were: 3.57 per 100,000 in individuals 40–49 years, 14.67 per 100,000 in individuals 50–59 years, 58.22 per 100,000 in individuals 60–69 years, 162.58 per 100,000 in individuals 70–79 years, and 258.47 per 100,000 in individuals over age 80 years.

Research on the genetic, epigenetic, and environmental causes of PD suggests multiple risk factors, including aging, environmental exposure, and genetic

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predisposition (Gao and Hong, 2011; Kwok, 2010). The peak incidence and prevalence of PD are consistently found in people 60–80 years of age. A consensus statement from a 2007 meeting of PD experts (Bronstein et al., 2009) concluded that, in addition to firm evidence that the toxicant 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine (MPTP) can induce PD, there is substantial evidence that men are at greater risk than women, while smoking and coffee consumption are associated with a reduced risk. Of note, it has been proposed that the latter factors—especially smoking—may not be protective but rather a case of reverse causation (Ritz et al., 2014). Specifically, the higher rate of quitting smoking and reduction in caffeine intake observed among PD patients may be prodromal behavior changes in the long premotor phase of PD when many non-motor symptoms occur, such as the loss of smell, digestive problems, and sleep disturbances. Further evidence of environmental exposures playing a role in the development of PD has continued to accrue (Chin-Chan et al., 2015; Mostafalou and Abdollahi, 2017; Ritz et al., 2016; Tanner et al., 2014).

Heredity has long been suspected as an important risk factor for PD. Although risk estimates of PD for first-degree relatives of a person with PD vary from study to study and from country to country, among large studies in the United States first-degree relatives of an affected individual are 2.7–3.5 times more likely to develop PD than an individual without a family history of it. For these first-degree relatives, the cumulative lifetime risk of developing PD is between 3% and 7% (Farlow et al., 2014). An estimated 5–10% of people with PD have monogenic forms of it, which exhibit a classical Mendelian type of inheritance, but the majority of PD cases are sporadic (Kalinderi et al., 2016). All known monogenic forms of PD combined explain only about 30% of familial and 3–5% of sporadic cases (K. R. Kumar et al. 2011). At least 13 gene mutations have been identified in autosomal dominant PD, including mutations in parkin and α-synuclein (Klein and Lohmann-Hedrich, 2007). Mutations associated with an autosomal recessive inheritance pattern have also been described; however, these disease genes are found in only a handful of familial cases worldwide. More complex genetics that act to increase susceptibility to PD in conjunction with environmental risk factors may play a much more important role, and this has been the focus of much study in the recent decade (Ritz et al., 2016, 2017).

Conclusions from *VAO* and Previous Updates

The committees responsible for *VAO*, *Update 1996*, *Update 1998*, *Update 2000*, *Update 2002*, *Update 2004*, and *Update 2006* all concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and PD. Five case-control studies reviewed by those committees had investigated the association between PD and "herbicide" exposure without providing further specificity and had reported mixed findings.

Two studies reviewed in *Update 2008* examined the association specifically with chlorophenoxy acid and ester herbicides and found increased odds ratios

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(Brighina et al., 2008; Hancock et al., 2008). The doubling in risk observed by Hancock et al. (2008), however, did not achieve formal statistical significance. Increases in the PD risk for the chemical class of chlorophenoxy acids or ester herbicides were reported by Brighina et al. (2008) in that patients with PD were 52% more likely than control subjects to have used these pesticides. In the Agricultural Health Study (AHS), incident PD was related in a dose–response manner to increasing days of pesticide use in general (Kamel et al., 2007b). In analyses of single pesticide exposures, 2,4-D did not increase PD incidence risk, but 2,4,5-T exposures did increase risk by 80% in adjusted hierarchical logistic regression models. On the basis of that evidence, the committee for *Update 2008* concluded that there was limited or suggestive evidence associating exposure to the COIs with PD. Additional studies considered by the committees responsible for *Update 2010* and *Update 2012* led them to affirm this conclusion.

The committee responsible for *Update 2014* was charged specifically with determining whether various diagnoses with Parkinsonian symptoms should be included in the presumptive service-related category for PD. Because diagnostic specificity is improbable in both the studies on which the conclusion of limited or suggestive association with exposure to military herbicides was based and in the documentation for the claims submitted to the Department of Veterans Affairs (VA) by Vietnam veterans, the committee clarified that the finding for PD should be interpreted by VA to include all diseases with Parkinson-like symptoms unless those symptoms can be definitively shown to be secondary to an external agent other than the herbicides sprayed in Vietnam.

Update 2014 included three studies of Vietnam veterans: one U.S. and two from the Korean Veterans Health Study. Among three cohorts of U.S. Vietnam-era military women—4,734 deployed to the theater of the war, 2,062 who served in countries near Vietnam, and 5,313 who were not deployed and served primarily in the United States—PD mortality, adjusted for age, race, duration of military service, officer status, and nursing status, was not different in those deployed to Vietnam from the non-deployed cohort, and there was no suggestion of an increase when this comparison was made for the subsets of only nurses (H. K. Kang et al., 2014a). In the Korean Veterans Health Study, 180,639 Korean veterans were followed for vital status and cause of death (Yi et al., 2014b). An exposure opportunity index (EOI) score was assigned to each veteran based on the proximity of his unit to the herbicide-sprayed areas. No association was found between PD mortality and the individual EOI scores or when the high-exposure group was compared with the low-exposure group. A second analysis compared the high- and low-exposure groups with respect to the prevalence of primary PD (*International Classification of Diseases*, 10th Revision [ICD-10] G20) and secondary Parkinsonism (ICD-10 G21). Effect estimates that were adjusted for age, rank, smoking, drinking, physical activity, the domestic use of herbicides, education, income, and BMI were less suggestive of an association with herbicide exposure than were the unadjusted results (Yi et al., 2014a).

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Environmental (Weisskopf et al., 2012) and occupational (van der Mark et al., 2014) exposure studies reviewed in *Update 2014* did not support a statistically significant association between exposure to the COIs and PD.

Update of the Epidemiologic Literature

One new study of Parkinson disease or Parkinsonism among Korean veterans who served in Vietnam was identified. No other studies of populations that specifically assessed exposure to the COIs have been published since *Update 2014*. Table 39, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to Parkinson disease and Parkinson-like conditions.

Vietnam-Veteran Studies Y. S. Yang (2016) conducted an analysis of the effect of exposure to Agent Orange on Korean veterans who served in Vietnam and had been diagnosed with PD. The study consisted of 143 PD patients with exposure and 500 PD patients without exposure to Agent Orange, as determined by self-report and a military record–verified "perceived exposure index." Comparing these patients' clinical characteristics and their radiolabeled 18F-FP-CIT PET uptake, this study found differences in clinical profile ($p < 0.05$) using motor subscales from the Unified Parkinson's Disease Rating Scale III: tremor at rest, rigidity, finger taps, and rapid alternating movement. Findings from the use of FP-CIT PET showed lower uptake in basal ganglia and higher asymmetry in exposed ($p < 0.05$) versus unexposed patients. The authors suggested the possibility that the PD in patients exposed to Agent Orange has a different pathophysiology from idiopathic PD.

Other Identified Studies The committee identified several additional studies that examined PD mortality or prevalence among occupational cohorts or in different populations with environmental exposures. However, each of them lacked the necessary exposure specificity to be considered further as contributing to the evidence base of the potential effect of the COIs. For example, although Ruder et al. (2014) examined U.S. workers exposed to mixed PCBs, the specific dioxin-like PCBs were not investigated separately, and no TEQs or other quantification of relevant exposures was presented. Likewise, a case-control study of clinically confirmed PD among French male farmers assessed occupational pesticide and herbicide use, duration, intensity, and cumulative exposure but did not report the specific chemicals or classes (Moisan et al., 2015). Two environmental studies were also identified—one among the residents of rural central California (Narayan et al., 2015) and the other conducted in the Netherlands (Brouwer et al., 2015), but neither specified the herbicides used in enough detail or used objective measures of exposure, such as serum concentrations, to contribute to the evidence base of exposure to the COIs and PD.

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Biologic Plausibility

McDowell and Chesselet (2012) reviewed the literature on the ability of both toxicant-induced (6-hydroxydopamine, MPTP, rotenone, cycad) and genetically based animal models to reproduce the non-motor symptoms of PD. The very clear PD-like toxicity resulting from human exposure to MPTP indicates that select chemicals can produce the same type of damage to dopaminergic neurons as occurs in classical PD, and MPTP has become an important toxicant in studies that use animal and in vitro models. It is notable that MPTP's bioactive metabolite, MPP+, is similar in chemical structure to paraquat (a commonly used herbicide, but not one that was used in Vietnam), but structurally unrelated to any of this report's COIs. Pesticides shown to produce PD-like toxicity in animal models include paraquat, rotenone, maneb, and dieldrin. Substantial research has gone into understanding the molecular mechanisms responsible for the toxicity, especially in connection with paraquat and rotenone (Blandini and Armentero, 2012; Di Monte et al., 2002; Drechsel and Patel, 2008; Duty and Jenner, 2011; Hatcher et al., 2008; Moretto and Colosio, 2013; Nunomura et al., 2007; Sherer et al., 2002; Yadav et al., 2012). The damage done to dopaminergic neurons in PD is probably caused by oxidative stress and inflammation and may well also involve damage to the mitochondria in the target cells (Anderson and Maes, 2014: Janda et al., 2012; Liang et al., 2007; Littleljohn et al., 2011; Sarnico et al., 2008).

The COIs are known to be distributed to the CNS. Cholanians et al. (2016) showed that arsenic exposure leads to an accumulation of α-synuclein in both cultured cells and adult rats. The accumulation of α-synuclein plays a key role in the pathogenesis of PD, suggesting that exposure to arsenical herbicides may have an influence on PD progression. Bongiovanni et al. (2007) found that rat cerebellar granule cells in culture (an in vitro model using cells not involved in PD pathology) produce increased concentrations of reactive oxygen species when exposed to 2,4-D. González-Barbosa et al. (2017) showed that TCDD exposure causes dysregulation of the UbcH7–parkin complex in the ventral midbrain of the mouse, but further studies are needed to characterize the consequences for dopaminergic cells. The COIs have not been investigated in experimental systems such as those that have shown that paraquat and other compounds cause inflammation and oxidative stress, so it is not known whether any of the COIs could produce these responses.

Research on the neurotoxicity of 2,4-D has been going on for a number of years, but most of it has focused on its effects on the developing rodent nervous system. The studies have often used high doses of 2,4-D that have resulted in adverse changes in the developing nervous system—both neurochemical (such as changes in D2 receptors, tyrosine hydroxylase, and dopamine beta-hydroxylase) and behavioral (for example, Bortolozzi et al., 1999, 2002, 2003, 2004; Duffard et al., 1996; Evangelista de Duffard et al., 1990, 1995; Garcia et al., 2004, 2006; Rosso et al., 2000a,b). The injection of 2,4-D directly into the rat brain results in

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toxicity in the basal ganglia (Bortolozzi et al., 2001), but this route of administration is highly artificial. The postpartum dietary exposure of females to 2,4-D results in adverse alterations in maternal behavior and neurochemical changes, including increases in dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid (Stürtz et al., 2008). Such an increase in dopamine is the reverse of what is seen in PD, in which a degradation of the dopaminergic system occurs. In addition, a study of mice and 2,4-D yielded no evidence of neurochemical damage to the dopaminergic system (Thiffault et al., 2001). Because most of the studies were on the developing nervous system, not the mature nervous system, and some studies yielded evidence of a lack of a role of 2,4-D in the development of PD, the existing studies do not support a role for the COIs in the etiology of PD.

Synthesis

Previously reviewed studies of PD in Vietnam veterans have not shown increased mortality or incidence of PD in U.S. or Korean Vietnam veterans. One new epidemiologic study of PD in Korean veterans who served in Vietnam was identified. Findings from the study suggested the possibility of a different pathophysiology for PD in patients exposed to Agent Orange than for idiopathic PD in terms of its clinical characteristics and brain ligand measures (fluororidopa PET). A biologic mechanism by which the COIs may cause PD has not been demonstrated. Nevertheless, the overall epidemiologic evidence continues to support an association between herbicide exposure and PD and to be consistent with an association with exposure to the phenoxy herbicides specifically.

Conclusion

On the basis of the lack of new evidence reviewed here supporting or refuting an association with PD, and given the evidence presented in previous VAO reports, the committee maintains the conclusion that there is limited or suggestive evidence of an association between exposure to the COIs and PD, including Parkinson-like conditions such as Parkinsonism, in the setting of dementia, multiple system atrophy, and progressive supranuclear palsy.

Amyotrophic Lateral Sclerosis

ALS is a progressive, adult-onset, motor neuron disease that presents with muscle atrophy, weakness, and fasciculations and with signs that indicate the involvement of motor neuron pathways in the CNS. The incidence of sporadic ALS is 1–2 per 100,000 person-years, and it peaks at the ages of 55–75 years (Brooks, 1996). The incidence of ALS in European populations is 2–3 people per 100,000 person-years (Johnston et al., 2006). The diagnosis of ALS is made through

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clinical examination and electrodiagnostic testing and has a high degree of accuracy when performed by experienced neurologists (Rowland, 1998; Rowland and Shneider, 2001). ALS is generally more common in men than in women by a factor ranging from 1.2 to 1.5, depending on the age group (Manjaly et al., 2010). Established risk factors to date are older age, male gender, and a family history of ALS (Couratier et al., 2016).

In most cases the cause of ALS is unknown, but about 5–10% of cases are recognized as resulting from the inheritance of autosomal dominant or recessive genes (Wood, 2014); 20% of familial-ALS patients have mutations in the gene that encodes for superoxide dismutase-1 (Rosen et al., 1993). Many other possible etiologic factors have been investigated (Breland and Currier, 1967; Gallagher and Sander, 1987; Hanisch et al., 1976; H. Kang et al., 2014b; Kurtzke and Beebe, 1980; Mitchell and Borasio, 2007; Roelofs-Iverson et al., 1984; Sutedja et al., 2009a,b; M. D. Wang et al., 2014), including military service (Weisskopf et al., 2005), but no conclusive evidence has been found of an association with any of the environmental exposures addressed. Pesticides have also been suggested as a risk factor for ALS, but the results of individual studies and meta-analyses have been mixed depending how exposure was defined, and herbicides were seldom specified and not reported as a separate category (Kamel et al., 2012; H. Kang et al., 2014b; Malek et al., 2012). H. Kang et al. (2014b) expanded inclusion criteria for their meta-analysis of pesticide exposures to include the occupation of "farmer" and a rural residence. Although some of the pesticide exposures included herbicides, they were not reported as separate estimates, and thus although an increased risk of ALS was associated with pesticide exposure and with having an occupation of farmer, these results are of limited usefulness.

Conclusions of *VAO* and Previous Updates

ALS was first evaluated as a disease that might be associated with the COIs by the committee for *Update 2002*. Pesticide or herbicide exposure has been associated with an increased risk of ALS, including a doubling of the risk after long-term occupational exposure to pesticides (Deapen and Henderson, 1986) and a tripling after exposure to agricultural chemical products (Savettieri et al., 1991) and herbicides (McGuire et al., 1997), but none of the risk estimates was statistically significant. A population-based case-control study demonstrated associations between an exposure to agricultural chemical products and ALS in men, with an OR of 2.4 and a trend with duration of exposure that were both statistically significant (McGuire et al., 1997). A mortality study of Dow Chemical Company employees exposed to 2,4-D found three deaths from ALS, which resulted in a statistically significant—but imprecise—positive association (C. J. Burns et al., 2001). Weisskopf et al. (2005) followed the vital status of subjects in the American Cancer Society's cohort for the Cancer Prevention Study II and found an increased risk of ALS in those who served in any of the armed services

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during times of conflict. They adjusted for a variety of confounding variables in their model, including exposure to herbicides, and found that none of them significantly altered their conclusions; thus, this large study indirectly suggests the lack of a strong effect of herbicide exposure on ALS risk. A latter analysis of the American Cancer Society's Cancer Prevention Study II also found no association between self-reported pesticide or herbicide exposure and ALS, but the lack of exposure specificity and the possibility of exposure estimation error limit the weight of this evidence (Weisskopf et al., 2009). Malek et al. (2014) compared 66 ALS patients to 66 controls, administering a questionnaire on occupational, vocational, and avocational exposures, and found self-reported pesticide exposure to be associated with ALS. The association was even stronger after controlling for smoking and education in a multivariate model but quite imprecise. Additional analyses conducted on occupational exposure to insecticides, to herbicides, or to fungicides and fumigants, individually, found no associations with ALS, but the sample sizes were very small. None of the results is based on sufficiently specific exposure metrics to be fully informative for VAO purposes.

Studies of veterans who served in Vietnam have reported weak and generally not statistically significant associations with the risk of ALS. A case-control study of Australian Vietnam veterans reported an association between deployment in Vietnam and ALS (ADVA, 2005c) but did not specifically study exposure to pesticides or herbicides. In the Korean Veterans Health Study, Yi et al. (2014b) calculated individual EOI scores based on the proximity of the veteran's unit to given areas when herbicides were sprayed and partitioned the veterans into high- and low-exposure groups. No association was found between spinal muscular atrophy and the EOI scores or when the high-exposure group was compared with the low-exposure group. However, in the analysis of disease prevalence among the same Korean Vietnam veteran cohort, after adjusting for age, rank, smoking, drinking, physical activity, domestic use of herbicides, education, income, and BMI, Yi et al. (2014a) found the risk for spinal muscular atrophy to be slightly elevated in both the analysis of the scores comparing high- and low-exposure group and as a continuous variable. The more specific diagnosis of motor neuron disease (ICD-10 G12.2), which includes ALS, had nearly the same risk estimate, but because these cases represented only about one-third of those in the entire spinal muscular atrophy grouping, estimates were more imprecise.

Update of the Epidemiologic Literature

Two studies of ALS in Vietnam veterans and one case-control study of occupational exposures and environmental toxicants on the odds of developing ALS have been identified since *Update 2014*. Table 40, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to ALS.

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Vietnam-Veteran Studies Beard et al. (2016) used the National Registry of Veterans with ALS to identify medical record–confirmed ALS cases from 2005 to 2010 for veterans who consented at the time of enrollment in the registry to participate in further studies. Controls were selected from the Veterans Benefits Administration's Beneficiary Identification and Records Locator System database. A total of 958 controls who were located, screened, and found to be eligible to participate were frequency-matched to 621 cases based on diagnosis age within 5 years and on socioeconomic status, as roughly estimated by the veteran's use of the VA health care system before the date of diagnosis. All participants were followed until 2013. Clinical characteristics were extracted from medical records, and standardized telephone interviews were used to collect self-reported information on military service (branch of the longest service, number of branches, rank, the total service time, and the end of the most recent service), deployments (number; locations; time in theater; ever received imminent danger pay, hardship duty, or combat zone tax exclusion benefits for deployment to 17 foreign countries or five sea regions [plus fill-in options]), and exposures that occurred before the diagnosis date (cases) or the interview date (controls) as well as on potential confounders. Self-reported information on 39 specific military exposures was also collected, some of which were conflict-specific (e.g., Agent Orange exposure during the Vietnam War). For 31 of 32 war-specific exposures, participants were asked whether they had ever been exposed, days exposed (not exposed, ≤ 5, 6–30, > 30), and whether they felt ill after exposure (not exposed, no, yes). Inverse probability weighting was used to adjust for potential bias from confounding, missing covariate data, and selection arising from a case group that disproportionately included long-term survivors and a control group that may or may not have differed from U.S. military veterans at large. The study population consisted of 302 cases and 3,793 veterans who were aged 18–25 years during the Vietnam War. War-specific exposures for these veterans were analyzed separately, although the authors did not present all such results independently from the analysis that included all veterans and not all veterans served in that war. For those results presented, 58 cases and 77 controls reported exposure to Agent Orange in the field (OR = 2.80, 95% CI 1.44–5.44), and 8 cases and 13 controls reported mixing and application of Agent Orange (OR = 1.15, 95% CI 0.38–3.44). Vietnam veterans diagnosed with ALS reported greater exposure to pesticides on clothing or bedding (OR = 1.83; 95% CI 0.99–3.40). Overall, ALS was positively associated with an exposure to herbicides for military purposes, nasopharyngeal radium, personal pesticides, exhaust from heaters or generators, high-intensity radar waves, contaminated food, explosions within 1 mile, herbicides in the field, mixing and application of burning agents, burning agents in the field, and Agent Orange in the field.

In a second study using the same registry population of ALS veteran cases, Beard et al. (2017) examined associations between military-related factors and

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ALS survival. A total of 616 medical record–confirmed cases were followed from enrollment in the registry (2005–2010) until death or July 25, 2013, whichever came first. Vital status information was obtained from several sources within VA, and information regarding military service, deployments, and 39 related exposures was collected from self-report via a standardized telephone interview (see details above in Beard et al. [2016]). Inverse probability weights were used to adjust for potential confounding and missing covariate data biases as well as to adjust for potential selection bias among a case group that included a disproportionate number of long-term survivors at enrollment. In the study population, 137 veterans diagnosed with ALS had reported service in Vietnam. The war-specific exposures for these veterans were analyzed separately, although the authors did not present all such results independently from the analysis that included all veterans. A total of 446 deaths occurred during 24,267 person-months of follow-up (median follow-up: 28 months). For exposures constrained to deployment in Vietnam, longer survival was associated with exposure to Agent Orange in the field (n = 39 deaths; hazard ratio [HR] = 0.66, 95% CI 0.42–1.05) and mixing and application of Agent Orange (n = 6 deaths; HR = 0.62, 95% CI 0.32–1.20); however neither exposure estimate is statistically significant.

Case-Control Studies F. C. Su et al. (2016) conducted a case-control study to evaluate the association of occupational exposures and environmental toxicants with the odds of developing ALS in Michigan. ALS cases (n = 156) with a diagnosis of definitive, probable, probable with laboratory support, or possible ALS by revised El Escorial criteria were recruited from a tertiary referral center for ALS. Controls (n = 128) were recruited from postings and the University of Michigan clinical research volunteer database and were excluded if they were diagnosed as having ALS or another neurodegenerative condition or if they had a family history of ALS in a first- or second-degree blood relative. Participants completed a self-administered written survey that collected information on demographics, occupational and residential exposures, military service, and smoking history. Specifically, there were 58 identified exposure risk factors, 20 occupational groups, and 20 industrial groups queried for each job. Blood samples were collected from 129 cases and 119 controls and measured for concentrations of 122 persistent environmental pollutants, including organochlorine pesticides, PCBs (and dioxin-like PCB 118, specifically), and brominated flame retardants. Odds ratios and 95% CIs were calculated using standardized chemical concentrations from the imputed sample (n = 284, 10 imputations) and adjusted for age, sex, and educational levels. An effect estimate for PCB 118 was calculated only for the model that used individual compounds as covariates, which showed exposure to be associated with a decreased risk of ALS (OR = 0.69, 95% CI 0.48–1.01).

Other Identified Studies One additional study of an occupationally exposed cohort and ALS was identified (Ruder et al., 2014), but it lacked the necessary

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exposure specificity or quantification to be considered further as contributing to the evidence base of the potential effect of exposure to the COIs on ALS.

Biologic Plausibility

Several studies have addressed the mechanisms of neurotoxicity that might be ascribed to COIs, notably 2,4-D and TCDD. Some of those effects suggest possible pathways by which the COIs could disrupt neuronal systems. A number of the studies suggest that the COIs have had neurologic effects in animal models when exposure occurred during development. There also are studies that suggest reactive oxygen species could alter specific signaling cascades and be involved in neurodegeneration. Although they do not specifically concern the COIs, such studies are potentially relevant inasmuch as TCDD and herbicides have been reported to elicit oxidative stress (Celik et al., 2006; Shen et al., 2005). The mechanistic studies suggest avenues that might be pursued to determine linkages between the COIs and the neurologic outcomes that could result in adult humans. No toxicology studies concerning exposure to the COIs and ALS have been published since *Update 2006*.

Synthesis

There is overall inadequate or insufficient evidence of an association between pesticides and ALS in broad terms, but the studies published to date have had low power to detect associations, which has resulted in numerous studies with wide CIs and non-significant ORs for exposure to the COIs. Three studies have been published since *Update 2014*. Two analyses of Vietnam veterans used data from the National Registry of Veterans with ALS (Beard et al., 2016, 2017). Exposure to Agent Orange in the field was statistically significantly associated with increased odds of ALS, but mixing and application of Agent Orange was not associated with ALS. Overall, ALS was positively associated with exposure to herbicides for military purposes, contaminated food, explosions within 1 mile, herbicides in the field, mixing and application of burning agents, burning agents in the field, and Agent Orange in the field. The second analysis of this population examined associations between military-related factors and ALS survival; longer survival was associated with exposure to Agent Orange in the field and mixing and application of Agent Orange, but neither of these exposure estimates was statistically significant. The third study examined occupational exposures and 122 environmental toxicants, including organochlorine pesticides and PCB 118 as measured in serum, on the odds of developing ALS. Exposure to PCB 118 was associated with a decreased risk of ALS in this population. The many epidemiologic studies reviewed in the series do not indicate a consistent association between ALS and herbicides as a class or, specifically 2,4-D or 2,4,5-T or dioxin-like PCBs, and the toxicology studies do not support an ALS mechanism specifically.

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Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that the evidence of an association between exposure to the COIs and ALS remains inadequate or insufficient.

Alzheimer Disease

AD is a progressive, neurodegenerative form of dementia that is characterized by memory loss, confusion, mood changes, social withdrawal, and deteriorating judgment. The course of the disease is divided into four stages—pre-dementia, early, moderate, and advanced—depending on the level of cognitive and functional impairment. Diagnosis typically occurs in people over 60 years old as the symptoms develop, although pre-dementia and early AD are occasionally seen in people as young as 30 years old. AD is the sixth leading cause of death in the United States and the fifth leading cause of death in people over 65 years old (S. Singh et al., 2012). In 2017 an estimated 5.5 million Americans were living with the diagnosis. Mean life expectancy is 7 years after an AD diagnosis; about 3% of people who receive the diagnosis live 14 years or more (Alzheimer's Association, 2018). Although the etiology of the disease remains elusive, suspected risk factors for AD include diet, exposure to aluminum or solvents, and genetics (Chin-Chan et al., 2015; de la Monte and Tong, 2014; Tanner et al., 2014).

Similar to PD diagnosis, mortality and hospitalization records are poor instruments for identifying AD cases (Désesquelles et al., 2014; Romero et al., 2014). AD is usually mentioned as contributing cause instead of the underlying cause of death, and therefore, is more prone to under-reporting. Death certificates for AD are further limited for diagnostic and research purposes because many times the type of dementia (vascular, AD, other) is not specified on death certificates. Like PD, hospitalizations for AD do not occur until very late in the disease process, if at all, and thus even though the accuracy of hospital records in terms of diagnosis might be better than death certificates, they may miss most of the less severe cases or those institutionalized in long-term care facilities who might avoid hospitalizations (Wilson and Truman, 2004). However, as long as the specificity of the AD diagnosis is close to perfect, the effect estimate would not be biased. The gold standard of AD diagnosis is pathology of the protein aggregates in the brain (amyloid beta and tau aggregates) and this standard is rarely, if ever, achieved in an epidemiologic project due to the low rate of autopsies or brain collection. However, one can assume that the longer the disease duration the more accurate the AD diagnosis. Also it is known that patients are diagnosed more accurately when they develop the diseases before the age of 80 years and the youngest onset patients are likely the most accurately diagnosed. Clinical accuracy also is much higher if patients are diagnosed in specialty clinics of tertiary care facilities (by dementia specialists), which are not common outside of urban areas.

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Conclusions from *VAO* and Previous Updates

AD was first addressed directly in *Update 2012*. Until that time, literature searches had not identified epidemiologic studies that assessed the possible association of AD with exposure to the specific COIs. However, an association with exposure to the broad classification of "pesticides" had been investigated. Because AD is a condition of considerable interest to aging Vietnam veterans, studies with peripherally related available information (including those with nonspecific exposure assessment) on this outcome were included. On the basis of two studies of nonspecific herbicide exposure or mixed exposure to herbicides and other pesticides and AD (Baldi et al., 2003; Gauthier et al., 2001) that found inconsistent associations, the *Update 2012* committee concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and AD.

Two studies of Vietnam veterans and one environmental study of AD were reviewed in *Update 2014*. The analyses of mortality in the Korean Veterans Health Study found no association between AD (ICD-10 G30) and the EOI scores analyzed as a continuous variable or between the high- and low-exposure groups (Yi et al., 2014b). However, a separate investigation of disease prevalence among the Korean Vietnam veterans found the risk for AD to be elevated in both the analysis of the scores as a continuous variable and the high- versus low-exposure comparison after adjusting for age, rank, smoking, drinking, physical activity, domestic use of herbicides, education, income, and BMI (Yi et al., 2014a). A subanalysis using the Canadian Study of Health and Aging examined 2,023 subjects for whom blood was available and who had a firm diagnosis of having dementia (n = 574, of which 399 were specifically diagnosed with AD) or not (n = 1,449) (Medehouenou et al., 2014). Among the 10 PCB congeners measured in the serum analyses were the mono-ortho PCBs 105, 118, and 156, which exhibit dioxin-like activity only to a modest extent. Two adjusted models were applied to the data, and of the 10 PCBs analyzed, only PCBs 105 and 118 showed an inverse association with dementia overall in the first model, but adjustment for additional confounders in the second model eliminated that negative association. Thus, no relationship with AD specifically was seen for any of the PCBs using either model. The Update 2014 committee reiterated that the evidence for an association between exposure to at least one of the COIs and AD is inadequate or insufficient.

Update of the Epidemiologic Literature

No studies of AD among Vietnam veterans or other populations that specifically assessed exposure to the COIs have been published since *Update 2014*.

Environmental Studies D. H. Lee et al. (2016) measured PCBs and organochlorine pesticides in the participants of the PIVUS (Prospective Investigation

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of the Vasculature in Uppsala Seniors) cohort of residents 70 years and older in Uppsala, Sweden, to examine cognitive impairment. Of 2,025 eligible subjects, only half (n = 1,016) agreed to participate and completed a questionnaire to assess medical history, smoking history, and medication use. In a subcohort of 989 men and women, the investigators determined whether PCB or organochlorine exposures were associated with cognitive impairment during 10 years of follow-up, relying on medical record review and death certificate information to ascertain disease status. General exposure to 16 PCBs and 3 organochlorine pesticides (p,p′-DDE, trans-nonachlor, and hexachlorobenzene) was quantified using high-resolution gas chromatography/mass spectrometry from stored serum samples collected at the time of entry into the PIVUS study, and these measure were normalized using individual lipid levels. The authors analyzed exposures using a compound summary score for the three organochlorine pesticides and the PCBs, grouping them according to the < 25th, 25th–75th, and > 75th percentile of exposure. Adjusted hazard ratios for the higher summary score PCB exposures compared with exposure < 25th percentile showed no associations with cognitive impairment, but the study did not distinguish between dioxin-like and non-dioxin-like PCBs. For the highest exposure (> 75th percentile) of hexachlorobenzene, a chemical with dioxin-like activity, the weight-loss-adjusted HR was 2.4 (95% CI 1.0–5.8) in those who developed cognitive impairment after age 75, and the p-trend was statistically significant. These results suggest that exposure to hexachlorobenzene may predispose to the development of cognitive impairment or AD.

Other Identified Studies Two additional studies of occupational exposures and mortality from AD and dementia (Ruder et al., 2014) or mortality from non-vascular dementia (Koeman et al., 2015) were identified. However, both studies lacked the necessary exposure specificity or quantification to be considered further as contributing to the evidence base of the potential effect of exposure to the COIs on AD or dementia.

Biologic Plausibility

There has been little toxicologic investigation of adult exposure to the COIs and endpoints relevant to AD.

Synthesis

The findings in the Korean Veterans Health Study (Yi et al., 2014a,b) remain the first in which the risk of AD has been investigated in association with a fully relevant exposure, but the findings were not consistent. Except for the analysis of the PIVUS cohort (D. H. Lee et al., 2016), which only suggested an association with cognitive impairment for hexachlorobenzene and only in subgroup analyses,

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there are no new relevant epidemiologic studies on AD to add to the evidence base since the last update, and the toxicologic data remains sparse.

Conclusion

On the basis of the lack of new evidence reviewed here and that reviewed in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and AD.

CHRONIC PERIPHERAL NERVOUS SYSTEM DISORDERS

Peripheral neuropathies are an array of disorders caused by damage to nerve fibers (axonal neuropathies) or to the myelin sheath that surrounds many fibers (demyelinating neuropathies). The manifestations of neuropathy can include a combination of sensory changes, weakness, and autonomic instability. Clinically, various forms of peripheral neuropathy can be characterized by the distribution of nerve abnormalities and their patterns of progression.

Peripheral neuropathy resulting from toxic exposure usually affects nerve fibers in a symmetric pattern, beginning distally in the longest fibers (in the toes) and moving proximally (toward the spine). This kind of neuropathy is called symmetric axonal sensorimotor polyneuropathy. Sensory deficits begin at the toes, progress above the ankles, and only later affect the hands. Motor symptoms show the same general pattern. Physiologically, various forms of peripheral neuropathy can be characterized by the results of electrodiagnostic testing to indicate which neural structures are affected. Most toxicant-induced neuropathies involve injury to the nerve-cell bodies or the axons, giving rise to changes in the amplitude of a nerve's response to an electric stimulus. The clinical manifestations of most symmetric axonal neuropathies are similar except for variations in the rates of progression and in whether pain is prominent. No specific signature distinguishes a toxicant-related neuropathy from one induced by other causes. As many as 30% of neuropathies are "idiopathic," that is, no etiology is determined despite exhaustive clinical evaluation.

The most common toxicant-induced neuropathy occurs as a result of chronic alcohol exposure. Peripheral neuropathy also occurs commonly as a complication of diabetes; its reported prevalence in people who have chronic diabetes is up to 50%. Thus, it is important to include an assessment of alcohol use and diabetes as covariates in epidemiologic studies because the neuropathies that are related to these conditions are clinically and physiologically indistinguishable from other toxicant-induced neuropathies.

Toxicant exposure can result in early onset (immediate) peripheral neuropathy or delayed-onset peripheral neuropathy, which occurs years after the external exposure has ended. For classification purposes, the committee considers a neuropathy early onset if abnormalities appear within 1 year after external exposure

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ends and delayed-onset if abnormalities appear more than 1 year after external exposure ends. Because the exposures of interest for Vietnam veterans are long past (more than 40 years ago), the immediate effects of the COIs are no longer pertinent for this cohort. Such outcomes would include early onset peripheral neuropathy and porphyria cutanea tarda. Because early onset peripheral neuropathy is not necessarily a transient condition, it may become a chronic condition that should be distinguished from delayed-onset peripheral neuropathy. The focus of this section is on data related to delayed-onset peripheral neuropathy.

Conclusions from *VAO* and Previous Updates

Several studies of Vietnam veterans have examined peripheral neuropathy. A study by the Centers for Disease Control and Prevention (CDC, 1988b) reported a slight excess in the signs or symptoms of peripheral neuropathy among deployed versus non-deployed Vietnam-era veterans. Decoufle et al. (1992) reported no association between self-reported exposure to herbicides in Vietnam and peripheral neuropathy. There was no indication of an increased incidence of peripheral neuropathy in the first examination of the Air Force Health Study (AFHS), which established the baseline for Ranch Hand veterans (AFHS, 1984a). Michalek et al. (2001c) described peripheral neuropathy in the AFHS cohort. In a primary analysis, the investigators had included diabetes as a potential confounder in the statistical model. In a secondary analysis, the subjects who had conditions that were known to be associated with neuropathy were excluded, and the subjects who had diabetes were enumerated. In both analyses, there were strong and significant associations between serum dioxin concentrations and possible and probable neuropathy, and significant trends were found with increasing concentrations of dioxin. However, there were too few nondiabetic subjects to produce useful estimates of risk in the absence of the contribution of diabetes. Thus, questions remained about the specific association between an exposure to the COIs and peripheral neuropathy in the absence of any effect of diabetes. The large veteran studies are limited by the confounding nature of concurrent diabetes and alcohol exposure, both of which are also related to neuropathy.

In the study of disease prevalence among the Korean Vietnam veterans, after adjusting for age, rank, smoking, drinking, physical activity, the domestic use of herbicides, education, income, and BMI, Yi et al. (2014a) found that the risk for polyneuropathies of the PNS (ICD-10 G60–G64) was slightly elevated in both the analysis of the EOI scores as a continuous variable and in the high- versus low-exposure comparison.

D. H. Lee et al. (2008) evaluated the association of exposure to a variety of toxicants to the presence of neuropathy in subjects who had either frank diabetes or impaired glucose tolerance. No evidence of an increased incidence of neuropathy or of a dose–response relationship was found for either group when stratified by high- versus low-hemoglobin A1c level that suggested a concentration-dependent

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risk of neuropathy. However, this study was limited by the small sample size and a lack of information regarding the duration of diabetes.

Update of the Epidemiologic Literature

No studies of exposure to the COIs and chronic peripheral system disorders were identified for the current update.

Biologic Plausibility

No new toxicity studies directly pertinent to the COIs and peripheral neuropathy were identified for the present update. However, it is worth reiterating findings from earlier updates. Neuronal cell cultures treated with 2,4-D showed decreased neurite extension associated with intracellular changes, including a decrease in microtubules, an inhibition of the polymerization of tubulin, disorganization of the Golgi apparatus, and an inhibition of ganglioside synthesis (Rosso et al., 2000a,b). The normal activity of those target processes is important for maintaining synaptic connections between nerve cells and for supporting the mechanisms involved in axon regeneration during recovery from peripheral neuropathy. Grahmann et al. (1993) and Grehl et al. (1993) reported observation of, respectively, electrophysiologic and pathologic abnormalities in the peripheral nerves of rats treated with TCDD. When the animals were sacrificed 8 months after exposure, there was pathologic evidence of persistent axonal nerve damage and histologic findings typical of toxicant-induced injury. These results constitute evidence of biologic plausibility for an association between exposure to the COIs and peripheral neuropathy.

Synthesis

The epidemiologic studies relating industrial or individual exposure to acute neuropathy were judged by the committee for *Update 1996* and later updates to provide limited or suggestive evidence of an association between exposure to the COIs and early onset transient peripheral neuropathy. Beginning with *Update 2010*, these acute outcomes were removed from this section to keep the focus on chronic and delayed-onset conditions. That committee concluded that no data suggest that exposure to COIs can lead to the development of delayed-onset chronic neuropathy many years after the termination of exposure of those who did not originally complain of early onset neuropathy and concluded the evidence to be inadequate or insufficient. Since *Update 2010*, only one relevant study was identified and reviewed, and that study was reviewed in *Update 2014*. Among Korean Vietnam veterans, the prevalence of polyneuropathies was slightly elevated in high- versus low-exposure comparisons. Given the lack of new epidemiologic studies and toxicologic information, the committee agrees with the prior two

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committees and concludes that the evidence does not support an association between exposure to the COIs and the development of delayed-onset chronic neuropathy.

Conclusion

On the basis of the lack of new evidence to date, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and delayed-onset chronic neuropathy.

