

**INTERACTION PROFILE FOR:  
PERSISTENT CHEMICALS FOUND IN FISH  
(CHLORINATED DIBENZO-*p*-DIOXINS,  
HEXACHLOROBENZENE, *p,p'*-DDE, METHYLMERCURY, and  
POLYCHLORINATED BIPHENYLS)**

**U.S. Department of Health and Human Services  
Public Health Service  
Agency for Toxic Substances and Disease Registry**

May 2004



## **ACKNOWLEDGMENT**

The Agency for Toxic Substances and Disease Registry (ATSDR) wishes to thank the U.S. Environmental Protection Agency (EPA) for its support in the production of this Interaction Profile.



## PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found.

To carry out the legislative mandate, ATSDR's Division of Toxicology (DT) has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists in collaboration with mixtures risk assessors and laboratory scientists have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what

influence the interactions may have when they do occur.

The assessments in the document are not intended to trigger a regulatory action, but rather to serve as screening tools to assess the potential for joint toxic action of chemicals in the mixture of concern.

Literature searches for this Interaction Profile were conducted in 1999–2000, with limited updating in 2001. This final version of the document, released in 2004, includes changes made in response to public comments. However, no new literature searches were done.

## CONTRIBUTORS

### CHEMICAL MANAGER(S)/AUTHORS:

Hana Pohl, M.D., Ph.D.  
ATSDR, Division of Toxicology, Atlanta, GA

Peter McClure, Ph.D., DABT  
Syracuse Research Corporation, Syracuse, NY

Marc Odin, M.S., DABT  
Syracuse Research Corporation, Syracuse, NY

Joan Colman, Ph.D.  
Syracuse Research Corporation, Syracuse, NY





## PEER REVIEW

The following expert panel was assembled to review this document on May 30–31, 2000.

**Vladimir Bencko, M.D., Ph.D.**

Institute of Hygiene and Epidemiology  
Charles University of Prague  
Studnickova 7  
CZ 128 00 Prague 2  
Czech Republic

**Ingvar Eide, Ph.D.**

Statoil Research Centre  
N-7005 Trondheim, Norway

**Henry Gardner, Ph.D.**

3407 Rolling Green Drive  
Ft. Collins, CO 80525

**Peter Grevatt, Ph.D.**

U.S. Environmental Protection Agency  
Office of Solid Waste & Emergency Response  
Washington, DC

**John Groten, Ph.D.**

Department of Explanatory Toxicology  
TNO Nutrition and Food Research Institute  
Zeist, Netherlands

**Richard Hertzberg, Ph.D.**

Waste Management Division  
U.S. Environmental Protection Agency  
Atlanta, GA

**Kannan Krishnan, Ph.D.**

Human Toxicology Research Group  
University of Montreal  
Montreal, PQ, Canada

**Scott Masten, Ph.D.**

Environmental Toxicology Program  
National Institute of Environmental  
Health Sciences  
Research Triangle Park, NC

**Mark McClanahan, Ph.D.**

Centers for Disease Control and Prevention  
National Center for Environmental Health  
Health Studies Branch  
Atlanta, GA

**Harihara Mehendale, Ph.D.**

Department of Toxicology  
College of Pharmacy  
University of Louisiana  
Monroe, LA

**Joel Pounds, Ph.D.**

Department of Molecular Biosciences  
Pacific Northwest National Laboratory  
Richland, WA

**Jane Ellen Simmons, Ph.D.**

U.S. Environmental Protection Agency  
Research Triangle Park, NC

**Madhusudan Soni, Ph.D.**

Burdock and Associates, Inc.  
Vero Beach, FL

**Els van Vliet, Ph.D.**

Health Council of the Netherlands  
2500 BB Den Haag  
The Netherlands

**Raymond Yang, Ph.D.**

Center for Environmental Toxicology  
and Technology  
Colorado State University  
Fort Collins, CO

**Technical Reviewer:**

**Patrick Durkin, Ph.D.**

Syracuse Environmental Research Associates  
Fayetteville, NY

All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

## SUMMARY

Chlorinated dibenzo-*p*-dioxins (CDDs), hexachlorobenzene, *p,p'*-DDE (the predominant metabolite of *p,p'*-DDT), methylmercury, and polychlorinated biphenyls (PCBs) occur with high frequency in water, sediment, and fish from the North American Great Lakes and occur, to varying degrees, in other dietary components including fish from other parts of the world (e.g., the Baltic Sea), human milk, dairy products, and meat. The purposes of this profile are (1) to evaluate data (if available) on health hazards, and their dose-response relationships, from oral exposure to this five-component mixture, (2) to evaluate data on the joint toxic actions of components of this mixture, and (3) to make recommendations for exposure-based assessments of the potential impact of joint toxic action of the mixture on public health.

No studies were located that examined health effects in humans or animals exposed to mixtures exclusively containing CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs, and no physiologically based pharmacokinetic/pharmacodynamic models (PBPK/PD) for this mixture have been developed.

Studies of possible associations between health effects and frequent consumption of Great Lakes and Baltic Sea fish containing the components of this mixture (and other persistent chemicals) were reviewed to determine the degree to which available data may identify pertinent health hazards. Frequent dietary consumption of contaminated Great Lakes fish by child-bearing-aged women has been associated in two prospective epidemiological studies with neurological deficits in their children, but other studies provide no consistent evidence that consumption of Great Lakes fish presents obvious risks for impaired reproduction, impaired immune capabilities, or physical birth defects. Low birth weight was reported in children of mothers who frequently ate Baltic Sea fish, and impaired immunological competence was reported in seals fed Baltic Sea fish, but the data do not clearly demonstrate dose-response relationships.

The weight of evidence for an association between Great Lakes fish consumption and effects on neurological development is greater than that for associations between frequent consumption of contaminated Baltic Sea fish and impaired immune capabilities or low birth weight, but none of the weights are sufficient to establish causal relationships between fish consumption and adverse health effects in humans. PCBs have been proposed as toxicants involved in the possible association between maternal fish consumption and altered childhood neurological development based on statistically significant associations between specific PCB levels in maternal fluids and neurological deficits in

children. Other hypotheses, however, have been proposed, including the possible involvement of other persistent chemicals in contaminated fish or synergistic interactions between PCBs and other neurotoxicants in fish.

The concentrations of persistent chemicals in fish are likely to be highly dependent on species and location, and a minimum risk level (MRL) for fish consumption based on responses in one population may not be applicable to another population. To facilitate exposure-based assessments of possible health effects associated with oral exposures to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs, available data on the joint toxic action of mixtures of these chemicals were reviewed, and the weights of evidence were assessed concerning the mode of joint toxic action of pairs of the five components. In this analysis, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) was taken as a representative of CDDs in accordance with the Toxicity Equivalence Factor (TEF) approach to assessing hazards from mixtures of CDDs. PCB mixtures were assessed as an entity in accordance with ATSDR's PCB MRLs which are derived for exposure to complex mixtures of PCBs.

The weight-of-evidence analysis indicates that only a limited amount of evidence is available to support the possible existence of greater-than-additive or less-than-additive joint actions of a few pairs of the components: (1) hexachlorobenzene potentiation of 2,3,7,8-TCDD reduction of body and thymus weights; (2) PCB antagonism of TCDD immunotoxicity and TCDD developmental toxicity; and (3) synergism between PCBs and methylmercury in disrupting regulation of brain levels of dopamine that may influence neurological function and development. Confidence in these assessments of deviations from additivity was low. For a few of the remaining pairs, additive joint action at shared targets of toxicity is supported by data, and for the rest, data were not available to characterize the modes of joint toxic action.

Component-based approaches that assume additive joint toxic action are recommended for exposure-based screening assessments of possible noncancer or cancer health hazards from oral exposure to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs, because there are no direct data available to characterize health hazards (and dose-response relationships) from the five-component mixture. The weight-of-evidence analysis indicated that data are inadequate to characterize the modes of joint action of the components, but the additivity assumption appears to be suitable in the interest of protecting public health since the components have several shared toxicity targets.

In making the recommendation, it is acknowledged that results from two epidemiological studies identify

altered neurological development as a possible health hazard from frequent consumption of fish contaminated with biopersistent chemicals. However, the results do not establish a causal relationship and are not directly useful for exposure-based assessments of hazards that are specific to a community or an exposure-scenario. The recommended approaches allow assessment of the possibility of altered neurological development as well as other potential health hazards including cancer.