HEARING LOSS

Hearing loss increases markedly with age, from approximately 3% among adults aged 20–29 years to an estimated 49% among people 60–69 years of age and more than 80% among people 85 years and older (NASEM, 2016b). These estimates are based on NHANES data and are likely to underestimate the true population prevalence of hearing loss because the NHANES sampling frame does not include people living in assisted care facilities, group homes, or nursing homes or those unable to come to the mobile examination center. Hearing loss is somewhat higher in men than in women (NASEM, 2016b). The most common forms of hearing impairment in adults are presbycusis and tinnitus. Heritable factors may influence the susceptibility to hearing loss, but external agents can also contribute. Aspirin at high doses can cause reversible tinnitus, and permanent hearing loss may be induced by certain pharmaceuticals (particularly antibiotics and antineoplastic drugs) and by some environmental and industrial chemicals (primarily solvents and metals) (Cannizzaro et al., 2014). In occupational medicine, hearing loss is most often regarded as noise induced. Farmers and migrant or seasonal agricultural workers have also been found to have high rates of hearing loss compared with those who had the lowest levels of pesticide exposure (Crawford et al., 2008; Rabinowitz et al., 2005).

Conclusions from *VAO* and Previous Updates

Epidemiologic results on hearing loss in relation to service in Vietnam or to herbicide exposure more generally were first discussed in *Update 2010*, when two citations that addressed this health outcome were identified. O'Toole et al. (2009) re-examined the health status of a cohort of Australian Vietnam veterans; as for almost every health endpoint surveyed in that group, the incidences of self-reported complete or partial deafness and of tinnitus showed statistically significant increases compared to the general population, but these results are likely compromised by methodologic problems with the study. Excesses in self-reported hearing loss were also found among licensed pesticide applicators in the AHS at the time of the 5-year follow-up interview (Crawford et al., 2008), but this effect was associated with insecticide exposure, not with herbicide use.

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Update of the Epidemiologic Literature

No epidemiologic studies addressing exposure to the COIs and hearing loss have been published since *Update 2014*.

Biologic Plausibility

Although no studies of hearing loss in adult animals directly exposed to the COIs were found, Crofton and Rice (1999) reported that perinatal maternal exposure to PCB 126 (a dioxin-like PCB) resulted in low-frequency hearing deficits in the offspring of exposed maternal rats. Increased auditory thresholds occurred in the group treated at 1.0 µg/kg/day for 0.5- and 1-kHz tones, but higher frequencies were not significantly affected. The frequency-specific deficit was hypothesized to be secondary to a postnatal hypothyroxinemia that occurred during a sensitive period for the development of the low-frequency regions of the cochlea, which was consistent with the finding that the pups had decreased T4 concentrations in their sera on postnatal day 21.

Synthesis

Two prior studies observed an increased prevalence of hearing loss in Vietnam veterans and pesticide applicators, but neither was able to examine exposure specifically to the COIs or to confirm hearing loss clinically. Furthermore, the report from the AHS (Crawford et al., 2008) observed an association only in insecticide applicators, not in herbicide applicators. Hearing loss among Australian Vietnam veterans was compared with the general Australian population, not veterans from the same era who were not deployed to Vietnam, so it could not distinguish between hearing loss that may be associated with noise that was related to military service and hearing loss potentially associated with exposures to toxic chemicals. In the absence of new studies, the conclusion remains unchanged since *Update 2010*.

Conclusion

On the basis of the evidence reviewed here, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and hearing loss.

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10

Metabolic and Cardiovascular Disorders

Chapter Overview

Based on new evidence and a review of prior studies, the current committee found that new scientific evidence combined with previously reviewed studies has led to changes in the categorizations of association for two metabolic and cardiovascular outcomes outcomes: type 2 diabetes and hypertension.

- *After extensive deliberation concerning the new evidence and the results of studies reviewed in previous updates, the committee was unable to reach consensus as to whether the evidence of an association between exposure to the chemicals of interest (COIs) and diabetes met the criteria for being considered sufficient or whether concerns about chance, bias, and confounding lead to the conclusion that the evidence regarding diabetes is limited or suggestive.*
- *There is sufficient evidence of an association of hypertension with the COIs.*

The committee found that the current evidence supports the conclusions reached by committees responsible for earlier updates concerning the other metabolic and cardiovascular outcomes reviewed in the Veterans and Agent Orange (VAO) *series of reports:*

- *There is limited or suggestive evidence of an association between the COIs and ischemic heart disease and stroke.*
- *There is inadequate or insufficient evidence to determine whether there is an association between the COIs and any other adverse metabolic or cardiovascular outcome examined.*

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This chapter summarizes and presents conclusions about the strength of the evidence from epidemiologic studies regarding an association between exposure to the COIs—2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), picloram, and cacodylic acid—and metabolic and cardiovascular disorders. The committee also considers studies of exposure to polychlorinated biphenyls (PCBs) and other dioxin-like chemicals to be informative if their results were reported in terms of TCDD toxic equivalents (TEQs) or concentrations of specific congeners. Studies that report TEQs based only on mono-ortho PCBs (which are PCBs 105, 114, 118, 123, 156, 157, 167, and 189) are considered even though their TEQs are several orders of magnitude lower than those of the non-ortho PCBs (77, 81, 126, and 169), based on the revised World Health Organization (WHO) toxicity equivalency factor (TEF) scheme of 2005 (La Rocca et al., 2008; van den Berg et al., 2006). The lower TEQs of the mono-ortho PCBs, however, may be counterbalanced by their abundance, which is generally many orders of magnitude higher than that of the non-ortho PCBs (H. Y. Park et al., 2010).

The first part of the chapter covers the metabolic disorder type 2 diabetes and the second part reviews a variety of cardiovascular and blood disorders. Cardiovascular conditions explored in this chapter are based on the categories in the 9th and 10th revisions of the *International Classification of Diseases* (ICD; ICD-9 390–459 and ICD-10 I00–I99, respectively) and include hypertension (ICD-9 401–404; ICD-10 I10–I13), ischemic heart disease (ICD-9 410–414; ICD-10 I20–I25), heart failure (ICD-9 428; ICD-10 I50), and cerebrovascular disease (ICD-9 430–438; ICD-10 I60–I69).

TYPE 2 DIABETES

Diabetes mellitus is a group of heterogeneous metabolic disorders characterized by hyperglycemia and a quantitative or qualitative deficiency in insulin action (Orchard et al., 1992) and classified as E08–E13 by ICD-10. Although all forms of diabetes share hyperglycemia, the pathogenic processes involved in the development of the various types of diabetes differ. Most cases of diabetes mellitus are classified as being in one of two categories: type 1 diabetes or type 2 diabetes. The long-term complications of both types can include cardiovascular disease (CVD), nephropathy, retinopathy, neuropathy, and increased vulnerability to infections.

When referring to diabetes, most research will not distinguish between type 1 and type 2 because type 2 diabetes accounts for 90% to 95% of all cases. Type 1 diabetes (ICD-10 E10) is characterized by a lack of insulin caused by the immunologically mediated destruction of insulin-producing cells in the pancreas (β cells), which often occurs during childhood but can occur at any age. As with many autoimmune diseases, genetic and environmental factors both influence its pathogenesis. Some viral infections are believed to be important environmental

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triggers for the autoimmunity associated with type 1 diabetes. Type 1 diabetes most often occurs in children, which is only of relevance in offspring of adults exposed to the COIs.

Type 2 diabetes exhibits both resistance to the actions of insulin and inadequate secretion of insulin (called relative insulin deficiency). The prevalence of diabetes in the United States has been increasing among nearly all races and ethnic groups. In 2015 there were approximately 30.3 million people ages 18 years and older who had diabetes in the United States, or 9.4% of the U.S. population. Of these, 23.1 million had officially been diagnosed, whereas 7.2 million (23.8%) were considered to be prediabetic (CDC, 2017c). Although the modern ICD-10 classification system recognizes that type 2 diabetes can occur in children and can require insulin treatment, most cases of type 2 diabetes occur in adults.

Onset can occur before 30 years of age, and the incidence increases with age. The main risk factors for type 2 diabetes are age, obesity, abdominal fat deposition, a history of gestational diabetes (in women), physical inactivity, ethnicity, and family history, but the relative contributions of each of those risks to the overall prevalence are not known. Type 2 diabetes affects women and men nearly equally (11.7 million versus 11.3 million, respectively); however, the Centers for Disease Control and Prevention (CDC) estimates that there are more undiagnosed diabetes cases among men than among women (4.0 million versus 3.1 million, respectively). Table 10-1 shows the estimated prevalence of diagnosed and undiagnosed diabetes among adults over 18 years by age and sex. The prevalence of type 2 diabetes among U.S. adults aged 18 years and older is greatest in American Indians/Alaska Natives (15.1%), followed by non-Hispanic blacks (12.7%) and Hispanics (12.1%). The lowest prevalence rates are for non-Hispanic whites (7.4%) and Asians (8.0%). As of 2015, an estimated 1.5 million new cases of diabetes are diagnosed annually, for an incidence rate of approximately 6.7 per 1,000 persons. In non-Hispanic blacks the incidence was 9.0 per 1,000 persons and 8.4 per 1,000 persons among people of Hispanic origin (CDC, 2017c).

TABLE 10-1 Estimated Number and Percentage of Diagnosed and Undiagnosed Diabetes Among Adults, Aged ≥ 18 years, United States, 2015

Characteristic	Diagnosed Diabetes # in Millions (95% CI)	Undiagnosed Diabetes # in Millions (95% CI)	Total Diabetes
Age in years			
18–44	3.0 (2.6–3.6)	1.6 (1.1–2.3)	4.6 (3.8–5.5)
45–64	10.7 (9.3–12.2)	3.6 (2.8–4.6)	14.3 (12.7–16.1)
≥65	9.9 (9.0–11.0)	2.1 (1.4–3.0)	12.0 (10.7–13.4)
Sex			
Women	11.7 (10.5–13.1)	3.1 (2.4–4.1)	14.9 (13.5–16.4)
Men	11.3 (10.2–12.4)	4.0 (3.0–5.5)	15.3 (13.8–17.0)
Total	23.0 (21.1–25.1)	7.2 (6.0–8.6)	30.2 (27.9–32.7)

SOURCE: CDC, 2017c.

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The etiology of type 2 diabetes is unknown, but three major components have been identified: peripheral insulin resistance in target tissues (muscle, adipose tissue, and liver), a defect in β-cell secretion of insulin, and the overproduction of glucose by the liver. When a person enters a state of insulin resistance, insulin secretion is initially higher for each concentration of glucose than in people who are not insulin resistant. This hyperinsulinemic state compensates for peripheral resistance and in many cases keeps glucose concentrations normal for years. Eventually, however, β-cell compensation becomes inadequate, and there is a progression to overt diabetes with concomitant hyperglycemia. Why the β cells cease to produce sufficient insulin is not known.

Pathogenic diversity and diagnostic uncertainty are some important problems in conducting epidemiologic studies of diabetes mellitus. There are multiple pathogenic mechanisms that are likely to play a role in the development of diabetes mellitus, including various genetic susceptibilities (as varied as autoimmunity and obesity) and a variety of potential environmental and behavioral factors (such as viruses, nutrition, and physical activity). Because in some populations up to half of all cases of diabetes remain undiagnosed for an extended period of time, the potential for ascertainment bias in population-based surveys is high, and groups that are more intensively followed for signs and symptoms of diabetes or those with more frequent health care contact, in general, are more likely to be diagnosed in time to receive appropriate treatment; this points to the need for formal standardized testing to detect cases in epidemiologic studies.

Metabolic syndrome is a cluster of at least three of the following—increased blood pressure or hypertension, insulin resistance, excess body fat around the waist, and abnormal serum cholesterol or triglyceride levels—that occur together, increasing the risk of heart disease, stroke, and diabetes. Although it is not a disease entity itself, metabolic syndrome is associated with a five-fold increased risk of developing type 2 diabetes and a doubling of the risk of developing CVD (Alberti et al., 2009). Swaminathan et al. (2015) found that pesticide exposure was a contributing factor to hyperglycemia and type 2 diabetes even after adjusting for body mass index (BMI). There is a growing literature on the association between the COIs and metabolic syndrome and its components. Given its strong linkage with type 2 diabetes, studies on metabolic syndrome will be discussed in this section.

Conclusions from *VAO* and Previous Updates

The committee responsible for the first report in the *VAO* series (IOM, 1994) concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and diabetes mellitus. Additional information available to the committees responsible for *Update 1996* (IOM, 1996) and *Update 1998* (IOM, 1999) did not change that conclusion.

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In 1999, in response to a request from the Department of Veterans Affairs, the Institute of Medicine convened a committee to conduct an interim review of the scientific evidence regarding exposure to the COIs and type 2 diabetes. That review focused on information published after the deliberations of the Update 1998 committee and resulted in the report *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes* (IOM, 2000b). The committee responsible for that report determined that there was limited or suggestive evidence of an association between exposure to at least one COI and type 2 diabetes, based on positive associations reported in several mortality studies (Pesatori et al., 1998; Steenland et al., 1992, 1999; Vena et al., 1998), which may underestimate the incidence of diabetes, and positive associations reported in most of the morbidity studies (AFHS, 2000; Calvert et al., 1999; CDVA, 1998a,b; Cranmer et al., 2000; Longnecker and Michalek, 2000; Michalek and Tripathi, 1999) reviewed by that committee. However, that committee also noted that the studies indicate that the increased risk, if any, from herbicide or dioxin exposure appeared to be small and that the known predictors of diabetes risk—family history, physical inactivity, and obesity—continued to greatly outweigh any suggested increased risk from wartime exposure to herbicides. The committees responsible for updates since that report have upheld that finding. Reviews of the considered studies are found in the earlier reports.

Several epidemiologic studies have been published that examined type 2 diabetes in different populations of Vietnam veterans: Air Force Health Study (AFHS), female U.S. Vietnam-era veterans, and Korean Vietnam veterans. Publications from the AFHS have found a statistically significant increased risk of diabetes among the Ranch Hands (Henriksen et al., 1997; Kern et al., 2004; Longnecker and Michalek, 2000; Michalek and Pavuk, 2008). Most analyses of the Army Chemical Corps (ACC) veterans did not find increased risk of diabetes. Using mortality data on U.S. women veterans, Kang et al. (2014a) found that the adjusted relative risk of diabetes mortality was non-significantly lower for the female veterans deployed to Vietnam than for their non-deployed counterparts, and when the analysis was restricted to nurses only, the adjusted relative risk of diabetes mortality was effectively the same for deployed and non-deployed nurses.

No statistically significantly increased risk of diabetes was found in several studies of Australian Vietnam veterans. In an exceptionally large epidemiologic study of Korean veterans who served in the Vietnam War, using data from the Korea National Health Insurance Service, the risk of type 2 diabetes mellitus was found to be nominally higher for those with a high potential for herbicide exposure than for those with low–exposure opportunity index scores, and there was a small, but statistically significant association for veterans with non-insulin-dependent diabetes mellitus (Yi et al., 2014a). In the mortality study of 180,639 Korean Vietnam veterans, Yi et al. (2014b) found that after adjustment, high exposure (compared with low exposure) was not associated with mortality from all forms of diabetes (ICD-10 E10–E14).

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Occupational studies of diabetes have found slightly elevated but generally not statistically significant associations between exposure to the phenoxy herbicides or TCDD and a risk of diabetes. Results from the Agricultural Health Study (AHS) found an increased risk of developing diabetes in the wives of pesticide applicators who themseleves had reported personally mixing or applying pesticides or the ever use of 2,4,5-T or 2(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP). Ever use of 2,4-D was much more prevelant, but it was not associated with a risk of diabetes (Starling et al., 2014). No difference or decreased risk of diabetes was found among the AHS pesticide applicators in both morbidity and mortality studies.

Several studies of environmental exposures to the COIs and diabetes have been reviewed in the VAO series. The findings are mixed, and the studies have used different designs and methods for adjusting effect estimates for confounding.

Update of the Epidemiologic Literature

Two new studies of Vietnam veterans and diabetes have been identified since *Update 2014*. Several occupational, environmental, and case-control studies of diabetes and exposure to the COIs were also identified for this update. Table 41, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to diabetes and related health outcomes.

Vietnam-Veteran Studies

Using data from the AFHS, Mazur et al. (2014) studied 991 Ranch Hands and comparison veterans who completed all six medical examinations over the 20 years of data collection (1982, 1985, 1987, 1992, 1997, 2002) to see if low testosterone is a risk factor for high fasting glucose and for a type 2 diabetes diagnosis. Subjects ranged from 32 to 68 years of age at the onset of the study. Fasting glucose was tested in the morning for all men at each exam. Hemoglobin A1c (HbA1c), which is indicative of blood glucose levels over the preceding 120 days, was measured for all men in cycles 4 and 5. Among the men, gradual increases in obesity, fasting glucose, and type 2 diabetes and a decline in mean testosterone levels occurred over the 20 years of study. The authors noted that men who would not be diagnosed with type 2 diabetes until cycles 5 or 6 already showed by cycle 1 that their mean fasting glucose was significantly higher than normal. A statistically significant ($p < 0.001$) correlation was found between fasting glucose in all six cycles and both HbA1c in the fourth and fifth cycles and a diagnosis of type 2 diabetes in any of the six cycles. Men who had high testosterone levels and low BMI had a mean fasting glucose level of 100 mg/dl, whereas men with a high BMI and low testosterone levels had a fasting glucose level of 116 mg/dl. The multivariate analyses showed that testosterone was inversely related to glucose, independently of BMI and age, but its effect on glucose was small. The

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study showed that levels of low testosterone were related to high fasting glucose, independent of the participant's age or obesity. Results also indicated that low testosterone is a relatively poor predictor of a type 2 diabetes diagnosis. However, this study is of limited value to this update because the results were not stratified by exposure status (Ranch Hand versus comparison) and because serum concentrations of dioxin were not included in the analysis.

Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify prevalent health conditions in 2,783 male New Zealand veterans who served in Vietnam. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% confidence intervals (CIs) were calculated for the veteran cohort and the general population and reported for diabetes and other outcomes. Although the risk estimate was slightly elevated for diabetes (standardized hospitalization ratio [SHR] = 1.14, 99% CI 0.90–1.38), it was not statistically significant compared with the general population. However, diabetes is not usually a condition that requires hospitalization, thus likely only those with the most severe disease were identified, and the number of cases available for study would be expected to be low, limiting the statistical power for this type of analysis considerably. Exposure to the COIs was not validated through serum measurements, and the study did not control for smoking or ethnicity or other potentially important risk factors.

Occupational Studies

Collins et al. (2016) added follow-up time for a retrospective cohort of 2,192 workers exposed to dioxins during trichlorophenol (TCP) and pentachlorophenol (PCP) production at a chemical manufacturing plant in Midland, Michigan. Workers were compared to the U.S. population in order to calculate standardized mortality ratios (SMRs), and work history records provided information about the length of exposure. Serum samples used to measure levels of six types of dioxins were collected from 431 TCP and PCP workers. Concentrations for each dioxin congener were calculated based on the median concentration in the serum samples and the known half-lives associated with each congener. Complete vital status follow-up was achieved for the cohort, and there were 1,198 deaths during the entire study period (1979–2011). Compared with the standardized U.S. population, no statistically significant difference in mortality for diabetes was found for the TCP workers (n = 19; SMR = 0.92, 95% CI 0.55–1.43) or the PCP workers (n = 9; SMR = 0.97, 95% CI 0.44–1.84). Among the combined 1,198 TCP and PCP workers, there were 28 deaths from diabetes (SMR = 0.97, 95% CI 0.64–1.42). Additionally, there are other concerns with the diabetes outcome in this analysis. First, mortality data for diabetes likely under-reports disease prevalence because diabetes is often a contributing factor to but not the actual cause of a death. Second, using the general U.S. population as the comparison

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is problematic because of the "healthy worker effect," that is, some of the comparison subjects are not in the workforce and will typically have, on average, poorer general health than individuals in the workforce. In this case, it would imply a control group with rates of diabetes greater than those in the workforce. In contrast, an internal comparison of workers with high- versus low-dioxin concentrations, which was not presented by the authors, would have avoided this type of selection bias.

't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and workers were potentially exposed to 2,4,5-T, intermediates of TCP and other chlorophenols, and TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by the International Agency for Research on Cancer (IARC) (Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. This study extended the follow-up period of these workers to approximately 30 years from the last 2,4,5-T production exposure. From the original cohort of 1,025 workers, 631 were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current follow-up, 430 of the 631 workers were randomly selected and invited to participate in the morbidity survey, of which 245 (57%) participated. The survey was administered in 2007–2008 by face-to-face interview, and it collected information on demographic factors and health information, including doctor-diagnosed conditions and the year of diagnosis. A blood sample was also collected at that time and analyzed for TCDD, lipids, thyroid hormones, and other parameters. For 111 participants, a neurological examination was conducted. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two methods: working in a TCDD-exposed job (based on occupational records) and serum TCDD concentration $\geq$ 10 pg/g lipid (18%). Mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy/TCP production and 6 pg/g lipid in the 141 men and 43 women who worked in other parts of the plant. Compared with the people in the non-highly exposed jobs, the people who had ever worked in a highly exposed job at the plant had an elevated risk of doctor-diagnosed diabetes (n = 13; odds ratio [OR] = 3.98, 95% CI 1.03–15.4), although the estimate was imprecise. When compared by serum TCDD concentration, no difference in risk of diabetes was found for workers in the high- versus low-exposure groups (n = 7; OR = 3.05, 95% CI 0.87–10.67), although the estimate was again imprecise.

Cappelletti et al. (2016) performed a retrospective study of 331 male electric arc foundry workers at a single plant in Trentino, Italy, to determine if they experienced excess mortality from all causes or were at increased risk for several other diseases due to occupational exposures to foundry dust. An analysis of the

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dust emissions found that it contained metals (including iron, aluminum, zinc, manganese, lead, chromium, nickel, cadmium, mercury, and arsenic), polycyclic aromatic hydrocarbons (PAHs), PCBs, and polychlorinated dibenzo-*p*-dioxin/dibenzofurans (PCDD/Fs) (reported as TEQs). Therefore, the authors could not determine which of the agents were associated with a specific outcome or to what extent. The men had worked at the factory for at least 1 year and, for the diabetes analysis, were compared with 32 presumed non-exposed workers (clerks, managers, and watchmen) or the general population of Region Trentino-Alto Adige (where the factory was located) because there were few non-exposed foundry workers and high attrition rates. Company and medical records were used to determine vital status; the cause of death was determined from death certificates or other registries. Requests for exemption health care fees were used as a surrogate measure to identify the most prevalent morbid conditions in the general population, which were then applied to the cohort to compute relative risks for each of the conditions, which included diabetes. The workers were followed from March 19, 1979 (or their first day of employment), through December 31, 2009, or date of death. The analysis for diabetes was limited to the 235 living workers, and effect estimates (prevalence ratios) were calculated using Mantel-Haenszel estimator adjusted for age group (20–64, 65–74, $\geq$ 75 years). Compared with the age-adjusted provincial population, a statistically significant increased risk of diabetes was found among electric arc furnace workers (n = 25; relative risk [RR] = 2.39, 95% CI 1.67–3.41). This study is most limited by the fact that foundry dust is a complex mixture, resulting in an inability to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those exposed workers. Estimates were adjusted only for age group and not for other risk factors, such as tobacco use, BMI, or other jobs or activities that could result in similar exposures. Possible exposure to foundry dust by the general population that was used for comparison is not discussed, although the foundry appears to be in the local vicinity, and emissions from it were reported to be present within a 2-kilometer radius of it.

Yamamoto et al. (2015) performed a cross-sectional study to investigate the health outcomes that 698 male workers sustained while employed at 36 municipal and private waste incineration plants in Japan; serum dioxin measurements were obtained for 678 of the workers. These workers were employed from 2000 to 2007. First, a questionnaire was distributed to the participants to determine their medical history. Blood samples were taken from each subject to evaluate serum dioxin and PCB levels. Both clinical and physiological examinations were also performed. Participants were then categorized into four groups: workers whose jobs did not involve working directly in an incineration facility, workers whose jobs did involve work inside the incineration facility (but only handling solidified fly ash and slag or residues that were nonflammable), workers whose jobs involved helping with incineration-related work inside an incineration facility, and workers whose jobs mainly involved operation and maintenance of an

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incinerator including a furnace, electric dust collector, and wet scrubber inside an incineration facility. Subjects were tested for diabetes, hypertension, hyperlipidemia, and liver dysfunction. The total serum dioxin concentration level for incinerator workers was 13.7 (median) or 17.2 (arithmetic mean) pg TEQ/g lipid, while the (arithmetic mean) average total dioxin concentration was 19.4 pg TEQ/g lipid for the general population in Japan, indicating no real difference between the two groups. However, in the exposed workers, the duration of employment was positively associated with TEQ levels (9.8 and 21.7 pg TEQ/g lipid for those employed 9 years and 15.5 years, respectively). Coplanar PCBs were significantly correlated ($p < 0.05$) with 14 parameters of laboratory and physiological tests. Diabetes was statistically significantly associated with PCDDs, PCDFs, coplanar PCBs, and total dioxins based on adjusted odds ratios between the first and fourth quartile, and tests for trend were also significant for each of these COIs. Positive associations were found between serum levels of total dioxins and the prevalence of diabetes in the incinerator workers even though, as the healthy worker effect would predict, the overall diabetes prevalence in the workers was not as high as in the general Japanese population (6.6% versus 9.0%, $p = 0.07$).

Environmental Studies

Everett and Thompson (2016) measured three chlorinated dibenzo-*p*-dioxins, one chlorinated dibenzofuran, and four dioxin-like PCBs in human blood and assessed their cross-sectional associations with nephropathy, specifically diabetes-related nephropathy using data collected between 1999 and 2004 from the National Health and Nutrition Examination Survey (NHANES). Two groups of people were included: the teen and young adult sample (12–30 years old) and the all-adult sample (≥ 20 years old). Nephropathy was defined as a urinary albumin-to-creatinine ratio > 30 mg/g, representing both microalbuminuria and macroalbuminuria. Diagnoses of diabetes were determined by individual, self-reported answers to the NHANES question: "*Other than during pregnancy, have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?*" Those who answered "borderline" were considered to not have diabetes. Having undiagnosed diabetes was defined as participants who had A1c levels ≥ 6.5% but had not been diagnosed as having diabetes. Total diabetes was defined as either diagnosed or undiagnosed diabetes. Several differences were noted between the teen/young adult and the all-adult samples. One or more of the eight dioxin-like chemicals were elevated among 9.9% of the teen/young adult sample (at-risk population) and among 53.5% of the all-adult sample. The proportions for nephropathy and pre-diabetes were, respectively, 7.8% and 1.8% in the teen/young adult sample versus 5.5% and 9.7% in the all-adult sample. The unweighted number of people with nephropathy in the teen/young adult sample was 87 females and 35 males. Only three people had pre-diabetes with nephropathy in the teen/young adult sample, which was too few for analyses.

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None of the eight dioxin-like chemicals were associated with pre-diabetes, but in most instances there were no cases of pre-diabetes in the elevated concentration range. In the highest TEQ8 category, which included 2.6% of the sample, there was a strong but very imprecisely estimated association with pre-diabetes among females (OR = 11.9, 95% CI 1.6–87.2), but no associations with pre-diabetes at any level of elevated TEQ8 in the entire sample.

Two reports from a study conducted by Aminov et al. (2016a,b) examined diabetes among 601 people of the Mohawk Nation between the ages of 18 and 84 years living at Akwesasne, which spans New York, Ontario, and Québec. The most detailed reporting of this data is found in Aminov et al. (2016a). Serum samples were taken from the subjects to test for PCB and pesticide concentrations as well as to determine each individual's fasting glucose levels. A subject in this study was considered to have diabetes if he or she had a fasting glucose concentration of > 125 mg/dL or if it was reported that a physician had made a diabetes diagnosis. Several models were used to estimate risks. Model 1 adjusted for sex, age, BMI, and serum lipid concentrations. In model 2, the total concentrations of PCBs were adjusted for the variables in model 1 and also for total pesticides (the sum of the concentrations of 101 PCB congeners, DDE, hexachlorobenzene [HCB], and mirex). Multiple analyses were undertaken, and in the minimally adjusted model 1, both total PCBs (highest quartile OR = 3.45, 95% CI 1.4–8.5) and total pesticides (highest quartile OR = 3.12, 95% CI 1.12–8.65) showed statistically significant associations with diabetes. These associations were attenuated in model 2, which adjusted for total pesticides, with total dioxin-like PCBs estimates having an odds ratio that was elevated but did not reach statistical significance (highest quartile OR = 1.82, 95% CI 0.61–5.40), while the total pesticides estimate (highest quartile OR = 5.01, 95% CI 1.76–14.24) was statistically significantly elevated but quite imprecise. In model 3, PCB concentrations were divided into three groups by numbers of chlorines (3–4, 5–6, and ≥ 7) and by numbers of ortho-substitute chlorines and each pesticide was considered alone. In addition to adjustment for sex, age, BMI, and total lipids, each result was also adjusted for the concentrations of all other groups of contaminants. Results using model 3 suggested a low-dose effect. In general, there was an association between PCB exposure and diabetes, but this was reported to be mainly due to low-chlorinated, non-dioxin-like congeners.

Grice et al. (2017) assessed the association of type 2 diabetes with persistent organic pollutants (POPs), including dioxin-like PCBs (PCB 105, 118, 156, 157, and 167) and HCB, using a nested case-control design from a relatively small prospective study conducted in American Indians in Arizona. A sample of 100 men and 200 women who were at high risk of diabetes were enrolled between 1965 and 1974. Participants underwent a baseline examination that included a blood draw and took an oral glucose tolerance test. The oral glucose test was repeated at each follow-up. Levels of PCBs and pesticides in the serum samples were assessed by CDC. Over 8 years of follow-up, 149 people developed diabetes

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(cases), and 151 remained non-diabetic (controls). Cases were followed from diabetes onset to end stage renal failure, death, or 2013. When models were adjusted for age, sex, BMI, 2-hour postload plasma glucose concentration, sample water loss, sample storage time, cholesterol, and triglycerides, the odds of diabetes were statistically significantly decreased for HCB exposure (OR = 0.64, 95% CI 0.41–0.99). In fully adjusted models, most dioxin-like PCBs were positively associated with incident diabetes, but none was statistically significant: PCB 105 (OR = 1.14, 95% CI 0.81–1.60), PCB 118 (OR = 1.16, 95% CI 0.82–1.65), PCB 156 (OR = 1.06, 95% CI 0.73–1.53), PCB 157 (OR = 1.06, 95% CI 0.75–1.49), and PCB 167 (OR = 1.11, 95% CI 0.80–1.55). This study is limited by the small sample size and the fact that serum concentrations of COIs were measured only once, yet the participants were followed for diabetes incidence for 8 years.

During 2007–2008, Singh and Chan (2017) conducted a cross-sectional survey study of 33 Canadian Inuit coastal communities and three inland communities. The survey included questions about health status, chronic diseases, and such behaviors as alcohol consumption, smoking, and exercise habits. Clinical tests such as fasting glucose and blood levels of PCBs and organochlorine pesticides supplemented the survey. In total, 2,172 Inuit people aged 18 years or older participated and provided blood samples. Of the PCB congeners measured in the blood, the dioxin-like mono-ortho PCBs 105, 118, and 156 are of interest for the committee's charge. Associations of diabetes with individual PCB congeners, the sum of dioxin-like PCBs, the sum of non-dioxin-like PCBs, and total PCBs were determined. Of the 2,172 respondents, 147 (5.7%) self-reported diabetes, with nearly the same proportions among males and females (5.5% and 5.8%, respectively). As expected, the percentage of people with diabetes increased as age increased (2.9% in the 31- to 50-year-old category and 22.1% in the 71- to 90-year-old category), and diabetes was clustered within families (either parent having diabetes: 19.7% in diabetics versus 10.4% in non-diabetics; or having a sibling with diabetes: 20.4% in diabetics versus 4.5% in non-diabetics). All contaminant concentrations were higher in respondents with diabetes. The concentrations of congeners were divided into quartiles, and odds ratios—adjusted for age, sex, BMI, high density lipoprotein-C, omega-3/omega-6 ratio, and education—were calculated for quartile 4 versus quartile 1 of self-reported diabetes. Statistically significant ($p < 0.05$) positive ORs were found for PCB 105, PCB 118, and PCB 156 as well as for some of the non-dioxin-like PCBs. With lipid adjustment, statistically significant positive ORs were found for dioxin-like PCB 118 and PCB 156 (information taken from graph; exact numbers not reported). The study also reported fasting glucose β coefficients for PCB congeners that were statistically significant based on lipid standardization or lipid adjustment; the congeners included PCB 105 and PCB 118. These models were adjusted for age, sex, BMI, high density lipoprotein-C, triglycerides, alcohol intake, smoking, omega-3/omega-6 ratio, selenium, and education. The β coefficients for wet-weight analyses were positive and were statistically significantly associated with

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an increase in fasting glucose for PCB 105 (6%, 95% CI 3%–9%) and PCB 118 (7%, 95% CI 4%–10%). The primary limitation of this study is its cross-sectional design, but the half-lives of these two congeners are estimated at 4.5–5.5 years (Grandjean et al., 2008), and the results of this study agree with other similar studies that found positive associations between PCBs and diabetes in different exposed populations.

Using data collected as part of cross-sectional health study of Taiwan residents living near a closed PCP-producing factory, C. Y. Huang et al. (2015) conducted an analysis of exposure to PCDD/Fs in order to evaluate the association between such exposures and type 2 diabetes. More details on the factory, exposure, recruitment, and collection of health information are provided in Chapter 5, but, briefly, the environmental contamination took place 25–40 years prior to the study. Of the 2,898 participants included in this analysis, 1,143 had a serum dioxin level between 20 and 63 pg WHO98-TEQ_{DF}/g lipid, which was considered a "high dioxin level"; and 284 subjects had a dioxin level of 64 pg WHO98-TEQ_{DF}/g lipid or higher. Of the 2,898 participants included in this analysis, 425 had diabetes, defined as having a fasting plasma glucose level above 126 mg/dL or an existing diagnosis. Subjects who had diabetes were compared with those who did not have diabetes; these two groups were comparable with regard to sex and reported family history of diabetes, but differed in age and BMI (high BMI was defined as $\geq$ 24 kg/m^2), factors that were controlled in analyses. Serum dioxin levels of 20–63 pg WHO98-TEQ_{DF}/g were associated with statistically significantly increased odds of diabetes (OR = 4.4, 95% CI 3.4–5.7), and the association was even stronger for serum dioxin levels above 64 pg WHO98-TEQ_{DF}/g lipid (OR = 7.8, 95% CI 5.6–10.9). After adjusting for age and BMI in multiple logistic regression models, high dioxin levels of 20–63 pg WHO98-TEQ_{DF}/g lipid (OR = 2.1, 95% CI 1.5–2.8) and $\geq$ 64 pg WHO98-TEQ_{DF}/g lipid (OR = 2.7, 95% CI 1.9–4.0) were found to increase the risk for diabetes. When the dioxin level was included as quartiles in the model, adjusted ORs remained elevated and statistically significant, with a statistically significant and steep upward trend ($p < 0.001$). Additional analyses replaced age with the duration of residency in the endemic area in the model, and after adjustment for the duration of residency and BMI, a high dioxin level of 20–63 pg WHO98-TEQ_{DF}/g lipid (OR = 2.2, 95% CI 1.6–3.0) and $\geq$ 64 pg WHO98-TEQ_{DF}/g lipid (OR = 3.6, 95% CI 2.4–5.4) remained strongly associated with risk of diabetes. The duration of residency in the endemic area was also an independent risk factor for diabetes, with a positive trend ($p = 0.01$). The strengths of this study include its large population (including a substantial proportion with diabetes, n = 425, the largest number of cases in studies newly reviewed in this volume); serum measurements of dioxin concentration; adjustments for age, BMI and residency; and a clear definition of diabetes and control groups. General limitations for this study and all studies that used the cross-sectional data collected from this population include an unknown age at first exposure to PCDDs and PCDFs, unknown

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cumulative exposure dose, the cross-sectional design, and the lack of additional data on other potentional confounders, such as waist circumference, dietary intake, and socioeconomic status.

Van Larebeke et al. (2015) describes how dioxin-like activity and serum HCB affected the risk of diabetes in both men and women (n = 1,583; 775 men and 808 women) enrolled by biomonitoring programs organized by the Flemish Centre for Environment and Health. Associations were based on self-reported health outcomes obtained in questionnaires administered in 2011. Blood and urine samples provided measures of internal exposure to organochlorine pollutants, PCB 118, and cadmium; samples had been collected from these subjects between September 2004 and June 2005. The researchers also assessed dioxin-like activity in pg TEQ/g fat. Among the participants, 30 men reported having diabetes (6.4%), and 62 men (13.2%) reported having either diabetes or a related condition; while 29 (5.8%) women reported having diabetes, and 55 (10.9%) women reported having diabetes or a related condition. A significant association ($p \leq 0.05$) was found between internal exposure to the above mentioned pollutants and diabetes or a related condition when both men and women were combined; however, when the estimates were adjusted for additional covariates and stratified by sex, serum dioxin-like activity showed a significantly positive association with diabetes and with diabetes or a related condition for men and women separately. Results from this study suggest that exposure to dioxin-like chemicals increases the risk of diabetes or a related condition.

Other Identified Studies

Two other studies of diabetes were identified but either lacked exposure specificity (Swaminathan and Thangavel, 2015) or examined the association of diet in diabetics on serum levels of persistent organic pollutants (Kahleova et al., 2016), which is not considered relevant to the committee's charge.

Biologic Plausibility

Data from cell culture and animal models support the potential diabetogenic effects of TCDD in humans. TCDD is known to modify the expression of genes related to insulin transport and signaling and to inflammation (Ambolet-Camoit et al., 2015; M. J. Kim et al., 2012), which play a pivortal role in diabetes progression. In previous VAO updates, several studies have been reviewed that support the postulate that TCDD is mechanistically implicated in an increased risk of insulin resistance and in the development of diabetes. C. Wang et al. (2011) found that mice that lacked the aryl hydrocarbon receptor (Ahr) had enhanced insulin sensitivity and enhanced glucose tolerance, suggesting that Ahr has a physiologic function in glucose metabolism. This is supported by studies that found that sustained activation of Ahr by dioxin-like chemicals could contribute to diabetes,

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including a study by Kurita et al. (2009), who found that exposing mice to dioxin significantly reduced insulin secretion after a glucose challenge. Recent data also demonstrate the importance of Ahr in glucose and fat metabolism, showing that the chemical inhibition of Ahr leads to decreased obesity and fatty livers in both male and female mice (Moyer et al., 2017). In an in vitro study of differentiated adipocytes, TCDD was found to significantly reduce insulin-stimulated glucose uptake (Hsu et al., 2010). Furthermore, P. Lu et al. (2015) found that activation of the AHR leads to dysregulation of the link between fatty liver and insulin resistance, suggesting a mechanism involving fibroblast growth factor 21. A recent study used a transcriptomic approach to identify potential molecular mechanisms of TCDD-induced changes in the insulin secretion of pancreatic islets and β cells (Lai et al., 2017). Taken together, these data provide potential mechanims for the metabolic-modulating effects of AHR and dioxins that are critical for the progreassion of metabolic diseases, including diabetes.

Among the studies included in the current literature review, K. S. Kim et al. (2014) explored the relationships of diabetes and insulin resistance with 14 organochlorine insecticides and 22 PCBs in visceral adipose tissue and subcutaneous adipose tissue in 50 patients with or without type 2 diabetes who underwent surgery for either cancer or benign liver or gallbladder lesions. The researchers reported that persistent organic pollutants in visceral or subcutaneous fat were significantly associated with both diabetes and insulin resistance. These findings are consistent with experimental animal studies that have reported that exposure to persistent organic pollutan mixtures through contaminated fish oil induces a severe impairment of whole-body insulin action (e.g., Ibrahim et al., 2011). Thus, on balance, there is biological plausibility for the COIs being causally implicated in the development of insulin resistance and diabetes. No new content has been identified since *Update 2014*.

Synthesis

The considerable amount of new evidence regarding type 2 diabetes reviewed and considered by the committee in forming its judgment included studies on male Vietnam veterans from the United States and New Zealand and studies of occupational cohorts and residential population-based studies of exposure. The analysis of AFHS data on testosterone levels and incident diabetes was considered to be of limited value to the committee's charge because the results were not stratified by exposure status and because serum concentrations of dioxin were not included in the analysis (Mazur et al., 2014). Cox et al. (2015) calculated annual rates of hospital discharge records for New Zealand Vietnam veterans (presumed to be exposed to herbicides based on deployment to Vietnam) compared with the general residential population of New Zealand. Although slightly elevated among the veterans, the standardized hospitalization ratio for diabetes was not statistically significant. However, because diabetes is not usually a condition that

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requires hospitalization, it is likely that only those with the most severe form of the disease were identified, and the prevalence of diabetes is expected to be higher. Moreover, this analysis is limited because exposure was not confirmed through serum measurements, and hospitalization estimates were adjusted only for age and not for other important risk factors, such as BMI or ethnicity.

The four occupational studies that were examined presented mixed results concerning an association between exposure to the COIs and diabetes mortality or prevalence. Among U.S. workers who were exposed to dioxins through the manufacturing of TCP or PCP, no difference in the rate of death from diabetes was found between either group of workers and the general U.S. population (Collins et al., 2016). Although serum dioxin measurements were collected, no results based on those measurements were presented for diabetes; instead the authors used employment records to categorize exposure, which may have introduced exposure misclassification, and use of the general U.S. population as a comparison likely introduced selection bias. Moreover, mortality is a poor measure of diabetes prevalence since while diabetes is often a contributing factor, it is not the actual cause of death and so may not be listed on the death certificate. Three international cohorts of workers examined the prevalence of diabetes. New Zealand workers who were employed in a phenoxy herbicide production plant that produced 2,4,5-T were also potentially exposed to the intermediates of TCP and other chlorophenols, and TCDD. 't Mannetje et al. (2018) extended the follow-up period of the 245 men and women in this cohort to approximately 30 years from their last 2,4,5-T production exposure. Comparisons of workers by job title (high- versus low-exposure jobs) found that workers with highly exposed jobs had an elevated risk of doctor-diagnosed diabetes after controlling for age, gender, smoking, BMI, and ethnicity, but when the comparison of diabetes prevalence was based on serum TCDD concentration, no difference in the risk of diabetes was found for workers in the high- versus low-exposure groups, although both of the estimates were imprecise. A cross-sectional study of Japanese incinerator plant workers that included serum dioxin measurements found that the duration of employment was positively associated with TEQ levels (Yamamoto et al., 2015). Diabetes was statistically significantly associated with PCDDs, PCDFs, coplanar PCBs, and total dioxins based on adjusted odds ratios between the first and fourth quartile, and tests for trend were also significant for each of these COIs. Finally, among 235 Italian electric arc furnace workers exposed to multiple agents including metals and PCDD/Fs, PAHs, and PCBs, as sampled in the foundry dust, the prevalence of diabetes among the workers was statistically significantly increased compared with the general population; however, this study is quite limited because foundry dust is a complex mixture, and the authors were unable to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those exposed workers. Estimates were adjusted only for age group and were not adjusted for other risk factors or activities that could affect the association (similar to Cox et al., 2015). It is likely

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that many of the occupationally exposed study populations received co-exposures to metals and chemicals other than those that the committee was charged with specifically reviewing. Co-exposure to metals is a possible confounder that may affect the estimates and associations reported in those studies but none of them attempted to adjust for this factor.

Several studies that examined environmental exposures to the COIs or related chemicals were also reviewed. Four of the studies used U.S. populations. An analysis of NHANES data that examined serum concentrations of three chlorinated dibenzo-*p*-dioxins, one chlorinated dibenzofuran, and four dioxin-like PCBs and diabetes-related nephropathy found that none of the eight dioxin-like chemicals were associated with pre-diabetes (Everett and Thompson, 2016). Two other studies, both of U.S. American Indian populations, were examined for diabetes prevalence (Aminov et al., 2016a,b) and incidence (Grice et al. 2017) with exposure to the COIs. Aminov et al. (2016a,b) found an association between pesticide (including HCB, a dioxin-like chemical) and PCB exposures and diabetes among a sample of people from the Mohowk Nation; however, analyses of individual dioxin-like chemcials or congeners were not presented. In a study of American Indian men and women in Arizona, Grice et al. (2017) examined incident type 2 diabetes with a one-time baseline measurement of serum concentrations of POPs, including dioxin-like PCBs and HCB, and found that the adjusted odds of diabetes were statistically significantly decreased for HCB exposure and that none of the measured dioxin-like PCBs were statistically significantly associated with incident diabetes.

Three international studies of environmental exposures to the COIs and diabetes were reviewed for the current volume. The largest study of diabetes reviewed in this volume was a population-based study of diabetes using residents living in close proximity to a closed PCP-producing factory in Taiwan that had contributed to a high dioxin (PCDD/Fs) contamination of the environment between 1965 and 1979 (C. Y. Huang et al., 2015). Blood samples and health information were collected on 2,898 residents in 2005–2007; 1,143 participants had dioxin levels between 20 and 63 pg WHO98-TEQ_{DF}/g, and 284 subjects had a dioxin level of 64 pg WHO98-TEQ_{DF}/g lipid or higher (considered to be the high-exposure groups). Serum dioxin levels of 20–63pg WHO98-TEQ_{DF}/g were associated with statistically significantly increased odds of diabetes in both crude and adjusted models (adjusted for age, BMI, and years of residence in the contaminated area), and the association was even stronger for serum dioxin levels above 64 pg WHO98-TEQ_{DF}/g lipid. When the dioxin level was analyzed in terms of quartiles in the model, the adjusted estimates remained elevated and statistically significant, with a statistically significant and steep upward trend ($p < 0.001$). In a cross-sectional survey of health outcomes that included a blood draw to measure the serum concentrations of PCBs (including dioxin-like mono-ortho congeners 105, 118, and 156) and organochlorine pesticides in 2,172 Canadian Inuits aged 18 years and older, 147 respondents self-reported diabetes

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(Singh et al., 2017). All contaminant concentrations were higher in respondents with diabetes. The concentrations of congeners were divided into quartiles, and the adjusted odds ratios for diabetes were found to be statistically significant for quartile 4 versus quartile 1 for PCB 105, PCB 118, and PCB 156. In the third international environmental study, Van Larebeke et al. (2015) conducted a general population study in Beligium and described how dioxin-like activity and serum HCB affected the risk of diabetes in both men and women. They assessed dioxin-like activity in pg TEQ/g fat and found a statistically significant association for both sexes between internal exposure to the COIs as well as dioxin-like activity in pg TEQ/g fat and diabetes or a related condition.

Several lines of toxicologic evidence support mechanisms by which dioxins and dioxin-like chemicals could increase risk for diabetes. First, these chemicals modify the expression of genes related to insulin transport and signaling and to inflammation (Ambolet-Camoit et al., 2015; Kim et al., 2012). Studies in Ahr knockout mice demonstrate increased insulin resistance and glucose tolerance (J. Wang et al., 2011), findings that are consistent with an experiment that found dioxin-exposed mice to have significantly reduced insulin secretion after a glucose challenge (Kurita et al., 2009). More recently, Lai et al. (2017) used a transcriptomic approach to identify the potential molecular mechanisms of TCDD-induced changes in the insulin secretion of pancreatic islets and β cells.

In the aggregate, the epidemiologic studies provide new evidence supporting the previously observed associations between dioxins or dioxin-like PCBs and increased risk for type 2 diabetes. Although some studies had substantial limitations or weaknesses, the Taiwanese study was large and demonstrated that even after adjustment for the factors associated with diabetes in their study, the odds ratios for dioxin-like chemicals equivalents had a strong monotonic trend for higher risk. The committee was divided on whether the newly reviewed studies were adequate to move the level of evidence of association of the COIs and diabetes to sufficient versus leaving the conclusion as limited or suggestive.

Conclusion

Following extensive deliberations regarding the strengths and weaknesses of the new evidence and evidence from studies reviewed in previous *VAO* reports, the current committee could not reach a consensus on whether the body of literature continues to constitute limited or suggestive evidence of an association between exposure to the COIs and diabetes or whether it now meets the criteria, shown below, for sufficient evidence of an association:

> Epidemiologic evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between exposure to herbicides and the outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example, if several small studies

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> that are free of bias and confounding show an association that is consistent in magnitude and direction, then there could be sufficient evidence of an association.

Studies quite consistently show a relationship between exposure to dioxin and dioxin-like chemicals, characterized via serum levels, occupation, or subject self-report, and measures of diabetes health outcomes. These studies include multiple, independent studies of Vietnam veteran populations as well as studies of diverse cohorts of men and women with occupational or environmental exposure to the COIs. Much is known about the risk factors for diabetes, such as age, obesity, and family history, and these have by and large been controlled for in the analyses of most studies reviewed. The studies of Ranch Hand veterans reviewed by previous committees and the Taiwan study examined by this committee demonstrate strong dose–response trends and excellent control for confounding. The disease is, unfortunately, common enough that it has been feasible for a number of investigators to conduct epidemiological investigations in worker or general populations with sufficient statistical power to allow for conclusions to be drawn from the results. Importantly, there is a separate scientific literature that has identified candidate biologic mechanisms that would account for the observed health outcomes in humans.

However, the human evidence base also has its weaknesses. Although positive associations have been observed, some of the relative risks reported are low. A number of studies examined cohorts exposed to mixtures of both dioxin and dioxin-like chemicals and, importantly, a number of other chemicals that could plausibly influence diabetes outcomes. This lack of exposure specificity complicates any attribution of the outcome to the COIs. While most studies adjusted for the primary risk factors for diabetes, several investigations relied on self-reported information that might affect the development of the disease, rendering any adjustment for confounders possibly less effective. The studies of diabetes mortality are of limited utility because death from diabetes, either as a primary or a contributing cause, is underdiagnosed, which could introduce bias. Finally, some committee members felt that it is not yet possible to dismiss the notion that an as yet unidentified systematic bias, including confounding, may be influencing the observed results.

Given these observations, it was not clear to all committee members that a category change was appropriate. Thus, the committee was unable to reach consensus as to whether the evidence regarding exposure to the COIs and diabetes should be classified as "limited or suggestive" or "sufficient."

CIRCULATORY DISORDERS

Circulatory diseases are a group of diverse conditions, of which hypertension, coronary heart disease, and stroke are the most prevalent. CDC reports that

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the number one cause of death for people 65 years of age and older is diseases of the heart. In the United States, 28.4 million adults (11.7% of the population) have a physician-diagnosed heart disease. American Indian and Alaska Natives have the highest incidence of heart disease, estimated at 13.7%, followed by whites with 11.3% and blacks with 9.5% (CDC, n.d.).

In 2016 more than 630,000 people died as a result of diseases of the heart, and more than 140,000 people died as a result of cerebrovascular disease and stroke (CDC, 2016). In addition to family history, the major risk factors for circulatory diseases include age, male gender, smoking, hyperlipidemia, diabetes, and hypertension (World Heart Federation, 2018). Ideally, epidemiologic investigations of circulatory diseases would consider the conditions in this category separately rather than as a group because they all have different patterns of occurrence, and many have different etiologies. However, many mortality studies follow the ICD-9 rubric and report deaths from circulatory diseases together as a single category. Deaths from coronary or ischemic heart disease (IHD), heart failure, and, to a lesser extent, stroke predominate. Many of the reports also break out subcategories such as cerebrovascular disease and hypertension. The American Heart Association reports mortality related to coronary heart disease, not to its symptoms, which include angina and myocardial infarction. The relative importance of heart failure is determined by the age of the cohort. In younger age groups, most of the deaths in this category are expected to be from IHD. In most cases, cerebrovascular deaths are deaths from strokes, which can be classified as either ischemic or hemorrhagic. In the U.S. population, the great majority of strokes are of the ischemic type.

The methods used in morbidity studies of circulatory diseases may involve a direct assessment of the circulatory system, including an analysis of symptoms or history, a physical examination of the heart and peripheral arteries, ultrasound measurements of the heart and arteries, electrocardiography (ECG), chest radiography, cardiac computed tomography (CT), and, more recently, cardiac magnetic resonance imaging (MRI). Carotid ultrasound, echocardiogram, CT, MRI, and nuclear medicine studies can be used to visualize the heart and related vasculature directly as well as to assess function. ECG can be used to detect heart conditions and abnormalities, such as arrhythmias (abnormal heart rhythms), heart enlargement, and ischemia (acute or past myocardial infarctions). Chest radiography can be used to assess the consequences of IHD and hypertension, such as the enlargement of the heart often seen in heart failure. It is sometimes difficult to determine the time of onset of clinical findings, making the temporal relationship between exposure and disease occurrence uncertain. Although the ideal way to study cardiovascular outcomes would be to prospectively following a well-characterized cohort and monitor for the incidence of discrete clinical events, researchers often must rely instead on medical records (which may or may not have all CVD-related information and outcomes) or death certificates (which may list nonspecific causes of death [e.g., respiratory arrest] and may not

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contain all comorbid or contributing factors of death). New-onset angina or the performance of a revascularization procedure in a person who has no history of disease is also used as evidence of incident disease.

The committee responsible for *Update 2006* began evaluating hypertension separately from other circulatory diseases, and the committee responsible for *Update 2008* began the practice of evaluating IHD separately from other cardiovascular outcomes. Beginning in *Update 2012*, stroke and cerebrovascular disease were also considered separately from discussions of "other circulatory diseases." The current committee has continued the practice of evaluating the literature related to these outcomes as independent subsections.

A number of studies of different populations that received potentially relevant exposures were identified in the literature search, but the studies did not characterize exposure with sufficient specificity for their results to meet the committee's criteria for inclusion in the evidentiary database (see Chapter 3), and they are only briefly mentioned under the heading of "Other Identified Studies." For instance, this rubric would apply to the occupational study conducted by Ruder et al. (2014) in which 24,865 eligible workers from capacitor manufacturing, repair, and maintenance sites in the United States were exposed to arochlor 1254, 1242, and 1016, among others (mixed PCBs), and the authors sought to examine the relationship between PCB exposure and different causes of mortality. However, the study did not name specific dioxin-like PCBs, and no TEQs or other quantification of relevant exposures was presented. Likewise, Ljunggren et al. (2014) assessed serum concentrations of dioxin-like chemicals in patients diagnosed with CVD but did not define how this determination was made, and therefore the work is of little utility to the commmittee.

Table 42, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to circulatory disorders.

Biologic Plausibility

Studies have demonstrated that both the vasculature and the adipose tissue are targets of TCDD toxicity, and these studies have provided a mechanistic understanding of how TCDD exposure increases the risk of circulatory diseases, such as hypertension, IHD, and stroke. Exposing cultured endothelial cells or cultured adipocytes to TCDD induces major changes in gene expression and leads to substantial increases in oxidative stress and inflammatory markers (Andersson et al., 2011; S. G. Han et al., 2012; Ishimura et al., 2009; Kerley-Hamilton et al., 2012a; M. J. Kim et al., 2012; Kopf and Walker, 2010; Majkova et al., 2009; Puga et al., 2004; Qin et al., 2015; Zhou et al., 2017). Studies also indicate that the exposure of cultured endothelial cells to TCDD results in the down-regulation of genes involved in blood pressure regulation and the up-regulation of genes involved in the myocardial infarction pathways (Qin et al., 2015) and can promote endothelial cell apoptosis (Q. Liu et al., 2017). In animal models, developmental

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exposure to TCDD has been shown to cause structural, molecular and functional cardiac abnormalities, and altered heart physiology in mouse embryos and to predispose adults to cardiac disease (Carreira et al., 2015). Furthermore, the loss of AHR, as happens in Ahr knockout mice, is associated with decreases in blood pressure (modeling hypotension), while sustained activation of AHR resulting from dioxin exposure leads to increases in blood pressure (Agbor et al., 2011). N. Zhang et al. (2010) showed that the genetic loss of AHR from all tissues or solely from endothelial cells results in hypotension. In contrast, Kopf et al. (2010) demonstrated that the chronic exposure of mice to TCDD induces hypertension that is associated with significant increases in vascular oxidative stress and decreases in vascular relaxation. Those changes in vascular function and blood pressure could be mediated in part by increases in the metabolism of arachidonic acid to vasoconstrictive and inflammatory eicosanoids (Bui et al., 2012; Diani-Moore et al., 2014). Studies have also demonstrated that exposure to AHR agonists, including TCDD and benzo[*a*]pyrene, increases the incidence, severity, and progression of atherosclerosis, a primary cause of IHD and stroke (Dalton et al., 2001; Kerley-Hamilton et al., 2012a; D. Wu et al., 2011). Furthermore, D. Wu et al. (2011) demonstrated that TCDD mediates those effects in part by increasing vascular inflammation, a finding that is suppored by a recent study in chick embryos showing that TCDD-cardiotoxicity is eliminated with exposure to a COX-2 inhibitor (Fujisawa et al., 2014). Another study in human coronary artery smooth muscle cells indicates that the AHR pathway can cooperate with the Tcf21 pathway to initiate the expression of pro-inflammatory genes (J. B. Kim et al., 2017). In addition to the vasculature, studies also suggest that the heart is a target of TCDD. TCDD exposure increases the hypertrophy of rat cardiac cells in culture (Zordoky and El-Kadi, 2010) and impairs the differentiation of mouse embryonic stem cells into cardiomyocytes (Neri et al., 2011).

In addition to the direct effects of TCDD on the vasculature and heart, there is evidence that TCDD influences other CVD risk factors, for example, by promoting obesity (Brulport et al., 2017; Kerley-Hamilton et al., 2012b), accumulating macrophage lipid, inducing lipid mobilization, and altering lipid metabolism. Thus, on the basis of animal models, there appear to be several overlapping and potentially contributing pathways that link TCDD exposure and increased CVD risk.

Arsenic exposure has also been linked to heart effects in a study examining the relationship between the expression of AHR and CYP1A1 in humans exposed to arsenic in drinking water. Data from this study indicate that arsenic exposure was associated with an increased expression of AHR and CYP1A1 in the blood and that increased CYP1A1 was associated with a prolonged corrected QT interval following long-term exposure to arsenic (Cui et al., 2016).

Long-term exposure to oxidative stress is suspected to be etiologic to many chronic diseases, including CVDs. A variety of data, some discussed above, demonstrates a link between TCDD exposure and oxidative stress (reviewed in

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Mohsenzadeh et al., 2018). A recent small study by Lerro et al. (2017) indicates a link between 2,4-D and biologic markers of oxidative stress in 30 farmers who participated in the AHS and who applied pesticides occupationally and in 10 non-farming controls who did not apply pesticicides; all were non-smoking men ages 40–60 years. Multivariate linear mixed-effect models for repeated measures were used for each pesticide–oxidative stress marker combination, and all estimates were adjusted for age, farmer or control subject, study time point, and creatinine to account for pesticide metabolites and oxidative stress markers in urine. Other confounders included were BMI, smoking history and duration, alcohol use, regular physical activity, multivitamin or vitamin C supplementation, infection or symptoms at sample collection, allergy symptoms at sample collection, and history of cancer. Farmers had significantly higher urinary 2,4-D levels compared with controls, and this was associated with elevated levels of 8-OHdG, a marker of oxidative stress ($\beta = 0.066$, 95% CI 0.008–0.124), and 8-isoPGF, a product of lipoprotein peroxidation ($\beta = 0.088$, 95% CI 0.004–0.172). Thus, this study provides one route to plausibility for the 2,4-D association with cardiovascular outcomes, although studies will need to be specifically conducted in individuals with known clinical endpoints (e.g., hypertension) in order to implicate this mechanism directly.

Hypertension

Hypertension, typically defined as blood pressure above 140/90 mmHg, affects more than 70 million adult Americans and is a major risk factor for coronary heart disease, myocardial infarction, stroke, and heart and renal failure. The major quantifiable risk factors for hypertension are well established and include family history, age, sex, race, obesity, reduced nephron number, high dietary salt intake, tobacco use, excessive alcohol intake, and physical inactivity (CDC, 2014b). The strongest conclusions regarding a potential increase in the incidence of hypertension come from studies that have controlled for these risk factors. CDC estimates that in the United States, 64% of men and 69% of women ages 65–74 years have hypertension. When stratified by race/ethnicity and sex, the prevalence of diagnosed hypertension is highest among African American men and women (43.0% and 45.7%, respectively), followed by white men and women (33.9% and 31.3%, respectively). For the groups reported, hypertension is lowest among Mexican Americans (27.8% for men and 28.9% for women) (CDC, 2018).

Conclusions from *VAO* and Previous Updates

The committee responsible for *Update 2006* first concluded that the available evidence placed hypertension in the limited or suggestive category, based primarily on consistent evidence from several studies of Vietnam veterans that used measured serum concentration of dioxin. Additional evidence reviewed

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in *Update 2008*, *Update 2010*, *Update 2012*, and *Update 2014* reaffirmed this conclusion.

Several studies of hypertension among Vietnam veterans have been reviewed in the VAO series. These have included well-designed studies of incidence, prevalence, or mortality in the U.S. ACC (Cypel and Kang, 2010; Kang et al., 2006) and the AFHS (AFHS, 1995, 2000, 2005) cohorts that have consistently reported increased hypertension with increasing levels of serum dioxin. Other studies of U.S. Vietnam veterans that did not use serum dioxin concentrations as markers of exposure also reported an increased prevalence of hypertension associated with presumed exposure to herbicides.

Among international cohorts of Vietnam veterans, the prevalence of and mortality due to hypertension have been assessed among Australians and South Koreans. A statistically significant increased prevalence was found among the Australian veterans compared with standardized population controls. Two prevalence studies of hypertension among the Korean Vietnam veteran cohort did not find an increased prevalence of hypertension (Yi, 2013; Yi et al., 2014a), but the studies were limited because they do not include veterans who died or relocated between their Vietnam service and the start of the investigation. Thus, the validity of the calculated exposure–outcome relationship is based on the strong assumption that the observed relationships in those included are similar to those who were not included, which is doubtful. In some cases, 40% of the relevant data for the population are missing. In addition, the determination of hypertension was either by self-report or through health insurance claims. It cannot be certain that all participants with hypertension were detected because no standardized blood pressure assessment was done.

Mortality studies that report hypertension are rarely informative because hypertension is so prevalent in the adult population and many more people die with hypertension than from hypertension. For those with hypertension listed as the cause or a contributing cause of death, it is uncertain how representative those who died from hypertension are of all people who may have developed it. Several mortality studies that included hypertension have been reviewed by VAO committees, but they have shown inconsistent findings, likely because they suffer from the limitations listed above as well as from a lack of adjustment for many important confounding factors. A decreased, but not statistically significant, risk of mortality from hypertension was found in the study of U.S. women veterans as well as when the population was limited to nurses only (Kang et al., 2014a), whereas a slight, but again, not statistically significant, increase was reported among Korean veterans (Yi et al., 2014b).

The studies of occupational cohorts reviewed by previous VAO update committees rarely reported hypertension as a discrete outcome; among those that did, the results were mixed, with reports of both increased and decreased risk, but none of the risk estimates were statistically significant. Several studies did not define hypertension, therefore making it difficult to draw conclusions on its

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association with the COIs. Similar mixed and not statistically significant findings were reported for the environmental studies that have been reviewed.

Update of the Epidemiologic Literature

Six new studies of exposure to the COIs and hypertension have been published since *Update 2014*.

Vietnam-Veteran Studies Cypel et al. (2016) analyzed the results of a 2013 survey of 3,086 (80.3% response rate) ACC veterans that compared outcomes in both Vietnam-deployed (n = 1,477) and non-Vietnam-deployed (n = 1,609) herbicide sprayers and non-sprayers. Eligibility for the ACC cohort was restricted to men who had a minimum of 18 months of active U.S. Army service from July 4, 1965, to March 28, 1973, and who were alive in October 2011 and whose health allowed them to participate. ACC veterans were specifically involved in chemical operations in Vietnam and were directly exposed to herbicides and other chemicals, including tear gas and napalm (see description of the ACC veterans in Chapter 5). Participants self-reported physician-diagnosed hypertension, but the diagnosis was evaluated and confirmed by blood pressure measurements taken by trained medical technicians and by medical record reviews for a subset of 468 individuals. Overall agreement between the medical records review and self-reported hypertension was 89%. Additionally, individual exposure to herbicides was collected by self-report to questions of mixing, handling, and spraying herbicides while in the military and was verified by using measured serum TCDD levels for a subset of 636 individuals who had participated in an earlier health survey of the cohort (1999–2000) and who had had blood drawn and serum TCDD (ppt or pg/g lipid) measurements made at that time. A history of herbicide spraying was much higher among the Vietnam-deployed (62.0%) than non-Vietnam-deployed veterans (28.0%) ($p < 0.0001$). A greater percentage of Vietnam-deployed veterans were current or former smokers (72.3%) than non-Vietnam-deployed veterans (64.0%) ($p < 0.0001$). Hypertension was highest in Vietnam-deployed sprayers (81.6%), followed by non-Vietnam-deployed sprayers (77.4%), Vietnam-deployed non-sprayers (72.2%), and non-Vietnam-deployed non-sprayers (64.6%). Overall, self-reported hypertension was found in 78.0% of all participants who served in Vietnam and in 68.2% of all participants who did not deploy to Vietnam, indicating a possible effect of Vietnam tour of duty over and above exposure to herbicides. To demonstrate a dose–response relationship, the authors showed that the mean serum TCDD levels were significantly higher among Vietnam herbicide sprayers (mean = 3.5 ppt, range: 0.5–30.6) than among Vietnam non-sprayers (mean = 2.5 ppt, range: 0.7–17.7) ($p < 0.0001$), but no differences in mean TCDD level were observed between non-Vietnam-deployed sprayers (mean = 2.4 ppt, range: 0.7–9.6) and non-sprayers (mean = 2.2 ppt,

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range: 0.4–12.5) (p = 0.69). Using logistic regression and adjusting for Vietnam service status, rank, age at the time of the survey, tobacco use, alcohol use, race, and BMI, having sprayed herbicides (OR = 1.74, 95% CI 1.44–2.11) and having deployed to Vietnam (OR = 1.26, 95% CI 1.05–1.53) were both strongly associated with self-reported hypertension. The association was strongest when comparing Vietnam-deployed sprayers to non-Vietnam-deployed non-sprayers (OR = 2.21, 95% CI 1.76–2.77). Finally, among Vietnam-deployed veterans, a significantly elevated association between the odds of hypertension for sprayers and non-sprayers remained after an adjustment was made for potential confounders (OR = 1.77, 95% CI 1.35–2.30). Similarly, for those veterans who did not deploy to Vietnam, self-reported hypertension was significantly elevated when sprayers were compared with non-sprayers (OR = 1.72, 95% CI 1.31–2.26).

This was a well-designed study with a large sample size and conducted among the most relevant population (Vietnam veterans with known herbicide exposure) which included several levels of exposure (herbicide sprayers and non-sprayers and Vietnam-deployed and non-Vietnam-deployed) and an attempt to quantify it in the participants. Although serum TCDD concentrations were not available for all participants and were collected at least 25 years after Vietnam-era service, for those with serum TCDD levels available, self-reported herbide spray status had high agreement with the measured levels. The highest mean serum TCDD level was observed among sprayers deployed to Vietnam, and the lowest mean TCDD level was found for non-Vietnam non-sprayers, as would be expected, with a significant dose–response association. Likewise, there was high agreement (89%) between self-reported hypertension and in-person blood pressure measurements and medical records review for a subsample of study participants. The analyses controlled for important risk factors for hypertension, including age, race (white versus others), BMI, tobacco smoking status, rank, Vietnam service status, and alcohol intake, but did not collect information on (and therefore did not control for) other risk factors such as diabetes, a family history of hypertension, and dietary intake of sodium and fat. A major strength of this analysis was using the non-Vietnam-deployed ACC veterans as a comparison group because they were similar to members of the study group with respect to branch, length and time period of service, military occupation, and duties except for deployment in Vietnam, which has the effect of minimizing unmeasured exposures and confounders of concern and bias. Additionally, because all of the men who served in ACC units were stationed at Fort McClellan for at least some time, and Fort McClellan is in close proximity to Anniston, Alabama, where Monsanto operated a plant that produced PCBs, all ACC veterans were likely exposed to at least low levels of these and other chemicals. Therefore, comparisons using deployed and non-deployed ACC men are likely to be biased toward the null due to this baseline of increased exposure, but despite that, the adjusted effect estimate when Vietnam-deployed sprayers were compared with non-Vietnam-deployed non-sprayers was still more than twice as high, precise, and statistically

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significant. Although the exact types and quantities of the various chemicals these ACC veterans were possibly exposed to during the Vietnam War are unknown and may include chemicals other than the herbicides (such as insecticides, diesel and jet fuels, cleaning solvents, tear gas, napalm, and antimalarial medications), there is statistically significant support for an association between herbicide exposure and self-reported, physician-diagnosed hypertension.

Occupational Studies Cappelletti et al. (2016) performed a retrospective study of 331 male electric arc foundry workers at a single plant in Trentino, Italy, to determine if they experienced excess mortality from all causes or were at an increased risk for several other disases including complicated (with end organ damage) and uncomplicated (no end organ damage) hypertension, diabetes, and CVD due to their occupational exposure to foundry dust. An analysis of the dust emissions found that the dust contained metals (including iron, aluminum, zinc, manganese, lead, chromium, nickel, cadmium, mercury, and arsenic), PAHs, PCBs, and PCDD/Fs (reported as TEQs). Because foundry dust is a mixture, it is not known which of the agents were associated with a specific outcome or to what extent. The men included in the study had worked at the factory for at least 1 year. For the hypertension analysis, 235 living workers, were compared with the standardized general population of Region Trentino-Alto Adige (where the factory was located) because there were few non-exposed foundry workers and high attrition rates. Company and medical records were used to determine vital status. Requests for exemption health care fees were used as a surrogate measure to identify the most prevalent morbid conditions in the general population, which were then applied to the cohort to compute relative risks for each of the conditions. The workers were followed from March 19, 1979 (or their first day of employment), through December 31, 2009 or date of death. Effect estimates (prevalence ratios) were calculated using Mantel-Haenszel estimator adjusted for age group (20–64, 65–74, ≥ 75 years). Compared with the age-adjusted provincial population, statistically significantly elevated risks of both non-complicated hypertension (n = 30; RR = 2.44, 95% CI 1.75–3.40) and complicated hypertension (n = 14; RR = 2.22, 95% CI 1.35–3.65) were found among the electric arc furnace workers. The strengths of the study include its long-term follow-up of the workers. This study is most limited by the fact that foundry dust is a complex mixture, which made it impossible to discern the impacts of the specific contaminants of the foundry dust on the health outcomes of the exposed workers. Estimates were adjusted only for age group and were not adjusted for other risk factors such as tobacco use, BMI, or other jobs or activities that could result in similar exposures. The possible exposure to foundry dust by the general population that was used for comparison is not discussed, although the foundry appears to be in the local vicinity and emissions from it were reported to be present within a 2-kilometer radius of it.

Yamamoto et al. (2015) performed a cross-sectional study to investigate the health outcomes that 698 male workers sustained while employed at 36 municipal

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and private waste incineration plants in Japan; serum dioxin measurements were obtained for 678 of the workers. These workers were employed from 2000 to 2007. First, a questionnaire was completed by participants on lifestyle and medical history. Blood samples were taken from each subject to evaluate serum dioxin, PCDD, PCDF, and coplanar PCB levels (77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, and 189). Serum concentrations of dioxin isomers were expressed as pg/g lipid, converted to TEQ, and expressed as TEQ/g lipid. Both clinical and physiological examinations were also performed. Participants were then categorized into four groups: workers whose jobs did not involve working directly in an incineration facility, workers whose jobs did involve work inside the incineration facility (but only handling solidified fly ash and slag or residues that were nonflammable), workers whose jobs involved helping with incineration-related work inside an incineration facility, and workers whose jobs mainly involved the operation and maintenance of an incinerator including a furnace, electric dust collector, and wet scrubber inside an incineration facility. Subjects were tested for diabetes, hypertension, hyperlipidemia, and liver dysfunction. The total serum dioxin concentration level for incinerator workers was 13.7 (median) or 17.2 (arithmetic mean) pg TEQ/g lipid, while the (arithmetic mean) average total dioxin concentration was 19.4 pg TEQ/g lipid for the general population in Japan, indicating no real difference between the two groups. However, in the exposed workers, the duration of employment was positively associated with TEQ levels (9.8 and 21.7 pg TEQ/g lipid for those employed 9 years and 15.5 years, respectively). Coplanar PCBs were significantly correlated ($p < 0.05$) with 14 parameters of laboratory and physiological tests. Serum concentrations of total dioxins were higher in workers whose jobs involved operation, maintenance, and other incinerator work inside the facilities regardless of the duration of their employment as compared with workers without these job duties. Serum concentrations of dioxin congeners (PCDDs, PCDFs, coplanar PCBs, and total dioxins) were used to divide the participants into quartiles, and odds ratios for hypertension were computed for each category of dioxin congener by comparing each quartile to the lowest quartile (referent) and adjusting for age, survey year, BMI, smoking, and alcohol consumption. Although the adjusted associations between quartiles 2 and 3 and the referent were not consistently significantly elevated, the comparisons of quartile 4 with the referent group for PCDDs (OR = 1.66, 95% CI 1.00–2.78, $p < 0.05$), PCDFs (OR = 1.90, 95% CI 1.12–3.25, $p < 0.05$), coplanar PCBs (OR = 2.31, 95% CI 1.33–4.02, $p < 0.01$), and total dioxins (OR = 1.92, 95% CI 1.12–3.28, $p < 0.05$) showed elevated, statistically significant associations between the serum levels of PCDD/Fs and total dioxins with the prevalence of hypertension. The test for trend was also significant ($p < 0.05$) for each of these COIs. Overall, there was no difference in the prevalence of hypertension among the workers of all ages and the Japanese population (44.8% versus 46.2%, respectively). However, when stratified by age groups and compared with the general Japanese population, the prevalence of hypertension was

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statistically significantly increased in in workers between the ages of 29 to 49 years. Nonetheless, there were no statistically significant differences in any of the age groups in the total dioxin concentrations between the incinerator workers and the general population, suggesting that something other than dioxin may be contributing to the increased risk for hypertension among this younger population. The strengths of this study include a large sample size, the homogeneity of study subjects with respect to ethnicity and workplace, the measurement of exposure for individuals, significance for each congener, and the adjustments of multiple confounders in the analysis.

Environmental Studies Shiue et al. (2014) used the U.S. population-based NHANES study data from 2011–2012 to examine whether different sets of environmental chemical concentrations were risk factors of high blood pressure. The study used previously collected demographics and blood pressure readings along with the concentrations of various environmental chemicals (14 heavy metals and 20 industrial chemicals, including arsenic compounds and 2,4-D) measured in urine. The urine samples were available for only a subsample of the study population (20–30%). High blood pressure was defined as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg. The analysis used a total sample size of 9,756 participants who were 20 years of age or older, of whom 3,035 (31.1%) had high blood pressure. This sample is quite young, with 63% of participants being under age 39 years, a group in whom hypertension would be uncommon. The associations of the environmental contaminents with high blood pressure were adjusted for urinary creatinine, age, sex, ethnicity, and BMI and were weighted for the survey design using logistic regression. After full adjustment, even though a number of heavy metals and chemicals were associated with high blood pressure, none of them were the COIs. Of note, however, although total arsenic was marginally associated with high blood pressure in the weighted model (OR = 1.13, 95% CI 0.99–1.29, p = 0.066), trimethylarsine oxide (OR = 2.47, 95% CI 1.27–4.81, p = 0.011) and dimethylarsonic acid concentrations (OR = 1.42, 95% CI 1.12–1.79, p = 0.006) were associated with high blood pressure. Thus, in the general U.S. population, arsenic and its related compounds were found to elevate the risk for hypertension, even in this low at-risk NHANES sample, and the correlation was likely detected because of the large sample size and accurate determinations of exposure concentrations.

Van Larebeke et al. (2015) prospectively studied the associations of exposure to organochlorine pollutants, HCB, dioxin-like (PCB 118) and non-dioxin PCBs, and cadmium with a variety of self-reported health conditions, including diabetes and hypertension, as part of a Flemmish biomonitoring program. In 2004–2005, height, weight, urine, and serum were collected from the participants. Dioxin-like activity in pg TEQ/g fat was also assessed. Subjects filled out a survey collecting demographic data and information on education and tobacco and alcohol use. In 2011, participants in the program were mailed a second survey to collect updated

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information on general health, smoking and alcohol consumption behaviors, medical conditions, and current medications. There were 973 respondents, 504 women and 469 men (response rate 65.6%), whose information was used for the current analysis. Serum was assayed for HCB, dioxin-like PCB 118, and other chemicals. Medical diagnoses were all self-reported and not confirmed. For the diagnosis of hypertension, subjects were asked, "Do you suffer or have you suffered from arterial hypertension in the past 10 years?" Although 162 (34.5%) men and 172 (34.1%) women reported hypertension, it is likely underreported in this population of adults aged 50–65 years when recruited in 2002–2006. Overall, there were no differences in the levels of dioxin-like activity (pg/TEQ/g fat) or PCB 118 (ng/g fat) between participants and non-participants of the follow-up survey, emphasizing the representativeness of the sample. However there was a difference for HCB ($p = 0.051$). In the combined sample, after adjusting for BMI, exercise in minutes per week, level of education, and glasses of alcoholic beverages per week, dioxin-like activity was positively associated with a risk of hypertension (OR = 1.61, $p = 0.014$), as were HCBs (OR = 1.99, $p = 0.0005$); PCB 118 was not reported. PCBs with dioxin-like activity approached statistical significance in men only ($p = 0.066$), while HCBs were significant for both men and women in at least one model. In a crude model, the serum concentration of PCB 118 was not associatied with hypertension. In a model accounting for confounders and with additional adjustments made for correlated exposures (HCB, p,p′-DDE, cadmium, and non-dioxin PCBs), there was a significant association between serum PCB 118 levels and hypertension in men, but not women. The odds ratio for men for the 90th percentile vs. 10th percentile was 3.27 ($p = 0.0011$). In a model with additional adjustments for covariates and correlated exposures (HCB, p,p′-DDE, cadmium, and non-dioxin PCBs), the association with hypertension was marginally significant for men: the OR for 90th percentile versus 10th percentile was 1.95 ($p = 0.09$). The strengths of the study included its prospective design, its large sample size, the representativeness of the older adult general population, and the use of objective measures of exposure. However, the findings are limited by the absence of validated medical diagnoses. Although HCB was associated with hypertension in several models, mono-ortho dioxin-like PCB 118 was not associated with hypertension.