A target-organ toxicity dose (TTDs) modification of the Hazard Index approach is recommended for conducting exposure-based screening assessments of noncancer health hazards. TTDs for several toxicity targets have been derived for each of the components including TTDs for hepatic, endocrine, immunological, neurological, reproductive, and developmental. For a screening assessment of cancer risks from joint toxic action of the mixture, a similar component-based approach is recommended that involves multiplication of intakes of the components by EPA cancer slope factors and summation of the resultant risk estimates. If the screening assessment indicates a potential hazard, further evaluation is needed, using biomedical judgment and community-specific health outcome data, and taking into account community health concerns.



## CONTENTS

ACKNOWLEDGMENT .....	iii
PREFACE .....	v
CONTRIBUTORS .....	vii
PEER REVIEW .....	ix
SUMMARY .....	xi
CONTENTS .....	xv
LIST OF TABLES .....	xvii
LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS .....	xix
1. Introduction .....	1
2. Joint Toxic Action Data for the Mixture of Concern and Component Mixtures .....	7
2.1 Mixture of Concern .....	7
2.1.1 Studies of Health Effects Associated with Consumption of Great Lakes Fish ...	7
2.1.2 Studies of Health Effects Associated with Consumption of Baltic Sea Fish ...	25
2.2 Component Mixtures .....	30
2.2.1 2,3,7,8-TCDD and Hexachlorobenzene .....	30
2.2.2 2,3,7,8-TCDD and <i>p,p'</i> -DDE .....	35
2.2.3 Hexachlorobenzene and <i>p,p'</i> -DDE .....	39
2.2.4 2,3,7,8-TCDD and Methylmercury .....	40
2.2.5 Hexachlorobenzene and Methylmercury .....	42
2.2.6 <i>p,p'</i> -DDE and Methylmercury .....	46
2.2.7 PCBs and 2,3,7,8-TCDD .....	46
2.2.8 PCBs and Hexachlorobenzene .....	67
2.2.9 PCBs and <i>p,p'</i> -DDE .....	67
2.2.10 PCBs and Methylmercury .....	68
2.3 Relevance of the Joint Toxic Action Data and Approaches to Public Health .....	78
3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture .....	113
4. Conclusions .....	119
5. List of References .....	121
Appendix A: Background Information for Chlorinated Dibenzo- <i>p</i> -Dioxins (CDDs) .....	149
A.1 Toxicokinetics .....	149
A.2 Health Effects .....	150
A.3 Mechanisms of Action .....	151
A.4 Health Guidelines .....	153
A.5 Derivation of Target-Organ Toxicity Dose (TTD) Values .....	155

Appendix B: Background Information for Hexachlorobenzene .....	161
B.1 Toxicokinetics .....	161
B.2 Health Effects .....	161
B.3 Mechanisms of Action .....	162
B.4 Health Guidelines .....	164
B.5 Derivation of Target-Organ Toxicity Dose (TTD) Values .....	165
Appendix C: Background Information for <i>p,p'</i> -DDE .....	169
C.1 Toxicokinetics .....	169
C.2 Health Effects .....	170
C.3 Mechanisms of Action .....	171
C.4 Health Guidelines .....	172
C.5 Derivation of Target-Organ Toxicity Dose (TTD) Values .....	172
Appendix D: Background Information for Methylmercury .....	177
D.1 Toxicokinetics .....	177
D.2 Health Effects .....	177
D.3 Mechanisms of Action .....	179
D.4 Health Guidelines .....	180
D.5 Derivation of Target-Organ Toxicity Dose (TTD) Values .....	181
Appendix E: Background Information for PCBs .....	185
E.1 Toxicokinetics .....	185
E.2 Health Effects .....	188
E.3 Mechanisms of Action .....	191
E.4 Health Guidelines .....	210
E.5 Derivation of Target-Organ Toxicity Dose (TTD) Values .....	211
Appendix F: Chemical Structures of Mixture Components .....	215



## LIST OF TABLES

Table 1.	Health Effects Observed in Humans or Animals after Oral Exposure to Chemicals of Concern	3
Table 2.	Mean and Range of Persistent Chemicals in Blood of Consumers of Great Lakes Fish	8
Table 3.	Daily Intake of Major Contaminants in Female F1 Rats Exposed to Diets Containing 0, 5, or 20% (w/w) Lyophilized Lake Ontario Salmon Flesh	20
Table 4.	Median Levels of Persistent Chemicals in High Consumers and Nonconsumers of Baltic Sea Fatty Fish (Source: Svensson et al. 1994)	26
Table 5.	Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of Hexachlorobenzene and the Influence of Hexachlorobenzene on Toxicity/Carcinogenicity of 2,3,7,8-TCDD by Sequential Exposure	34
Table 6.	Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of <i>p,p'</i> -DDE and the Influence of <i>p,p'</i> -DDE on Toxicity/Carcinogenicity of 2,3,7,8-TCDD by Simultaneous Exposure	38
Table 7.	Summary of Available Data on the Influence of Hexachlorobenzene on Toxicity/Carcinogenicity of Mercuric Chloride and the Influence of Mercuric Chloride on Toxicity/Carcinogenicity of Hexachlorobenzene by Simultaneous Exposure	45
Table 8.	Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD	54
Table 9.	Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs	62
Table 10.	Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of Methylmercury	75
Table 11.	Summary of Available Data on the Influence of Methylmercury on Toxicity/Carcinogenicity of PCBs	76
Table 12.	Health Effects Forming the Basis of ATSDR Oral MRLs for Chemicals of Concern	78
Table 13.	Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions	84
Table 14.	Effect of <b>2,3,7,8-TCDD</b> on <b>Hexachlorobenzene</b>	85
Table 15.	Effect of <b>Hexachlorobenzene</b> on <b>2,3,7,8-TCDD</b>	86
Table 16.	Effect of <b>2,3,7,8-TCDD</b> on <i>p,p'</i> -DDE	88
Table 17.	Effect of <i>p,p'</i> -DDE on <b>2,3,7,8-TCDD</b>	89
Table 18.	Effect of <i>p,p'</i> -DDE on <b>Hexachlorobenzene</b>	90
Table 19.	Effect of <b>Hexachlorobenzene</b> on <i>p,p'</i> -DDE	91
Table 20.	Effect of <b>2,3,7,8-TCDD</b> on <b>Methylmercury</b>	92
Table 21.	Effect of <b>Methylmercury</b> on <b>2,3,7,8-TCDD</b>	94
Table 22.	Effect of <b>Hexachlorobenzene</b> on <b>Methylmercury</b>	95
Table 23.	Effect of <b>Methylmercury</b> on <b>Hexachlorobenzene</b>	96
Table 24.	Effect of <i>p,p'</i> -DDE on <b>Methylmercury</b>	98
Table 25.	Effect of <b>Methylmercury</b> on <i>p,p'</i> -DDE	99
Table 26.	Effect of <b>PCBs</b> on <b>2,3,7,8-TCDD</b>	100
Table 27.	Effect of <b>2,3,7,8-TCDD</b> on <b>PCBs</b>	102
Table 28.	Effect of <b>PCBs</b> on <b>Hexachlorobenzene</b>	104
Table 29.	Effect of <b>Hexachlorobenzene</b> on <b>PCBs</b>	105
Table 30.	Effect of <b>PCBs</b> on <i>p,p'</i> -DDE	106
Table 31.	Effect of <i>p,p'</i> -DDE on <b>PCBs</b>	107
Table 32.	Effect of <b>PCBs</b> on <b>Methylmercury</b>	108
Table 33.	Effect of <b>Methylmercury</b> on <b>PCBs</b>	110

Table 34. Matrix of BINWOE Determinations for Repeated Simultaneous Oral Exposure to Chemicals of Concern ..... 112

Table 35. MRLs and TTDs for Repeated Oral Exposure to Chemicals of Concern ..... 115

## LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

Ah	arylhydrocarbon	RfC	Reference Concentration
AHH	arylhydrocarbon hydroxylase	RfD	Reference Dose
ATSDR	Agency for Toxic Substances and Disease Registry	SD	standard deviation
		SRBC	sheep red blood cells
BINWOE	binary weight-of-evidence	T4	thyroxin
BROD	benzoxylresorufin-O-deethylase	TT3	total triiodothyronine
CDD	chlorinated dibenzo- <i>p</i> -dioxin	TT4	total thyroxine and free thyroxine
CDF	chlorinated dibenzofuran	TAO	triacytyleandomycin
CI	confidence interval	TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
CYP	cytochrome P450	TCDF	tetrachlorodibenzofuran
		TCHQ	tetrachlorohydroquinone
DNA	deoxyribonucleic acid	TEF	Toxic Equivalency Factor
DTH	delayed-type hypersensitivity	TEQ	toxic equivalents
		TGF	transforming growth factor
EGF	epidermal growth factor	TSH	thyroid stimulating hormone
EPA	Environmental Protection Agency	TTD	target-organ toxicity dose
EROD	ethoxyresorufin O-deethylase		
		UDP	uridine-5'-diphosphate
HCB	hexachlorobenzene	UF	uncertainty factor
		U.S.	United States
IARC	International Agency Research on Cancer	WOE	weight-of-evidence
IRIS	Integrated Risk Information System		
		>	greater than
kg	kilogram	≥	greater than or equal to
		=	equal to
LOAEL	lowest-observed-adverse-effect level	<	less than
LSE	Levels of Significant Exposure	≤	less than or equal to
mg	milligram		
MRL	Minimal Risk Level		
mRNA	messenger ribonucleic acid		
NOAEL	no-observed-adverse-effect level		
OR	odds ratio		
PBB	polybrominated biphenyl		
PBPK	physiologically based pharmacokinetic		
PCB	polychlorinated biphenyl		
ppb	parts per billion		
ppm	parts per million		
ppt	parts per trillion		



## 1. Introduction

The primary purpose of this Interaction Profile for chlorinated dibenzo-*p*-dioxins (CDDs), hexachlorobenzene, *p,p'*-DDE, methylmercury, and polychlorinated biphenyls (PCBs) is to evaluate data on the toxicology of the “whole” mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture MRL, and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR DT’s recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.

The Great Lakes basin comprises one-fifth of the total fresh water on the earth’s surface; it is a valuable national resource for both the United States and Canada. For over 200 years, the Great Lakes basin has been used as a resource for industry, agriculture, shipping, and recreation. Researchers have identified almost 400 contaminants in the water, sediment, and biota in quantifiable amounts. Based on criteria such as persistence in the environment, bioconcentration, bioaccumulation, and toxicity, the International Joint Commission Water Quality Board identified 11 of these substances as critical Great Lakes pollutants. They are polychlorinated biphenyls (PCBs), mirex, hexachlorobenzene, dieldrin, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT) and its metabolite 1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethylene (*p,p'*-DDE), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF), benzo[*a*]pyrene, alkylated lead, toxaphene, and mercury (GLWQB 1985).

In 1990, Congress amended the Federal Water Pollution Control Act and mandated the Environmental Protection Agency (EPA), in consultation with the Agency for Toxic Substances and Disease Registry (ATSDR) and the Great Lakes states, to submit a research report on the adverse human health effects related to water pollutants in the Great Lakes. Since then, ATSDR awarded several research grants and established cooperative agreements to coordinate basin-wide human health effects research. The primary interests of ATSDR's Great Lakes Research Program are to document and characterize the exposure, identify populations at higher risk, identify association between the consumption of contaminated Great Lakes fish and short and long-term harmful health effects, identify the most sensitive endpoints, establish registries and surveillance cohorts, and identify ways to prevent or mitigate exposure and resulting health effects.

This Interaction Profile for CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs serves as a support document for the ATSDR's Great Lakes Research Program and represents a first step in evaluating possible joint toxic actions of the International Joint Commission Water Quality Board's 11 critical Great Lakes pollutants. These five chemicals were selected because (1) each occurs with high frequency in Great Lakes water, sediment, and biota; (2) each has been associated with a wide array of overlapping toxic effects leading to concerns that they may jointly act on several similar targets of toxicity (see Table 1); (3) neurological development (associated with pre- or post-natal oral exposure) is a critical toxicity target for each (Table 1); and (4) two prospective studies (Jacobson and Jacobson 1996; Stewart et al. 2000b) reported that deficits in neurological development of children were associated with frequent maternal consumption of contaminated Great Lakes fish and increasing levels of PCBs in maternal body fluids (see Section 2.1).

The profile begins with a review of studies of possible health effects associated with the consumption of fish from the North American Great Lakes or fish from the Baltic Sea (Section 2.1). The results of the fish consumption studies are reviewed to evaluate their utility in identifying health problems in human populations that may be associated with exposure to fish likely to have been contaminated with CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, PCBs, and other chemicals. A companion Interaction Profile on Persistent Chemicals Found in Breast Milk (ATSDR 2001b) similarly reviews studies on whether or not health problems may be associated with exposure to mixtures of persistent chemicals in human breast milk.

**Table 1. Health Effects Observed in Humans or Animals after Oral Exposure to Chemicals of Concern. See Appendices A, B, C, D, and E for More Details**

Effects	Chemicals of concern <sup>a</sup>				
	2,3,7,8-TCDD	Hexachlorobenzene	<i>p,p'</i> -DDE	Methylmercury	PCBs
Wasting syndrome	x	x			x
Kidney damage				x	
Liver damage	<b>X</b>	<b>X</b> <sup>b</sup>	x <sup>b</sup>		x
Immunosuppression	x <sup>b</sup>	x	x	x	x <sup>b</sup>
Thyroid hormone disruption	<b>X</b>	<b>X</b>			x
Female reproductive organ disruption	x	<b>X</b> <sup>b</sup>		x	x
Male reproductive organ disruption	<b>X</b>		x	x	x
Neurological impairment	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	x
Altered neurological development (pre- and/or post-natal)	x <sup>b</sup>	<b>X</b> <sup>b</sup>	x <sup>c</sup>	<b>X</b> <sup>b</sup>	<b>X</b> <sup>b</sup>
Altered female reproductive organ development	x				x
Altered male reproductive organ development	x		x <sup>b</sup>		x
Other developmental effects (malformations or fetotoxicity)	x	<b>X</b>	x	x	x
Cancer <sup>d</sup>	x	x	x	x	x

<sup>a</sup>Upper case and bolded **X** indicates that effects have been observed in humans. Lower case and non-bolded x indicates that effects have been observed only in animals.

<sup>b</sup>Indicates that these are the most sensitive noncancer health effects from oral exposure (i.e., they occur at lower dose levels than other noncancer effects).

<sup>c</sup>No data are available for *p,p'*-DDE effects on this endpoint, but altered neurobehavior was observed in adult rats following exposure to single oral doses of 0.5 mg *p,p'*-DDT/kg on postnatal day 10 (Eriksson et al. 1990, 1992).

<sup>d</sup>Carcinogenic responses have been demonstrated in animals exposed to each of the chemicals. EPA has derived oral slope factors for humans exposed to 2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, and PCBs based on tumor responses in animals (see Appendices A, B, C, and E). EPA did not derive a slope factor for humans exposed to methylmercury based on evidence that effects on the nervous system and its development would occur at exposure levels much lower than those necessary to produce cancer (see Appendix D).

In preparing the profile, no studies were located regarding the toxicology of the whole 5-component mixture, but some studies are available that examined joint toxic actions of binary mixtures of the components (Section 2.2). Following the evaluation of data on the joint toxic actions (e.g., additivity, greater-than-additive interactions, or less-than-additive interactions) of the components, the relevance of the data to public health concerns associated with exposures to the mixture is discussed (in Section 2.3), and recommendations are made for approaches to exposure-based assessments of joint toxic action of the mixture (in Section 3).

Each component of the mixture is a persistent chemical that bioaccumulates in higher food-chain organisms including humans, and has been demonstrated to produce a wide range of health effects in humans and animals (see Appendices A–E and Table 1). The ranges of health effects produced by these chemicals overlap, leading to concern that, following regular consumption of mixtures of the chemicals in food such as contaminated fish, all five may jointly act to produce altered neurological development, suppression of immune competence, or cancer, and three (CDDs, *p,p'*-DDE, and PCBs) may jointly act to alter development of reproductive organs (Table 1). In addition to being found in fish from the Great Lakes, these chemicals are found, to varying degrees, in other dietary components including fish from other parts of the world (e.g., the Baltic Sea), human milk, dairy products, and meat (ATSDR, 1996, 1998, 1999a, 2001b). The profile focuses on data relevant to concurrent oral exposure scenarios that are of most interest to public health concerns for these chemicals.