P. M. Lind et al. (2014) examined hypertension in participants of the PIVUS (Prospective Investigation of the Vasculature in Uppsala Seniors) cohort, who were men and women aged 70 years or older and residents of Uppsala, Sweden. Of 2,025 eligible subjects, only half (n = 1,016) agreed to participate. Participants completed a questionnaire to assess their medical history, smoking history, and medication use. A clinical exam including blood pressure measurement was performed, and fasting blood work was obtained for lipid and glucose analysis. Hypertension was defined as having a systolic blood pressure > 140 mmHg, a diastolic blood pressure > 90 mmHg, or using antihypertensive medication. General exposure to 23 persistent organic pollutants (POPs) was quantified from

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stored serum samples collected at the time of entry into the PIVUS study using high-resolution gas chromatography/mass spectrometry. Normalized POPs were estimated using individualized lipid levels and TEQs measured for dioxin-like PCBs and OCDD combined and separately for the dioxin-like coplanar non-ortho PCBs (PCB 126 and PCB 169) and the dioxin-like mono-ortho PCBs (105, 118, 156, 157, and 189). As the participation rate in this cohort was only 50%, an evaluation of cardiovascular disorders and medications in 100 consecutive non-PIVUS participants was also performed to serve as a control. Although both PIVUS participants and non-participants were broadly similar, the prevalence of diabetes, congestive heart failure, and stroke was higher among non-participants. Overall, 72% of the participants in this study had hypertension. When exposures were adjusted for gender, BMI, smoking status, exercise habits, and education levels, none of the dioxin-like PCBs were associated with prevalent hypertension: PCB 105 (OR = 1.23, 95% CI 0.96–1.60; p = 0.11), PCB 118 (OR = 1.26, 95% CI 0.95–1.67; p = 0.11), PCB 156 (OR = 0.9, 95% CI 0.63–1.3; p = 0.58), PCB 157 (OR = 0.9, 95% CI 0.65–1.26; p = 0.55), PCB 189 (OR = 0.87, 95% CI 0.69–1.09; p = 0.22), PCB 126 (OR = 1.1, 95% CI 0.94–1.3; p = 0.24), and PCB 169 (OR = 0.86, 95% CI 0.61–1.2; p = 0.36). When exposure was classified by individual POPs, PCB 105 (OR = 1.5, 95% CI 1.19–1.89) and PCB 118 (OR = 1.56, 95% CI 1.2–2.01) were significant when adjusted for gender only. This was a large and well-designed and analyzed study of elderly adults that when adjusted for gender, BMI, smoking staus, exercise habits, and education showed no associations between hypertension and dioxin-like PCBs.

Other Identified Studies One other occupational study was identified that reported deaths from hypertension with underlying heart disease, but it was limited by a lack of exposure specificity (Ruder et al., 2014). An environmental study was also identified (Akahane et al., 2017) that examined the prevalence of self-reported long-term health outcomes (including high blood pressure) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further.

Biologic Plausibility

The biological mechanism for dioxin's impact on hypertension is being investigated in animal models and human cell cultures, and it has shown clear effects on gene expression, vascular function, and lipid glucose metabolism. The exposure of human endothelial cells to TCDD causes the down-regulation of genes involved in blood pressure regulation (Qin et al., 2015), suggesting a potential molecular mechanism for TCDD's impact on hypertension. Recent data also indicate that the increase in hypertension following AHR activation is linked

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to the inactivation of endothelial nitric oxide synthases, which is expressed in endothelial cells and functions to modulate vascular blood pressure (C. C. Chang et al., 2017). Data also demonstrate a link to the Ahr pathway using mouse models, demonstrating that sustained Ahr activation by dioxins results in increased blood pressure, which is associated with significant increases in vascular oxidative stress and decreases in vascular relaxation (Kopf et al., 2010). Conversely, hypotension is associated with Ahr loss in knockout models, either knocked out in the whole animal or specifically in endothelial cells, (Agbor et al., 2011; N. Zhang et al. 2010). Those changes in vascular function and blood pressure could be mediated in part by increases in the metabolism of arachidonic acid to vasoconstrictive and inflammatory eicosanoids (Bui et al., 2012; Diani-Moore et al., 2014).

A recent study on genetic polymorphisms of the AHR signaling pathway genes in a human cohort recruited from central Russian (Kursk) cardiology and neurology clinics also suggests a plausible link between exposure to TCDD and dioxin-like chemicals and essential hypertension (Polonikov et al., 2017). Seven common polymorphisms in the AHR pathway genes *AHR*, *ARNT*, *AHRR*, *CYP1A1*, *CYP1A2*, *CYP1B1*, and *NQO1* were genotyped from venous blood samples of 1,341 cases and 819 controls. A polymorphism in ARNT was shown to be associated with increased essential hypertension, whereas a single nucleotide polymorphism in CYP1A2 showed a decreased risk of essential hypertension in a recessive genetic model. Additional log-likelihood ratio tests showed epistatic interactions on essential hypertension susceptibility for all single nucleotide polymorphisms. These results are generally consistent with the suggestion that the AHR pathway is involved in hypertension and that TCDD activation of this pathway can influence hypertension in the human population.

Synthesis

Hypertension, defined as a systoloic/diastolic blood pressure exceeding 140/90 mmHg, affects approximately 75 million Americans, or one in every three adults. This trait remains one of the main contributing risk factors to cardiovascular, peripheral vascular, and cerebrovascular disease. Risk factors include family history, age, sex, race, obesity, reduced nephron number, high dietary salt intake, tobacco use, excessive alcohol intake, and physical inactivity. Owing to its frequency, assessing whether there is increased risk with exposure to the COIs has been challenging. However, the committee for the current update believes that there are enough new data to move the category of association to sufficient evidence.

Cypel et al. (2016) offers the most compelling evidence for the change. The well-designed study was conducted in the population of interest, U.S. Vietnam veterans, had a large sample size, appropriate controls (non-Vietnam-deployed sprayers, Vietnam non-sprayers, and non-Vietnam-deployed non-sprayers), and validated endpoints (self-reported physician-diagnosed hypertension that was

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confirmed with measurements and medical record reviews). The study also quantified exposures using serum TCDD measurements that were validated in a subset of participants. Finally, the statistical analyses conducted are robust, used state-of-the art methods, and adjusted for appropriate confounders. This study clearly demonstrated that self-reported physician-diagnosed hypertension rates were the highest among Vietnam-deployed sprayers (81.6%) compared with non-Vietnam-deployed sprayers (77.4%), Vietnam-deployed non-sprayers (72.2%), and non-Vietnam-deployed non-sprayers (64.6%), representing a significant association with exposure.

The five additional studies reviewed were occupational (Cappeletti et al., 2016; Yamamoto et al., 2015) and environmental (Lind et al., 2014; Shiue et al., 2014; Van Larebeke et al., 2015) exposure investigations. Each of these has one or more significant study design deficiencies as compared to Cypel et al. (2016) and would not be considered adequate to change the level of association individually. However, at least a portion of the effect model results corroborate the positive, elevated risk between exposure to the COIs and hypertension using a variety of study designs, populations, and measurements of exposure.

Briefly, among the occupational cohorts, Yamamoto et al. (2015) found hypertension to be statistically significantly associated with serum concentrations of PCDDs, PCDFs, dioxin-like PCBs, and total dioxins based on adjusted odds ratios between the first and fourth quartile of each of those COIs among incinerator workers, and tests for trend were also significant for each of these COIs. Among 235 Italian electric arc furnace workers exposed to multiple agents, including metals and PCDD/Fs, PAHs, and PCBs, as sampled in the foundry dust, the prevalence of both complicated and non-complicated hypertension among the workers compared with the general regional population showed statistically significant increased risks; however, this study is quite limited because foundry dust is a complex mixture, and the authors were unable to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those exposed workers (Cappelletti et al., 2016). Estimates were only adjusted for age group, and were not adjusted for other risk factors or activities that could affect the association. It is likely that workers of both of these occupationally exposed study populations received co-exposures to metals and chemicals other than those that the committee was charged with specifically reviewing that may be possible confounders that may affect the true estimate of association.

The results of the three environmental exposure studies had mixed findings. In their analysis of 2011–2012 NHANES data, Shiue and colleagues (2014) found an increased risk of hypertension with urinary levels of a number of heavy metals and other chemicals, including trimethylarsine oxide and dimethylarsonic acid, after adjusting for several risk factors of hypertension. Among the participants of a Flemish biomonitoring program, Van Larebeke et al. (2015) found an elevated risk of self-reported (and unconfirmed) hypertension for dioxin-like activity and HCB after adjusting for BMI, exercise in minutes per week, level of education,

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and glasses of alcoholic beverages per week that was significant for men and women combined. However, when stratified by sex, the risk of hypertension was only marginally significant for men exposed to PCBs with dioxin-like activity, while HCBs were significant for both men and women in at least one model. In the third environmental exposure study of elderly residents of Upsala, Sweden, significant positive associations between hypertension and dioxin-like PCBs were found only for PCB 105 and PCB 118 when analyses were adjusted for gender only. When models were fully adjusted for the risk factors of hypertension, no association between dioxin-like PCBs and hypertension was found (Lind et al., 2014).

In addition to the new studies, the committee re-examined the studies reviewed from the previous VAO reports, specifically those among Vietnam veterans (AFHS, 1995, 2000, 2005; CDC, 1988a; Cypel and Kang, 2010; Kang et al., 2006, 2014; J. S. Kim et al., 2003; O'Toole et al., 2009; Yi et al., 2014a,b) when making its decision of the strength of the evidence of exposure to the COIs and hypertension. Furthermore, data from animal and human cell-culture models support the hypothesis that TCDD activation of AHR increases the development of hypertension, and suggest plausible molecular mechanisms for hypertension.

Conclusion

After an examination of the literature that had been previously reviewed with the additional new evidence, the committee determined that there is sufficient evidence of an association between at least one of the COIs and hypertension.

Ischemic Heart Disease

IHD refers to a loss of blood flow and lack of oxygen to the heart muscle. It is also referred to as cornonary heart disease or myocardial ischemia and includes the conditions of stable angina, unstable angina, myocardial infarction, and sudden cardiac death. It is often the result of an atherosclerotic narrowing of the blood vessels that supply the heart muscle. Risk factors include smoking, hypertension, hyperlipidemia, obesity, family history, age, and male sex. It is one of the leading causes of death in the United States, at a rate of 97.2 per 100,000 deaths (Healthypeople.gov, 2018a). IHD was first addressed as a separate oucome in *Update 2008*.

Conclusions from *VAO* and Previous Updates

The committee responsible for *Update 2008* revisited the entire body of evidence on TCDD exposure and heart disease and concluded that the evidence supported moving IHD to the limited or suggestive category. That conclusion was based on evidence of a dose–response relationship in the occupational cohorts,

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evidence of an increased risk of myocardial infarction in Vietnam veterans, supporting cross-sectional survey data, and a strong biologic rationale. Evidence reviewed for *Update 2010* and *Update 2012* continued to support that classification. A number of studies of potential relevance were reviewed for *Update 2014*, including several studies of Vietnam veterans. The studies of New Zealand, Korean, and female U.S. veterans did not find an increase in IHD mortality (Kang et al. 2014a; McBride et al. 2013; Yi et al. 2013a, 2014a,b). However, because IHD is not a uniformly fatal disease, mortality rates may not be the most accurate marker of prevalence. Information on IHD gleaned from death certificates represents not only disease occurrence but also disease severity—the validity of which depends on those dying of the disease being a fair representation of all persons developing IHD. For example, in the Korean Veterans Health Study of veterans who served in Vietnam, the mortality analysis included 843 deaths from IHD, but analyses of disease prevalence in the same population identified more than 20,000 persons with this condition.

Studies comparing mortality among veteran populations to that among the general population may also be biased by the so called "healthy warrior effect," in which veterans have a health advantage over the general population across a range of health outcomes. Furthermore, only the study of Korean veterans quantified possible herbicide exposure, whereas the New Zealand and U.S. veteran studies assumed deployment to be synonymous with herbicide exposure. Each of the Vietnam veteran cohort studies was limited by not adjusting the estimates for various relevant confounding variables. Additional studies of CVD, which includes IHD, in well-conducted and nationally representative samples, such as NHANES (Lin et al., 2012), found an increase in the risk of CVD death in those with higher levels of dioxin TEQs, but other studies of CVD did not demonstrate such relationships; thus IHD has remained in the limited or suggestive category.

Update of the Epidemiologic Literature

Results of new studies of IHD and exposures to the COIs are summarized below. No case-control studies of exposure to the COIs and IHD have been published since *Update 2014*.

Vietnam-Veteran Studies Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify prevalent health conditions in 2,783 male New Zealand veterans who served in Vietnam. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% CIs were calculated for the veteran cohort and the general population and reported for CVD overall as well as for acute myocardial infarction, coronary atherosclerosis, chest pain, cardiac arrest, dysrhythmia, and congestive heart failure. CVD was broken down into

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acute myocardial infarction (SHR = 1.16, 99% CI 1.00–1.33), coronary atherosclerosis (SHR = 1.27, 99% CI 1.14–1.39), chest pain (SHR = 1.35, 99% CI 1.16–1.53), cardiac arrest (SHR = 1.54, 99% CI 0.22–2.86), dysrhythmia (SHR = 1.27, 99% CI 1.06–1.48), and congestive heart failure (SHR = 1.02, 99% CI 0.78–1.26). Acute myocardial infarction, coronary atherosclerosis, and chest pain were further examined by 4-year periods of time. In each case, the time period 2006–2009 had the highest SHR, which was also statistically significant. The authors concluded that there was a small but significant increase in the number of hospitalizations for New Zealand Vietnam veterans, with modest increases in hospitalization for common conditions such as CVD. Exposure to the COIs was not validated through serum measurements, and the study did not control for smoking or ethnicity or other potentially important risk factors.

Occupational Studies Collins et al. (2016) offers additional follow-up time to a retrospective analysis of a cohort of 2,192 workers exposed to dioxins during TCP and PCP production at a chemical manufacturing plant in Michigan. The U.S. population was used as the comparator for standardized mortality ratios. Work history records were used to determine the length of exposure. Serum samples to measure levels of six types of dioxins, which were collected from 431 TCP and PCP workers. The historical concentrations for each dioxin congener were calculated from the median concentrations from the serum samples and the known half-lives associated with each congener. Complete vital status follow-up was achieved for the cohort, and there were 1,198 decedents through the entire study period (1979–2011). For IHD, there were 371 deaths among all workers combined (SMR = 1.10, 95% CI 0.99–1.22). No difference in mortality from IHD was found among the TCP workers (n = 256; SMR = 1.07, 95% CI 0.95–1.21), but there was a slight increase of death from IHD among the PCP workers (n = 150; SMR = 1.20, 95% CI 1.01–1.41). When the SMRs for IHD were evaluated by exposure levels of each constituent congener, only the middle estimates for HpCDD and OCDD were statistically significantly elevated, but the trend was not.

't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and workers were potentially exposed to 2,4,5-T, intermediates of TCP and other chlorophenols, and TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by IARC (Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. This study extended the follow-up period of these workers to approximately 30 years from the final 2,4,5-T production exposure. From the original cohort of 1,025 workers, 631 were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current 't Mannetje et al. follow-up, 430 or the

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631 workers were randomly selected and invited to participate in a morbidity survey, of whom 245 (57%) participated. The survey was administered in 2007–2008 by face-to-face interviews and collected information on demographic factors and health information, including doctor-diagnosed conditions and the year of diagnosis. A blood sample was also collected at that time and analyzed for TCDD, lipids, thyroid hormones, and other parameters. A neurological examination was conducted for 111 of the participants. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two methods: working in a TCDD-exposed job (based on occupational records) and serum TCDD concentration ≥10 pg/g lipid (18%). Mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy/TCP production and 6 pg/g lipid in the 141 men and 43 women who worked in other parts of the plant. Compared with the people in the not highly exposed jobs, the people who had ever worked in a highly exposed job at the plant did not have any difference in risk of doctor-diagnosed heart disease (n = 10; OR = 2.71; 95% CI 0.65–11.4), although the estimate was imprecise. When compared by serum TCDD concentration, again there was no difference in the risk of heart disease for workers in the high- versus low-exposure groups (n = 5; OR = 1.64; 95% CI 0.41–6.57), although the estimate was again imprecise.

Cappelletti et al. (2016) performed a retrospective study of 331 male electric arc foundry workers at a single plant in Trentino, Italy, to determine if they experienced excess mortality from all causes or were at an increased risk for several other disases including CVD, due to an occupational exposure to foundry dust. An analysis of the dust emissions found that the dust contained metals (including iron, aluminum, zinc, manganese, lead, chromium, nickel, cadmium, mercury, and arsenic), PAHs, PCBs, and PCDD/Fs (reported as TEQs). Because foundry dust is a mixture, it is not known which of the agents were associated with a specific outcome or to what extent. The men included in the study had worked at the factory for at least 1 year. Company and medical records were used to determine vital status. Requests for exemption health care fees were used as a surrogate measure to identify the most prevalent morbid conditions in the general population, which were then applied to the cohort to compute relative risks for each of the conditions. The workers were followed from March 19, 1979 (or their first day of employment), through December 31, 2009, or date of death. Effect estimates (prevalence ratios) were calculated using Mantel-Haenszel estimator adjusted for age group (20–64, 65–74, ≥ 75 years). Compared with the age-adjusted provincial population, no difference in death from IHD (n = 4; SMR = 1.27, 95% CI 0.35–3.26) was found among the electric arc furnace workers. However, a statistically significantly elevated risk of CVD (n = 5; RR = 1.74, 95% CI 1.07–2.82) was found. This study is most limited by the fact that foundry dust is a complex mixture, which makes it impossible to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those

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exposed workers. Estimates were adjusted only for age group and were not adjusted for other risk factors such as tobacco use, BMI, or other jobs or activities that could have resulted in similar exposures. Exposure to foundry dust by the general population that was used for comparison is not discussed, although the foundry appears to be in the local vicinity and emissions from it were reported to be present within a 2-kilometer radius of it.

Environmental Studies Van Larebeke et al. (2015) carried out a prospective study of the associations of exposure to organochlorine pollutants, HCB, dioxin-like (PCB 118) and non-dioxin-like PCBs, and cadmium with a variety of self-reported health conditions, including diabetes, hypertension, and atheromata (the results for diabetes and hypertension were described earlier) as part of a Flemmish biomonitoring program. In 2004–2005, height, weight, urine, and serum were collected from the participants. Dioxin-like activity in pg TEQ/g fat was also assessed. Subjects filled out a survey that collected demographic data and information on education, tobacco use, and alcohol consumption. In 2011, participants in the program were mailed a second survey to collect updated information general health, smoking and alcohol consumption behaviors, medical conditions, and current medications. There were 973 respondents, 504 women and 469 men (response rate 65.6%), whose information was used for the current analysis. The serum was assayed for HCB, dioxin-like PCB 118, and other chemicals. Medical diagnoses were all self-reported and not confirmed. For the diagnosis of atheromata, subjects were asked, "Did you have a problem with the blood circulation in the brain (cerebrovascular incident, ischemic episode)?" "Have you ever experienced a heart infarction?" and "Do you sometimes suffer from pain in the thorax during physical effort?" A positive response to at least one of these questions was considered a diagnosis. A total of 71 men (15.1%) and 62 women (12.3%) reported atheromata, and 27 men (5.8%) and 10 women (2%) reported myocardial infarction. Overall, there were no differences in the levels of dioxin-like activity (pg/TEQ/g fat) or PCB 118 (ng/g fat) in participants versus non-participants of the follow-up survey, but there was a difference for HCB ($p = 0.051$). In the combined sample, after adjusting for BMI, the level of education, and cholesterol concentration in blood, the researchers found that dioxin-like activity was not associated with the risk of atheromata (OR = 1.60, $p = 0.083$); estimates for HCBs and PCB 118 were not provided. In a model accounting for confounders and making additional adjustments for correlated exposures (HCB, p,p'-DDE, cadmium, and non-dioxin PCBs), the odds of atheromata in the 90th versus the 10th percentiles were increased in men only (OR = 1.83, $p = 0.031$). No individual analyses were performed for myocardial infarction. The strengths of the study include its prospecitive design, its large sample size, the representativeness of the older adult population of Flanders, and the use of objective measures of exposure. However, the findings are limited by the study's lack of validated medical diagnoses, and the data were collected based on self-report from a survey that was not validated.

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Bergkvist et al. (2015) performed a prospective population-based cohort study of 33,446 women who participated in the Swedish Mammography Cohort and who were free of CVD, diabetes, and cancer at baseline. The women were followed for 12 years to determine the association between dietary PCB exposure and the risk of myocardial infarction. Fish fatty acid consumption was also measured to determine any protective effects of the consumption of eiconsapentaenoic acid and docosahexaenoic acid. The food frequency questionnaire-based dietary PCB estimates have been extensively validated against serum PCBs in women from this cohort. During the follow-up period, 1,386 myocardial infarctions occurred, 276 of which were fatal. Cases were ascertained through a computerized linkage to the National Hospital Discharge and Cause of Death Registers in Sweden using personal identification numbers. PCB exposure was grouped into quartiles, and women in the highest quartile of dietary PCB exposure were found to be more likely to report high cholesterol levels, to use fish oil supplements, to have a slightly higher consumption of alcohol and red and processed meat, and to have up to a six-times-higher consumption of fish than those in the lowest quartile. Multivariate-adjusted analysis was used to determine the association between risk of myocardial infarction and PCB exposure. Models were adjusted for several factors, including postsecondary education, a family history of myocardial infarction before the age of 60 years, ever use of postmenopausal hormones, the use of aspirin, the use of fish oil supplements, and a weight loss of $\geq$ 5 kg within 1 year, which were treated as dichotomous variables (yes/no). Additional variables were also included in Cox proportional hazards regression models: smoking status (never, past, or current); waist circumference (< 80, 80–87, $\geq$ 88 cm); parity (0, $\geq$ 1 children); total physical activity (quartiles, MET-h); alcohol consumption (0, > 0–4.9, 5.0–14.9, > 15.0 g/day); energy intake (continuous, kcal/day); consumption of fruit and vegetables (quartiles, servings/week), dairy products (quartiles, servings/day), and red and processed meat (quartiles, servings/week); dietary intake of saturated fatty acids (quartiles, g/day); and dietary methyl mercury exposure (quartiles, µg/day). In additional adjustments, the dietary sum of both eiconsapentaenoic acid and docosahexaenoic acid (quartiles, g/day) was also included. Models were not adjusted for hypertension or high cholesterol levels because the authors reasoned that these intermediate risk factors may be in the causal pathway between PCB exposure and CVD. Dietary PCB exposure was associated with total myocardial infarction in the multivariable-adjusted model (RR = 1.21, 95% CI 1.01–1.45; p for trend = 0.012), and this estimate increased after adjusting the model for the sum intake of eiconsapentaenoic acid and docosahexaenoic acid (RR = 1.58, 95% CI 1.10–2.24; p for trend = 0.007). There are several limitations to the study. Although the survey was validated with a serum PCB analysis in this population, recall bias is of concern, as is the fact that this information was collected only at baseline, yet the cohort was followed for 12 years. Using a prospective design eliminates concerns of temporal ambiguity. Methyl mercury is a potentially confounding factor. Though adjustments were made for dietary intake, it is not possible to ascertain the role of

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mercury in risk of CVD. Most importantly, because exposure to PCBs is grouped, it is not possible to know if any of the increased risk is due to dioxin-like PCBs and which specifically those might be.

Other Identified Studies One other study that reported deaths from IHD was identified, but it was limited by its lack of exposure specificity (Ruder et al., 2014).

Biologic Plausibility

Experimental studies demonstrate that exposure to AHR agonists, including TCDD and benzo[*a*]pyrene, increase the incidence, severity, and progression of atherosclerosis, a primary cause of IHD (Dalton et al., 2001; Kerley-Hamilton et al., 2012a; D. Wu et al., 2011). There is also evidence that TCDD influences other IHD risk factors by promoting obesity (Brulport et al., 2017; Kerley-Hamilton et al., 2012b), accumulating macrophage lipid, inducing lipid mobilization, and altering lipid metabolism. Thus, on the basis of animal models there appear to be several overlapping and potentially contributing pathways that link TCDD exposure and increased IHD risk.

Synthesis

This committee considered six new studies in addition to the multiple studies reviewed by prior VAO committees when determining its conclusion of the level of association. Among New Zealand veterans who had served in Vietnam, Cox et al. (2015) calculated hospitalization ratios for CVD and found elevated rates for the outcomes of acute myocardial infarction, chest pain, and coronary atherosclerosis among the veterans. The two occupational studies of heart disease mortality—one among U.S. workers producing TCP and PCP (Collins et al., 2016) and the other among Italian electric arc foundry workers exposed to dioxins (Cappelletti et al., 2016)—both found no difference in mortality from heart disease compared with their resepective general populations; however the use of general populations as a comparator likely introduced selection bias. A third occupational study of morbidity among people who had worked in a New Zealand plant that produced phenoxy herbicides also found no increased risk of heart disease based on high versus low exposure ('t Mannetje et al., 2018). Two studies of populations with environmental exposures were also examined. Bergkvist et al. (2015) followed a cohort of women for 12 years who were free of CVD at baseline and who were exposed to PCBs via dietary exposure. Even though adjustments were made for dietary intake, PCBs were grouped together, making it difficult to determine the role of individual dioxin-like PCBs in the CVD risk. Regardless, these data are not immediately generalizable, given that very few women were exposed to the herbicides. The occupational studies are further limited because

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these populations of workers likely received co-exposures to metals and chemicals other than those that the committee was charged with specifically reviewing that may be possible confounders that may affect the true estimate of association. Van Larebecke et al. (2015) studied the association of exposure to organochlorine pollutants, HCB, and dioxin-like and non-dioxin-like PCBs with a self-reported history of atheromata and myocardial infarction. While there was a significant association for men reporting atheromata, this association lost significance when the analysis corrected for other risk factors known to cause atheromata. Furthermore, based on a review of available experimental data, there appear to be several overlapping and potentially contributing pathways that link TCDD exposure and an increased risk of IHD.

The above studies were limited by relatively small numbers of cases, a lack of consistent case definitions for the various types of CVD (IHD versus angina versus chest pain versus atheromata), the use of non-validated surveys for diagnosis, and the presence of mixtures of chemicals in addition to the COIs. Most of the new studies used mortality or hospitalization data, which, as discussed above, may not be the best endpoint for determining an increased risk for a particular condition. Given these considerations, the committee reaffirmed the decision of previous VAO committees to keep IHD in the limited or suggestive category.

Conclusion

After reviewing the new evidence, the present committee concurred with those for previous updates that there is limited or suggestive evidence that IHD is associated with exposure to the COIs.

Cerebrovascular Disease and Stroke

Cerebrovascular disease refers to a disorder that affects the circulation in the brain. The most common conditons are ischemic stroke, hemorrhagic stroke, and transient ischemic attack. This disease is one of the top 10 causes of death in the United States and was responsible for 5.2% of deaths overall in 2015 (CDC, 2017b). Presented by race, in 2015 whites had the greatest number of deaths from cerebrovascular disease (~117,000) followed by blacks (~18,000), Asian and Pacific Islanders (~5,000), and American Indians/Alaska Natives (~700) (CDC, 2017b). Mortality rates from cerebrovascular disease and stroke were declining, but have remained stagnant in recent years (CDC, 2017d).

Conclusions from *VAO* and Previous Updates

Among U.S. Vietnam veterans, stroke and cerebrovascular disease have not been found to be elevated in the AFHS, ACC, or female veteran cohorts (Cypel and Kang, 2010; Kang et al., 2014; Ketchum and Michalek, 2005). Similar to

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the analysis of deployed and non-deployed U.S. female veteans, analyses of Australian Vietnam veterans compared with the general Australian population have found a decreased risk of stroke and cerebral hemorrhage. Among Korean Vietnam-era veterans, after adjustment for multiple behavioral, demographic, and service-related factors, stroke prevelance was statistically significantly elevated among the more highly exposed cohort members (Yi et al., 2014a). The association was seen for both major stroke types: cerebral infarction (ICD-10 I63) and cerebral hemorrhage (ICD-10 I60–I62). In the mortality study in the same cohort, after adjusting for only age and rank, no differences were found in mortality from cerebrovascular disease (ICD-10 I60–I69) between the groups with high and low potential for herbicide exposure or when individual exposure potential was used as a continuous variable (Yi et al., 2014b).

Few occupational cohorts have examined stroke and cerebrovascular diseases, but those that have, have reported very few cases and very few differences in mortality rates. Such studies have included Dutch herbicide production workers (Boers et al., 2010), the U.S. National Institute for Occupational Safety and Health mortality cohort (Steenland et al., 1999), PCP production workers in Midland, Michigan (Collins et al., 2009a), and Italian licensed pesticide users (Gambini et al., 1997). One study of AHS applicators and their wives (Waggoner et al., 2011) found a statistically significantly decreased risk of cerebrovascular disease.

Only two environmental studies have reported on stroke or cerebrovascular disease. Y. S. Lin et al. (2012) reported a positive, but not significant association between dioxin-like compounds in the blood and mortality from cerebrovascular disease (defined as a stroke) in an analysis of NHANES data. In the 25-year follow-up of the Seveso cohort, Consonni et al. (2008) found no difference in cerebrovascular disease between people exposed in Zone A and nonexposed residents of 11 surrounding municipalities, but slight statistically significant increases in cerebrovascular disease were found for people residing in Zones B and R compared with the nonexposed residents of 11 surrounding municipalities.

Therefore, based on these studies the evidence to determine an association between exposure to the COIs and cerebrovascular disease and stroke remains limited or suggestive.

Update of the Epidemiologic Literature

Four new studies of exposure to at least one COI and cerebrovascular disease and stroke outcomes have been identified since *Update 2014*.

Vietnam-Veteran Studies Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify prevalent health conditions in 2,783 male New Zealand veterans who served in Vietnam. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized

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hospitalization rates and 99% CIs were calculated for the veteran cohort and the general population and reported for several noncancerous conditions. A total of 170 cases of acute cerebrovascular disease were found in the veteran population, which was statistically significantly elevated compared with the standardized hospitalization rate of New Zealand (SHR = 1.31, 99% CI 1.05–1.56). In addition, a total of 45 cases of aneurysm within the veteran population were identified (SHR = 1.47, 99% CI 0.91–2.04). The study was limited by its assumption that deployment to Vietnam was synonomous with herbicide exposure, which was not validated through serum measurements, and by a lack of adjustment for smoking or ethnicity or other potentially important risk factors.

Han et al. (2016) conducted a small hospital-based study of Korean Vietnam veterans in Seoul who had presumed Agent Orange exposure (determined by deployment to Vietnam) to examine whether exposure resulted in differences in the vascular features of acute ischemic stroke. A total of 91 veterans with preseumed exposure were compared with 288 male patients from other area general hospitals (and presumed to be unexposed to Agent Orange). Both groups of patients were compared on lifestyle factors, clinical history, and the clinical manifestations of stroke at admission and discharge. Vascularity was examined within 7 days of acute ischemic stroke onset, and stroke was confirmed by MRI within 1 week after onset. The subtype of the stroke was assessed using the acute stroke treatment protocol and the modified Rankin Scale at discharge and at 3 months after onset. The two groups were comparable for many demographic and lifestyle factors except that veterans (exposed) had higher rates of smoking and comorbid diabetes and the unexposed males had higher hyperlipidemia and history of stroke. Stroke subtypes were statistically significantly different ($p = 0.014$) between the two groups, and characterized by small vessel occlusion in exposed subjects and large artery atherosclerosis in unexposed subjects. These features may have led to a somewhat better short-term prognosis of exposed than unexposed members. The exposed veterans had lower scores on the stroke scale at admission than the control group ($p = 0.003$), but there was no significant difference between the two groups at discharge. This study was small and exposure was assumed based on deployment to Vietnam and not otherwise objectively measured or validated. Only simple comparison testing was used, but the study does indicate that studying stroke as a single category may lose statistical power especially when attempting to study the effects of potential exposure to the COIs.

Occupational Studies Collins et al. (2016) offered additional follow-up time to a retrospective analysis of a cohort of 2,192 workers exposed to dioxins during TCP and PCP production at chemical manufacturing plant in Michigan. The U.S. population was used as the comparator for standardized mortality ratios. Work history records were used to determine the length of exposure. Serum samples were collected from 431 TCP and PCP workers to measure the levels of six types of dioxins. The historical concentrations for each dioxin congener

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were calculated from the median concentrations from the serum samples and the known half-lives associated with each congener. Complete vital status follow-up was achieved for the cohort, and there were 1,198 deaths through the entire study period (1979–2011). For cerebrovascular disease, there were 68 deaths among all workers combined and no difference in the mortality rate compared with the general population (SMR = 1.03, 95% CI 0.80–1.30). No difference in mortality from cerebrovascular disease was found among the TCP workers (n = 49; SMR = 1.07, 95% CI 0.79–1.41) or the PCP workers (n = 27; SMR = 1.07, 95% CI 0.701–1.55). Although serum dioxin measurements were collected, no results based on those measurements were presented for cerebrovascular disease; instead the authors used employment records to categorize exposure, which may have introduced exposure misclassification, and use of the general U.S. population as a comparison likely introduced selection bias.

Environmental Studies Van Larebeke et al. (2015) carried out a prospective study of the associations of exposure to organochlorine pollutants, HCB, dioxin-like (PCB 118) and non-dioxin-like PCBs, and cadmium with a variety of self-reported health conditions, including atheromata, as part of a Flemmish biomonitoring program. Because a diagnosis of atheromata was assessed based on three questions, one of which was "Did you have a problem with the blood circulation in the brain (cerebrovascular incident, ischemic episode)?" the results for atheromata were described in the section on IHD.

Other Identified Studies One other study that reported deaths from cerebrovascular disease was identified, but it was limited by a lack of exposure specificity (Ruder et al., 2014).

Biologic Plausibility

Although no papers were identified that specifically addressed cerebrovascular disease and stroke following exposure to the COIs, many of the mechanisms that influence stroke and cerebrovascular disease incidence and progression have been shown to be targets of TCDD and the AHR pathway, as noted in the introduction of the biologic plausibility section under the circulatory diseases heading. These mechanisms include impacts on endothelial cell function and proliferation, inflammation, and blood vessel blockage.

Synthesis

Four new studies on cerebrovascular disease or stroke were reviewed for the current update. Two studies followed Vietnam veterans. Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify the health conditions that had affected the veterans. Among New Zealand veterans, 170 cases

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of cerebrovascular disease or stroke were observed, resulting in a significant increase in the standardized hospitalization rate for acute cerebrovascular disease compared with the general population. Han et al. (2016) published a hospital-based case-control study of 91 Korean veterans presumed to have been exposed to Agent Orange during their deployment to Vietnam who presented for medical attention within 7 days of the onset of an acute ischemic stroke. The key finding was a statistically different type of stroke between the presumed exposed and unexposed groups, with small vessel occlusion more common in the exposed subjects and large artery atherosclerosis more common in the unexposed subjects, resulting in a somewhat better short-term prognosis for the exposed versus the unexposed patients. While this study is small and requires replication as well as exposure validation, it raises an important issue about including all stroke subtypes in future studies of stroke and exposure to the COIs. Among a long-term follow-up of U.S. workers exposed to dioxins during TCP and PCP production, Collins et al. (2016) found no difference in the mortality rate from cerebrovascular disease between the workers and the standardized general population, but the study is limited by likely exposure misclassification and selection bias. In a prospective study of environmental exposure to organochlorine pollutants, HCB, and dioxin-like and non-dioxin-like PCBs in Belgium, Van Larebeke et al. (2015) assessed levels of exposure with a variety of self-reported health conditions, including atheromata. A small but statistically significant increase was found between the 90th versus the 10th percentile of exposure, but this was found for men only. The strengths of the study include its prospecitive design and large sample size, the representativeness of the older adult population of Flanders, and the use of objective measures of exposure. However, the findings are limited by the study's lack of validated medical diagnoses and by the fact that the data were collected based on self-report from a survey that was not validated. No new data specifically addressing the biological plausibity of the impact of exposure to the COIs on cerebrovascular disease and stroke were identified. Therefore, on the basis of the new studies, the committee maintained the previous conclusion of limited or suggestive evidence between exposure to the COIs and cerebrovascular disease or stroke.

Conclusion

After carefully reviewing the previous evidence as well as the new evidence related to exposure to the COIs and cerebrovascular disease and stroke, the committee maintains the prior conclusion of limited or suggestive evidence of association that cerebrovascular disease and stroke are associated with exposure to the COIs.

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Other Cardiovascular Outcomes

The previous sections in this chapter cover hypertension, IHD, and cerebrovascular disease and stroke. This section covers additional cardiovascular and blood disorders that have been examined in studies that have measured exposure to the COIs.

Vietnam-Veteran Studies

Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify health conditions in 2,783 male New Zealand veterans who served in Vietnam. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% CIs were calculated for the veteran cohort and the general population and reported for cardiovascular and other outcomes. Syncope (n = 100; SHR = 1.47, 99% CI 1.18–1.76) and phlebitis (n = 51; SHR =1.67, 99% CI 1.07–2.28) were both elevated among the veterans compared with the standardized New Zealand population. No difference in the hospitalization rate for peripheral atherosclerosis (n = 70; SHR = 1.17, 99% CI 0.81–1.53) was found. Asymptomatic peripheral arterial vascular disease is more common than symptomatic disease and does not often result in death or hospitalization. This study was limited by its assumption that deployment to Vietnam was synonomous with herbicide exposure, which was not validated through serum measurements, and by the fact that important risk factors, such as smoking and ethnicity, were not controlled for in the analysis.

Occupational Studies

Laverda et al. (2015) assessed the association between weight gain (using BMI) and pesticide use using data on 8,365 male pesticide applicators from the AHS. The goal was to assess the effect of common factors beyond high-calorie diet and physical activity on BMI; pesticides were studied because many include endocrine disruptors and thus have a biological rationale for why they might affect BMI. Details of the AHS study design, exposure measurement, and data collection are presented in Chapter 5. Multivariate linear regression was used to estimate the unit change in BMI ($kg/m^2/d$) associated with a unit change in exposure to a pesticide class/individual pesticide. To assess validity and the strength and direction of the associations, partial Spearman correlation coefficients controlling for all covariates were calculated for BMI at follow-up in relation to cumulative exposure days to each pesticide class/individual pesticide. Ordinal logistic regression was used to evaluate the exposure–response associations between BMI and pesticide exposures. Models were adjusted for BMI at age 20 years (from enrollment data), age, smoking, energy input, and energy output (from follow-up data). Results

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were also adjusted for the state of residence and used multiple test corrections. In this analysis, 1,262 (15.1%) applicators reported exposure to phenoxy herbicides (2,4,5-T and 2,4,5-TP), and 1,214 (14.9%) applicators reported exposure to 2,4-D. The adjusted parameter estimate for unit change in BMI ($kg/m^2/d$) was statistically significant for both phenoxy herbicides and 2,4-D in the entire sample ($p = 0.02$ for both), but when stratified by state, the two parameter estimates remained statistically significant for Iowan applicators only.

Environmental Studies

S. A. Kim et al. (2015) used serum concentrations of persistent organic pollutants, including dioxin-like and non-dioxin-like PCBs and organochlorine pesticides collected within the 1999–2004 NHANES and adjusted for fat mass in order to make associations with overall mortality, mortality from all cancers combined, and mortality from CVDs in people 70 years and older. Among the 633 people for whom there was information on PCBs, 50 had died of cardiovascular disease during the follow-up, while 56 had died among the 675 people for whom there was information on organochlorine pesticides. Models were adjusted for age, sex, race, BMI, cigarette smoking, and physical activity. When fat mass was excluded from the analysis of PCB exposure by tertiles, the adjusted hazard ratio for death from CVD in the second tertiles compared with the lowest exposure (tertile 1) was not different (HR = 1.06, 95% CI 0.47–2.40), but it was elevated for the third versus first tertile (HR = 2.37, 95% CI 1.10–5.09), and the trend for increasing exposure was statistically significant ($p = 0.02$). For organochlorine pesticides there was no difference in cardiovascular deaths between the second and third tertiles and the referent (p trend = 0.17). When individuals were stratified by body fat mass (< 25%, 25%–75%, and > 75%) and estimates were adjusted for the factors listed above, there were statistically significantly more deaths from CVD with exposure to PCBs only for the comparison of the third tertile with the first in the intermediate fat mass group (25– < 75%) (HR = 3.42, 95% CI 1.02–11.4), but this estimated is imprecise. This study was limited by the relatively low numbers of deaths, especially when stratified by exposure levels. This study measured death, not the incidence of disease, and it is possible that the POPs affect development of disease differently than they affect survival once the disease has been acquired.