For the purposes of this profile, 2,3,7,8-TCDD, the best studied CDD, is taken to be representative of other CDDs based on assumptions that CDDs display joint additive toxic actions that are mediated by a common initial mechanism involving binding to the arylhydrocarbon (Ah) receptor (Appendix A; ATSDR 1998), and that interactions between 2,3,7,8-TCDD and other non-CDD chemicals are representative of interactions between other CDDs and other non-CDD chemicals. Although no data were located to directly support the second assumption, there are several observations supporting the first assumption, including: (1) acute or subchronic exposure of rats to individual CDDs produce a similar spectrum of toxic effects (Kociba et al. 1978; Viluksela et al. 1998a, 1998b); (2) acute oral exposure of rats to a mixture of four CDDs with chlorination in the 2,3,7,8-positions produced decreased body weight and deaths in rats at dose levels equivalent to dose levels of the individual components producing similar effects (Stahl et al. 1992); and (3) 13-week oral exposure of rats to a mixture of four CDDs produced a spectrum of effects (e.g., decreased body weight, increased mortality, induction of hepatic ethoxy-



resorufin *O*-deethylase [EROD]) similar to effects produced by the individual CDDs at equipotent dose levels (Viluksela et al. 1998a, 1998b).

Like CDDs, oral exposure of animals to PCB mixtures elicits a broad array of effects, including a body weight wasting syndrome involving thymic atrophy, induction of hepatic Phase I (CYP oxygenases) and Phase II (e.g., UDP-glucuronyltransferases) enzymes, liver damage and enlargement, porphyria, kidney damage, immunosuppression, thyroid hormone disruption, disruption of female and male reproductive organs, altered development of female and male reproductive organs, neurological impairment, altered neurological development (associated with pre- or post-natal exposure), and cancer (Appendix E; ATSDR 2000). In contrast to CDDs, Ah-receptor mediation may account for only a subset of the wide array of PCB-induced effects. There is increasing evidence from animal studies that several PCB-induced effects may involve multiple mechanisms (ATSDR 2000; Fischer et al. 1998; Hansen 1998; Li and Hansen 1997; Safe 1994). PCB-induced effects that appear to predominately involve Ah-receptor dependent mechanisms include: induction of hepatic activities of CYP1A1, 1A2, and 1B1 (Connor et al. 1995; Hansen 1998; Safe 1994); body weight wasting and thymic atrophy from acute exposure (Hori et al. 1997; Safe 1994); and porphyria and porphyria cutanea tarda (Smith et al. 1990b). PCB-induced effects involving Ah-receptor independent mechanisms include: induction of hepatic activities of CYP2B1, 2B2, 2A1, and 3A (Connor et al. 1995; Hansen 1998); neurological and neurodevelopmental effects involving changes in brain dopamine levels (Seegal 1996b, 1998) and/or changes in brain cell intracellular calcium homeostasis and related signal transduction processes (Tilson and Kodavanti 1997; Tilson et al. 1998; Wong and Pessah 1996, 1997; Wong et al. 1997); and tissue injury related to activation of neutrophils (Brown and Gamey 1995; Gamey et al. 1993; Tithof et al. 1995).

The profile does not focus on a representative PCB congener (or congeners) or subclasses of PCBs to discuss interactions with the other components of the subject mixture, because it is likely that:

(1) multiple mechanisms are involved in PCB-induced health effects; (2) different PCB congeners may produce effects by different and multiple mechanisms, and (3) humans are exposed to complex mixtures of PCB congeners with differing biological activities. PCB mixtures are discussed as the entity of concern in parallel with ATSDR's PCB MRLs, which are derived for exposure to PCB mixtures (see Appendix E).



## 2. Joint Toxic Action Data for the Mixture of Concern and Component Mixtures

### 2.1 Mixture of Concern

No studies were located that examined health effects in humans or animals exposed to mixtures exclusively containing CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs. No physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models were found for mixtures of these five chemicals. There are, however, several studies designed to examine whether or not consumption of Great Lakes or Baltic Sea fish containing biopersistent chemicals may be associated with detrimental effects on the health and/or development of humans or animals. A review of these studies follows to determine the degree to which data identify health hazards associated with exposure to complex mixtures of contaminants in fish that are likely to contain CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs.

#### 2.1.1 Studies of Health Effects Associated with Consumption of Great Lakes Fish

People whose diets include Great Lakes fish are exposed to complex mixtures of biopersistent chemicals, including the chemicals of concern in this profile. Table 2 shows that a group of Great Lakes fisheaters who consumed an average of 49 meals containing Great Lakes sportfish per year for an average of 33 years had higher mean blood levels of CDDs, chlorinated dibenzofurans (CDFs), PCBs, *p,p'*-DDE, and mercury than did reference groups of subjects whose diets did not contain Great Lakes fish (Anderson et al. 1998). The large amount and long duration of Great Lakes fish consumption by this sample of Great Lakes fisheaters suggests that they are within the most highly exposed group of Great Lakes fisheaters. Although mean concentrations of biopersistent contaminants in blood or serum were generally higher in this group of fisheaters than in reference groups, there was considerable overlap in the ranges between fisheater and reference groups for many of the contaminants. The lipophilicity of the chemicals of concern is likely to limit serum concentrations, and differences (between fisheaters and non-fisheaters) in fatty tissue concentration may be more dramatic. This study, however, did not sample and analyze contaminant concentrations in fatty tissue.

**Table 2. Mean and Range of Persistent Chemicals in Blood of Consumers of Great Lakes Fish (Source: Anderson et al. 1998)**

Chemical	Great Lakes Fisheaters <sup>a</sup> (n=30 or 31)	Reference Group <sup>b</sup>
CDDs in serum (ppt TEQs)	27.5 (8.2–58.7)	15.5 (range NA; n=70)
CDFs in serum (ppt TEQs)	11.9 (3.4–25.3)	4.9 (range NA; n=70)
Coplanar PCBs in serum (ppt TEQs)	17.4 (ND–56)	1.8 (range NA; n=70)
Total PCBs in serum (ppb)	5.2 (1.2–15.2)	1.2 (0.46–2.9; n=41)
Hexachlorobenzene in serum (ppb)	0.1 (0.02–0.2)	0.1 (ND–0.3; n=180)
Lead in whole blood (µg/dL)	4.0 (0.9–13.0)	2.8 (2.7–3.0; n=13,201)
Mercury in whole blood (µg/L)	3.8 (0.9–13.0)	2.5 (0.6–8.5; n=13,201)
<i>o,p'</i> -DDT in serum (ppb)	0.06 (0.03–0.3)	ND (n=180)
Oxychlordane in serum (ppb)	0.3 (0.04–0.8)	0.2 (0.02–0.5; n=180)
<i>p,p'</i> -DDE in serum (ppb)	5.2 (0.6–23.9)	2.8 (ND–38.5; n=180)
<i>p,p'</i> -DDT in serum (ppb)	0.3 (0.05–0.8)	ND (n=180)

<sup>a</sup>Subjects had no known occupational exposure to the compounds analyzed and consumed, in the previous year, an average of 49 meals (range 10–172) containing sportfish (trout, salmon, carp, catfish, perch, smelt, or walleye) from Lake Michigan (n=10), Lake Huron (n=11), and Lake Erie (n=11). Subjects consumed Great Lakes sportfish for a mean of 33 years (range 11–58). Concentration values were not obtained for all chemicals in each of the subjects.

<sup>b</sup>Comparison groups were chosen from previous studies in which analyses were conducted by the same laboratory and were taken to represent the general population with no apparent exposure to the compounds that were analyzed [see Anderson et al. (1998) for more details].

NA = not available; ND = not detected; ppb = parts per billion; ppt = parts per trillion; TEQ = toxic equivalents; µg = micrograms

There are several studies examining reproductive or physical developmental endpoints (Buck et al. 1997; Courval et al. 1999; Dar et al. 1992; Fein et al. 1984; Kostyniak et al. 1999; Lonky et al. 1996; Mendola et al. 1995, 1997; Vena et al. 1996) and neurodevelopmental or neurobehavioral endpoints (Fein et al. 1984; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b; Lonky et al. 1996; Mergler et al. 1998; Schantz et al. 1999; Stewart et al. 1999, 2000b) in people whose diets included North American Great Lakes fish contaminated with complex mixtures of the chemicals of concern and other chemicals (most notably CDFs and chlorinated pesticides such as mirex and chlordane). Studies of animals exposed to Great Lakes fish in their diet include: a two-generation study of reproductive, immunological, neurobehavioral, and neurochemical endpoints in rats fed diets containing varying amounts of similarly contaminated Great Lakes fish (Feeley et al. 1998; Seegal 1999; Seegal et al. 1998;

Stewart et al. 2000a); a two-generation reproductive study of minks fed diets containing varying amounts of Great Lakes fish (Restum et al. 1998); and other studies that examined neurobehavioral endpoints in rats fed diets with Great Lakes fish (Daly 1991; Hertzler 1990).

### ***Reproductive Effects and Great Lakes Fish Consumption: Human Studies***

Between 1993 and 1995, Courval et al. (1999) surveyed 626 married couples (aged 17–34 years old) concerning conception delay (i.e., ever having failed to conceive after 12 months of trying). At least one person in each couple was a licensed angler residing in 1 of 10 Michigan counties bordering a Great Lake (Lake Erie, Lake Huron, or Lake Michigan). Subjects were categorized into four exposure classes based on the estimated number of sportfish meals consumed since 1970: 0, 1–114, 115–270, and 271–1,127 for men, and 0, 1–54, 55–138, and 139–1,127 for women. For men, unadjusted odds ratios (ORs) for conception delay increased with increasing exposure class as follows 1.2, 1.3, and 2.0; results of a trends test were statistically significant. After adjustment for potential confounding variables of age, race, region of Michigan, household income, smoking, alcohol consumption, and partner's sportfish consumption, the ORs (and 95% confidence intervals) for men were 1.4 (0.5–3.9), 1.8 (0.6–5.0), and 2.8 (1.0–8.0). For women, unadjusted ORs were 0.9, 1.0, and 1.4; adjusted ORs were 0.8 (0.4–1.9), 0.8 (0.4–1.8), and 1.0 (0.4–2.4). The findings provide suggestive evidence that highest frequency of consumption of Great Lakes sportfish in this study may be associated with an increased risk of conception delay for men, but not for women. Analysis of the data controlled for several potential confounders, but no information was collected regarding the subjects' frequency or timing of sexual intercourse during the period of attempting to conceive, the working status of the women, or the levels of biopersistent chemicals, such as PCBs, CDDs, *p,p'*-DDE, hexachlorobenzene, or mercury, in biological samples from the subjects.

The New York State Angler Cohort Study assessed, by interviews and self-administered questionnaires, sportfish consumption, health status, and reproductive endpoints in groups of subjects who held New York State fishing licenses in 1990–1991 and resided in counties adjacent to Lake Ontario (Vena et al. 1996). Findings for different reproductive or developmental endpoints from this study have been reported in several reports (Buck et al. 1997; Kostyniak et al. 1999; Mendola et al. 1995, 1997).

In a study of menstrual cycle length and fish consumption among 2,223 women in the New York State Angler Cohort, women who consumed more than one Lake Ontario fish meal per month in 1991 reported a statistically significantly shorter mean menstrual length than did referent women in the cohort who never ate Lake Ontario sportfish (Mendola et al. 1997). The mean difference (adjusted for age and ever having smoked) was 1.11 days when considering all of the surveyed women and 0.51 days when only women who reported having regular menstrual cycles were included in the analysis (n=2,080). No significant differences in menstrual cycle length were found between the referent group and women who consumed  $\leq 1$  Lake Ontario fish meal per month in 1991 or women who consumed Lake Ontario fish in the past. When the women were assigned indices of PCB exposure based on species-specific PCB levels and 1991 fish consumption histories, similar results were found, with significantly shorter mean menstrual cycle lengths (of a similar magnitude) only in the highest exposure category. No significant differences in mean menstrual cycle length were found when the subjects were classified into groups based on the number of years during which Lake Ontario fish were consumed. Limitations of the study include the reliance on self-reported exposure data (biological samples were not collected and analyzed for expected contaminants) and outcome data, and the lack of information on potential confounders such as current smoking status, stress, and use of contraceptives. Mendola et al. (1997) noted that, although the small decreases in menstrual cycle length are not likely to be clinically significant or of major public health concern, they may indicate potential endocrine effects on a population level.

Buck et al. (1997) found no statistically significant associations between several measures of dietary exposure to Lake Ontario fish and time-to-pregnancy, a measure of fecundity, among 874 women of the New York State Angler Cohort who were pregnant between 1991 and 1993 and reported a known time-to-pregnancy. Exposure measures were self-reported and included years of Lake Ontario fish consumption, number of meals per month in 1991, and a PCB index based on the amount, duration, and type of fish consumed. As with other New York State Angler Cohort studies, limitations include the reliance on self-reported exposure data and outcome data, and the lack of information on potential confounding factors such as occupational exposures and current smoking status. In addition, women with unplanned pregnancies were necessarily ruled out from the analysis; Buck et al. (1997) noted that this may be a potential bias inherent in the study.

Kostyniak et al. (1999) reported that increasing levels of PCBs or DDE in breast milk (normalized for lipid content) were significantly associated with decreasing number of months of lifetime lactation in a sample of 98 lactating women from the New York State Angler Cohort. In this sample, PCB levels in breast milk were significantly associated with self-reported measures of fish consumption, but DDE levels

were not. The observed association is likely to be important in estimating dose rates for these chemicals in nursing neonatal populations, but the relevance of the association to reproductive performance is not clear.

Mendola et al. (1995) found no statistically significant associations between increased risk for spontaneous fetal death and four measures of dietary exposure to Lake Ontario fish among 1,820 multigravid, fertile women from the New York State Angler Cohort. Four measures of exposure were constructed for each subject based on fish consumption histories: (1) lifetime PCB exposure based on species-specific PCB levels (subjects were placed in no, low, moderate, or high exposure classes); (2) number of years of sportfish consumption; (3) kilograms of sportfish consumed in the 1990–1991 season; and (4) lifetime kilograms of sportfish consumed (no, low, moderate, or high exposure classes). Spontaneous fetal death histories (ever having a pregnancy end in a miscarriage, spontaneous abortion, or stillbirth) were obtained from New York State live-birth certificates. Logistic regression analyses to examine possible associations were stratified by number of prior pregnancies and were controlled for smoking and maternal age. Strengths of the study included sufficient statistical power to detect fairly small increases in ORs and the reliability of reproductive history data on birth certificates. Limitations included the focus on clinically recognized fetal deaths, which may not detect early pregnancy loss, the self-reported nature of both exposure and outcome data, and the lack of biomarker monitoring to validate the self-reported exposure data.

### ***Developmental Effects and Great Lakes Fish Consumption: Human Studies***

Three studies that examined physical variables such as birth weight in infants of mothers who consumed Great Lakes fish before and during pregnancy reported variable results (Dar et al. 1992; Fein et al. 1984; Lonky et al. 1996). Fein et al. (1984) reported that infants (n=242) of mothers who reported consuming an average about two or three Lake Michigan fish meals per month (about 15 pounds per year) for an average of about 16 years had statistically significant deficits in mean birth weight (5.2%) and head circumference (1.6%), compared with infants (n=71) of non-fisheating Michigan mothers. In contrast, Dar et al. (1992) found no statistically significant associations between self-reported prepregnancy consumption of Lake Michigan fish in Green Bay, Wisconsin women and adverse reproductive outcomes (e.g., still births, decreased birth weight, decreased head circumference), and found that birth weights were often higher for mothers who claimed to eat more Lake Michigan fish prior to pregnancy. Dar et al. (1992) noted that all of the subjects in the Michigan study exposed group (Fein et al. 1984) ate much more Lake Michigan fish than most of the participants in the Wisconsin study. It was calculated that the

exposure necessary to be selected for the Michigan exposed group was equivalent to the 96<sup>th</sup> percentile of exposure in the Wisconsin study. Lonky et al. (1996) reported that no significant differences in birth weight or head circumference were found in infants of groups of Oswego, New York women who reported consuming, before giving birth, >40 pounds (n=152), <40 pounds (n=243), or no Lake Ontario sportfish (n=164). Lonky et al. (1996) suggested that behavioral measures in infants may be more sensitive to contaminants in Great Lakes fish than measures of physical development (see following discussion of studies examining neurodevelopmental endpoints in groups of Great Lakes fish eaters).

A prospective study of children whose mothers consumed Lake Michigan fish before and during pregnancy found small, but statistically significant, changes in neurological endpoints at several stages of development compared with children of non-fisheating mothers (Fein et al. 1984; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b). The basis of the study was a 1980–1981 screening interview of newly delivered mothers in four hospitals located near Lake Michigan in which women reported the amount of each species of Lake Michigan fish they consumed before and during pregnancy (Fein et al. 1984). Exposed mothers were those who had consumed at least 11.8 kg of fish over 6 years; controls were selected at random from among women who did not eat Lake Michigan fish. Median frequency of consumption in the exposed group was about two or three Lake Michigan fish meals per month (average of 6.7 kg/year, standard deviation [SD]=5.8; [14.8 pounds/year]) for an average of about 16 years. Maternal fish consumption values were not significantly correlated with cord serum PCB levels, but were significantly correlated with maternal serum PCB levels, which in turn were correlated with cord serum PCB levels (Fein et al. 1984). Jacobson and Jacobson (1996) noted that due to analytical limitations in the early 1980s, PCBs were below the level of detection in 70% of cord serum samples and 22% of maternal serum samples. The lack of consistency of correlations among these three variables may be related to a lack of precision of the analytical methods at such low serum concentrations.