Penell et al. (2014) investigated whether baseline circulating levels of POPs can predict future changes in circulating lipid levels. They measured lipid levels and the levels of 23 different POPs in 598 individuals in the PIVUS study at ages 70 and 75 years. No subjects were on lipid-lowering medications. POPs included non-dioxin-like PCBs, organochlorine pesticides, one dioxin (OCDD), and one brominated flame retardant compound. There were no significant difference related to the level of dioxin. The authors propose that the increase in low-density lipoproteins could strengthen the link between POP exposure and CVD.

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Other Identified Studies

Zong et al. (2015) conducted a methodological study to assess the relationship between POPs and the fat mass percentage in the trunk, leg, and whole body, so the study does not have direct relevance to a health outcome. Their rationale was that since fat biopsies are used for assessing long-term lipophilic pollutant exposure, the anatomical location of the biopsy may affect the estimated body burden of these chemicals. The analysis included 2,358 men and women 20 years of age and older who had participated in NHANES 1999–2004 and who had stored serum samples. Serum measurements of 49 to 68 POPs were measured using gas chromatography/isotope dilution mass spectrometry. X-ray bone densitometry was used for dual-energy X-ray absorptiometry scans and fat mass percentage measurement. Demographics and information on lifestyle, medical history, and the history of lactation and parity for women were collected using questionnaires. Ethnicity, educational attainment, smoking and alcohol use, and physical activity were used as covariates. β-hexachlorocyclohexane, heptachlorodibenzo-*p*-dioxin, octachlorodibenzo-*p*-dioxin, and PCB 126 showed stronger positive correlations with trunk fat mass percentage than with leg fat mass percentage, suggesting a more important or greater role of trunk fat in the action and pharmacokinetics of persistent organic pollutants as endocrine disrupters. This study highlights one explanation for why studies may provide varying results on a metabolic or cardiovascular effect of COI exposure depending on which anatomical site was used for measurement.

Biologic Plausibility

Stea et al. (2016) suggest that the inconsistent associations of chronic low-dose exposure to arsenic on CVD may be due to high inter-individual variability in susceptibility to arsenic's toxicity, a part of which is genetic. To assess this relationship, 214 healthy Italian volunteers between the ages of 20 and 46 years were studied for arsenic and related compounds in first voided urine, intima-media thickness, and genotypes for arsenic-metabolizing genes (*ASIIIMT Met287Thr*, *GSTT1*+/−, and *GSTM1*+/−). The samples were tested for inorganic arsenics (including trivalent and pentavalent), for methylated metabolites (i.e., mono-methylarsinic [MMA] and dimethylarsinic acid [DMA]), and for total arsenic using high-performance liquid chromatography and mass spectrometry. Intima-media thickness was measured on the common carotid artery on both sides using carotid ultrasound by a trained cardiologist. All genotypes were obtained by PCR and RFLP analysis. The main finding was that in both men and women with chronic low dose exposure to arsenic, intima-media thickness increases significantly faster with age than in the healthy population. The findings also suggest that *GSTT1* and *hOGG1* gene polymorphisms might play an important role in the

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individual risk of arsenic-induced carotid atherosclerosis, with the same exposure providing differential outcomes.

Synthesis

In addition to outcomes related to hypertension, IHD, and cerebrovascular disease, several studies presented other cardiovascular and circulatory outcomes. However, most of these findings were of limited use because they examined different outcomes and different measures, and some of the outcomes were more methodologic or surrogates in the pathway of disease. For example, Penell et al. (2014) examined whether POPs can predict changes in lipid levels because higher blood lipid levels are a risk factor for CVD; however, no association was found for dioxin-like chemicals. S. A. Kim et al. (2015) conducted an analysis using NHANES data to look for an association between the concentrations of POPs (both dioxin-like and non-dioxin-like) and organochlorine pesticides and mortality from CVD. While there were statistically significant trends in death from cardiovascular disease among several subgroups classified by body fat, the estimates were imprecise. Stea et al. (2016) examined how exposure to arsenic may be a possible contributing factor to the individual risk of arsenic-induced carotid atherosclerosis, but a genetic component appears to play a part. The study of hospitalization risk among New Zealand Vietnam veterans used deployment to Vietnam as an indicator of presumed exposure to herbicides, and in analyses that did not control for smoking or other important risk factors of CVDs they found elevated rates of syncope and phlebitis but not peripheral atherosclerosis. Although there is similarity in the mechanisms by which atherosclerosis develops regardless of where it occurs (e.g., the heart, central nervous system, or periphery), asymptomatic peripheral arterial vascular disease is more common than symptomatic disease. The peripheral vascular disease literature is limited by high rates of asymptomatic disease, the requirement for specific and often costly testing to document clinically silent disease, death due to myocardial infarction and stroke before diagnosis, and infrequency of death or hospitalization from peripheral vascular disease alone.

In an assessment of weight gain (using BMI) and pesticide use from the AHS, Laverda et al. (2015) found that the parameter estimate for unit change in BMI ($kg/m^2/d$) was statistically significant for both phenoxy herbicides and 2,4-D in the entire sample. However, based on the myriad studies and outcomes examined, no conclusion can be made concerning an association of exposure to the COIs and other cardiovascular disorders.

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Conclusion

After carefully reviewing the previous evidence as well as the new evidence related to exposure to the COIs, the committee maintains the prior conclution that there is inadequate or insufficient evidence that blood disorders and other cardiovascular disorders are associated with exposure to the COIs.

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Other Chronic Health Outcomes

Chapter Overview
*Based on new evidence and a review of prior studies, the current committee did not find any new associations between exposure to the chemicals of interest (COIs; 2,4-dichlorophenoxyacetic acid [2,4-D], 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], picloram, dimethylarsinic acid [DMA or cacodylic acid], and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]) and other chronic, non-malignant outcomes including respiratory disorders, gastrointestinal and digestive diseases, adverse effects on thyroid homeostasis, kidney and urinary disorders, chronic skin conditions, eye problems, and bone conditions. Current evidence supports the results of earlier updates that:*

- *There is sufficient evidence of an association between the COIs and chloracne.*
- *There is limited or suggestive evidence of an association between exposure to the COIs and hypothyroidism, early onset peripheral neuropathy, and porphyria cutanea tarda.*
- *There is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and respiratory disorders, gastrointestinal and digestive disease (including liver toxicity), kidney and urinary disorders, adverse effects on endocrine function (other than hypothyroidism), chronic skin conditions, eye problems, or bone conditions.*

This chapter discusses data on the possible association between exposure to the COIs and several non-cancer health outcomes: respiratory disorders, gastrointestinal and digestive disease (including liver toxicity), adverse effects on thyroid

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homeostasis, kidney and urinary disorders, chronic skin conditions, eye problems, and bone conditions. The committee also considers the results of studies of exposure to polychlorinated biphenyls (PCBs) and other dioxin-like chemicals to be informative if they were reported in terms of TCDD toxic equivalents (TEQs) or as concentrations of dioxin-like specific congeners. Although all studies reporting TEQs based on PCBs were reviewed, TEQs based only on mono-ortho PCBs (which are PCBs 105, 114, 118, 123, 156, 157, 167, and 189) are several orders of magnitude lower than those of the non-ortho PCBs (77, 81, 126, and 169), based on the revised World Health Organization (WHO) toxicity equivalency factor (TEF) scheme of 2005 (La Rocca et al., 2008; van den Berg et al., 2006). The lower TEQs of the mono-ortho PCBs, however, may be counterbalanced by their abundance, which is generally many orders of magnitude higher than that of the non-ortho PCBs (Park et al., 2010).

In previous updates, chloracne and porphyria cutanea tarda were considered with the chronic non-cancer conditions. They are accepted as being associated with dioxin exposure, but they are considered acute outcomes and are no longer considered specifically in this chapter. For each type of health outcome, background information is followed by a brief summary of the findings described in earlier *Veterans and Agent Orange* (*VAO*) reports. In the discussion of the most recent scientific literature, the studies are grouped by exposure type (Vietnam veteran, occupational, or environmental). For articles that report on the health outcomes of a previously studied population, the detailed design information is summarized in Chapter 5. A synopsis of toxicologic and clinical information related to the biologic plausibility of the COIs influencing the occurrence of a health outcome is presented next and is followed by a synthesis of all the material reviewed. Each health-outcome section ends with the committee's conclusions regarding the strength of the evidence that supports an association with the COIs. The categories of association and the committee's approach to categorizing the health outcomes are discussed in Chapter 3.

NON-CANCEROUS RESPIRATORY DISORDERS

For the purposes of this report, "non-cancerous respiratory disorders" are defined as all acute and chronic lung diseases other than cancers; the variety of conditions are described by the *International Classification of Diseases* (ICD), 9th Revision (ICD-9 460–519) or 10th Revision (ICD-10 J00–J99). Acute non-cancerous respiratory disorders include pneumonia and other respiratory infections; they can increase in frequency and severity when the normal defense mechanisms of the lower respiratory tract are compromised.

Chronic non-cancerous respiratory disorders generally take two forms: airways diseases and parenchymal diseases. Airway diseases are disorders characterized by an obstruction of the flow of air out of the lungs; common examples

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are asthma and chronic obstructive pulmonary disease (COPD). COPD includes such disorders as emphysema and chronic bronchitis. Parenchymal disease, or interstitial disease, generally includes disorders that cause inflammation and scarring of the deep lung tissue, including the air sacs and supporting structures. Parenchymal disease is less common than airway diseases and is characterized by a reduction in lung capacity, although it can also include a component of airway obstruction. Some severe chronic lung disorders, such as cystic fibrosis, are hereditary. Because Vietnam veterans received health screenings before entering military service, few severe hereditary chronic lung disorders are expected in that population.

More than 25 million people in the United States are thought to be living with asthma. As of 2015, the mortality rate for asthma among children and adults in the United States was highest among African Americans with 13.2 deaths per 100,000 in people less than 35 years old. Non-Hispanic whites had the second highest mortality rate, with 4.3 deaths per 100,000 in people less than 35 years old. There are nearly 14.8 million people who have physician-diagnosed COPD, but 12 million people are estimated to have undiagnosed COPD. The mortality rate for COPD was highest among non-Hispanic whites, with 127.8 deaths per 100,000 people. Native Americans had a death rate of 81.1 per 100,000 in individuals 45 years and older. African Americans ranked third, with a rate of 78.4 deaths per 100,000 people 45 years and older. Mortality rates were lowest among Asian or Pacific Islanders with 30.3 per 100,000 (healthypeople.gov, 2018b).

The most important risk factor for many non-cancerous respiratory disorders is the inhalation of cigarette smoke. Although exposure to cigarette smoke is not associated with every disease of the lungs, it is the major cause of many airway disorders, especially COPD; it also contributes to some interstitial disease, and it compromises host defenses in such a way that people who smoke are generally more susceptible to some types of pneumonia. Cigarette smoking also makes almost every respiratory disorder more severe and symptomatic than it would otherwise be. The incidence rates of habitual cigarette smoking vary with occupation, socioeconomic status, and generation. For those reasons, cigarette smoking can be a major confounding factor in interpreting the literature on risk factors for respiratory disease. Vietnam veterans are reported to smoke more heavily than non-Vietnam veterans (Kang et al., 2006; McKinney et al., 1997).

The causes of death from respiratory diseases, especially chronic diseases, are often misclassified on death certificates (Mieno et al. 2016). Grouping various respiratory diseases for analysis, unless they all are associated with a given exposure, will lead to an attenuation of the estimates of relative risk and to a diminution of statistical power. Moreover, the diagnosis of the primary cause of death from respiratory and cardiovascular diseases is often inconsistent. In particular, when a person had both conditions concurrently and both contributed to the death, there may be some uncertainty about which cause should be selected as the primary underlying cause. In other instances, errors may arise in selecting

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one underlying cause in a complex chain of health events (for example, if COPD leads to congestive heart failure and then to respiratory failure).

Many study populations are small, so investigators often group deaths from all non-cancerous respiratory diseases into one category that combines pneumonia, influenza, and other diseases with COPD and asthma. The committee notes that an association between the group of all non-cancerous respiratory diseases with any of the COIs would be too nonspecific to be clinically meaningful; at most, such a pattern would be an indication that within this broad classification, the incidence of some particular disease entity might be affected by an exposure to a COI.

Conclusions from *VAO* and Previous Updates

The committee responsible for the initial VAO report (IOM, 1994) concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and respiratory disorders. Additional information available to the committees responsible for *Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), *Update 2000* (IOM, 2001), *Update 2002* (IOM, 2003c), and *Update 2004* (IOM, 2005) also maintained the original conclusion of an inadequate or insufficient level of association.

A number of studies of non-malignant respiratory diseases in Vietnam veterans have since been reviewed. However, the majority of these studies were not able to control for major risk factors, such as smoking or tobacco use. Mortality from respiratory diseases was not found to be higher than expected in the Centers for Disease Control and Prevention's Vietnam Experience Study (Boehmer et al., 2004), in the Air Force Health Study (AFHS) (Ketchum and Michalek, 2005), or in two Australian studies of Vietnam veterans (ADVA, 2005b,c), but it is possible that the use of death certificates may have introduced some misclassification of respiratory deaths (Drummond et al., 2010). In contrast, in the Army Chemical Corps (ACC) cohort of Vietnam veterans, the prevalence of self-reported non-cancerous respiratory problems diagnosed by a doctor was significantly increased by about 40–60%, although no differences in the prevalence of respiratory problems were found in the subset of veterans whose serum TCDD was above 2.5 parts per trillion (Kang et al., 2006). Further study of cause-specific mortality in the ACC cohort by Cypel and Kang (2010) found a statistically significant excess of mortality from COPD when comparing the deployed and non-deployed groups. In a later analysis, Cypel and Kang were able to control for self-reported herbicide exposure, body mass index (BMI), and smoking status but found no statistically significant differences in respiratory diseases between sprayers and nonsprayers. Deaths due to COPD were lower in non-deployed ACC veterans than in males in the U.S. population; this is noteworthy because the prevalence of smoking in the non-deployed ACC veterans was about twice that in men in the U.S. population (Kang et al., 2006). Kang et al. (2014a) found lower risks

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of respiratory system disease deaths and COPD deaths in women veterans who were deployed to Vietnam compared with non-deployed women veterans, but these associations were not statistically significant.

Comparing New Zealand veterans who served in Vietnam with the standardized general male population of New Zealand, the risk of death from all non-malignant respiratory diseases (excluding COPD) was statistically significantly lower, but for COPD specifically no difference was found (McBride et al., 2013). Researchers of the Korean Veterans Health Study applied the Stellman exposure model and found no statistically significant difference for deaths from respiratory disease (Yi et al., 2014a,b). A comparison of deaths in the low-exposure category with the deaths in the high-exposure category found a statistically significant risk for non-malignant respiratory diseases that was statistically significantly elevated for COPD but not for pneumonia or asthma. A separate analysis of disease prevalence in the Korean Veterans Health Study used insurance claim data and found no difference in the risk of diseases of the respiratory system overall between the high-exposure and the low-exposure groups, but it did report statistically significant differences in the risks for COPD, pneumonia not due to influenza, chronic bronchitis, bronchiectasis, and asthma (Yi et al., 2014a).

Several studies of occupational cohorts have also been reviewed by the many VAO Update committees. *Update 2000* (IOM, 2001) drew attention to findings in the Seveso cohort that suggested a higher mortality from non-cancerous respiratory disorders in study subjects, particularly males, who were more heavily exposed to TCDD. Additional follow-up of mortality in the Seveso cohort found some increase in mortality from COPD at the 15- and 20-year points (Bertazzi et al., 1998, 2001) as well as at the 25-year follow-up (Consonni et al., 2008).

Occupational and industrial cohorts in the United States, the United Kingdom, New Zealand, and Australia did not find increased mortality from non-cancerous respiratory diseases overall (Boers et al., 2010; Collins et al., 2009a,c; McBride et al., 2009a), though they were unable to control for smoking. An update of the National Institute for Occupational Safety and Health (NIOSH) cohort of pentochlorophenol (PCP) workers (Ruder and Yin, 2011) did not find an association between exposure to the COIs and deaths from all non-malignant respiratory diseases; no association with COPD deaths was found for the subgroup exposed to both TCDD and PCP. Updated mortality data on workers in two chlorphenoxy herbicide plants in the Netherlands were reanalyzed by Boers et al. (2012) using serum measurements, but no association of TCDD exposure with respiratory diseases was observed. An updated mortality study of workers in a pesticide factory with TCDD contamination (Manuwald et al., 2012) showed no association of non-malignant respiratory disease with exposure.

No associations were observed with respiratory mortality in a small subcohort of New Zealand phenoxy herbicide sprayers ('t Mannetje et al., 2005) or with mortality from COPD in private applicators or their spouses in the Agricultural Health Study (AHS) (Blair et al., 2005a). Several other studies using the

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AHS cohort that examined morbidity from particular self-reported respiratory health problems, including wheeze, asthma, "farmer's lung" or hypersensitivity pneumonitis, and chronic bronchitis were examined by update committees, but most findings were not statistically significant.

Based on the many studies considered in various cohorts that applied different designs, measures of exposure, and definitions of non-malignant respiratory outcomes, the conclusion of inadequate or insufficient evidence of an association between exposure to the COIs and mortality from all non-cancerous respiratory diseases or from COPD specifically, has remained unchanged. There also remains inadequate or insufficient evidence of an association between exposure to the COIs and the prevalence of respiratory diseases, such as wheeze or asthma, COPD, and farmer's lung.

Update of the Epidemiologic Literature

This section summarizes the results of the relevant studies on respiratory disorders and exposures to the COIs. One new study of Vietnam veterans and respiratory disorders, one occupational study, and three other relevant studies of respiratory disorders and the COIs have been identified since *Update 2014*. Tables 43 and 44, which can be found at www.nap.edu/catalog/25137, summarize the results of studies related to non-cancer respiratory disease and COPD and pulmonary function, respectively.

Vietnam-Veteran Studies

Since *Update 2014*, one follow-up study of 2,783 male New Zealand Vietnam veterans, who served during 1964 to 1972 was identified and reviewed. Cox et al. (2015) used hospital discharge records from 1988 to 2009 to report the prevalent health conditions among this population. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% confidence intervals (CIs) were calculated for both the veteran cohort and the general population, and a standardized hospitalization ratio (SHR) was calculated using the two rates. Results showed a statistically significant increase in the hospitalization risk for COPD (n = 300; SHR = 1.68, 99% CI 1.43–1.93) and pneumonia (n = 174; SHR = 1.36, 99% CI 1.09–1.63). No difference in the ratio was found for asthma, although the estimate was decreased (n = 33; SHR = 0.84, 99% CI 0.46–1.21). Overall, the results of this study showed a small increase in hospital admissions of Vietnam veterans for COPD and pneumonia compared with the standardized population of New Zealand. Because smoking is a major risk factor for respiratory conditions, the lack of smoking-adjusted ratios raises concerns about the validity of

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the estimates. Moreover, exposure to the COIs was not validated and was simply assumed based on deployment to Vietnam.

Occupational Studies

Collins et al. (2016) added follow-up time for a retrospective cohort of 2,192 workers exposed to dioxins during trichlorophenol (TCP) and PCP production at chemical manufacturing plant in Michigan. Workers were compared with the U.S. population in order to calculate standardized mortality ratios, and work history records provided information about the length of the exposure. Serum samples used to measure levels of six types of dioxins were collected from 431 TCP and PCP workers. The historical concentrations for each dioxin congener were calculated based on the median concentration in the serum samples and the known half-lives associated with each congener. Complete vital status follow-up was achieved for the cohort, and there were 1,198 deaths during the entire study period (1979–2011). For the 1,198 TCP and PCP workers, there were 110 deaths from respiratory system disorders, with an SMR of 0.94 (95% CI 0.77–1.13).

Henneberger et al. (2014) examined the exacerbation of asthma among pesticide applicators with asthma who were enrolled in the AHS; details of the AHS study design, data collection, and cohort are found in Chapter 5. In this analysis, participants were selected for inclusion based on completing both the baseline and an additional take-home questionnaire and having reported a doctor diagnosis of asthma and also having reported active asthma based on having had at least one episode of wheezing or whistling in the previous 12 months and having had breathing problems in the same time period. The final study sample included 926 adult pesticide applicators with active asthma. Exacerbation was defined as having visited a hospital emergency room or doctor for an episode of wheezing or whistling in the previous 12 months. AHS participants were asked about their use of individual pesticides, and this analysis focused on the 36 pesticides used in the 12 months before enrollment (current exposure) by at least 10 applicators with at least two exacerbation cases. Logistic regression was used to estimate odds ratios for pesticide exposure, controlling for age, state, type of pesticide applicator (private or commercial), cigarette smoking status, allergy status based on self-reports of doctor-diagnosed hay fever or eczema, and adult onset of asthma based on onset at >20 years of age. No association with an elevated odds of asthma exacerbation was found for either 2,4-D (OR = 0.8; 95% CI 0.5–1.3) or dicamba (OR = 1.0; 95% CI 0.6–1.6). Interaction models for pesticide exposure and allergic status were not significant for 2,4-D and dicamba. The authors interpreted the inverse associations as related to the possibility that asthmatic farmers avoided certain activities and exposures. The AHS includes a well-characterized cohort with self-reported pesticide exposure data that has been shown to be reliable and the asthma and exacerbation outcomes were carefully

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defined. However, the data are cross-sectional, and the sequence of exposure and events cannot be determined.

't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and workers were potentially exposed to 2,4,5-T, the intermediates of TCP and other chlorophenols, and TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by the International Agency for Research on Cancer (IARC) (Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. This study extends the follow-up period of these workers to approximately 30 years from their last 2,4,5-T production exposure. From the original cohort of 1,025 workers, 631 were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current follow-up, 430 of the 631 workers were randomly selected and invited to participate in the morbidity survey, of which 245 (57%) participated. The survey was administered in 2007–2008 by face-to-face interview, and information was collected on demographic factors and health information, including doctor-diagnosed conditions and the year of diagnosis. A blood sample was also collected at that time and analyzed for TCDD, lipids, thyroid hormones, and other substances. For 111 participants, a neurological examination was conducted. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two different methods of exposure: having worked in a TCDD-exposed job (based on occupational records) and having serum TCDD concentration ≥10 pg/g lipid (18%). Mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy/TCP production, and 6 pg/g lipid in the 141 men and 43 women who worked in other parts of the plant. Compared with the 124 people in the non-highly-exposed jobs, the 121 people who had ever worked in a highly exposed job were no more likely to have doctor-diagnosed asthma (n = 8; OR = 1.13; 95% CI 0.40–3.22) or chronic bronchitis (n = 3; OR = 2.39; 95% CI 0.17–34.0). When compared by serum TCDD concentration ≥10 pg/g lipid, there were few cases of asthma or chronic bronchitis, and no difference in risk was found. Diagnoses of tuberculosis, pleurisy, or pneumonia were also examined, but there were too few cases to present valid estimates.

Cappelletti et al. (2016) performed a retrospective study of 331 male electric arc foundry workers at a single plant in Trentino, Italy, to determine if they experienced excess mortality from all causes or were at an increased risk for several other diseases, including asthma, due to occupational exposures to foundry dust. Analysis of the dust emissions found that the dust contained metals (including iron, aluminum, zinc, manganese, lead, chromium, nickel, cadmium, mercury, and arsenic), polycyclic aromatic hydrocarbons (PAHs), PCBs, and

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polychlorinated dibenzo-*p*-dioxin/dibenzofurans (PCDD/Fs) (reported as TEQs). Therefore, the authors could not determine which of the agents were associated with a specific outcome or to what extent. The men had worked at the factory for at least 1 year and, for the asthma analysis, were compared with 32 presumed non-exposed workers (clerks, managers, and watchmen) or with the standardized general population of Region Trentino-Alto Adige (where the factory was located) because there were few non-exposed foundry workers and high attrition rates. Company and medical records were used to determine vital status; the cause of death was determined from death certificates or other registries. Requests for exemption health care fees were used as a surrogate measure to identify the most common morbid conditions in the general population, which were then applied to the cohort to compute the relative risks for each of the conditions. The workers were followed from March 19, 1979 (or their first day of employment), through December 31, 2009, or date of death. The analysis for asthma was limited to 235 workers, and effect estimates were calculated using Mantel-Haenszel tests. Only two cases of asthma were found among the workers, and there was no difference in risk compared with the age-adjusted provincial population, although the effect estimate was imprecise (RR = 1.08, 95% CI = 0.27–4.31). This study is most limited by the fact that foundry dust is a complex mixture, which made it impossible to discern the impact of the specific contaminants of the foundry dust on the health outcomes of those exposed workers. Estimates were adjusted for only the age group and were not adjusted for other risk factors such as tobacco use, BMI, or other jobs or activities that could result in similar exposures. Exposure to foundry dust by the general population that was used for comparison was not discussed, although the foundry appears to be in the local vicinity, and emissions from it were reported to be present within a 2-kilometer radius.

Other Identified Studies

Five other studies of non-malignant respiratory diseases were identified, but all lacked sufficient exposure specificity to be included as contributing to the evidence base of the potential effect of the COIs. One study of U.S. capacitor manufacturer workers exposed to mixed PCBs that reported on deaths from respiratory diseases was identified, but it was limited by its lack of exposure specificity (Ruder et al., 2014). An analysis of data from the AGRICAN French cohort study examined the prevalence of respiratory diseases in adult men and women who were active or retired farm workers or owners, but all exposures and outcomes, which included asthma, were self-reported, and exposures to specific pesticides were not ascertained (Baldi et al., 2014). Butinof et al. (2015) conducted a cross-sectional study of male pesticide applicators who were licensed in Argentina and who were directly exposed to pesticides (n = 880). All participants completed a self-administered questionnaire that was adapted from

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the U.S. AHS, which collected information about herbicides, insecticides, and fungicides used, but no pesticide-specific exposure assessment was conducted, which limited the study's utility. A study of occupational exposure to pesticides in Ethiopian farmers and farm workers and respiratory health effects was conducted by Negatu et al. (2016) but was not considered further since data on specific pesticides were not collected. Finally, Akahane et al. (2017) examined the prevalence of self-reported long-term health effects (including respiratory disorders) in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of relevant exposures were presented, the study was not considered further.

Biologic Plausibility

Several recent studies explored the pro- and anti-inflammatory activity of TCDD exposure and of aryl hydrocarbon receptor (Ahr) in lungs in mice and in mouse and human lung cells. These studies addressed such health effects as lung response to viral infections, asthma, COPD, and allergen-induced lung inflammation. Cho et al. (2015) found that nuclear coactivator 7 (NCOA7) showed differential activity in a cell-culture study of TCDD exposure in normal lung epithelial and lung cancer cells with NCOA7 increasing CYP1A1 activity and the levels of inflammatory cytokines in the diseased cells but not in normal cells. S. M. Lee et al. (2017) found that AHR activation by the endogenous ligand kynurenine plays a role in immune regulation in inflammatory responses in a mouse model of lung injury.

Q. Liu et al. (2014) exposed juvenile zebrafish to TCDD in diet and reported a novel finding of lesions in nasal epithelium. Boule et al. (2015) exposed mice to TCDD during development and investigated their response to viral infection as adults. Treated mice had increased bronchopulmonary inflammation with an enhanced CD4+ T-cell response.

X. M. Li et al. (2016) found that TCDD-Ahr activation prevented inflammation and airway hyper-responsiveness in a non-eosinophilic asthma model in mice. The lungs of treated mice showed reduced eosinophil and neutrophil infiltration, and Th17 cytokine IL-17 was reduced in serum and bronchio-alveolar lavage fluid (with Th2 cytokine IL-4 also reduced). In a study comparing *Ahr* knockout and wild-type mice, T. Xu et al. (2015) found that TCDD treatment enhanced mesenchymal stem cell recruitment, thereby suppressing allergen-induced lung inflammation.

Sarill et al. (2015) explored the role of AHR and suggested that AHR has a role regulating anti-oxidant defenses in lung structural cells. In *Ahr* knockout mouse lung fibroblasts and human lung adenoma cells that were deficient in AHR expression and exposed to cigarette smoke extract, they found increased

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oxidative stress compared to wild-type cells. They also showed that anti-oxidant gene induction was significantly less in the *Ahr* knockout mouse lung fibroblasts. In lung fibroblasts from mice with an *Ahr* mutation (incapable of DNA binding), they showed that anti-oxidant sulfiredoxin 1 induction after cigarette smoke-extract exposure was independent of dioxin-response element binding (i.e., a role for AHR independent of exogenous ligand). In sum, the findings of Sarill et al. (2015) suggest that low AHR expression may facilitate the development or progression of COPD. Zago et al. (2017) studied the role of AHR and Cox-2 protein suppression and also concluded that low AHR contributes to increased inflammation, based on their study in human lung fibroblasts.

Synthesis

Update 2014 included new data that did not add any compelling evidence of an association of exposure to the COIs and respiratory disorders. The current update still does not display a coherent body of epidemiologic evidence to support conclusions concerning whether the risks of other particular respiratory problems are associated with exposure to the COIs. Cox et al. (2015) showed an increase in hospitalization risk rates for COPD and pneumonia, but not asthma, in New Zealand Vietnam veterans. However, smoking was not controlled for in the analysis and exposure to the COIs was not verified. In a 30-year follow-up of workers who produced 2,4,5-T in New Zealand, highly exposed workers did not have an elevated risk of doctor-diagnosed asthma, chronic bronchitis, tuberculosis, pleurisy, or pneumonia compared with lower-exposed workers based on job duties or serum TCDD concentration. The study of electric arc foundry workers in Italy (Cappelletti et al., 2016) reported no difference in the risk of asthma, but this was based on two cases. Moreover, the primary exposure, foundry dust, is a complex mixture, and it is not possible to isolate any potential COI exposure. In a follow-up of TCP and PCP workers at the Dow Midland, Michigan, plant, Collins et al. (2016) did not find elevated mortality for respiratory system disorders. Henneburger et al. (2014) found no association of asthma exacerbation with exposure to the COIs in the AHS. Therefore, the prior assessment cannot be altered since the new findings are mixed, and the study designs have limitations.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence of an association between exposure to the COIs and mortality from all non-cancerous respiratory diseases or from COPD specifically. There is also inadequate or insufficient evidence of an association between exposure to the COIs and the prevalence of respiratory disorders.

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GASTROINTESTINAL AND DIGESTIVE DESEASES, INCLUDING LIVER TOXICITY

This section discusses a variety of conditions specified by ICD-9 520–579 or ICD-10 K00–K95: diseases of the esophagus, stomach, intestines, rectum, liver, and pancreas. Details on peptic ulcer and liver disease, the two conditions most often discussed in the literature reviewed, are provided below. The symptoms and signs of gastrointestinal disease and liver toxicity are highly varied and often vague.

The essential functions of the gastrointestinal tract are to absorb nutrients and to eliminate waste. Those complex tasks involve numerous chemical and molecular interactions on the mucosal surface and complex local and distant neural and endocrine activity. One common condition of the gastrointestinal tract is motility disorder, which is present in about 15% of adults. The most convenient way to categorize diseases that affect the gastrointestinal system is according to the affected anatomic segment. Esophageal disorders predominantly affect swallowing, gastric disorders are related to acid secretion, and conditions that affect the small and large intestines are reflected in alterations in nutrition, mucosal integrity, and motility. Some systemic disorders (inflammatory, vascular, infectious, and neoplastic conditions) also affect the gastrointestinal system.

Peptic-Ulcer Disease

Peptic-ulcer disease refers to ulcerative disorders of the gastrointestinal tract that are caused by the action of acid and pepsin on the stomach or duodenal mucosa. Peptic-ulcer disease is characterized as gastric or duodenal ulcer, depending on the site of origin. Peptic-ulcer disease occurs when the corrosive action of gastric acid and pepsin overcomes the normal mucosal defense mechanisms that protect against ulceration. About 10% of the population has clinical evidence of having had a duodenal ulcer at some time in their lives; a similar percentage is affected by gastric ulcer. The incidence of duodenal ulcer peaks in the fifth decade, and the incidence of gastric ulcer about 10 years later.

Evidence increasingly indicates that the bacterium *Helicobacter pylori* is linked to peptic-ulcer disease (both duodenal and gastric). *H. pylori* colonizes the gastric mucosa in 95–100% of patients who have a duodenal ulcer and in 75–80% of patients who have a gastric ulcer. Healthy people in the United States under 30 years old have gastric colonization rates of about 10%. Over the age of 60 years, colonization rates exceed 60%. Colonization alone, however, is not sufficient for the development of ulcer disease; only 15–20% of subjects who have *H. pylori* colonization will develop ulcers in their lifetime. Other risk factors include genetic predisposition (such as some blood and human leukocyte antigen types), cigarette smoking, and psychologic factors (chronic anxiety and stress).

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Liver Disease

Blood tests of liver function are the mainstay of diagnosing liver disease. Increases in serum bilirubin and in the serum concentrations of some hepatic enzymes—aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyltransferase (GGT)—are commonly noted in liver disorders. The relative sensitivity and specificity of those enzymes for diagnosing liver disease vary, and a diagnosis can require several tests. The only regularly reported abnormality in liver function associated with TCDD exposure in humans is an increase in GGT. The estimated serum activity of that enzyme is a sensitive indicator of a variety of conditions, including alcohol and drug hepatotoxicity, infiltrative lesions of the liver, parenchymal liver disease, and biliary tract obstruction. Increases are noted after many chemical and drug exposures that are not followed by evidence of liver injury. The confounding effects of alcohol use (often associated with increased GGT) make it difficult to interpret changes in GGT in exposed people (Calvert et al., 1992). An increase in GGT can be considered a normal biologic adaptation to chemical, drug, or hormone exposure.

Cirrhosis is the most commonly reported liver disease in epidemiologic studies of herbicide or TCDD exposure. Cirrhosis is an irreversible chronic injury of the liver with extensive scarring and a resulting loss of function. Clinical symptoms and signs include jaundice, edema, abnormalities in blood clotting, and metabolic disturbances. Cirrhosis can lead to portal hypertension with associated gastroesophageal varices, an enlarged spleen, abdominal swelling attributable to ascites, and, ultimately, hepatic encephalopathy that can progress to coma. It is generally impossible to distinguish the various causes of cirrhosis through the clinical signs and symptoms or pathologic characteristics. The most common cause of cirrhosis in North America and many parts of western Europe and South America is excessive alcohol consumption. Other causes are chronic viral infection (hepatitis B or hepatitis C), the poorly understood condition primary biliary cirrhosis, chronic right-sided heart failure, and a variety of less common metabolic and drug-related conditions.

Conclusions from *VAO* and Previous Updates

Several studies reviewed by previous VAO committees have reported on several non-cancerous digestive system outcomes, and for that reason this section and the update of new epidemiologic literature presents all results for digestive and hepatobiliary outcomes by publication. Some studies that have been reviewed focused on liver enzymes, while others reported on specific liver diseases. An evaluation of the effects of herbicide and TCDD exposure on non-cancerous gastrointestinal ailments is challenging in that clinical experience suggests that medical history and physical examination are undependable diagnostic tools for some ailments, so incidence data are sometimes problematic. The strong

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interdependence among the characteristics of a given person (such as weight and the laboratory indexes of hepatic function and health) and TCDD body burden complicates the already difficult task of assessing association.

Studies of Vietnam veterans have generally examined different laboratory endpoints and clinical conditions, making comparisons and overall conclusions difficult. An analysis of the AFHS found that a significantly higher percentage of Ranch Hand veterans in the high-dioxin category had excesses of transaminase and other nonspecific laboratory measures of liver function than their comparison subjects. The data were consistent with an interpretation of a dose–response relationship, but other explanations were also plausible (AFHS, 2000). Later analyses also reported some abnormalities in liver enzymes among the Ranch Hands, including decreasing levels of C4 complement as dioxin increased and abnormal triglyceride concentrations that increased as the 1987 dioxin concentration increased (AFHS, 2005). However, mortality studies of the Ranch Hand cohort have not found an increased mortality related to gastrointestinal or liver disease (Ketchum and Michalek, 2005). Among ACC veterans, an increased rate of hepatitis was associated with Vietnam service but not with a history of spraying herbicide (Kang et al., 2006), and an 80% excess of digestive system or cirrhosis deaths was observed in veterans who handled or sprayed herbicides in Vietnam compared with their non-Vietnam-veteran peers (Cypel and Kang, 2010).

Studies of digestive and liver disease among other Vietnam veterans cohorts have not found associations with presumed herbicide exposure. The Australian Vietnam-veterans study (ADVA, 2005b) did not find an increase in liver disease in military personnel who served in Vietnam compared with the general population of Australia. The relationship between possible herbicide exposure and liver and gastric-ulcer disease was described in a sample of Korean Vietnam-era veterans in three publications by Yi et al. (2013, 2014a,b). Based on health insurance claims data, the adjusted prevalence of peptic-ulcer disease was found to be 3% higher in those with high exposure than in those with low exposure after adjusting for several behavioral, demographic, and service-related factors. For liver cirrhosis, there was likewise a small elevation in the prevalence and a significant log-linear relationship between an exposure opportunity score and the odds of having cirrhosis. In the mortality study in the same cohort of Korean Vietnam veterans, there was no association between putative log-transformed exposure and mortality from peptic ulcers (Yi et al., 2014b). However, highly exposed veterans were 17% more likely to die from cirrhosis than those with low exposure. Deaths from alcoholic liver disease were also statistically significantly elevated in the more highly exposed veterans.

Most of the analyses of occupational or environmental cohorts have had insufficient numbers of cases to support confident conclusions. Gastric ulcer was not associated with serum levels of dioxin-like PCDD/Fs or dioxin-like PCBs or with total TEQs after adjusting for possible confounders in a general population sample of 2,264 Japanese adults (Nakamoto et al., 2013). An analysis of National

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Health and Nutrition Examination Survey (NHANES) data on blood levels of 37 environmental pollutants and alanine aminotransferase levels in 1,345 persons aged 12 years and older found that, in general, blood levels of dioxin-like chemicals were not significantly correlated with alanine aminotransferase levels. The exception was 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin, which was associated with only a slight elevation (< 1%) (Yorita Christensen et al., 2013).

A study of the IARC cohort of phenoxy-herbicide and chlorophenol production workers and sprayers (Vena et al., 1998), the only study that had a relatively large number of observations, found less digestive system disease and cirrhosis mortality in exposed workers than in non-exposed controls. The mortality results through 2001 for the Seveso, Italy, cohort (Consonni et al., 2008) found no excess of deaths related to digestive diseases or specifically to cirrhosis. Several mortality studies of various occupational cohorts exposed to the COIs have been inconsistent but generally found no statistically significant increases in deaths from either ulcers or cirrhosis (Boers et al., 2010, 2012; Collins et al., 2010a,b; Manuwald et al., 2012; McBride et al., 2009a,b; Ruder and Yiin, 2011).