Fein et al. (1984) reported that, at birth, children (n=242) of all exposed mothers had statistically significant deficits, compared with children (n=71) of non-fisheaters, in mean birth weight (5.2%), head circumference (1.6%), and scores in the Ballard Examination for Fetal Maturity for gestational age (1.8%) and neuromuscular maturity index (5.4%). These variables were adjusted for several potentially confounding factors including maternal prepregnancy weight and consumption of alcohol and caffeine prior to and during pregnancy.

Jacobson et al. (1984) reported that high maternal levels of fish consumption were associated with increased likelihood of infants showing decreased scores on three of six clusters (response decrement,



orientation, tonic, range of state, regulation of state, and autonomic maturity) of the Neonatal Behavioral Assessment Scale administered on the third day after birth: autonomic maturity, range of state, and reflexes. Regression analyses indicated that levels of fish consumption were significantly associated with two of the three autonomic items (decreased motor maturity and increased propensity to startle), one of the four range of state items (limited lability of states, i.e., depressed responsiveness), and increased number of weak reflexes. Incidence of infants with performance scores classified as “worrisome” in autonomic maturity, reflexes, and range of state clusters were compared between non-exposed mothers and a subset of exposed mothers who consumed >6.5 kg fish per year. Statistically significant increased incidences of “worrisome” performance were found in the highly exposed group for reflexes (24/72) and range of state (7/63), compared with respective non-exposed incidences (10/65 and 1/65, respectively); but the increased incidence for autonomic maturity “worrisome” performance was of marginal ( $p < 0.1$ ) statistical significance (6/73 compared with 1/65).

In a follow-up examination of 123 of these infants at the age of 7 months (92 exposed and 31 non-exposed), scores in a test of visual recognition memory showed a statistically significant decline (before or after adjustment for a number of potentially confounding variables) with increasing maternally reported fish consumption or with increasing cord serum PCB levels (Jacobson et al. 1985). No significant relationship was found between declining visual recognition scores at 7 months of age and increasing measures of PCB exposure from nursing (Jacobson et al. 1985).

At 4 years of age, 236 of the 313 children assessed at birth were evaluated for cognitive functioning using McCarthy’s Scales of Children’s Abilities: verbal, perceptual-performance, quantitative, memory, motor, and general cognitive index (Jacobson et al. 1990a). A step-wise multiple regression analysis with several potentially confounding variables (maternal age, gravidity, and examiner) indicated that verbal and memory scores significantly declined with increasing cord serum PCB levels. A similar analysis indicated that memory scores also significantly declined with increasing measures of PCB exposure from nursing. Further analysis, based on five levels of maternal milk PCBs and three nursing periods, indicated that only the most exposed group significantly differed from the other groups. Jacobson et al. (1990a) noted that maternal milk PCB level was correlated with maternal PCB body burdens (and thus prenatal exposure) and that the memory deficit appeared to be unrelated to the amount of contaminated breast milk that was consumed. Jacobson et al. (1990a) suggested that the effect of maternal milk PCB level on memory performance reflected greater prenatal, rather than nursing, exposure. No significant associations were found between cognitive functioning and concurrent PCB serum levels in the 4-year-olds. Jacobson et al. (1990b) also reported that body weight in female children at 4 years of age

significantly declined with increasing cord serum PCB levels after adjustment for maternal and paternal weight and height, maternal age, and gravidity, but was not significantly associated with maternal milk PCB level, weeks of nursing, or serum PCB levels at 4 years of age. Composite ratings for reduced behavioral activity provided by mothers and two independent examiners were significantly associated with increasing concurrent serum PCB levels in the 4-year-olds and maternal milk PCB levels, but not with cord serum PCB level or weeks of nursing (Jacobson et al. 1990b).

At 11 years of age, 212 of the original 313 children were evaluated in a battery of IQ and achievement tests (Wechsler Intelligence Scales for Children, Wide Range Achievement Tests for Spelling and Arithmetic, and Woodcock Reading Mastery test) (Jacobson and Jacobson 1996). Composite estimates of prenatal exposure to PCBs were derived from concentrations in cord serum, maternal serum shortly after delivery, and/or maternal milk samples obtained within 0.2–4.5 months of delivery. This composite estimate was used because PCB levels were below detection limits for 70% of cord serum samples and 22% of maternal serum samples due to analytical limitations during the early 1980s. For dose-response analysis, children were grouped into one of five estimated prenatal exposure classes based on this composite, expressed in units of  $\mu\text{g}$  PCB per gram of fat in milk:  $<0.5$ ,  $0.5\text{--}0.74$ ,  $0.75\text{--}0.99$ ,  $1\text{--}1.24$ , and  $\geq 1.25$ . Multiple regression analysis, which controlled for potential confounding factors such as socioeconomic status and maternal education and vocabulary, indicated that prenatal exposure to PCBs from maternal consumption of Lake Michigan fish was significantly associated with lower full-scale and verbal IQ scores. Forty percent of the 30 children with the highest estimated prenatal exposure levels had full-scale and verbal IQ scores that were below one standard deviation of the sample mean score compared with 11% of the 148 children with lower prenatal exposure levels. These differences were statistically significant. Using the same definition of poor performance in the reading mastery-word comprehension test, 23% of high-exposure children had poor performances compared with 10% of the less exposed children. No statistically significant associations were found between poor performance in the IQ and achievement tests and measures of post-natal PCB exposure (PCB concentrations in milk, number of weeks of nursing, or serum PCB levels of the children at 4 or 11 years of age).

The Michigan prospective study reported finding statistically significant associations between prenatal exposure, and possibly lactational exposure, to PCBs from maternal consumption of Lake Michigan fish and deficits in neonatal behavioral development (Jacobson et al. 1984), in short-term memory during infancy (Jacobson et al. 1985), in short-term memory during early childhood (Jacobson et al. 1990a), and in general intellectual ability during early school years (Jacobson and Jacobson 1996). Due to the likelihood of co-exposure to a number of other biopersistent contaminants in Lake Michigan fish, the

possible contribution of other potentially neurotoxic chemicals such as CDDs, CDFs, methylmercury, DDE, and hexachlorobenzene is plausible and cannot be ruled out.

Causal relationships between PCB exposure and deficits in cognitive function in infants and children and between Lake Michigan fish consumption and cognitive deficits are not definitively established by the results due to several study limitations and a limited amount of corroborative results from other studies (see following review of a study of Lake Ontario fish consumption and neurological development of children). Strengths of the study include the prospective design and the collection and chemical analysis of biological samples from the mothers and children to attempt to validate self-reported fish exposure. Although the studies assessed a broad range of control variables, several reviewers (Danse et al. 1997; Expert Panel 1994; Kimbrough 1997; Seegal 1996b) have suggested that the statistical analysis and study design had a limited ability to control for potentially confounding variables and differences in characteristics (such as prepregnancy weight and alcohol consumption) between the exposed and non-exposed groups. Seegal (1996b) suggested that a matched, rather than a random, control design may have provided a better approach for controlling confounding variables. Several reviewers also have criticized the interpretation of the PCB prenatal exposure data (Danse et al. 1997; Expert Panel 1994; Seegal 1996b). These reviewers questioned the validity of statements that the data demonstrate dose-response relationships for cognitive deficits in children and prenatal PCB exposure from maternal fish consumption, because PCB levels were below the detection limit in most of the cord serum samples (the most direct available measure of prenatal exposure) and PCB levels in cord serum samples were not significantly correlated with self-reported levels of maternal fish consumption. The results suggest an association between exposure to contaminants in Lake Michigan fish and long-lasting subtle deficits in cognitive function and activity in infants and children exposed *in utero* and via lactation, but do not establish causal relationships.

The Oswego Newborn and Infant Development Project is a prospective study designed to examine possible relationships between consumption of Lake Ontario fish by mothers before and during pregnancy and behavioral effects in children at birth, 3, 6, 12, 24, 36, and 48 months; Lonky et al. (1996) reported initial results from the assessment at birth. Volunteers were recruited at 20-week sonogram visits to an obstetrics practice and interviewed between June 1991 and June 1994 to obtain information on Lake Ontario fish consumption, health history, smoking and alcohol use, and other demographics. Children were assigned to high- (>40 pounds of fish consumed in mothers' lifetime), low- (<40 pounds of fish consumed in mothers' lifetime), and no-exposure groups using an exposure index that was based on maternal species-specific fish consumption and a weighting factor based on PCB levels in Lake Ontario

fish species. Means of 388 (SD=859), 10 (SD=18), and 0 (SD=0) lifetime PCB-weighted pounds of fish consumed were calculated for the high-, low-, and no-exposure groups. The Neonatal Behavioral Assessment Scale was administered to newborns at 12–24 and 25–48 hours after birth. Scores (adjusted for potential confounding factors by components analyses of demographic data; data on substances consumed during pregnancy, chronic medical conditions, and other toxic exposure; and data on labor and delivery complications and birth characteristics) for the high-exposure group were significantly lower on the reflex, autonomic, and habituation clusters of the tests compared with the no-exposure group. No significant differences were found between groups in birth weight, head circumference, or on the orientation, range of state, regulation of state, or motor clusters of the behavioral tests. Strengths of the Lake Ontario study include the prospective design, the controlling of potential confounding factors, and the apparent similarities of the three groups in demographic characteristics. Limitations include the lack of exposure data based on biological samples from the mothers or children, but Lonky et al. (1996) reported that cord blood and maternal hair samples were collected, as were breast milk samples shortly after birth, and were to be analyzed for total PCBs, PCB congeners, DDE, hexachlorobenzene, lead, and mercury. Discussion of these results follows.

Analyses of the biological samples indicated that average total PCB concentrations in cord blood were low (below 1 ppb) and were not significantly different between self-reported fish eaters and controls (Stewart et al. 1999). In contrast, cord blood concentrations of PCB congeners with 7–9 chlorines, expressed either as mole percentage or ppb, were significantly higher in fish eaters compared with controls, but no significant differences in cord blood concentrations of less chlorinated PCBs (1–3 or 4–6 chlorines per molecule) were found between fish eaters and controls. Blood cord concentrations of PCBs with 7–9 chlorines were significantly and positively correlated with breast milk concentrations, but no significant relationships between blood cord and breast milk concentrations were found for PCBs with 1–3 or 4–6 chlorines (Stewart et al. 1999).

Linear trend analysis identified statistically significant relationships between lower scores on the Habituation and Autonomic clusters of the Neonatal Behavioral Assessment Scale (given at 25–48 hours after birth) and increasing cord blood levels of PCBs with 7–9 chlorines (Stewart et al. 2000b). For the analysis, parent/child pairs were divided into four exposure groups based on cord blood concentrations of PCBs with 7–9 chlorines: no detectable PCBs (n=173); bottom 33<sup>rd</sup> percentile (n=39); middle 33<sup>rd</sup> percentile (n=40); and upper 33<sup>rd</sup> percentile (n=40). The analysis included potential confounding covariates identified in an initial analysis of variance. Similar linear trend analyses found no significant relationships between lower scores and levels of the following blood cord contaminants: PCBs with

1–3 or 4–6 chlorines, DDE, hexachlorobenzene, lead, or mercury.

### *Neurological Effects and Great Lakes Fish Consumption: Human Studies*

Mergler et al. (1998) examined possible associations between consumption of fish from two lakes of the Upper St. Lawrence River and performance in a neurofunctional test battery in a group of 63 matched pairs of fisheating and non-fisheating adult residents of Southwest Quebec. Matching was based on sex, alcohol consumption, age, and education. Participants provided information on a questionnaire regarding socio-demographic information, residential and medical history, and dietary practices including whether they ate fish from Lake St. Francois and Lake St. Louis on a scale of never/ occasionally/ a few times a month/ a few times a week. Paired analysis indicated that fisheaters had small, but significantly higher mean levels of blood lead (4.4 µg/dL) and organic mercury (0.97 µg/L) than non-fisheaters (3.1 µg/dL and 0.58 µg/L, respectively). Chemical analysis of blood samples did not include other organic chemicals, such as PCBs or DDE, that are expected to be present in the fish. Paired t-test analysis indicated that fisheaters had small (generally <10%), but statistically significant, performance deficits in four of five tests of auditory learning or recall, three of three tests of cognitive flexibility, two of three grapho-motor tests, one of three visual recognition and recall tests, two of three reaction time tests, and one of three quantified neurological motor examination tests. No significant differences between group performances were found in two fine motor movement tests or in three sensory tests. Strengths of the study include the matched pair analysis to adjust for potential confounding factors and the extensive battery of tests applied. The study was limited in that exposure data on fish consumption was limited and self-reported and that chemical analysis of blood samples was restricted to metallic components. The study was part of a larger study designed to examine neurotoxic effects from environmental exposure to manganese; fish consumption was surveyed as a potential confounding factor. Mergler et al. (1998) concluded that the two groups were similar in performance on motor and sensory tasks and tests of visual recognition and recall, and that the fisheaters had small performance deficits in neurological tests involving “higher levels of information processing”, i.e., auditory recall and word naming.

In 1993–1995, Schantz et al. (1999) examined hand steadiness and visual-motor coordination in a group of 50- to 90-year-old Michigan residents who were part of a cohort of fisheaters and non-fisheaters who reported in 1980–1982 that they consumed one or more Lake Michigan fish per week (>24 pounds/year; fisheaters) or less than 6 pounds/year (non-fisheaters). Fisheaters’ median consumption was 38.5 pounds/year (range 24–270 pounds). Blood samples were collected from 180 of the 188 participants (49 men and 55 women fisheaters and 34 men and 50 women non-fisheaters) and analyzed for PCBs,

DDE, polybrominated biphenyls (PBBs), hexachlorobenzene, oxychlorane, dieldrin, mirex, mercury, and lead. Median concentrations of PCBs, DDE, lead, and mercury were significantly higher in fish eaters compared with non-fish eaters. Schantz et al. (1996, 1999) noted that the elevations in serum lead and mercury, although statistically significant, were most likely not of toxicological significance, and that levels of PCBs and DDE were markedly higher than values for non-fish eaters and the general population. Serum levels of PCBs and DDE were significantly correlated and were thus used as a single exposure variable to place subjects into three categories: low = PCB and DDE levels at or below respective medians; intermediate = PCB or DDE levels in third quartile of distributions; and high = PCB or DDE levels in upper quartile. Multiple regression analysis, including age and gender as additional explanatory variables, indicated that performances in the Static Motor Steadiness Test or the Grooved Pegboard Test were not significantly associated with the PCB/DDE exposure variable. Performance in the Static Motor Steadiness Test showed a marginally significant ( $p=0.052$ ) trend test for improved performance with the dominant hand (but not the non-dominant hand) with increasing exposure measure, but pairwise comparison indicated no significant differences in adjusted mean scores for the three groups. Strengths of the study include the single-blinded administration of the motor function tests, the adjustment for potential confounding variables, and the collection and analysis of biological samples to verify fish consumption exposure data. Limitations of the study include the cross-sectional design of the health outcome data, the modest sample size that may have restricted statistical power to detect very subtle changes in motor functions, and the restriction of neurological function assessment to motor functions. Schantz et al. (1999) noted that subjects were also assessed on tests of memory and learning, executive functions, and visual-spatial functions, and that results would be forthcoming. Schantz et al. (1999) concluded that the findings suggest that PCB/DDE exposure from Great Lakes fish consumption has not significantly impaired hand steadiness or visual-motor coordination in this sample of older adults who were heavy consumers of Lake Michigan fish in the 1970s.

### ***Health Effects in Animals Consuming Great Lakes Fish***

A multigenerational rat study provided groups of rats (through 3 generations) with diets containing 0, 5, or 20% (w/w) of lyophilized protein from chinook salmon harvested from either Lake Huron or Lake Ontario (Feeley and Jordan 1998; Feeley et al. 1998; Pappas et al. 1998; Seegal et al. 1998; Tryphonas et al. 1998b). The rats' consumption of fish was reported to be approximately 15- or 60-fold greater than that of average Great Lakes sportfish anglers. Daily intakes of major contaminants were calculated based on chemical analysis of the diet and food intake data. Data for F1 female rats (Table 3) show that intakes of CDDs, CDFs, PCBs, total DDT (including  $p,p'$ -DDE), and mercury increased with increasing intakes

of Lake Huron or Lake Ontario salmon, but that intakes of lead did not (Feeley and Jordan 1998). These data indicate that lead is not a contaminant of concern in salmon from these lakes.