Thus, the reports have been inconsistent, and interpreting individual studies is difficult because of a lack of information on alcohol consumption and other risk factors. In the studies that showed the strongest association between potential exposure and gastrointestinal disease (specifically cirrhosis), there was strong evidence that excess alcohol consumption was the cause of the cirrhosis. The committee responsible for *VAO* concluded that there was inadequate or insufficient information to determine whether there is an association between exposure to the COIs and gastrointestinal and digestive disease, including liver toxicity. Additional information available to the committees responsible for subsequent updates has not changed that conclusion.

Update of the Epidemiologic Literature

One new study of gastrointestinal diseases among Vietnam veterans and two occupational studies of workers in herbicide-producing plants have been identified since *Update 2014*.

Vietnam-Veteran Studies

Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify health conditions that affected 2,783 male New Zealand veterans who had served in Vietnam during 1964–1972. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% CIs were calculated for the veteran cohort and the general population and reported for 14 conditions related to gastrointestinal and hepatobiliary outcomes. Those conditions were: esophageal disorders,

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gastroduodenal ulcer, gastritis, appendicitis, abdominal hernia, intestinal obstruction, diverticulosis, anal/rectal disorders, biliary disorders, alcoholic liver disease, other liver disease, pancreatic disease, abdominal pain, and gastrointestinal hemorrhage. The risk of hospitalization for gastroduodenal ulcer was elevated for veterans (n = 44; SHR = 1.80, 99% CI 1.10–2.50). Few cases of alcoholic liver disease were found, but the risk of hospitalization was not different between veterans and the general population (n = 11; SHR = 1.07, 99% CI 0.24–1.90). The risk of hospitalization from other liver disease (not further defined) was higher in veterans (n = 48; SHR = 1.86, 99% CI 1.17–2.55). No other increased or decreased risks of gastrointestinal and hepatobiliary outcomes were found. Exposure to the COIs was not validated and was assumed based on deployment to Vietnam. Moreover, the analysis did not control for smoking or ethnicity or other potentially important risk factors.

Occupational Studies

Collins et al. (2016) included additional follow-up time in a retrospective analysis of a cohort of 2,192 workers exposed to dioxins during TCP and PCP production at chemical manufacturing plant in Michigan. The U.S. population was used as the comparator for estimating standardized mortality ratios. Work history records were used to determine the length of the exposure. Serum samples for measuring the levels of six types of dioxins were collected for 431 TCP and PCP workers. The historical concentrations for each dioxin congener were calculated from the median concentrations from the serum samples and the known half-lives associated with each congener. Complete vital status follow-up was achieved for the cohort, and there were 1,198 deaths through the entire study period (1979–2011). Deaths from ulcer of the stomach and duodenum (K25–K27) and cirrhosis of the liver (K70–K74) were also included. No difference in the mortality of ulcer of the stomach and duodenum was found for the TCP workers (n = 2; SMR = 0.78, 95% CI 0.10–2.82), but an increased risk was found for the PCP workers (n = 5; SMR = 3.38, 95% CI 1.10–7.89). However, the interpretation of this finding is limited because the number of deaths from ulcers was small and the resulting risk estimates were imprecise. There were 16 total reported deaths from liver cirrhosis, but no difference in mortality was found for the TCP workers (n = 8; SMR = 0.44, 95% CI 0.19–87.2) or the PCP workers (n = 8; SMR = 0.97, 95% CI 0.42–1.91) compared with the standardized U.S. population. The committee notes that there is a possible mistake in the confidence interval reported for the TCP risk estimate, but there is no compelling data to indicate that cirrhosis of the liver is associated with exposure to dioxin. The committee also notes that no new deaths from stomach or duodenum ulcer have been reported since the last update of this cohort in 2003 (Collins et al., 2009a,b) at which time a statistically significant increase in stomach and duodenal ulcer deaths was found

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for PCP workers but not TCP workers. Since 2003, two additional deaths from liver cirrhosis among TCP workers were reported.

't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and workers were potentially exposed to 2,4,5-T, the intermediates of TCP and other chlorophenols, and TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by IARC (Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. This study extends the follow-up period of these workers to approximately 30 years from their last 2,4,5-T production exposure. From the original cohort of 1,025 workers, 631 were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current follow-up, 430 of the 631 workers were randomly selected and invited to participate in the morbidity survey, of which 245 (57%) participated. The survey was administered in 2007–2008 by face-to-face interview, and information was collected on demographic factors and health information, including doctor-diagnosed conditions and the year of diagnosis. A blood sample was also collected at that time and analyzed for TCDD, lipids, thyroid hormones, and other substances. For 111 participants, a neurological examination was conducted. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two different methods of exposure: having worked in a TCDD-exposed job (based on occupational records) and having serum TCDD concentration ≥10 pg/g lipid (18%). Mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy/TCP production and 6 pg/g lipid in the 141 men and 43 women who worked in other parts of the plant. Compared with the 124 people in the non-highly-exposed jobs, the 121 people who had ever worked in a highly exposed job were no more likely to have a doctor-diagnosed liver function problem (n = 10; OR = 0.84; 95% CI 0.30–2.34). When compared by serum TCDD concentration ≥10 pg/g lipid, there were few cases of liver function problem, leading to imprecise effect estimates (n = 3; OR = 0.54; 95% CI 0.12–2.36).

Other Identified Studies

Ruder et al. (2014) assessed cause-specific mortality including grouped diseases of the digestive system among 24,865 workers exposed to dioxin-like and non-dioxin-like PCBs from three U.S. electrical capacitor manufacturing plants who were employed 3 months or more from as early as 1939 through 1977. However, a lack of exposure specificity precluded further consideration. Akahane et al. (2017) examined the prevalence of self-reported long-term health effects in people exposed to PCBs, dioxins (e.g., PCDD/Fs), and dioxin-like chemicals through the ingestion

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of contaminated rice bran oil (Yusho accident) compared with an age-, sex- and residential-area-matched group. Because no TEQs or other quantification of the relevant exposures was presented, the study was not considered further.

Yamamoto et al. (2015) studied 678 male workers at a waste incineration plant in Japan. Blood samples were measured to determine the amounts of TCDD and PCBs in the blood, and several markers of hematology and blood chemistry profiles were also measured. Although some of these tests may be able to detect liver dysfunction, it was not linked to a health outcome, and the study was not considered further.

Biologic Plausibility

The liver is the first organ that encounters chemicals absorbed from the gastrointestinal tract, and it is responsible for metabolizing them to water-soluble chemicals that can be excreted in the urine. Because the liver has many detoxifying enzymes that efficiently metabolize many chemicals, liver toxicity is usually associated only with high-dose acute exposure or lower-dose chronic exposure. The liver can be damaged if the metabolism of a chemical results in the production of a reactive intermediate that is more toxic than the parent chemical. Changes in the serum concentrations of liver enzymes are biomarkers of liver toxicity, and their magnitudes correlate with the degree of liver damage. The exposure of laboratory animals to high doses of 2,4-D, 2,4,5-T, and TCDD is known to cause liver damage. The mechanisms by which the phenoxy herbicides damage the liver are based on the inhibition of mitochondrial function by the blocking of oxidative phosphorylation; this leads to a loss of generation of adenosine triphosphate, the death of cells, and hepatic necrosis and fibrosis. TCDD-induced hepatotoxicity is mediated by the activation of the AHR, which leads to changes in gene transcription and associated changes in cell function. Changes in gene expression are associated with several physiologic processes, oxidative stress, and apoptosis (Boverhof et al., 2005, 2006). TCDD-mediated hepatic steatosis is characterized by the accumulation of triglycerides caused by the combined up-regulation of CD36/fatty acid translocase and fatty acid transport proteins, the suppression of fatty acid oxidation, the inhibition of the hepatic export of triglycerides, an increase in peripheral fat mobilization, and an increase in hepatic oxidative stress (J. H. Lee et al., 2010). Recent evidence suggests that hepatic steatosis produced by TCDD might be mediated by the mitochondria (He et al., 2013). The exposure of rats to TCDD over a 2-year period (NTP, 2004) also produced several changes in the liver, including hepatocyte hypertrophy, multinucleated hepatocytes, inflammation, pigmentation, diffuse fatty change, necrosis, bile duct hyperplasia, bile duct cyst, nodular hyperplasia, portal fibrosis, and cholangiofibrosis. Lamb et al. (2016) studied a mouse model of liver injury and found that TCDD exposure increased

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liver damage and inflammation, suggesting that TCDD-activated AHR enhanced hepatic stellate cell activity.

AHR displays species differences; for example, amino acid sequences in the C-terminal region of human and mouse AHR are only 58% identical. Compared with the mouse Ahr, the human AHR has about a 10-fold lower relative affinity for TCDD; the difference has been attributed to the amino acid residue valine 381 in the ligand-binding domain of the human AHR (Flaveny et al., 2009; Ramadoss and Perdew, 2004). The existence of species differences associated with AHR activation is supported by the divergence in the transcriptomic and metabolomic responses to TCDD in mouse, rat, and human liver (Boutros et al., 2008, 2009; Carlson et al., 2009; Forgacs et al., 2012, 2013; Kim et al., 2009; Nault et al., 2013; Watson et al., 2017). In a recent study, gene-expression changes were compared in adult female primary human and rat hepatocytes that had been exposed to TCDD in vitro (Black et al., 2012). Whole-genome microarrays found that TCDD produced divergent gene-expression profiles in rat and human hepatocytes, both on an ortholog basis (conserved genes in different species) and on a pathway basis. For commonly affected orthologs or signaling pathways, the human hepatocytes were about 15-fold less sensitive than rat hepatocytes. Another microarray study examining species-specific transcriptomic differences in primary hepatocytes from humans, mice, and rats identified 16 orthologous genes that were dysregulated by TCDD in all three species (Forgacs et al., 2013). Such findings are consistent with epidemiologic studies that have shown humans to be less sensitive to TCDD-induced hepatotoxicity. However, it should be noted that in vitro human hepatocyte studies may not reflect the in vivo response of human liver to TCDD.

There have been few reports of health-relevant effects of phenoxy herbicides or TCDD on the gastrointestinal tract, even after high exposure. A series of recent papers demonstrated the influence of TCDD and the Ahr pathway on the development of the gut microbiome in mice (Stedtfeld et al., 2017a,b,c), which is thought to influence immune response that may affect gastrointestinal disorders. However, the available animal data do not support a plausible link between herbicide exposure and gastrointestinal toxicity in Vietnam veterans.

Synthesis

Previous updates indicated that there have been inconsistent findings for the COIs and gastrointestinal and liver disease and that interpreting individual studies is difficult because of a lack of information on alcohol consumption (especially for liver cirrhosis) and other risk factors. The recent studies of Vietnam veterans from New Zealand (Cox et al., 2015) and occupational cohorts of phenoxy herbicide producers (Collins et al., 2016; 't Mannetje et al., 2018) have not provided informative evidence for an association with the COIs. Although a few animal studies have shown an influence of TCDD and the Ahr pathway on the development of the gut microbiome in mice (Stedtfeld et al., 2017a,b,c), which may

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modify the immune response in gastrointestinal disorders. The available animal data do not support a plausible link between herbicide exposure and gastrointestinal toxicity in Vietnam veterans.

Conclusion

On the basis of the evidence reviewed here and in previous VAO reports, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and gastrointestinal and digestive diseases.

KIDNEY AND URINARY DISORDERS

Update 2014 was the first update for which the literature search identified studies reporting results concerning a possible association between exposure to the COIs and kidney diseases (ICD-9 580–589; ICD-10 N00–N29). The kidneys are located in the lower back region; their main function is to filter wastes and excess water out of the blood, which results in the production of urine. The kidneys are also responsible for helping maintain the body's chemical balance, helping control blood pressure, and synthesizing hormones. When problems arise with kidney function, it is often the result of damaged nephrons, which may leave the kidneys unable to filter blood and, thus, unable to remove wastes, which can then accumulate in the body. Chronic kidney disease is characterized by a gradual and usually permanent loss of kidney function that often results in renal failure. Diabetes, hypertension, and glomerulonephritis (acute inflammation) can all increase the risk of kidney disease.

Conclusions from *VAO* and Previous Updates

Publications from the Korean Veterans Health Study included findings for non-malignant kidney disease. Yi et al. (2013a) examined the prevalence of self-reported exposure based on six questions and self-reported kidney failure using a postal survey of 114,562 Korean veterans who had served in the Vietnam War. The incidence of kidney failure was compared by defining high and low categories of exposure based on the six exposure questions and also by exposure opportunity index (EOI) scores that were calculated. Self-reported kidney failure was statistically significantly increased when the analysis was based on perceived exposures, but it was not significant when the analysis was based on the EOI scores. In a study of cause-specific mortality through 2005 for 180,639 Korean veterans who were alive in 1992, Yi et al. (2014b) found that after adjustment for age in 1992 and rank, no differences in the hazard ratios were observed for acute renal failure or for chronic renal failure when the analyses were based on the EOI scores.

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Two environmental studies of non-U.S. populations were also considered. In a cross-sectional study of 2,264 Japanese men and women who had not been occupationally exposed to dioxins, self-reported kidney disease (not otherwise specified) was not associated with serum levels of dioxin-like PCDD/Fs or dioxin-like PCBs or with total TEQs after adjusting for possible confounders (Nakamoto et al., 2013). The second study sought to determine the factors contributing to a form of kidney disease not related to diabetes, hypertension, or any other recognized cause in adults in Sri Lanka (Jayatilake et al., 2013). Urine samples were taken from 57 cases and from 39 controls who were from non-endemic areas, and the samples were analyzed for 11 biomarkers of pesticides, including the COIs 2,4-D, 2,4,5-T, and 2,4,5-TCP. Of these, only 2,4-D was among the seven biomarkers found at concentrations above the limit of detection; 3.5% of the cases had 2,4-D concentrations above the reference limit of 0.3 µg/L. Since the urinary pesticide results were presented for only the cases, no inference can be made about the relative risk for this kidney condition in association with 2,4-D.

Based on these four studies, the committee for *Update 2014* concluded that there was inadequate or insufficient evidence of exposure to the COIs and non-malignant kidney diseases.

Update of the Epidemiologic Literature

One new study of Vietnam veterans and kidney and urinary disorders has been identified since *Update 2014*, as well as supporting occupational, environmental, and case-control studies of kidney and urinary disorders and exposure to the COIs.

Vietnam-Veteran Studies

Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify health conditions that affected 2,783 male New Zealand veterans who had served in Vietnam during 1964–1972. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% CIs were calculated for the veteran cohort and the general population and the two rates were used to calculate an SHR for several conditions including kidney and urinary outcomes. An elevation in chronic renal failure risk was identified (SHR = 1.21, 95% CI 1.07–1.36), with an acceleration in the risk for later time periods. No significant associations were identified with urinary tract infections (OR = 1.06, 95% CI 0.66–1.46), benign prostatic hypertrophy (OR = 1.11, 95% CI 0.79–1.42), or urinary stones (OR = 1.06, 95% CI 0.81–1.31). The exposures were not validated through serum measurements and

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were assumed based on deployment to Vietnam, and the study did not control for smoking status, ethnicity, or other potentially important risk factors.

Occupational Studies

Two studies using data from the AHS were reviewed. Using a prospective cohort design with an average of 16 years of follow-up, Lebov et al. (2016) evaluated the association between use of 41 specific pesticides and end-stage renal disease in 55,580 male pesticide applicators. Significant associations were found with several non-COIs (p for trend < 0.05), but there were no statistically significant associations with the phenoxy herbicides (2,4-D p for trend = 0.32; 2,4,5,T p for trend = 0.55). An education level greater than high school and obesity at enrollment were also associated with end-stage renal disease, as were diabetes, high blood pressure, and kidney disease.

Lebov et al. (2015) evaluated the use of 50 pesticides and factors of use and exposure, including frequency and duration, duration of residence on a farm, specific farm tasks performed, household practices of pesticides, and end-stage renal disease in the wives of pesticide applicators (n = 31,142). End-stage renal disease was higher in women who were obese, who used nonsteroidal anti-inflammatory drugs, or who had diabetes and hypertension. There was a protective effect associated with the personal use of any pesticide (hazard ratio [HR] = 0.42, 95% CI 0.28–0.64), but among women who personally mixed or applied pesticides, positive associations were observed only for the highest category of lifetime exposure days (HR = 4.22, 95% CI 1.26–14.20), although the estimate was imprecise. Wives with end-stage renal disease who reported having direct exposure to phenoxy herbicides (n = 9; HR = 1.10, 95% CI 0.50–2.39) or 2,4-D (n = 9; HR = 1.11, 95% CI 0.51–2.41) were found not to be at an elevated risk of the disease. Among wives who reported no direct use of pesticides but whose husbands used phenoxy herbicides (n = 47; HR = 0.86, 95% CI 0.49–1.51), 2,4-D (n = 45; HR = 0.82, 95% CI 0.47–1.43), 2,4,5-T (n = 12; HR = 0.69, 95% CI 0.36–1.32), or 2,4,5-TP (n = 5; HR = 0.88, 95% CI 0.36–2.15), the risks of end-stage renal disease were all decreased.

A third occupational exposure study was identified. 't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and the workers were potentially exposed to 2,4,5-T, the intermediates of TCP and other chlorophenols, as well as to TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by IARC (Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. This study extends the follow-up period of these workers to approximately 30 years from their last 2,4,5-T production exposure. From the original cohort of 1,025 workers, 631

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were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current follow-up, 430 of the 631 workers were randomly selected and invited to participate in the morbidity survey, of whom 245 (57%) participated. The survey was administered in 2007–2008 by face-to-face interview, and information was collected on demographic factors and health information, including doctor-diagnosed conditions and the year of diagnosis. A blood sample was also collected at that time and was analyzed for TCDD, lipids, thyroid hormones, and other substances. For 111 participants, a neurological examination was conducted. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two different methods of exposure: having worked in a TCDD-exposed job (based on occupational records) and having serum TCDD concentration ≥10 pg/g lipid (18%). Mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy/TCP production and 6 pg/g lipid in the 141 men and 43 women who worked in other parts of the plant. Compared with the people in the non-highly exposed jobs, the people who had ever worked in a highly exposed job at the plant were no more likely to have a doctor-diagnosed kidney function problem (n = 13; OR = 0.82, 95% CI 0.34–1.99). When compared by serum TCDD concentration, no difference in the risk of kidney function problems was found for workers in the high- versus low-exposure groups (n = 5; OR = 1.03, 95% CI 0.32–3.33).

Environmental Studies

Using data from the 1999–2004 cycles of NHANES, Everett and Thompson (2016) evaluated the association of blood levels of three chlorinated dibenzo-*p*-dioxins, one chlorinated dibenzofuran, and four dioxin-like PCBs with nephropathy (microalbuminuria or macroalbuminuria) among 1,505 adolescents and young adults (12–30 years of age) with normal glycohemoglobin (A1c < 5.7%). In logistic regression models 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (OR = 51.1, 95% CI 4.1–641.6), PCB 126 (OR = 8.9, 95% CI 2.0–39.7), PCB 169 (OR = 9.4, 95% CI 1.02–87.6), and PCB 156 (OR = 17.9, 95% CI 2.1–152.6) were associated with nephropathy (OR = 7.1, 95% CI 1.8–28.1) when one or more of these four dioxin-like chemicals was elevated; however, the effect estimates are quite imprecise. The effect was driven by females (OR = 17.4, 95% CI 3.4–88.6), as among males there were no cases of nephropathy when one or more of the four dioxin-like chemicals were elevated. These results were verified by TEQs; TEQ8 ≥ 50.12 fg/g was associated with nephropathy among females (OR = 11.9, 95% CI 1.6–87.2) but not males. Thus, in a cross-sectional study, dioxin-like chemicals were associated with nephropathy among young females, but not males, though reverse causality cannot be excluded, and the effect estimates were very imprecise.

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Two analyses using data from the cross-sectional, community-based study in the Annan District of Tainan City, Taiwan, where a former PCP factory had operated, and had released PCDD/Fs into the surrounding area, were identified that examined outcomes related to kidney disease (J. W. Chang et al., 2013; C. Y. Huang et al., 2016). As described in Chapter 5, people who were 18 years of age and older and who were residents of the exposure area were asked to participate in the study. Health examinations were performed on each participating individual, and serum samples had been previously collected and measured for levels of dioxins by the Tainan City Bureau of Health. A self-administered questionnaire, which was administered at the same time as the examination, was used to collect demographic information and medical history. C. Y. Huang et al. (2016) examined chronic kidney disease, defined as having an estimated glomerular filtration rate ≤ 60 mL/min/1.73m^2 or having been diagnosed by a physician. People diagnosed with congenital kidney disease, IgA nephropathy, post-infectious kidney disease, or medicine-induced kidney disease were excluded from the study. Of the 2,828 participating individuals, 1,427 had high dioxin levels (defined as > 20 pg WHO98-TEQ_{DF}/g lipid in the serum), and 156 had chronic kidney disease. High dioxin levels were associated with an increased prevalence of chronic kidney disease compared with low dioxin levels (10.9% versus 1.6%, respectively, $p < 0.001$). After adjustment for PCDD/Fs, gender, mercury, metabolic syndrome, age, fasting glucose, insulin, and uric acid, a high dioxin level was found to be significantly associated with chronic kidney disease (OR = 1.74, 95% CI 1.02–2.97). The strengths of this study include a large population, adjustments for age, fasting glucose, insulin, and uric acid, as well as serum measurements of exposure and a clear definition of chronic kidney disease. However, this study is limited by having had no follow-up of renal function measurements, the fact that the serum PCDD/Fs levels were collected over an extended period of time that ended about 3 years before the interview and health examination, unknown age at first exposure to PCDD/Fs, unknown duration of exposure, unknown cumulative exposure dose, the cross-sectional design, and a lack of additional data collection. Data on other potential confounders, such as waist circumference, dietary intake, and socioeconomic status, were not available.

Case-Control Studies

Raines et al. (2014) examined agricultural behaviors and health outcomes via questionnaire in a Nicaraguan community. Of the 424 total participants, 151 reported an occupational history of agriculture. The pesticides that were reported by participants as commonly used included 2,4-D. Decreased glomerular filtration rate was found in 9.8% of the women and 41.9% of the men. Glomerular filtration rate was associated with cutting sugarcane during dry season (OR = 5.86, 95% CI 2.45–14.01) and sugarcane chewing (OR = 3.24, 95% CI 1.39–7.58). Glomerular filtration rate was also associated with non-deliberate pesticide inhalation

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(OR = 3.31, 95% CI 1.32–8.31). This study is limited by its lack of exposure validation through serum or other measures.

Other Identified Studies

Two other studies of kidney and urinary disorders were identified, but both were limited by a lack of exposure specificity (Orantes et al., 2015; Ruder et al., 2014). In a study of women in agricultural communities in El Salvador (Orantes et al., 2015), the authors examined exposures to various agrochemicals, which may have included organophosphate insecticides as well as phenoxy herbicides (2,4,-D, hedonal), but the results were not stratified by chemical exposures, and thus the study was not considered further.

A third study was also identified, but instead of being limited by exposure specificity, it was limited by the fact that the outcomes examined were not diagnosed health outcomes but rather indicators of biologic effects. In a separate analysis of people residing near the former PCP factory in Taiwan, J. W. Chang et al. (2013) evaluated associations between PCDD/Fs and the risk of hyperuricemia (too much uric acid in the blood) in a subset of healthy subjects from the community health study (n = 1,531). Serum levels of 17 2,3,7,8-substituted PCDD/Fs were measured, and associations were tested between the serum TEQ_{DF}-2005 (total PCDD/Fs 2005 WHO TEQ) and various factors, including uric acid, glomerular filtration rates, and hyperuricemia risk. Hyperuricemia is a measure of disturbed metabolism, not a health outcome, and therefore this study was not considered relevant to the committee's task.

Biologic Plausibility

Studies of mice, rats, goldfish, and zebrafish have documented kidney toxicity from TCDD exposure. Studies of 2,4-D in goldfish and TCDD in rats report oxidative stress in kidneys (Matviishyn et al., 2014; Palaniswamy et al., 2014). Q. Liu et al. (2014) investigated the effects of developmental exposure to TCDD in zebrafish and found kidney lesions and the dysregulation of the genes involved in renal necrosis and cell death as well as decreased hematopoietic cells in the kidney marrow. Aida-Yasuoka et al. (2014) reported a chance finding that C57BL/6J mouse pups are more susceptible to TCDD-induced hydronephrosis than BALB/cA mice; in pups exposed to TCDD on postnatal days 1–7, the prevalence of hydronephrosis was 64% in the C57BL/6J pups and 0% in BALB/cA pups. In both strains of mice, the Ahr receptors are highly responsive to TCDD; however, genetic differences were found in expression of renal m-Prostaglandin E synthase-1 and early growth response 1 (Egr-1). In a study of mice, Bu et al. (2017) found that Ahr activation up-regulates glucose transporter 9 (Glut9), which plays a role in maintaining uric acid homeostasis.

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Synthesis

Since *Update 2014*, seven studies have been reviewed for kidney disease and urinary disorder outcomes related to exposure to the COIs. A hospitalization study of New Zealand Vietnam veterans found that chronic renal failure risk was statistically significantly increased among the veterans compared with the standardized general population of New Zealand, but there was no difference in the prevalence of other kidney or urinary outcomes (Cox et al., 2015); however, there was no exposure validation, some of the conditions (such as urinary tract infections) do not typically require hospitalization, and potentially important risk factors were not adjusted for in the analysis. In a 30-year follow-up study of New Zealand workers in a plant that produced 2,4,5-T, having a doctor-diagnosed kidney function problem was not different for workers in high- versus low-exposure groups, based on reported job and duties in the plant or on serum TCDD levels. Two analyses of end-stage renal disease in the AHS (Lebov et al., 2015, 2016) found no statistically significant associations with the phenoxy herbicides 2,4-D or 2,4,5-T among the male pesticide applicators or their wives. In an analysis of NHANES, Everett and Thompson (2016) evaluated the association of the blood levels of three chlorinated dibenzo-*p*-dioxins, one chlorinated dibenzofuran, and four dioxin-like PCBs with nephropathy among 1,505 adolescents and young adults and found that dioxin-like chemicals were associated with nephropathy among young females, but not males, although the effect estimates were very imprecise. An environmental exposure study of Taiwanese residents living in close proximity to a former PCP-producing factory found that those who had high serum dioxin levels had a statistically significantly elevated risk of chronic kidney disease (Huang et al., 2016). A second analysis of this population was identified (Chang et al., 2013), but because it examined hyperuricemia, which is a measure of disturbed metabolism and not a recognized health outcome, it was not considered as part of the evidence base of chronic kidney conditions related to exposure to the COIs. A cross-sectional study of agricultural behaviors, including the use of 2,4-D, and health outcomes in a Nicaraguan community (Raines et al., 2014) found a decreased glomerular filtration rate to be associated with cutting sugarcane, sugarcane chewing, and non-deliberate pesticide inhalation, but no serum or other objective measure of exposure were collected. Studies of mice, rats, goldfish, and zebrafish document kidney toxicity from TCDD exposure, but such outcomes do not present a consistent mechanism for the kidney dysfunction diseases in humans. Epidemiology studies concerning exposure to the COIs and kidney diseases were not reported prior to *Update 2014*. However, the new epidemiologic findings reviewed do not present a coherent pattern of an association between exposure to the COIs and kidney or urinary disorders.

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Conclusion

After reviewing the new evidence of exposure to the COIs and non-malignant kidney and urinary outcomes, the committee concludes that there remains inadequate or insufficient evidence of an association between exposure to the COIs and non-malignant kidney or urinary disorders.

THYROID HOMEOSTASIS AND OTHER ENDOCRINE FUNCTIONS

This section discusses a variety of conditions related to endocrine function, excluding diabetes and other pancreatic disorders, which were discussed in Chapter 10: Cardiovascular and Metabolic Outcomes. In particular, clinical disruptions of thyroid function are grouped as ICD-9 240–246 or as ICD-10 E00–E07, E20–21, while the remaining endocrine disorders are grouped as ICD-9 252–259 or as ICD-10 E22–E35. Thyroid homeostasis in humans was first addressed with respect to the COIs by the committee for *Update 2002*.

The thyroid secretes the hormones thyroxine (T4) and triiodothyronine (T3), which stimulate and help to regulate metabolism throughout the body. The thyroid also secretes calcitonin, a hormone that controls calcium concentration in the blood and the storage of calcium in bones. Secretion of T4 and T3 is under the control of thyroid-stimulating hormone (TSH), which is secreted by the anterior pituitary. Iodine operates in thyroid physiology both as a constituent of thyroid hormones and as a regulator of glandular function. Concentrations of those circulating hormones are regulated primarily by a negative-feedback pathway that involves three organs: the thyroid, the pituitary, and the hypothalamus. In the hypothalamus–pituitary–thyroid feedback scheme, the hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the pituitary to produce TSH, which in turn triggers the thyroid to produce T4 and T3. Cells in the hypothalamus and pituitary respond to concentrations of circulating T4 and T3. When T4 and T3 are low, the pituitary is stimulated to deliver more TSH to the thyroid, which increases T4 and T3 output. When circulating T4 and T3 are high, it triggers a reduction in the output of TRH and TSH. The negative-feedback loop maintains hormone homeostasis.

A disruption of thyroid homeostasis can be stimulatory (hyperthyroidism) or suppressive (hypothyroidism). Both conditions are diagnosed on the basis of blood concentrations of thyroid hormones, TSH, and other proteins (antithyroid antibodies). The prevalence of thyroid dysfunction in adults in the general population ranges from 1% to 10%, depending on the group, the testing setting, sex, age, the method of assessment, and the presence of conditions that affect thyroid function. People who have subclinical (biochemical) conditions may or may not show other signs or symptoms of thyroid dysfunction.

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It is important to distinguish between potential effects on adults and effects that may occur during development. In adults, the thyroid is able to compensate, within reasonable limits, for mild or moderate disruption (such as that caused by hyperplasia or goiter). In contrast, the fetus is highly sensitive to alterations in thyroid hormones, and alterations in thyroid homeostasis can hamper the development of many organ systems, including the nervous and reproductive systems; such findings are discussed in Chapter 8, which addresses the potential effects of Vietnam veterans' exposure to herbicides on their offspring. Only observations on adults are considered here.

Conclusions from *VAO* and Previous Updates

Thyroid homeostasis in humans was first addressed with respect to the COIs by the committee for *Update 2002*. After consideration of several new studies and because of the consistent observations of exposures to the COIs being related to perturbations of thyroid function—and to clinical hypothyroidism in particular—the committee for *Update 2014* considered the body of epidemiologic data, in combination with strong biologic plausibility, to represent limited or suggestive evidence of an association between exposure to the COIs and hypothyroidism. Additional endocrine effects have been observed in conjunction with exposure to the COIs in both humans and animals, but the evidence is inadequate or insufficient to establish an association with herbicide exposure for them.

An extensive assessment of endocrine function in clinical examinations, including a series of thyroid-function tests, failed to show systematic differences in thyroid function between Ranch Hands and comparison veterans (AFHS, 1991a). In analyzing individual TCDD readings obtained for subjects in the AFHS, however, Pavuk et al. (2003) found statistically significantly increased TSH measures from the 1985 and 1987 examinations in the high-exposure category and a significant increasing trend across the three TCDD categories during the 1982, 1985, 1987, and 1992 examinations. No increased risk of disorders of the thyroid was found among a study of Australian Vietnam veterans (O'Toole et al., 2009). Among Korean Vietnam veterans, two publications considered thyroid outcomes (Yi et al., 2014a,b). The first (Yi et al., 2014a) used claims data to report on the prevalence of disorders of the thyroid gland in 111,726 veterans and found an increased risk of thyroid conditions overall (ICD-10 E00–E07) with herbicide exposure, after adjustment for several factors. The pattern was very similar for both non-iodine-deficiency hypothyroidism (ICD-10 E03) and for other nontoxic goiter (ICD-10 E04). The risk of thyroiditis (ICD-10 E06) overall was not found to be significantly associated with herbicide exposure, but the strongest endocrine-related results were for autoimmune thyroiditis (ICD-10 E06.3), a subcategory of thyroiditis. No difference in the risk of hyperthyroidism (ICD-10 E05) was found between high- and low-exposure groups. Comparing the high-exposure group with the low-exposure group based on EOI scores, an elevated

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risk was observed for pituitary hypofunction ($p = 0.011$), while the risk of pituitary hyperfunction was not different between high- and low-exposure groups. The risk of hyperaldosteronism was also not elevated. A mortality analysis of the Korean Vietnam veteran cohort of 180,639 male veterans did not find any association between herbicide exposure and deaths from endocrine diseases when the cohort was analyzed as a group (Yi et al., 2014b).

Few studies of thyroid function have been conducted among occupational cohorts exposed to the COIs. Calvert et al. (1999) provided evidence of higher adjusted mean free-T4 concentrations in TCDD-exposed workers in the NIOSH Cross-Sectional Medical Study, but there was no dose–response relationship with serum TCDD. Bloom et al. (2006) found indications of an inverse relationship between the sum of dioxin-like chemicals and the concentration of free T4 in anglers in New York State, but no association between the sum of dioxin-like chemicals and TSH or T3. An analysis of the AHS that was restricted to male private pesticide applicators examined self-reported physician-diagnosed hyperthyroidism, hypothyroidism, and other thyroid conditions and the use of 50 specific agents, including 2,4-D, 2,4,5-T, and 2-(2,4,5-triphenoxy) proprionic acid (2,4,5-TP) (Goldner et al., 2013). After adjusting for age, education, and BMI, the odds of hypothyroidism for ever-use versus never-use were significantly elevated for 2,4-D, for 2,4,5-T, and for 2,4,5-TP. In comparison with those who had never used 2,4-D, an increased risk of hypothyroidism was seen in both those who had used 2,4-D for more than the median number of days and those whose days of 2,4-D use were fewer than the median (p-trend = 0.025). The use of 2,4,5-TP was found to be associated with a decreased risk of hyperthyroidism. None of the phenoxy herbicides were found to be related to having histories of other thyroid diseases. In an Italian study that compared urban and rural workers, Ciarrocca et al. (2012) found that the workers' urinary arsenic levels differed by a factor of between 2 and 4, and urinary arsenic was positively correlated with serum TSH and thyroglobulin and negatively with free T3 and T4.

The results from several environmental studies have also been reviewed by update committees, and no evidence was found of effects on thyroid function or disease in women exposed to pesticides (Chevrier et al., 2008) or, among women in the AHS, exposed to phenoxy herbicides (Goldner et al., 2010). Using data from NHANES III (1988–1994), Schreinemachers (2010) did not find associations between recent exposure to 2,4-D and T4 or TSH concentrations. An analysis of 1999–2002 NHANES data (Turyk et al., 2007) found total T4 to have a weak inverse relationship with serum TEQs; the effect was somewhat stronger in people over 60 years old and in women.

Among women exposed in the Seveso incident, a significant inverse association was found between serum concentrations of TCDD over a 20-year period (1976–1996) with serum total T4, but not with TSH or free T3, which were measured in 1996. This association was stronger for women who were exposed before menarche than for women exposed after menarche. When thyroid

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hormones were measured again in 2008 and compared with TCDD levels in 1976 and 1996, the association was no longer present (Chevrier et al., 2014). In a cross-sectional study of 2,264 Japanese men and women who had not been occupationally exposed to dioxins, self-reported thyroid disease (not otherwise specified) was not associated with serum levels of dioxin-like PCDD/Fs or dioxin-like PCBs or with total TEQs after adjusting for possible confounders (Nakamoto et al., 2013). Abdelouahab et al. (2008) described thyroid function in adult freshwater-fish consumers in Canada; dioxin-like congeners were associated with an increase in TSH and a decrease in T4 but below the threshold at which clinical symptoms would be present. Clear effects of dioxin-like chemicals on thyroid function were not apparent in Inuit adults (Dallaire et al., 2009) or in a cross-sectional study of a Chinese community exposed to an electronic-waste recycling plant (J. Zhang et al., 2010). Manh et al. (2013) and Kido et al. (2014) studied steroid hormone levels in the serum and saliva and dioxin concentrations in the breast milk of lactating Vietnamese women living in an "Agent Orange hot spot" (n = 51) or in an area with no suspected exposure (n = 58). Levels of cortisol and corticosterone in serum and saliva were higher in those women living in the hot spot area and were positively correlated with breast-milk dioxin concentrations. Trnovec et al. (2013) measured thyroid gland volume and free T4 in 320 adults from an organochlorine-contaminated area in Slovakia. Blood samples from these subjects produced readings above the limits of detection for all 7 dioxins, 8 of 10 furans, and all but 1 of the 12 PCBs on the 2005 WHO list of dioxin-like chemicals.

Update of the Epidemiologic Literature

One new epidemiological study of occupational exposures to the COIs and thyroid or endocrine effects has been identified for this update. Table 45, which can be found at www.nap.edu/catalog/25137, summarizes the results of studies related to thyroid homeostasis that have been reviewed in the VAO series.

Occupational Studies

't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and workers were potentially exposed to 2,4,5-T, the intermediates of TCP and other chlorophenols, and TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by IARC (Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. This study extends the follow-up period of these workers to approximately 30 years from their last 2,4,5-T production exposure. From the original cohort of 1,025 workers,

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631 were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current follow-up, 430 of the 631 workers were randomly selected and invited to participate in the morbidity survey, of which 245 (57%) participated. The survey was administered in 2007–2008 by face-to-face interview and information was collected on demographic factors and health information, including doctor-diagnosed conditions and the year of diagnosis. A blood sample was also collected at that time and analyzed for TCDD, lipids, thyroid hormones, and other substances. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two different methods of exposure: having worked in a TCDD-exposed job (based on occupational records) and having serum TCDD concentration ≥10 pg/g lipid (18%). Mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy/TCP production and 6 pg/g lipid in the 141 men and 43 women who worked in other parts of the plant. Compared with the people in the non-highly exposed jobs, the people who had ever worked in a highly exposed job at the plant were no more likely to have a doctor-diagnosed thyroid disorder (n = 6; OR = 0.95, 95% CI 0.19–4.67). When compared by serum TCDD concentration, no difference in the risk of thyroid disorder was found for workers in the high- versus low-exposure groups (n = 5; OR = 4.00, 95% CI 0.76–21.0), but the estimate is very imprecise owing to the small number of cases.

Other Identified Studies

Three additional epidemiologic studies were identified that presented outcomes on endocrine and metabolic effects. A cross-sectional study of endocrine effects from the use of pesticides was conducted using a random sample of agricultural workers aged 18–69 years old in Brazil (Piccoli et al., 2016). Although questionnaires that collected detailed information on exposures were administered by trained interviewers to the participants and blood samples were collected from all participants to test cholinesterase activity, serum residues of organochlorine pesticides, and levels of free T4, total T3, and TSH, the researchers did not offer results for specific herbicides and organochlorine pesticides, thereby limiting the usefulness of this study to provide evidentiary weight regarding a potential association between exposure to the COIs and thyroid functioning. The second study was a cross-sectional study that examined whether the presence of metabolic syndrome altered serum concentrations of dioxin-like and non-dioxin-like PCBs under conditions of weight loss (Dirinck et al., 2016). Given the cross-sectional nature of the work, it is of limited usefulness in assessing the association of metabolic syndrome with dioxin-like chemicals.

A study by X. Sun et al. (2014) collected blood samples to determine the dioxin concentration and levels of nine steroid hormones from 48 men in an herbicide-sprayed hot spot and 36 men from a non-sprayed area. Participants

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also completed a questionnaire about their health status. The levels of the steroid hormones, including testosterone, dehydroepiandrosterone, and estradiol, were measured and compared by exposure group. However, the differences in hormone levels are not surrogate measures of a health outcome, and, therefore, this study was not considered relevant to the committee's charge.

Biologic Plausibility

The influence of TCDD on thyroid-hormone homeostasis has been measured in numerous animal studies, and TCDD exposure has been associated with changes in serum concentrations of T4, T3, and TSH. In most studies, TCDD exposure is associated with a hypothyroid state, including reduced circulating T3 and T4 and increased TSH, especially after chronic exposure. The reduction in circulating T4 concentrations is robust and has recently been proposed as a biomarker of the effect of dioxin-like chemicals (J. M. Yang et al., 2010). Female rats exposed chronically to TCDD showed follicular-cell hyperplasia and hypertrophy of thyroid follicles, which were consistent with an overstimulation of the thyroid by TSH (TSH increases as a homeostatic response to low T4 levels) (Yoshizawa et al., 2010). TCDD enhances the metabolism of thyroid hormones primarily through an AHR-dependent induction of glucuronyl transferase activity (Gessner et al., 2012; Y. Kato et al., 2010; Martin et al., 2012; Nishimura et al., 2005). An enhanced accumulation of T4 in hepatic tissue of TCDD-treated mice may also contribute to the reduction in circulating T4 (Y. Kato et al., 2010).

The possibility that arsenic could act as an endocrine disruptor on thyroid hormone–mediated processes has been proposed on the basis of cell-culture studies and experiments with the ex vivo amphibian tail metamorphosis assay (Davey et al., 2008). In guinea pigs that were fed diets containing 50 ppm arsenic as sodium arsenite or arsenic trioxide for 11 weeks, serum (total) T3 and T4 were reduced compared to controls by about 20–25% and 33%, respectively (Mohanta et al., 2014). Recent data in zebrafish show that arsenite exposure increases thyroxine levels and alters the expression of genes in the hypothalamic–pituitary–thyroid axis, including thyroid receptors α and β, TSH, and corticotropin-releasing hormone (H. J. Sun et al., 2015). These data raise the possibility that cacodylic acid may also disrupt thyroid homeostasis, but there are no published epidemiologic studies that have addressed this.

Synthesis

Numerous animal experiments and several epidemiologic studies have shown that TCDD and dioxin-like chemicals exert some influence on thyroid homeostasis, with the findings being most consistently indicative of hypothyroidism (Boas et al., 2006, 2012; Chevrier et al., 2014). The underlying molecular mechanisms resulting in these effects on thyroid hormone and TSH concentrations in humans,

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however, are not yet fully characterized. In addition, there are some data to suggest the possibility that arsenic-based herbicides may also affect thyroid function.

Several previous studies of populations environmentally exposed to PCBs found some combination of elevated TSH concentrations and depressed T4 and T3 levels (Bloom et al., 2006; Hagmar et al., 2001a; Persky et al., 2001; Schell et al., 2004), although some (Hagmar et al., 2001b; Sala et al., 2001) found no significant effect. For example, Pavuk et al. (2003) reported a trend of higher TCDD serum concentrations being associated with increasing concentrations of TSH that was not accompanied by changes in circulating T4 or T3 (which would be interpreted as subclinical hypothyroidism) in participants in the AFHS. This finding in U.S. Vietnam veterans is complemented by the results from the Korean Veterans Health Study (Yi et al., 2014a), which found evidence of an increased occurrence of clinical hypothyroid disease, possibly associated with autoimmune hypothyroidism, in association with higher estimated potential herbicide exposure. In addition, the report from the AHS of increased physician-diagnosed hypothyroidism in herbicide applicators with phenoxy herbicide exposure (Goldner et al., 2013) supports the notion that this association is real. Results from the Korean Veterans Health Study suggest that adrenal and possibly pituitary function may also be affected by exposure to dioxin-like chemicals.

For the current update, in vitro and animal data continue to support previous findings that the COIs can alter thyroid homeostasis. There is additional new data (Sun et al., 2015) that raise the possibility that cacodylic acid might similarly disrupt the normal action of the thyroid. There is little additional epidemiologic data available regarding the association of the COIs with thyroid disease or other endocrine function. A follow-up of phenoxy herbicide producers in New Zealand did not find any difference in thyroid disorders between high- and low-exposure groups ('t Mannetje et al., 2018).

Conclusion

On the basis of the new evidence and that reviewed in previous VAO reports, the committee concludes that there is limited or suggestive evidence of an association between exposure to at least one of the COIs and hypothyroidism. There is inadequate or insufficient evidence for disruption of thyroid homeostasis or other endocrine disorders.

CHRONIC SKIN DISORDERS

In previous VAO reports, skin disorders such as chloracne were mentioned, but they were not consistently included as independent outcomes. However, due to the nature of new published epidemiologic literature, the committee has reviewed several studies of exposure to the COIs that have included skin conditions, and thus the committee has decided to include them as a distinct outcome in this chapter.

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Chloracne is a skin disease that is characteristic of high levels of exposure to TCDD and other diaromatic organochlorine chemicals. It is one of the few outcomes in humans that are consistently associated with such exposure, and it is a well-validated indicator of high-dose exposure to TCDD and related chemicals (Sweeney et al., 1997/1998). Chloracne shares some pathologic processes (such as the occlusion of the orifice of the sebaceous follicle) with more common forms of acne (such as acne vulgaris), but it can be differentiated by the presence of epidermoid inclusion cysts, which are caused by the proliferation and hyperkeratinization (horn-like cornification) of the epidermis and sebaceous gland epithelium. Although chloracne is typically distributed over the eyes, ears, and neck, it can also occur on the trunk, genitalia, and buttocks of chemical-industry workers exposed to TCDD (Neuberger et al., 1998).

If chloracne occurs, it appears within a few months after the chemical exposure, not after a long latent period; therefore, new cases of chloracne among Vietnam veterans would not be the result of exposure during the Vietnam War. Although it is resistant to acne treatments, it usually regresses.

The chronic skin conditions considered include skin infections, nuclear buds, karyolysis or karyorrhexis, comedones, scar formation, and skin pigmentation.

Conclusions from *VAO* and Previous Updates

The committee responsible for *VAO* (IOM, 1994) determined that there was sufficient evidence of an association between exposure to at least one COI (TCDD) and chloracne. Additional information available to the committees responsible for *Update 1996* (IOM, 1996), *Update 1998* (IOM, 1999), *Update 2000* (IOM, 2001), *Update 2002* (IOM, 2003c), *Update 2004* (IOM, 2005), and *Update 2006* (IOM, 2007) maintained that conclusion. Even in the absence of a full understanding of the cellular and molecular mechanisms that lead to the disease, several notable reviews (Panteleyev and Bickers, 2006; Sweeney and Mocarelli, 2000) have deemed the clinical and epidemiologic evidence of dioxin-induced chloracne to be strong. The occupational epidemiologic literature has many examples of chloracne in workers after reported industrial exposures (Beck et al., 1989; Bond et al., 1987, 1989a,b; Cook et al., 1980; Goldman, 1972; May, 1973, 1982; Oliver, 1975; Pazderova-Vejlupkova et al., 1981; Poland et al., 1971; Suskind and Hertzberg, 1984; Suskind et al., 1953; Zober et al., 1990). With relative risk estimates as high as 5.5 in exposed workers compared with referent non-exposed workers, Bond et al. (1989a) identified a dose–response relationship between probable exposure to TCDD and chloracne. Not everyone who is exposed to relatively high doses develops chloracne, and some with lower exposure may demonstrate the condition (Beck et al., 1989).

Almost 200 cases of chloracne were recorded among those residing in the vicinity of the accidental industrial release of dioxin in Seveso, Italy; most cases were in children and in those who lived in the highest-exposure zone, and most

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had resolved within 7 years (Assennato et al., 1989a,b; Caramaschi et al., 1981; Mocarelli et al., 1991).

Exposures of Vietnam veterans were substantially lower than those observed in occupational studies and in environmental disasters, such as in Seveso. The long period since the putative exposure has imposed methodologic limitations on the studies of Vietnam cohorts for chloracne. Nonetheless, the Vietnam Experience Study (CDC, 1988a) found that chloracne-like lesions were self-reported more often by Vietnam veterans than by Vietnam-era veterans (OR = 1.4; 95% CI 0.7–2.9). No excess risk was found in Vietnam versus era veterans among subjects who had dermatologic examinations (OR = 0.9, 95% CI 0.7–1.2 for comedones and acne from lesions; OR = 1.2, 95% CI 0.9–1.7 for hyperpigmentation). Compared with matched, non-exposed controls, Ranch Hand personnel reported a significant excess of acne (OR = 1.6, 95% CI 1.1–2.1) (Wolfe et al., 1990), but no cases of chloracne or post-inflammatory scars were found on physical examination 20 years after the potential herbicide exposure (AFHS, 1991b).

Update of Epidemiologic Literature

Three new studies of exposure to the COIs and chronic skin conditions have been identified since *Update 2014*. However, each study examined different outcomes, making comparisons among the studies difficult.

Vietnam-Veteran Studies

Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify health conditions that affected 2,783 male New Zealand veterans who had served in Vietnam during 1964–1972. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% CIs were calculated for the veteran cohort and for the general population for several noncancerous conditions, and an SHR was calculated using the two rates. This analysis found that hospitalization for skin infections (n = 140; SHR = 1.22, 99% CI 1.15–1.21) was greater in Vietnam veterans than what would be expected based on same-age New Zealand males. However, there was no difference in hospitalizations for other skin diseases (n = 60; SHR = 1.17, 99% CI 0.87–1.46). This analysis was restricted to the first hospitalization for each cause in order to account for chronic disease. This analysis did not include information on or control for lifestyle factors or ethnicity. Exposure was not validated through serum measurements and was assumed based on deployment to Vietnam.

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Occupational Studies

't Mannetje et al. (2018) conducted a morbidity survey among a subset of workers who were employed at the New Plymouth, New Zealand, phenoxy herbicide production plant for at least 1 month between 1969 and 1984. The plant produced 2,4,5-T, and workers were potentially exposed to 2,4,5-T, the intermediates of TCP and other chlorophenols, and TCDD. Workers had previously been recruited and examined as part of the international cohort of producers of phenoxy herbicides led by IARC (Kogevinas et al., 1997); see Chapter 5 for more details on the IARC cohort and the New Zealand phenoxy producers. This study extends the follow-up period of these workers to approximately 30 years from their last 2,4,5-T production exposure. From the original cohort of 1,025 workers, 631 were living, had a current address in New Zealand, and were below 80 years of age on January 1, 2006. For the current follow-up, 430 of the 631 workers were randomly selected and invited to participate in the morbidity survey, of which 245 (57%) participated. The survey was administered in 2007–2008 by face-to-face interview, and information was collected on demographic factors and health information, including doctor-diagnosed conditions and the year of diagnosis. A blood sample was also collected at that time and analyzed for TCDD, lipids, thyroid hormones, and other substances. For 111 participants, a neurological examination was conducted. Associations between exposure and health outcomes were assessed using logistic regression models that controlled for age, gender, smoking, BMI, and ethnicity using two different methods of exposure: having worked in a TCDD-exposed job (based on occupational records) and having serum TCDD concentration ≥10 pg/g lipid (18%). The mean TCDD concentrations were 19 pg/g lipid in the 60 men directly involved in phenoxy/TCP production and 6 pg/g lipid in the 141 men and 43 women who worked in other parts of the plant. Compared with the 124 people in the non-highly exposed jobs, the 121 people who had ever worked in a highly exposed job were no more likely to have doctor-diagnosed eczema (n = 17; OR = 0.57, 95% CI 0.27–1.21) or acne (n = 2; OR = 0.73, 95% CI 0.10–5.47). When compared by serum TCDD concentration ≥10 pg/g lipid, no difference in eczema (n = 8; OR = 1.31, 95% CI 0.47–3.61) or acne (based on 1 case) was found.

Environmental Studies

In 1968, bran rice oil was accidentally contaminated with high concentrations of PCBs, dioxins, and dioxin-like chemicals in Western Japan (the Yusho accident). People who were poisoned were recruited into a cohort (i.e., the Yusho Group) and received regular medical follow-up. Mitoma et al. (2015) examined skin symptoms in the Yusho Group (n = 352) who underwent dermatological check-ups in 2012. PCB and dioxin serum concentrations were still elevated in the Yusho Group compared to the general Japanese population, and skin diseases

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were still very common. Furthermore, black comedones and scar formation were significantly correlated with blood 2,3,4,7,8-PeCDF level, and black comedones (n = 115; 32.7% of all patients), scar formation (n = 111; 31.5% of all patients), and skin pigmentation (n = 57; 16.2% of all patients) were significantly positively associated with the total serum PCB concentration. These results show that the prevalence of skin conditions such as black comedones, scar formation, and skin pigmentation are correlated with exposure to PCBs, dioxins, and dioxin-like chemicals, and that chloracne was persistent in this population 44 years after the acute ingestion of dioxins and dioxin-like chemicals.

Other Identified Studies

Four additional studies that reported skin conditions were identified, but each lacked the necessary exposure specificity to be considered further. A second study of long-term health effects from the Yusho accident (Akahane et al., 2017) examined the prevalence of self-reported conditions including diseases of the skin compared with an age-, sex-, and residential area–matched group. Because no TEQs or other quantification of relevant exposures was presented, the study was not considered further. Butinof et al. (2015) conducted a cross-sectional study of male pesticide applicators (n = 880) who were issued applicator licenses by the Agriculture, Livestock, and Food Ministry of Argentina between 2007 and 2010. All participants completed a self-administered questionnaire that was adapted from the U.S. AHS. The average worker was exposed to 11 different chemicals, and no pesticide-specific exposure assessment was conducted. Ruder et al. (2014) examined skin conditions such as chloracne and hyperpigmentation in a U.S. cohort of capacitor manufacturers exposed to dioxin-like and non-dioxin-like PCBs, but it was limited by a lack of exposure specificity. C.Y. Yang et al. (2015) evaluated 240 Yucheng, Taiwan, subjects aged 9–65 years who were exposed to PCB-contaminated rice oil in 1979 and who completed a health-related quality-of-life questionnaire about 30 years following the exposure. Exposure was not verified by serum measurements or other means.

A fifth study (Carbajal-Lopez et al., 2016) involved a cross-sectional study that examined biomarkers of DNA damage in buccal cells in male agricultural workers in Mexico who were exposed to pesticides that likely included 2,4-D and dicamba (n = 111) and compared them with males who had no occupational history of pesticide exposure (n = 60). Although significantly ($p < 0.0001$) more evidence of DNA damage, as measured by comet tail length, micronuclei, binucleated cells, pycnosis, and condensed chromatin, was found among the agricultural workers who were exposed to pesticides than among the non-exposed workers, these differences are not tied to an observable health outcome, and the study was not considered further.

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Biologic Plausibility

Previous updates have reported that chloracne-like skin lesions have been observed in several animal species in response to exposure to TCDD but not to purified phenoxy herbicides. Data accruing over the past several decades demonstrated that TCDD alters the differentiation of human keratinocytes, and more recent studies have illuminated how. TCDD accelerates the events associated with early differentiation and also obstructs the completion of differentiation (Bock, 2017a,b; Fontao et al., 2017; Geusau et al., 2005; Mandavia, 2015). Panteleyev and Bickers (2006) proposed that the major mechanism of TCDD induction of chloracne is activation of the stem cells in the basal layer of the skin to differentiate and then an inhibition of their ability to commit fully to a differentiated status. De Abrew et al. (2014) elucidated the role of matrix metalloproteinase-10 in the histopathological changes that typify chloracne. Work by Tauchi et al. (2005) and K. J. Smith et al. (2017) implicated additional inflammation-related mechanisms by which TCDD exposure may lead to chloracne. The data provide biologically plausible mechanisms for the induction of chloracne by TCDD.

Synthesis

No epidemiologic data in the past decade have refuted the conclusion of prior VAO committees that the evidence of an association between exposure to dioxin and chloracne is sufficient. Because TCDD-associated chloracne becomes evident within a few months after exposure, there is no risk of new cases long after service in Vietnam. Given the established relationship of an association between TCDD and chloracne and the long period that has elapsed since service in Vietnam, the present committee concludes that the emergence of persistent additional biologic or epidemiologic evidence that would merit review and deliberation by later VAO committees is unlikely. The formation of chloracne lesions and other skin disorders after the administration of TCDD has been observed in some species of laboratory animals. The newly identified epidemiologic studies (Cox et al., 2015; Mitoma et al., 2015; 't Mannetje et al., 2018) examined different outcomes and had varying exposure specificity, thereby limiting the comparisons that could be made. Although some differences between exposed and unexposed populations were noted, each study had several limitations that did not change the evidence of association with exposure to the COIs and chronic skin conditions.

Conclusion

On the basis of numerous epidemiologic studies of occupationally and environmentally exposed populations and supportive toxicologic information, previous VAO committees have consistently concluded that there is sufficient evidence

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of an association between exposure to at least one COI and chloracne. After review of new literature, the committee concludes that there is inadequate or insufficient evidence of an association between exposure to the COIs and other chronic skin conditions.

EYE PROBLEMS

This section discusses eye problems (ICD-9 360–379; ICD-10 H00–H59). The loss of vision is increasingly common with advanced age, and about one-sixth of people over 70 years old have substantial impairment, with men and women being similarly affected (NCHS, 2010). The most prevalent ocular problems in the current age range of Vietnam veterans are age-related macular degeneration, cataracts, glaucoma, and diabetic retinopathy. Ocular problems involving chemical agents most often arise from acute, direct contact with caustic or corrosive substances that may have permanent consequences. Ocular impairment arising from systemic exposure to toxic agents may be mediated by nerve damage. Cataracts can be induced by a chronic internal exposure of the lens to such chemicals as 2,4-dinitrophenol, corticosteroids, and thallium; glaucoma may be secondary to a toxic inflammation or may result from topical or systemic treatment with anti-inflammatory corticosteroids (Casarett and Doull, 1995).

Conclusions from *VAO* and Previous Updates

Update 2010 considered one study of Australian Vietnam veterans that found that the veterans had a higher prevalence of all the eye conditions assessed—cataracts, presbyopia, color blindness, and other diseases of the eye—than the Australian population (O'Toole et al., 2009). However, the committee noted a lack of information on exposure to the COIs and a lack of clinical confirmation of the eye conditions, and it had serious concerns about the possibility that recall bias had played a role in the findings. On the basis of that one study, the committee for *Update 2010* concluded that there was inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and eye conditions. No new epidemiologic studies of exposure to the COIs and eye problems had been published for review in *Update 2012* and *Update 2014*.

Update of Epidemiologic Literature

Only one new study of eye conditions was identified. Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify prevalent health conditions in 2,783 male New Zealand veterans who had served in Vietnam during 1964–1972. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the

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average annual resident population. Standardized hospitalization rates and 99% CIs were calculated for the veteran cohort and the general population, and an SHR was calculated using the two rates for two conditions related to the eyes. Neither the risk of hospitalization for cataracts (n = 99; SHR = 1.11, 99% CI 0.82–1.40) nor retinal disease (n = 31; SHR = 1.35, 99% CI 0.73–1.98) was different between the veterans and general New Zealand population. Cataract and retinal disease are not generally conditions that require hospitalization, and therefore, the estimated prevalence may be higher. Exposure was not validated through serum measurements and was assumed based on deployment to Vietnam.

Biologic Plausibility

There have been several recent reports of ocular activity associated with Ahr activation or TCDD exposure of rats (Sugamo et al., 2009), mice (Takeuchi et al., 2009), and human non-pigmented ciliary epithelial cells (Volotinen et al., 2009). Hu et al. (2013) reported that mice harboring the null allele at the *Ahr* locus developed macular age-related degeneration-like pathology.

Woeller et al. (2016) investigated whether AHR activation had therapeutic potential for thyroid eye disease, which includes myofibroblast accumulation and orbital scarring. TGF-β induces myofibroblast formation, and AHR influences TGF-β signaling. Using orbital fibroblasts cultured from thyroid eye disease patients, Woeller et al. found that exposure to AHR ligands 6-formylindolo(3,2-b)carbazole2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (FICZ and ITE) prevented TGF-β-induced myofibroblast formation.

Synthesis

Hospital discharge data from New Zealand Vietnam veterans (Cox et al., 2015) do not suggest any association between exposure to the COIs and retinal disease or cataracts. The data generated in vitro or in vivo in animal models reveal a quite modest possibility of ocular activity for the COIs; these are limited data that do not assist the committee in determining an association between exposure to the COIs and eye conditions.

Conclusion

Given the lack of additional evidence, the committee concludes that there is inadequate or insufficient evidence to determine whether there is an association between exposure to the COIs and eye conditions.

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BONE CONDITIONS

This section discusses osteoporosis, or decreased bone density (ICD-9 733.0–733.1; ICD-10 M80–M81), and other joint and connective tissue disease, excluding arthritis, which is discussed in Chapter 6: Immune System Disorders. Osteoporosis is a skeletal disorder characterized by a decrease in bone mineral density and a loss of the structural and biomechanical properties of the skeleton, which leads to an increased risk of fractures. Although there are no practical methods for assessing overall bone strength, bone mineral density correlates closely with skeletal load-bearing capacity and fracture risk (Lash et al., 2009). WHO has defined osteoporosis based on bone mineral density measurements. The diagnostic T-score derived by dual energy X-ray absorptiometry is the number of standard deviations from the mean bone mineral density for healthy women. In women, readings greater than −1.0 are normal, whereas osteopenia is defined by a T-score between −1.0 and −2.5, osteoporosis is defined by a T-score between −2.5 and −5.0, and severe osteoporosis corresponds to a T-score of −5.0 or lower. Diagnostic criteria have not been standardized for osteoporosis in men. Although men have much higher baseline bone mineral density than women, they seem to have a similar fracture risk for a given bone mineral density (Lash et al., 2009), so most authorities apply the same WHO ranges for T-scores calculated relative to normal young women.

Sex is an important risk factor for osteoporosis; about 56% of postmenopausal women have decreased bone mineral density, and 6% have osteoporosis (CDC, 2002). The effects of aging on bone loss in women are well known, but many health care providers and patients are less familiar with the prevalence and effects of bone changes in older men (Orwoll et al., 2010). Individual patients have genetic and acquired risks of osteoporosis, and the osteoporosis disease process can be without symptoms for decades. It is well known that hormones, vitamins, and pharmaceuticals can have adverse effects on bone and that drug-induced osteoporosis occurs primarily in postmenopausal women, but premenopausal women and men are also significantly affected. Glucocorticoids are the most common cause of drug-induced osteoporosis (Mazziotti et al., 2010). Other risk factors for the loss of bone mineral density include the use of long-acting benzodiazepine or anticonvulsant drugs, previous hyperthyroidism, excessive caffeine intake, and routinely standing for less than 4 hours per day (Lash et al., 2009).

Several studies have described a link between organochlorine exposure and effects on bone growth, most notably reports of infants exposed in utero to high concentration of PCBs and PCDFs who developed irregular calcifications of their skulls (Miller, 1985) and reports of accidental organochlorine poisoning that resulted in osteoporosis (Cripps et al., 1984; Gocmen et al., 1989). However, epidemiologic studies of the association between environmental exposures to organochlorine compounds and bone disorders have had inconsistent results.

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Conclusions from *VAO* and Previous Updates

Update 2010 was the first report in the VAO series to review studies of the association between exposures to the COIs and a decrease in bone mineral density. Few studies have reported results in this category with enough exposure specificity to be fully informative. Hodgson et al. (2008) studied the relationship between environmental exposures and bone mineral density in a set of 325 members of the Osteoporosis Cadmium as a Risk Factor (OSCAR) cohort who were at least 60 years old. Forearm bone mineral density was measured, blood samples were analyzed for the five dioxin-like mono-ortho PCB congeners (PCB 105, 118, 156, 157, and 167), and TEQs were calculated. In men, PCB 118 was significantly negatively associated with bone mineral density, but there was no association between the TEQ for any of the five dioxin-like mono-ortho PCBs and bone mineral density. In women, PCB 118 and the TEQs for all five dioxin-like mono-ortho PCBs were positively associated with bone mineral density. When the risk of low bone mineral density was treated as a binary variable in an adjusted logistic model, there was a significant association with PCB 118 in men, but none of the measured compounds was predictive in women. A study of 350 women who were exposed to TCDD as a result of the chemical explosion in Seveso, Italy, examined the relationship of DEXA-assessed bone mineral density and TCDD serum levels (Eskenazi et al., 2014). The results suggested that TCDD levels were associated with some evidence of better bone structure in the 48 women for whom exposure occurred after peak bone mass, which is estimated to happen 2 years after menarche, but the findings did not support the hypothesis that postnatal TCDD exposure adversely affects adult bone health. Two cross-sectional studies that examined the association of exposure to dioxin-like chemicals and bone quality in residents of Canada's northern regions, who are known to be exposed to these chemicals as a result of their diet of marine mammals and predatory fish (Paunescu et al., 2013a,b), found an increase in dioxin-like PCBs 105 and 108 to be negatively associated with a "stiffness index," and neither total plasma dioxin-like chemicals levels nor any specific dioxin-like PCB level was associated with ultrasonography-assessed bone strength in Inuit women.

Update of the Epidemiologic Literature

Only one new study of bone conditions was identified. Cox et al. (2015) used hospital discharge records from 1988 to 2009 to identify health conditions that had affected 2,783 male New Zealand veterans who had served in Vietnam during 1964 to 1972. Age-specific hospitalization rates were calculated using the total number of annual hospitalizations published by the Ministry of Health and the average annual resident population. Standardized hospitalization rates and 99% CIs were calculated for the veteran cohort and the general population, and an SHR was calculated using the two rates for conditions related to other joint

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diseases and other connective tissue diseases. An elevated risk of hospitalization for other joint diseases (n = 77; SHR = 1.34, 99% CI 1.04–1.64) and other connective tissue disease (n = 169; SHR = 1.25, 99% CI 1.06–1.44), neither of which is further defined, was found for veterans when compared with the standardized New Zealand population. Since these disease categories were not clearly defined, it is difficult to interpret the findings. The study is also limited because the exposures were not validated through serum measurements and were assumed based on deployment to Vietnam.

Biologic Plausibility

Animal studies suggest that TCDD may have some influence on bone formation and maintenance. Recent work from Herlin et al. (2013) showed that the exposure of adult mice to TCDD resulted in harder bone matrix, thinner cortical bone, mechanically weaker bones, and, most notably, increased trabecular bone volume fraction in *Ahr* knockout mice. It is known that TCDD can induce chondrocyte apoptosis in culture, which could be an initial event leading to the sort of cartilage degradation observed in arthritis (Yang and Lee, 2010). Lee and Yang (2012) recently demonstrated that this is mediated by reactive oxygen species. In addition, TCDD exposure via the dam's milk impaired bone mineralization during postnatal development in mice because of a reduction in osteoblastic activity as a result of TCDD-induced up-regulation in the active form of vitamin D in serum (Nishimura et al., 2009). TCDD altered osteogenesis (bone formation) in an in vitro osteoblast model and led to alterations in the proteins associated with cytoskeleton organization and biogenesis, a decrease in the expression of calcium-binding proteins, and a decrease in osteoblast calcium deposition (Carpi et al., 2009). In adult rats, TCDD exposure reduced trabecular bone cross-sectional area, but it significantly increased total bone mineral density; it was further noted that TCDD decreased the expression of the bone-formation marker procollagen type I N-terminal propeptide and increased the expression of the bone-resorption marker carboxy-terminal collagen cross-link, suggesting a net loss of bone tissue (Lind et al., 2009). It is also known that exposure to polyaromatic hydrocarbons (such as those in tobacco smoke) can affect bone health, and some of these alterations have been shown to be mediated, at least in part, by the AHR. That implies that TCDD may alter or modify the effects of osteoblast formation and function as well as inhibit osteoclast formation and function (Kung et al., 2012; Yan et al., 2011). Iqbal et al. (2013) recently addressed this, studying genetically altered mice in order to understand the contributions of tobacco carcinogens and TCDD. In their work, mice in which the *Ahr* or *CYP1A1*, *CYP1A2*, and *CYP1B1* genes were deleted displayed reduced resorption and high bone mass. In contrast, *Ahr* activation by administering benzo[*a*]pyrene to wild-type mice increased osteoclastogenesis and bone resorption.

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Synthesis

The small amount of available epidemiologic information on the possible adverse effects of exposure to the COIs on bone structure has previously been based entirely on dioxin-like mono-ortho PCBs, which contribute a small percentage to total TEQs based on all dioxin-like PCBs. The recent data from New Zealand Vietnam veterans, while suggesting a slightly statistically elevated risk of "other" bone and joint conditions, has poorly described outcomes (with essentially no data on the exact bone conditions considered) and is further limited by the use of hospitalization rates for such outcomes, which are difficult to interpret. Biological data confirm that TCDD is active in bone metabolism, but the pattern of association of exposure to the COIs and subsequent disease is not consistent in the current literature.

Conclusion

There is inadequate or insufficient evidence of an association between exposure to the COIs and clinical or overt adverse effects of osteoporosis, loss of bone mineral density, or other bone conditions.

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12

Conclusions and Recommendations

Chapter Overview

This final chapter presents a synopsis of the conclusions drawn by the committee regarding statistical associations between diseases and possible exposure to dioxin and other chemical compounds in herbicides used in Vietnam. It also presents the committee's recommendations regarding future research on Vietnam veterans' health.

SYNOPSIS OF COMMITTEE CONCLUSIONS

Under the aegis of Public Law (PL) 102-4, the current committee reviewed and evaluated the available scientific evidence regarding the associations between diseases and exposure to dioxin and other chemical compounds in herbicides used during the Vietnam War.[1] In reaching its conclusions, it weighed the strengths and limitations of the epidemiologic evidence reviewed in its report and in previous Veterans and Agent Orange (VAO) reports. Although the studies published since *Update 2014* are the subject of a detailed evaluation here, the committee drew its conclusions in the context of the entire body of literature. The contribution of recent publications to the scientific evidence base is emphasized in this report, but the weight of the evidence in its totality was the primary consideration guiding the evaluation of health outcomes. Although the study subjects in much of the new literature reviewed here were not the male U.S. Vietnam veterans who

[1]These chemicals—2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, and cacodylic acid—and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) are collectively referred to as the "chemicals of interest" (COIs) in the report.

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constitute most of the population affected by the VAO reports, the new studies from U.S. and other Vietnam veteran populations provided pertinent information.

Epidemiologic methods and analytic capabilities have continued to improve over the period in which the previous VAO updates have been conducted. As has been the case for recent updates, a considerable number of the new studies presented their results in terms of serum TCDD concentrations or total toxic equivalents (TEQs), which are particularly useful for the committee's purpose because they provide a cumulative measure of exposure to all dioxin-like chemicals.

The committee also notes that considerable experimental data related to the biologic plausibility of the health conditions statistically associated with exposure to the components of the herbicides sprayed in Vietnam have emerged since the beginning of the VAO report series. These findings help to inform decisions about how to categorize the degree of association for individual conditions. On the basis of its evaluation of epidemiology studies of Vietnam-veteran populations and of occupationally and environmentally exposed populations, and aided by experimental studies on biologic plausibility, the committee assigned each health outcome to one of four categories of relative certainty of association with exposure to the herbicides used in Vietnam or to any of their components or contaminants.

Principal Changes from Previous Updates

The application of the committee's evaluation methodology led to three principal changes in the conclusions regarding exposure to the chemicals of interest (COIs) and adverse health outcomes reported in *Update 2014*. They are explicated in the preceding chapters and summarized below.

First, the current committee concluded that the information now assembled constitutes sufficient evidence of an association between exposure to at least one of the COIs and hypertension. As detailed in Chapter 10, the decision to change the classification from limited or suggestive evidence of an association was motivated in large part by the work of Cypel and colleagues (2016). These investigators conducted a study on the population of interest, U.S. Vietnam veterans (specifically, the Army Chemical Corps), that was characterized by a large sample size, appropriate controls (non-Vietnam-deployed sprayers, Vietnam-deployed non-sprayers, and non-Vietnam-deployed non-sprayers), and validated endpoints (self-reported physician-diagnosed hypertension that was confirmed by medical record reviews and serum TCDD measurements in a subset of participants). The statistical analyses conducted were robust, used state-of-the art methods, and adjusted for relevant confounders. The study clearly showed that self-reported hypertension rates were the highest among Vietnam-deployed sprayers (81.6%) followed by non-Vietnam-deployed sprayers (77.4%), Vietnam-deployed non-sprayers (72.2%), and non-Vietnam-deployed non-sprayers (64.6%), providing a significant association with exposure. Among Vietnam-deployed veterans, there was a significantly elevated association between the odds of hypertension for

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sprayers versus non-sprayers that remained after adjustment for potential confounders (odds ratio [OR] = 1.77, 95% confidence interval [CI] 1.35–2.30). Similarly, for those veterans who did not deploy to Vietnam, self-reported hypertension was significantly elevated when sprayers were compared with non-sprayers (OR = 1.72, 95% CI 1.31–2.26). When considered in light of other new research and earlier papers that demonstrated a consistency in the direction and magnitude of this effect—notably Kang et al. (2006), Cypel and Kang (2010), and multiple publications from the Air Force Health Study (AFHS; AFHS, 1995, 2000, 2005)—the committee found that this body of literature constituted sufficient evidence of an association.

The committee also concluded (as detailed in Chapter 7) that there was sufficient evidence of an association between exposure to at least one of the COIs and a previously unclassified health condition, monoclonal gammopathy of undetermined significance (MGUS). MGUS is a precursor to multiple myeloma, although only an estimated 1% of cases of it progress to multiple myeloma each year. It is a clinically silent condition defined by the presence of a monoclonal antibody, antibody heavy chain, or antibody light chain in the blood or urine of a person lacking symptoms or signs of a more serious plasma cell dyscrasia. The foundation of this finding was a well-conducted study by Landgren et al. (2015) that examined data and biospecimens from a population of veterans that included participants with known exposure to herbicides in Vietnam: the AFHS cohort. Landgren and colleagues used previously measured serum levels of TCDD and performed a new assay of 958 serum samples to detect MGUS. Known confounders, including age, race, body mass index, smoking and drinking history, and a history of radiation therapy or chemotherapy, were considered. The investigators found that the prevalence of MGUS in veterans involved in herbicide spray operations (7.1%) was higher than in comparison veterans (3.1%) (adjusted OR = 2.37, 95% CI 1.27–4.44; p = 0.007). The direct relevance of the exposure and exposed population, combined with the high quality of the study and underlying database, were persuasive in convincing the committee that there was sufficient evidence of an association.

Finally, the committee—after extensive deliberations regarding the strengths and weaknesses of the new evidence and evidence from studies reviewed in previous VAO reports—could not come to a consensus on whether the available scientific information regarding exposure to the COIs and type 2 diabetes should continue to be categorized as limited or suggestive or whether it meets the criteria for sufficient evidence of an association (see Chapter 10). Newly and previously reviewed studies consistently show a relationship between well-characterized exposure to dioxin and dioxin-like chemicals and measures of diabetes health outcomes in diverse cohorts, including Vietnam veteran populations. However, the lack of exposure specificity and the potential for uncontrolled confounding that characterized many of these studies complicates any attribution of the outcome to the COIs. It was therefore not clear to the committee as a whole whether a category change was appropriate.

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Health Outcomes Identified by the Department of Veterans Affairs for Specific Focus

The Department of Veterans Affairs (VA) also asked the committee to specifically focus on three health outcomes: possible generational health effects that may be the result of herbicide exposure among male Vietnam veterans, myeloproliferative neoplasms, and glioblastoma multiforme.

Chapter 8 summarizes the available literature on the effects of exposure to the COIs on the reproductive health of Vietnam veterans and on the health of their descendants. This is a burgeoning area of research, and there were several new studies for the committee to consider. Few of them address Vietnam veterans specifically, however, and almost all of those that were conducted on other populations have weaknesses—prominently, different exposures than those experienced by veterans and also poor exposure characterization—that limit their usefulness in assessing risks for veterans. Some find associations between exposures and various outcomes, but there are no circumstances for which there is a consistent and compelling body of evidence that would lead the committee to conclude that there might be limited or suggestive or sufficient evidence of an association between an exposure to a COI and a particular outcome. Transgenerational effects—those that might occur in veterans' grandchildren and subsequent generations in which gestational exposure did not take place—are of great interest to veterans, but no epidemiological literature exists to evaluate whether exposure to the COIs might lead to such outcomes.

As further delineated below, the committee strongly believes that more work in this area is warranted. It concurs with the Update 2014 committee that it is critical that such research include animal studies in order to elucidate whether and which mechanisms for intergenerational and transgenerational effects might exist. It is, in principle, possible to do studies on the health of children and grandchildren of veterans, but it must be understood up front that such complex studies will need to be carefully planned and conducted if they are to yield meaningful results. Voluntary participation surveys and registries relying on self-reported information will not be helpful. The 2018 National Academies of Sciences, Engineering, and Medicine report *Gulf War and Health*, *Volume 11*: *Generational Health Effects of Serving in the Gulf War* (NASEM, 2018) addresses how studies of health outcomes in the progeny of veterans might be conducted.

Myeloproliferative neoplasms (MPNs) and myelodysplastic syndromes, addressed in Chapter 7, are diseases of the blood cells and bone marrow. VA asked that MPNs be explicitly examined as part of the consideration of the literature concerning leukemias and related diseases. However, after conducting a targeted search of scientific and medical databases (delineated in Box 3-1), the committee was unable to identify any papers that addressed the outcome with the exception of Yi and Ohrr (2014) (reviewed in *Update 2014*), which assessed cancer incidence among Korean veterans who had served in Vietnam between 1964 and 1973.

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Those authors reported non-significant and imprecise increased risks of myeloproliferative disease and myelodisplastic syndrome in internal comparisons of high- and low-exposure groups, based on so-called exposure opportunity scores.[2] The organization MPN Advocacy and Education International presented anecdotal information on MPNs in Vietnam veterans for the committee's consideration, but this was not usable for drawing conclusions.

The committee observes that, in general, those studies that looked at exposure to the COIs and hematological outcomes have generated much more compelling results—including results on abnormalities of lymphoid development and immune function such as non-Hodgkin lymphoma, chronic lymphocytic leukemia, multiple myeloma, and MGUS—than have those that examined whether myeloid neoplasms or MPNs have some relation with the COIs.

Given the absence of new studies, the paucity of epidemiologic studies in general, and the lack of information on the biologic plausibility of a connection between exposures to the COIs and abnormalities of hematopoietic cells associated with nonmalignant bone marrow–derived diseases, the committee concluded that there was inadequate or insufficient evidence of an association between exposure to the COIs and MPNs. Because the outcome has not been subject to previous research attention and is of interest to veterans, **the committee recommends that investigators examine existing databases on myeloid diseases to determine whether there are data available that would allow for an evaluation of MPNs in Vietnam veterans and others who have been exposed to dioxin and the other COIs.**

The scientific literature regarding exposure to the COIs and brain and other nervous system cancers, including glioblastoma multiforme (often abbreviated as glioblastoma), has been examined since the first VAO report. The body of evidence that has been developed, which is summarized in Chapter 7, has not found statistically significant associations between exposure and any relevant outcome in studies performed on Vietnam-veteran, occupational, or environmental cohorts. These studies have by and large been underpowered because of the relative rarity of these cancers. Given the limited epidemiologic data available on glioblastoma, the committee heard invited presentations from two experts on the disease. While their presentations to the committee were helpful and impressive, demonstrating that the biological understanding of glioblastoma in particular is rapidly advancing, they reinforced the absence of clear data suggesting that the COIs are associated with the occurrence of brain cancers. Information on glioblastomas in Vietnam veterans submitted for the committee's consideration by the Sierra Valley Cancer Registry Services, Inc., was, in part, anecdotal and without documented levels of exposure and was therefore of limited usefulness for the purpose of drawing conclusions.

[2]In brief, an exposure opportunity score is a means of quantifying exposure that accounts for the quantity of herbicide sprayed and the distance in time and space from the spraying (S. D. Stellman and J. M. Stellman, 2004).

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Furthermore, the committee did not identify any animal studies that have reported an association between exposure to the COIs and any brain cancer. While some studies have put forward mechanisms that might explain why dioxin exposure would be associated with glioblastoma, the information reviewed by the committee along with the presentations it received from experts in the field were not sufficient to alter the conclusion of previous reports that the evidence is inadequate or insufficient to determine whether there is an association between exposure to the COIs and brain or other nervous system cancers. The committee believes it is appropriate for VA to be mindful of the concerns raised about the possible association between Vietnam service and glioblastoma, but it observes that the outcome is so rare and the information concerning herbicide exposures so imprecise that it is doubtful that any logistically and economically feasible epidemiologic study of veterans—no matter how well designed or executed—would produce meaningful results. **The committee therefore recommends that epidemiologic studies of glioblastoma in Vietnam veterans should not be pursued and that VA should instead focus on fostering advancements in other areas that may be used to inform improved treatment options.**

Other Health Outcomes

Table 12-1 summarizes the conclusions regarding exposure to the COIs and health outcomes, with changes to the categorizations determined by the committee for *Update 2014* noted in boldface. As mandated by PL 102-4, the distinctions among categories are based on statistical association and not on strict causality. The committee was directed to review the scientific data, not to recommend VA policy, and, therefore, the conclusions presented in Table 12-1 are not intended to imply or suggest policy decisions. Instead, the conclusions are based on observed associations between exposure and health outcomes in human populations. These categorizations do not address the likelihood that the health problems of any one individual are associated with or caused by the chemicals in question.

OTHER COMMITTEE RECOMMENDATIONS

As part of their charge, all VAO committees have been asked to offer recommendations concerning the need for additional scientific studies and research to resolve areas of continuing scientific uncertainty concerning the health effects of exposure to the COIs. The previous update of the VAO series (the tenth update; *Update 2014*) was originally understood to be the last of the reports mandated by the Agent Orange Act (PL 102-4, renewed in PL 107-103, and subsequently extended). The committee responsible for that report thus considered it appropriate to compile the recommendations made by prior VAO committees and to consider, in light of the lessons learned in this process, what would be the most important activities to undertake in the future. They produced two tables as part of this effort:

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TABLE 12-1 Summary of the *Eleventh Biennial Update* Findings on Vietnam-Veteran, Occupational, and Environmental Studies Regarding Scientifically Relevant Associations Between Exposure to Herbicides and Specific Health Outcomes[a]

Sufficient Evidence of an Association

Epidemiologic evidence is sufficient to conclude that there is a positive association. That is, a positive association has been observed between exposure to herbicides and the outcome in studies in which chance, bias, and confounding could be ruled out with reasonable confidence.[b] For example, if several small studies that are free of bias and confounding show an association that is consistent in magnitude and direction, there could be sufficient evidence of an association. There is sufficient evidence of an association between exposure to the chemicals of interest and the following health outcomes:

Soft-tissue sarcoma (including heart)
* Non-Hodgkin lymphoma
* Chronic lymphocytic leukemia (including hairy cell leukemia and other chronic B-cell leukemias)
* Hodgkin lymphoma
Chloracne
Hypertension (category change from Limited or Suggestive in *Update 2014*)
Monoclonal gammopathy of undetermined significance (MGUS) (newly considered condition)

The committee did not reach consensus on whether the evidence regarding type 2 diabetes (mellitus) was more properly classified as *Sufficient* or *Limited or Suggestive*.

Limited or Suggestive Evidence of an Association

Epidemiologic evidence suggests an association between exposure to herbicides and the outcome, but a firm conclusion is limited because chance, bias, and confounding could not be ruled out with confidence.[b] For example, a well-conducted study with strong findings in accord with less compelling results from studies of populations with similar exposures could constitute such evidence. There is limited or suggestive evidence of an association between exposure to the chemicals of interest and the following health outcomes:

Laryngeal cancer
Cancer of the lung, bronchus, or trachea
Prostate cancer
Cancer of the urinary bladder
* Multiple myeloma
* AL amyloidosis
Early-onset peripheral neuropathy
Parkinson disease (including Parkinsonism and Parkinson-like syndromes)
Porphyria cutanea tarda
Ischemic heart disease
Stroke
Hypothyroidism

The committee did not reach consensus on whether the evidence regarding type 2 diabetes (mellitus) was more properly classified as *Sufficient* or *Limited or Suggestive*.

continued

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TABLE 12-1 Continued

Inadequate or Insufficient Evidence to Determine an Association

The available epidemiologic studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an association. For example, studies fail to control for confounding, have inadequate exposure assessment, or fail to address latency. There is inadequate or insufficient evidence to determine association between exposure to the chemicals of interest and the following health outcomes that were explicitly reviewed:

Cancers of the oral cavity (including lips and tongue), pharynx (including tonsils), or nasal cavity (including ears and sinuses)
Cancers of the pleura, mediastinum, and other unspecified sites in the respiratory system and intrathoracic organs
Esophageal cancer
Stomach cancer
Colorectal cancer (including small intestine and anus)
Hepatobiliary cancers (liver, gallbladder, and bile ducts)
Pancreatic cancer
Bone and joint cancers
Melanoma
Non-melanoma skin cancer (basal-cell and squamous-cell)
Breast cancer
Cancers of reproductive organs (cervix, uterus, ovary, testes, and penis; excluding prostate)
Renal cancer (kidney and renal pelvis)
Cancers of brain and nervous system (including eye)
Endocrine cancers (thyroid, thymus, and other endocrine organs)
Leukemia (other than chronic lymphocytic leukemia, including hairy-cell leukemia and other chronic B-cell leukemias)
Other myeloid diseases (including myeloproliferative neoplasms)
Cancers at other and unspecified sites
Infertility
Spontaneous abortion (other than after paternal exposure to TCDD, which appears not to be associated)
Neonatal or infant death and stillbirth in offspring of exposed people
Low birth weight in offspring of exposed people
Birth defects in offspring of exposed people, including spina bifida
Childhood cancer (including acute myeloid leukemia) or other adverse health outcomes in offspring of exposed people
Neurobehavioral disorders (cognitive and neuropsychiatric)
Neurodegenerative diseases, excluding Parkinson disease
Chronic peripheral nervous system disorders
Hearing loss
Respiratory disorders (wheeze or asthma, chronic obstructive pulmonary disease, and farmer's lung)
Gastrointestinal, metabolic, and digestive disorders (changes in hepatic enzymes, liver disorders including cirrhosis, lipid abnormalities, and ulcers)
Immune system disorders (immune suppression, allergy, and autoimmunity)
Circulatory disorders (other than hypertension, ischemic heart disease, and stroke)
Endometriosis

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TABLE 12-1 Continued

Disruption of thyroid homeostasis (other than hypothyroidism)
Eye problems
Bone conditions
Kidney and urinary disorders (including chronic kidney disorder, differences in kidney function, nephropathy, and end stage renal disorder)
Chronic skin disorders (including skin infections and changes in skin pigmentation)

The committee used a classification that spans the full array of cancers. However, reviews for non-malignant conditions were conducted only if they were found to have been the subjects of epidemiologic investigation or at the request of the Department of Veterans Affairs. By default, any health outcome on which no epidemiologic information has been found falls into this category.

Limited or Suggestive Evidence of No Association

Several adequate studies, which cover the full range of human exposure, are consistent in not showing a positive association between any magnitude of exposure to a component of the herbicides of interest and the outcome. A conclusion of "no association" is inevitably limited to the conditions, exposures, and length of observation covered by the available studies. In addition, the possibility of a very small increase in risk at the exposure studied can never be excluded. There is limited or suggestive evidence of no association between exposure to the herbicide components of interest and the following health outcome:

Spontaneous abortion after paternal exposure to TCDD

[a]*Herbicides* indicates the following chemicals of interest: 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin), cacodylic acid, and picloram. The evidence regarding association was drawn from veteran, occupational, and environmental cohort studies in which people were exposed to the herbicides used in Vietnam, to their components, or to their contaminants.

[b]Evidence of an association is strengthened by experimental data supporting biologic plausibility, but its absence would not detract from the epidemiologic evidence.

*The committee notes the consistency of these findings with the biologic understanding of the clonal derivation of lymphohematopoietic cancers that is the basis of the World Health Organization classification system (Campo et al., 2011; see table here: www.ncbi.nlm.nih.gov/pmc/articles/PMC3109529/table/T1, accessed May 17, 2018).

- a compendium of the recommendations of prior committees condensed and sorted into topic areas, with comments on what response these recommendations received from VA, the Department of Defense, and other parties (*Update 2014*, Table 14-2, pp. 910–917), and
- a summary of the future activities the committee considered most important for monitoring and evaluating the health issues of Vietnam veterans and other veterans who might experience service-related health problems long after discharge (*Update 2014*, Table 14-3, pp. 918–919).

Generally speaking, the recommendations of previous VAO committees fell into four primary areas: better management of veterans' health information; additional epidemiologic studies; the improvement of exposure estimation; and

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priority areas for toxicologic research. Suggested future activities included initiatives in these areas plus other initiatives related to the collection and analysis of additional information on Vietnam veterans' service, exposures, and health. Complete details may be found in Chapter 14 of the *Update 2014* report.

While there have been a few laudable exceptions—notably, the initiation of additional epidemiologic studies on Vietnam veterans, the development of a herbicide exposure assessment model for use in studies of Vietnam veterans, and the fostering of additional research on the data and biospecimens collected in the course of the AFHS[3]—there has been no known follow-up to the vast majority of recommendations that have been offered. The current committee did not choose to revisit this issue in general, concluding that the Update 2014 committee had effectively covered it.

It does observe, though, that the very first *VAO* (1994) indicated that "carefully conducted epidemiologic studies—with adequate sample size to detect elevated associations—of the reproductive history of individuals with occupational or environmental exposure to herbicides and dioxin are . . . needed" (p. 731). Several subsequent volumes (*Update*s *2006*, *2008*, *2010*, *2012*, and *2014*; summarized in Table 12-2) have echoed and expanded on this. **The current committee** is in agreement with these sentiments and therefore **recommends further specific study of the health of offspring of male Vietnam veterans.**

The Update 2014 committee also offered suggestions for research activities that should follow the end of the VAO report series. Several of these, summarized in Table 12-3, addressed reproductive outcomes. As that committee noted, although progress has been made in understanding the health effects of exposure to the COIs and the mechanisms underlying these effects, significant gaps in our knowledge remain. Many additional opportunities for progress via continuing and new toxicologic, mechanistic, and epidemiologic research exist. Such work should include efforts to gain new knowledge through the integration of existing Department of Defense and VA databases. While such research opportunities were mentioned in previous VAO updates, the Update 2014 committee restated them to emphasize its conviction that more progress should be made in the fields noted. This committee concurs in this assessment and endorses the recommendations offered in Table 12-3, noting that research in the rapidly advancing field of epigenetics appears to hold particular promise. Furthermore, the committee observes that VA's Vietnam Era Health Retrospective Observational Study (VEHEROeS)—a study of the health and well-being of U.S. Vietnam and Vietnam-era veterans—was under way at the time this report was completed and that it, along with other ongoing Vietnam veteran research efforts, holds the promise of adding to the body of knowledge.

[3]The Institute of Medicine publications *Disposition of the Air Force Health Study* (IOM, 2006) and *The Air Force Health Study Assets Research Program* (IOM, 2015) provide details of this work.

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TABLE 12-2 Compendium of Research Recommendations from Previous *Veterans and Agent Orange* Series Reports Related to Effects on Veterans' Descendants (excerpted, adapted, and updated from *Update 2014*, Table 14-2)

Recommendation Focus Area	Volume Initially Recommended	Follow-Up?
EPIDEMIOLOGIC STUDIES		
Army Chemical Corps (ACC)		
Study paternally mediated effects on health outcomes in offspring.	*Update 2006*	No known follow-up
Air Force Health Study (AFHS)		
Study the potential for paternally mediated effects on health outcomes in offspring.	*Update 2006*	No known follow-up
Comprehensive longitudinal analysis of the AFHS data collected in the six intensive medical-cycle examinations particularly concerning birth defects in veterans' offspring, making use of the available exposure data.	*Update 2008*	No known follow-up
National Institute for Occupational Safety and Health (NIOSH)		
Conduct epidemiologic studies—with adequate sample size to detect elevated associations—of the reproductive history of individuals with occupational or environmental exposure to herbicides and dioxin.	*VAO*	*As noted in Chapter 5, NIOSH has, at its own instigation, collected data on and followed several groups of U.S. workers exposed to the committee's COIs since the early 1990s.*
International Agency for Research on Cancer (IARC)		
Carefully conducted epidemiologic studies—with adequate sample size to detect elevated associations—of the reproductive history of individuals with occupational or environmental exposure to herbicides and dioxin are recommended.	*VAO*	No known follow-up

continued

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TABLE 12-2 Continued

Recommendation Focus Area	Volume Initially Recommended	Follow-Up?
Other Recommended Epidemiologic Studies and Analyses:		
An ad hoc group should conduct a meta-analysis of the current epidemiologic studies of male populations exposed to COIs and the risk of birth defects in offspring.	*Update 2006*	No known follow-up (Given the paucity of studies of only paternal transmission and the extremely heterogeneous study designs and exposures, meta-analyses no longer seems a plausible approach to evaluating birth defects [or any other health outcome].)
Investigate possible effects in offspring of Vietnam veterans (especially for birth defects or developmental disease, including cognitive and developmental effects in children and possibly grandchildren), especially those associated with paternal exposures.	*Update 2006*	No known follow-up
Conduct studies of defined clinical health conditions in mature offspring following exposure of either parent, rather than more investigations of physiological biomarkers that may merely be predictive of disease development later in life.	*Update 2010*	No known follow-up
Develop epidemiologic protocols to address whether adverse effects are being manifested in later generations as a result of paternal exposure (in the absence of maternal exposure, focusing on those organ systems that have shown the greatest impact following maternal exposure, including neurologic, immune, and endocrine effects). Consideration must be given to the minimum sample size needed to detect changes, if present, and to which outcome measures would be most sensitive and reliable.	*Update 2010*	No known follow-up

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Case-control study should be used to explore whether information about Vietnam exposure or specific herbicide exposure could be ascertained in any of the many birth cohorts that have been established in the past several decades (especially for very uncommon health outcomes). To home in on a paternal effect, however, it will be necessary to establish that the mothers did not have the opportunity for other than background exposure to the chemicals of interest.	*Update 2010*	No known follow-up
TOXICOLOGIC STUDIES		
Animal models are needed to elucidate disease mechanisms and progression, including transgenerational or paternally mediated effects.	*Update 2006*	No known specific follow-up of the committee's recommendation, although numerous animal studies have been conducted since the report was published.
Toxicological investigations of the potential for the COIs (particularly TCDD) to induce epigenetic modifications, with special attention to the capacity for paternal transmission of such effects, should be conducted.	*Update 2010*	No known follow-up
Animal studies of the mechanisms of inhibition of fetal growth, particularly in male offspring, after maternal exposure could help to elucidate findings seen in some epidemiologic studies that examined maternal exposure and birth weight.	*Update 2012*	No known follow-up
Given the current concern among male veterans about the transmission of adverse effects to their descendants, focused use of animal models to investigate the possibility of paternal exposure contributing to the development of disease in offspring would be very informative.	*Update 2014*	No known follow-up

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TABLE 12-3 Suggested Activities to Follow the Completion of the *Veterans and Agent Orange* Report Series Mandated by the Agent Orange Act Related to Effects on Veterans' Descendants (excerpted from *Update 2014*, Table 14-3)

OVERSIGHT OF LONG-TERM HEALTH STATUS OF DEPLOYED SERVICE MEMBERS
Very careful review of evidence concerning whether **paternal exposure** to any toxicant has definitively been demonstrated to result in abnormalities in even the first generation of offspring. Careful assessment of the risks to offspring that may arise from **maternal exposure** is also merited, given the greatly increased number of women now serving in the military.
EPIDEMIOLOGIC STUDIES
Air Force Health Study (AFHS)
Comprehensive longitudinal analysis of the AFHS data collected in the six intensive medical-cycle examinations (including **birth defects in veterans' offspring**), making use of the available exposure data.
Use AFHS samples for study of **epigenetic changes** and definition of biomarkers of exposure and effect.*
Other Epidemiology Goals
Pursue development of protocols that could feasibly and efficiently **investigate paternal transmission of adverse effects to offspring at birth** or manifesting with maturation that have sufficient power for convincing findings. The logistics of attempting to detect adverse effects in the grandchildren of Vietnam veterans would be considerably more challenging.
TOXICOLOGIC RESEARCH
Foster investigation of **epigenetic changes in both somatic tissues and germ cells and during gestation.**
Without sophisticated and specific markers of environmentally induced epigenetic activity, epidemiologic investigations will not be able to distinguish the mechanisms inducing any observed adverse health effects in exposed people or their offspring.
Fully investigate whether **paternally transmitted adverse effects** occur in animal models.

*A pilot study by Boekelheide and colleagues—described in IOM (2005)—proposed to use a "new epigenome-wide molecular approach to detect dioxin exposure-related alterations in DNA methylation in biospecimens from the Air Force Health Study" (p. 112). Proof-of-concept work was conducted, but the project was not carried forward due to lack of funding.

FINAL OBSERVATIONS

In the course of carrying out its Statement of Task, the committee has offered myriad criticisms of the conduct of studies of Vietnam veterans' health, pointing out specific weaknesses and shortcomings in particular papers along with widespread (although not universal) issues such as poor exposure characterization, failure to fully control for confounding influences on outcomes, and sample sizes that are inadequate for drawing statistically meaningful results. The committee wishes to make clear, though, that the difficulty in conducting research on Vietnam veteran health issues should not act as a barrier to carrying out such work. There are many questions regarding veterans' health that cannot be adequately answered by examining superficially analogous exposures and outcomes in other populations. It is only through research on veterans themselves that the totality of the military service experience can be properly accounted for.

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Appendix A

Public Meeting Agendas

FIRST PUBLIC MEETING

March 2, 2017
Keck Center of the National Academies
500 Fifth Street, NW
Washington, DC 20001

Open Session

1:00–1:10 p.m. Welcome and introductions; conduct of the open session
Irva Hertz-Picciotto, *Ph.D.* – Committee Chair

1:10–2:00 p.m. Charge to the committee and discussion
Peter R. Rumm, *M.D.*, *M.P.H.* – Director, Pre-9/11 Era Environmental Health Program, Department of Veterans Affairs (VA), with Dr. R. Loren Erickson, Chief Consultant, Post-Deployment Health Services

2:00–2:45 p.m. Overview of VA's Vietnam Era Health Retrospective Observational Study (VE-HEROeS)
Victoria Davey, *Ph.D.*, *M.P.H.*, *R.N.* – Principal Investigator, VA

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2:45–3:30 p.m. VA Health Care Utilization for Vietnam Veterans
Aaron I. Schneiderman, *Ph.D.*, *M.P.H.*, *R.N.* – Director, Epidemiology Program Post-Deployment Health Services, VA

3:30–4:30 p.m. Public comments
Thomas Berger, Ph.D., Vietnam Veterans of America
Ann Brazeau, MPN Advocacy and Education International
Carla L. Dean, Bladder Cancer Foundation of Florida
Cindy Fabbri
MSgt LeRoy G. Foster
Raajit K. Rampal, M.D., Ph.D., Memorial Sloan Kettering Cancer Center
Rick Weidman, Vietnam Veterans of America

4:30 p.m. Open session adjourns

SECOND PUBLIC MEETING

September 7, 2017
Courtyard Marriott Bloomington by Mall of America
7800 Bloomington Avenue S.
Lake Hiawatha Room
Bloomington, MN 55425

Open Session

1:00–1:10 p.m. Welcome | Notes on the conduct of the open session | Introduction of participants
Irva Hertz-Picciotto, *Ph.D.* – Committee Chair

1:10–2:30 p.m. Presentations and comments by participants and questions from the committee
Ronald R. Bach, Ph.D.
Robert Behrens
Richard Bergling
Linda O. Bergum, M.D.
Jeremy Eberley and parents
Maynard Kaderlick
Lee McClary
Mokie Porter

2:30 p.m. Wrap-up and thanks to participants

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THIRD PUBLIC MEETING

November 30, 2017
Keck Center of the National Academies
500 Fifth Street, NW
Washington, DC 20001

Open Session

10:15–10:30 a.m. Welcome | Notes on the conduct of the open session | Introduction of participants
Irva Hertz-Picciotto, *Ph.D.* – Committee Chair

10:30–11:15 a.m. Exposures and Heritable Effects
Thaddeus (*Thad*) *Schug*, *Ph.D.* – Health Scientist Administrator, Population Health Branch, National Institute of Environmental Health Sciences and the National Toxicology Program

11:15 a.m.–12:00 p.m. Russian Children's Study: Dioxins and Semen Quality
Russ Hauser, *Sc.D.* – Frederick Lee Hisaw Professor of Reproductive Physiology. Chair, Department of Environmental Health, Harvard T.H. Chan School of Public Health

12:45–1:30 p.m. Multiple Myeloma and its Precursor Disease (MGUS)
C. Ola Landgren, *M.D.*, *Ph.D.* – Professor of Medicine and Chief Attending Physician of the Myeloma Service, Memorial Sloan Kettering Cancer Center

1:30–2:15 p.m. Epidemiology of Glioma
Quinn T. Ostrom, *Ph.D.*, *M.P.H.* – Research Coordinator, Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine; Research Manager and Primary Analyst, Central Brain Tumor Registry of the United States

2:15–3:00 p.m. Glioblastoma – State of the Science
Paul S. Mischel, *M.D.* – Member and Head, Laboratory of Molecular Pathology, Ludwig Institute for Cancer Research San Diego and Professor, Department of Pathology, University of California, San Diego

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3:00–3:30 p.m.	U.S. Department of Veterans Affairs: Vietnam Veterans and Brain Cancer *Ralph L. Erickson, M.D., Dr.P.H.*, Chief Consultant, Post-Deployment Health Services *Peter D. Rumm, M.D., M.P.H.*, Director, Pre-9/11 Era Environmental Health Program
3:30–4:00 p.m.	Public comments (spoken comments may not exceed 3 minutes per person; written submissions of any length are welcome) *Kathy-Lynn Carroll Josenhans* *Robert M. (Bob) Hunter, Ph.D.* *Pegi Scarlett* *Laurel Smith Holt*
4:00 p.m.	Open session adjourns

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Appendix B

Committee and Staff Biographies

COMMITTEE BIOGRAPHIES

Irva Hertz-Picciotto, Ph.D., M.P.H., M.A. (*Chair*), is a professor in the Department of Public Health Sciences in the School of Medicine and also at the Medical Investigations of Neurodevelopmental Disorders (MIND) Institute, University of California, Davis (UC Davis), and she is the chief of the Division of Environmental and Occupational Health. She is also the deputy director of the Center for Children's Environmental Health at UC Davis and the director of the Northern California Center for the National Children's Study. She has published widely on environmental exposures, including metals, pesticides, polychlorinated biphenyls, and air pollution and on their effects on pregnancy, the neonate, and early child development as well as on methods in epidemiologic research. She has also led several cohort studies of toxic chemicals and both pregnancy outcomes and early child development in Chile, eastern Europe, and Mexico. Recently she co-founded Project TENDR (Targeting Environment and Neuro-Developmental Risks), a collaborative effort of scientists, clinicians, policy makers, and advocates that aims to decrease the incidence of neurodevelopmental disorders by reducing the neurotoxicant exposures that contribute to them. Dr. Hertz-Picciotto has served on scientific advisory panels for the Environmental Protection Agency, the National Institutes of Health's (NIH's) National Toxicology Program, the National Institute for Occupational Safety and Health, the NIH Interagency Coordinating Committee on Autism Research, and the California Governor's Proposition 65 committee. She has served or currently sits on the editorial boards for the *American Journal of Epidemiology*, *Environmental Health Perspectives*, *Epidemiology*, and *Autism Research*. She served as the president of the Society for Epidemiologic

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Research and of the International Society for Environmental Epidemiology, and in 2011 she received the Goldsmith Lifetime Achievement Award from the International Society for Environmental Epidemiology. Dr. Hertz-Picciotto has previously chaired three National Academies' committees: one on breast cancer and the environment and two previous updates of Committees to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. She received a Ph.D. and an M.P.H. in epidemiology and an M.A. in biostatistics from UC Berkeley. Before joining the faculty at UC Davis, Dr. Hertz-Picciotto was a professor in the Department of Epidemiology at the School of Public Health at University of North Carolina at Chapel Hill. She is a National Associate of the National Academies.

Nancy Berliner, M.D., is the chief of the Division of Hematology at Brigham and Women's Hospital and a professor at Harvard Medical School. Her research focus is gene regulatory pathways of normal white blood cell development and how they are disrupted in leukemia and pre-leukemic syndromes as well as the pathogenesis of the anemia of aging and benign and malignant hematologic disorders. Most recently, her laboratory has studied the role of cellular stress responses in the disruption of hematopoietic cell differentiation in myelodysplasia. A second focus of her laboratory is the role of inflammatory cytokines in the anemia of the elderly and in modulating the natural history of myelodysplasia. Dr. Berliner is a member of the National Academy of Medicine (NAM; elected 2010). She received her M.D. from the Yale University School of Medicine.

Wendy B. Bernstein, M.D., is a staff physician in the Department of Hematology Oncology at Walter Reed National Military Medical Center and an associate professor of medicine at the Uniformed Services University of the Health Sciences (USUHS). Prior assignments included chairing the central scientific review committee at Walter Reed and serving as a staff clinician at the National Cancer Institute's Medical Oncology Branch. Her clinical interests include malignancies in immune-compromised hosts, and her research activities involve immune reconstitution in HIV-infected persons using CAR-T cells. Dr. Bernstein is a 28-year veteran of the Army Medical Corps, having retired with the rank of colonel. She received her M.D. from USUHS and is board certified in internal medicine, oncology, and hematology.

Michael J. Carvan III, Ph.D., M.S., is a Shaw Professor at the School of Freshwater Sciences and School of Public Health, both of the University of Wisconsin–Milwaukee. He earned his M.S. in biologic oceanography at the University of Miami's Rosenstiel School of Marine and Atmospheric Science in Coral Gables and his Ph.D. in veterinary anatomy and public health with a focus in toxicology from Texas A&M University's College of Veterinary Medicine in College Station. After obtaining his doctorate, Dr. Carvan held National Institute of Environmental

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Health Sciences molecular toxicology fellowships at the University of Cincinnati Medical Center. His research uses zebrafish as a genetic system for identifying genes that influence the susceptibility of response to xenobiotics. He has served on the National Academies of Sciences, Engineering, and Medicine's Board on Life Sciences and as a committee member on the ninth and tenth updates of Committees to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides.

Aravinda Chakravarti, Ph.D., is a professor of medicine and neuroscience at the New York University School of Medicine and the director of the Center for Human Genetics and Genomics. He has been a key participant and architect of the Human Genome, HapMap, and 1000 Genomes projects. His research focus is the genome-scale analysis of humans and the computational analysis of gene variation and function to understand the molecular genetic basis of complex human phenotypes, particularly disease. Dr. Chakravarti's discovery of genes and pathways contributing to Hirschsprung disease has served as a model for the genetic dissection of other multifactorial human disorders, such as autism spectrum disorders, hypertension, and sudden cardiac death. He was president of the American Society of Human Genetics in 2008 and received its William Allan Award in 2013. He is one of the founding editors-in-chief of *Genome Research* and the *Annual Reviews of Genomics and Human Genetics*, and has served and serves on the boards of numerous international journals, academic societies, the National Institutes of Health, and biotechnology companies. He is a member of the National Academy of Sciences (elected 2015) and the National Academy of Medicine (NAM) (elected 2007). Dr. Chakravarti is also an honorary fellow of the Indian Academy of Sciences. Currently he serves on the NAM Council nominating committee. He received his Ph.D. in human genetics from the University of Texas–Houston Health Science Center.

Dana C. Dolinoy, Ph.D., is the NSF International Chair of Environmental Health Sciences and a professor of environmental health sciences and nutritional sciences at the University of Michigan School of Public Health, and she leads the Environmental Epigenetics and Nutrition Laboratory, which investigates how nutritional and environmental factors interact with epigenetic gene regulation to shape health and disease. She serves on the editorial boards of the *Journal of Nutritional Biochemistry* and *Epigenetics* and is an associate editor for *Environmental Health Perspectives*, *Environmental Epigenetics*, and *Toxicological Sciences.* She is an active member of the Society of Toxicology, the Environmental Mutagen and Genomics Society, and the American Society for Nutrition, and she served as the chair of the 2015 Gordon Research Conference in Molecular and Cellular Mechanisms of Toxicity. In 2011 Dr. Dolinoy received the Norman Kretchmer Memorial Award from the American Society for Nutrition and the Classic Paper of the Year Award from *Environmental Health Perspectives*

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for Dolinoy et al. "Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome." In 2012 she was the recipient of the Association of Schools of Public Health/Pfizer Research Award for the article, "An expression microarray approach for the identification of metastable epialleles in the mouse genome." This work was cited as a model approach that may allow for directly assessing the role of early nutritional and environmental exposures in human adult disease. Dr. Dolinoy recently received the 2015 National Institutes of Health Director's Transformative Research Award to develop novel epigenome editing tools to reduce disease risk, and in 2016 served as the chair of the Society of Toxicology's Contemporary Concepts in Toxicology meeting, ToxicoEpigenetics: The Interface of Epigenetics and Risk Assessment. Dr. Dolinoy holds an M.Sc. in environmental sciences and engineering from the Harvard T.H. Chan School of Public Health and a Ph.D. in genetics and genomics and integrated toxicology from Duke University.

Mary A. Fox, Ph.D., is an assistant professor in the Department of Health Policy and Management and the co-director of the Risk Sciences and Public Policy Institute at the Johns Hopkins Bloomberg School of Public Health. She teaches courses in quantitative risk assessment methods and risk policy and management for the Risk Sciences and Public Policy Institute's Certificate Program. Dr. Fox's research is focused on human health risk assessment as a part of environmental policy making, particularly approaches to cumulative and chemical mixtures risk assessment. Dr. Fox has served on three National Academies' committees: Gulf War and Health, Volume 10: Update of Health Effects of Serving in the Gulf War, Long-Term Health Consequences of Exposure to Burn Pits in Iraq and Afghanistan, and Health Risks of Phthalates. Dr. Fox began her public health career conducting community health studies around hazardous waste sites as a research scientist in the New York State Department of Health. Dr. Fox received her M.P.H. from the University of Rochester School of Medicine and Dentistry and a Ph.D. from the Johns Hopkins Bloomberg School of Public Health.

Karl T. Kelsey, M.D., M.O.H., is a professor of epidemiology, pathology, and laboratory medicine at Brown University. Dr. Kelsey received his M.D. from the University of Minnesota and a master's degree in occupational health from Harvard University. Until 2007 he was on the faculty of the Harvard T.H. Chan School of Public Health and Harvard Medical School. He is interested in the application of laboratory-based biomarkers in chronic-disease epidemiology and tumor biology and in characterizing individual susceptibility to cancer. He is an author of more than 200 publications and has served on the National Academies' Committees on Toxicity Testing and Assessment of Environmental Agents, on Copper in Drinking Water, on the Evaluation of the Department of Veterans Affairs Uniform Case Assessment Protocol to Review the Health Consequences of Service During the Persian Gulf War, on Curriculum Development

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in Environmental Medicine, on the Health Effects of Mustard Gas and Lewisite, and, most recently, on the past three Committees to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides.

Molly L. Kile, Sc.D. is an associate professor in the College of Public Health and Human Sciences at Oregon State University and the director of its Environmental Exposure and Biomarker Lab, and she coordinates the program in environmental and occupational health. She has affiliations with the university's Center for Global Health and is a visiting scholar in the Department of Environmental Health at the Harvard T.H. Chan School of Public Health. She also leads the Community Engagement Core of Oregon State University's Superfund Basic Research Program, which works in collaboration with Native American tribes in the Pacific Northwest to investigate their environmental health concerns and is funded by the National Institute of Environmental Health Sciences on a research project examining the potential for developmental exposures to influence immune functioning in children. Dr. Kile's primary research interest is in environmental and molecular epidemiology. Her research has focused on the application of biological markers for studying exposures and the interaction between host factors (genetic polymorphisms, nutritional status, microbiome, and epigenetic markers) and environmental exposures. She serves as an editor for the *Journal of Environmental and Public Health*. Dr. Kile received her Ph.D. from the Harvard T.H. Chan School of Public Health in environmental health and continued her postdoctoral training at Harvard in molecular epidemiology.

Andrew F. Olshan, Ph.D., is the Barbara Sorenson Hulka Distinguished Professor in the Department of Epidemiology of the University of North Carolina (UNC) Gillings School of Global Public Health. He is also the associate director of population sciences at the UNC Lineberger Comprehensive Cancer Center. His research interests are the etiology of cancer and reproductive, perinatal, and pediatric outcomes. Recent work has focused on the role of environmental exposures, genetic factors, and adverse health effects in children and adults. He directs the National Institutes of Health–funded studies of head and neck cancer, breast cancer, and childhood cancer. He is director of the Centers for Disease Control and Prevention–funded North Carolina Center for Birth Defects Research and Prevention. He has served on several National Academies' committees, most recently as co-chair of the Committee to Review the Draft IRIS Assessment on Formaldehyde. He has also served as a member on four prior committees to review health effects in Vietnam veterans exposed to Agent Orange and other herbicides. Dr. Olshan received both his M.S. and Ph.D. in epidemiology from the University of Washington and was a postdoctoral fellow in medical genetics at the University of British Columbia.

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Beate R. Ritz, M.D., Ph.D., M.P.H, is a professor in and the former chair of the Epidemiology Department at the School of Public Health at the University of California, Los Angeles (UCLA), where she has been a faculty member since 1995. Dr. Ritz holds co-appointments in both the environmental health department at the UCLA School of Public Health and in neurology at the UCLA School of Medicine. She received both her M.D. and Ph.D. in medical sociology from the University of Hamburg, Germany, in 1987. Dr. Ritz was a research fellow and served her residency at the Psychiatric University Hospital in Hamburg. She went on to receive her doctoral training and a Ph.D. in epidemiology in 1995 from UCLA. Dr. Ritz's research has focused on environmental toxins and the health effects they may have on birth outcomes, neurodegenerative and neurodevelopmental disorders, cancers, and chronic diseases. For the past two decades, she has conducted research on the effects of air pollution on adverse birth outcomes and neurodevelopmental disorders in children who live in Southern California. She also studied the long-term effects of pesticides exposure on Parkinson disease and cancer and is working on establishing a Parkinson disease registry in California. Dr. Ritz previously served on National Academies' committees including two committees responsible for Gulf War and Health series reports, on the Committee on the Review of the Scientific Literature on Amyotrophic Lateral Sclerosis in Veterans, and, most recently, Using 21st Century Science to Improve Risk-Related Evaluations.

Lori A. White, Ph.D., M.S., is an associate professor in the Department of Biochemistry and Microbiology of the School of Environmental and Biological Sciences of Rutgers, the State University of New Jersey. She received a master's degree in zoology from the University of Maine, earned a Ph.D. in biochemistry from the Dartmouth Medical School, and did postdoctoral work at the University of Wisconsin. She has been active in Gordon Research Conference programs and was the chairperson for the Mechanisms of Toxicology summer session in 2008. Her past research focused on the elucidation of dioxin's carcinogenic activity, specifically how TCDD activates the aryl hydrocarbon receptor pathway resulting in altered gene expression in different biologically relevant targets. In addition to this project, her laboratory currently uses the zebrafish model to study the neurotoxicological and behavioral effects following exposure to environmental contaminants during development. She served on the ninth and tenth biennial updates of the Committees to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides.

STAFF BIOGRAPHIES

David A. Butler, Ph.D., is a scholar in and the director of the Office of Military and Veterans Health in the Health and Medicine Division of the National Academies of Sciences, Engineering, and Medicine. He earned his B.S. and M.S.

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degrees in engineering from the University of Rochester and his doctoral degree in public policy analysis from Carnegie Mellon University. Before joining the National Academies, Dr. Butler served as an analyst for the U.S. Congress Office of Technology Assessment, was a research associate in the Department of Environmental Health of the Harvard T.H. Chan School of Public Health, and conducted research at Harvard's John F. Kennedy School of Government. He has directed several National Academies studies on military and veterans health, environmental health, and risk assessment topics, including ones that produced *Research on the Health Effects of Low-Level Ionizing Radiation: Opportunities for the Armed Forces Radiobiology Research Institute*; *Future Uses of the DoD Joint Pathology Center Biorepository*; *Provision of Mental Health Counseling Services Under TRICARE*; *PTSD Compensation and Military Service*; *Veterans and Agent Orange: Update 1998* and *Update 2000*; *Disposition of the Air Force Health Study*; and the report series *Characterizing the Exposure of Veterans to Agent Orange and Other Herbicides Used in Vietnam.* Dr. Butler was also a co-editor of *Systems Engineering to Improve Traumatic Brain Injury Care in the Military Health System.*

Anne N. Styka, M.P.H., is a senior program officer in the Health and Medicine Division of the National Academies. Over her tenure she has worked on more than 10 studies on a broad range of topics related to the health of military and veteran populations. Studies have included mental health treatment offered in the Department of Defense and the Department of Veterans Affairs (VA); designing and evaluating epidemiological research studies using VA data for health outcomes related to deployment-related exposures, including burn pits and chemicals; and directing a research program of fostering new research studies using data and biospecimens collected as part of the 20-year Air Force Health Study. Before coming to the National Academies, Ms. Styka spent several years working as an epidemiologist for the New Mexico Department of Health and the Albuquerque Area Southwest Tribal Epidemiology Center, and she spent several months in Zambia as the epidemiologist on a study of silicosis and other nonmalignant respiratory diseases among copper miners. She has several peer-reviewed publications and has contributed to numerous state and national reports. She received her B.S. in cell and tissue bioengineering from the University of Illinois at Chicago and has an M.P.H. in epidemiology from the University of Michigan. Ms. Styka was the 2017 recipient of the Division of Earth and Life Sciences Mt. Everest Award, the 2015 recipient of the Institute of Medicine and National Academy of Medicine Multitasker Award, and a member of the 2011 National Academies' Distinguished Group Award.

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Office of Grants and Contracts Accounting, where she managed all grants-related issues, wrote for the Emory research newsletter, and conducted proposal training courses. Ms. Banks also spent time working with the Georgia Department of Health (GDPH) in Atlanta on the DPH13-1305 cooperative agreement funded by the Centers for Disease Control and Prevention. In her role with GDPH, Ms. Banks analyzed wellness policies for schools in various counties within Georgia and made recommendations for each county to improve their policy. She received her B.A. in psychology from Oglethorpe University and has an M.P.H. in prevention science from the Rollins School of Public Health–Emory University in Atlanta.

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Pamela Ramey-McCray, is an administrative assistant in the Health and Medicine Division. She has worked to support numerous studies on military and veterans health, malaria research, and U.S. veteran twins since coming to the National Academies in 1993. Ms. Ramey-McCray is a recipient of the Institute of Medicine's 2009 Veteran Award. She earned her bachelor's degree in human relations at Trinity Washington University in Washington, DC. Before coming to the National Academies, Ms. Ramey-McCray worked for the American Psychological Society and the Consumer Product Safety Commission.

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