No significant exposure-related adverse effects on reproductive parameters (mating, fertility, viability, lactation indices, litter size) were found in either generation fed Lake Huron or Lake Ontario salmon (Feeley et al. 1998). No consistent exposure-related effects were found across the generations on several immunological variables (thymus weights, plaque-forming cell assay, mitogen-induced lymphocyte proliferation, natural killer cell activity, and susceptibility to challenge with *Listeria monocytogenes*) (Tryphonas et al. 1998b). No exposure-related effects on spatial reference memory, activity, exploration, timidity, sensorimotor function, or behavioral stereotype were found in F1 and F2 offspring, but impairment in a test of working memory (radial arm maze) was reported for F1 males fed the 20% Lake Ontario fish diet and F2 males fed the 20% Lake Huron fish diet (Pappas et al. 1998). Decreased concentrations of frontal cortical and striatal dopamine were found in F1, but not F2, rats fed the 20% diets. These decreases in dopamine concentrations were hypothesized to be related to impaired working memory in F1 males, but the association was not seen in F2 males with impaired working memory (Seegal et al. 1998). Earlier studies found changes in behavioral responses to a novel situation (modified open-field test; Hertzler 1990) or an aversive situation (reward reduction; Daly 1991) in rats fed Lake Ontario fish diets (10–30%) for 20 or 60 days, but no significant behavioral response to reward reduction was observed in the multigenerational study of rats exposed to Lake Ontario or Lake Huron fish diets for up to 70 days (Feeley and Jordan 1998; Feeley et al. 1998; Pappas et al. 1998).

**Table 3. Daily Intake of Major Contaminants in Female F1 Rats Exposed to Diets Containing 0, 5, or 20% (w/w) Lyophilized Lake Ontario Salmon Flesh<sup>a</sup>**  
**Source: Feeley and Jordan 1998**

Contaminant	Control (0%)	5% diet	20% diet
Total CDD/CDF TEQ (ng/kg body weight/day)	0.019	0.238	0.918
Total CDD/CDF/coplanar PCB TEQ (ng/kg/body weight/day)	0.050	0.702	2.84
Total PCBs (µg/kg body weight/day)	0.22	23.20	82.37
Mirex (µg/kg body weight/day)	0.008	1.01	3.94
Total DDT (µg/kg body weight/day) <sup>b</sup>	0.127	4.36	18.55
Total chlordanes (µg/kg body weight/day)	0.028	0.491	1.71
Cadmium (µg/kg body weight/day)	0.318	0.340	0.496
Lead (µg/kg body weight/day)	2.83	2.62	2.67
Total mercury (µg/kg body weight/day) <sup>c</sup>	0.849	5.22	18.00
Arsenic (µg/kg body weight/day)	0.99	5.44	25.31

<sup>a</sup>Intakes were calculated based on food intake data for rats between 7 and 12 weeks of age. The complete data table in Feeley and Jordan (1998) shows that intakes were qualitatively similar, but generally were somewhat smaller, for males compared with females, and for F0 rats compared with F1 rats. Rats fed diets containing 0, 5 or 20% (w/w) lyophilized Lake Huron salmon flesh showed similar qualitative and quantitative aspects of contaminant intakes, except that intakes of mirex from the Lake Huron diet were about 10-fold less than mirex intakes from the Lake Ontario diets, and intakes of PCBs, dioxin TEQs, and DDT were about 1.5- to 3-fold less.

<sup>b</sup>The DDT complex (*p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD) accounted for 75 and 60% of organochlorine pesticide residues in the Lake Huron and Lake Ontario fish, respectively. The analysis also included quantification of a wide range of organochlorine compounds including hexachlorobenzene, toxaphene, endosulfan, and octachlorostyrene, but individual concentrations were not reported by Feeley and Jordan (1998). Total DDT was predominately comprised of *p,p'*-DDE; the ratio of *p,p'*-DDE concentration to *p,p'*-DDT concentration in the fish ranged from about 7 to 8.7 (Feeley and Jordan 1998).

<sup>c</sup>Metals were quantified as total metal; no attempt to quantify relative contributions from organic and inorganic forms was reported.

The significant decreases in rat brain regional concentrations of dopamine induced by the Lake Ontario or Lake Huron fish diets were associated with daily intakes between 13.9 and 74.3 µg/kg/day PCB, whereas dietary exposure to 2,2',4,4'-tetrachlorobiphenyl, one of the more potent neurotoxic PCBs in an *in vitro* assay, required doses as high as 10 or 20 mg/kg/day to effect similar changes in regional dopamine concentrations (Seegal 1999). Seegal (1999) hypothesized from this comparison that neurological deficits noted in offspring exposed *in utero* and during lactation to fish-borne contaminants may be due to either contaminants other than PCBs or to interactions between PCBs and other neurotoxicants in the fish.



Rats fed a 30% diet of Lake Ontario salmon for 20 or 65 days showed behavioral deficits (reduced persistence in a progressive-ratio reinforcement schedule), increased brain concentrations of PCBs, DDE, and mirex, and increased brain concentrations of highly chlorinated PCBs, compared with rats fed a standard rat chow diet or a 30% diet of Pacific Ocean salmon (Stewart et al. 2000a). Stewart et al. (2000a) noted that although the behavioral deficits were related to the increased PCB levels in the brain of the rats, other contaminants in Lake Ontario salmon may contribute to the observed deficits.

In a reproduction study of minks fed diets formulated to provide 0, 0.25, 0.5, or 1.0 ppm PCBs by substituting carp harvested from Saginaw Bay of Lake Huron for ocean fish in the control diet, survivability of F1 and F2 offspring was markedly decreased in the mid- and high-dose carp-fed groups compared with controls (Restum et al. 1998). Other chlorinated hydrocarbons detected in the carp used in this study included CDDs, CDFs, *p,p'*-DDT, *p,p'*-DDT, chlordane, nonachlor, and dieldrin. The reduced survivability of the F1 offspring predominately occurred after birth during the lactation period. For example, the first F1 litter produced by the F0 generation showed a 70.5% survivability at birth (compared with 94.6% in controls), but by the end of lactation, 6 weeks after birth, the average survivability was 23% (compared with about 73% in controls). In several exposure groups, there were decreased percentages of mated females that gave birth, but the decreases were not statistically significant. The failure to demonstrate statistical significance may have been due to small sample sizes (due to reduced survival in the exposed F1 generation) for several of these groups. For example, in a high-dose F1 group, 2/4 mated females gave birth (50%), compared with 11/14 (79%) in the F1 control group. Results from this mink study are contrasted by those from the multigenerational rat study showing no decrease in offspring survival in rats fed Lake Huron or Lake Ontario salmon through two generations of breeding (Feeley et al. 1998). From the available information, it is not possible to determine if the different results from the mink and rat studies are due to physiological or biochemical differences between minks and rats, qualitative or quantitative differences in chemical composition of the fish flesh, or some other cause.

### ***Health Effects and Great Lakes Fish Consumption: Summary***

No studies were located examining immunological endpoints in humans exposed to Great Lakes fish in the diet. No consistent exposure-related effects were found on several immunological variables (thymus weights, plaque-forming cell assay, mitogen-induced lymphocyte proliferation, natural killer cell activity,

and susceptibility to challenge with *Listeria monocytogenes*) in a multigenerational study of rats fed Lake Huron or Lake Ontario salmon (Feeley et al. 1998; Tryphonas et al. 1998b).

Studies examining reproductive endpoints in people whose diets contained Great Lakes fish have found suggestive evidence that consumption of Great Lakes fish may not be associated with increased risk for spontaneous fetal death (Mendola et al. 1995) and may be associated with increased risk of conception delay for men, but not for women (Buck et al. 1997; Courval et al. 1999) and slightly shorter length of menstrual cycle (Mendola et al. 1997) and duration of lactation in women (Kostyniak et al. 1999). However, the strength of the human evidence that consumption of Great Lakes fish may or may not be associated with adverse effects on reproductive abilities is weak given the small magnitude of effects when they have been detected and the limitations of the studies, such as the lack of information on potential confounding factors and the self-reported nature of the exposure data. In animals, no effects on reproductive ability, neonatal survival, or physical development of neonates were found in a multigenerational study of rats consuming diets containing up to 60-fold more Lake Ontario or Lake Huron salmon than diets of average Great Lakes sportfish anglers (Feeley et al. 1998). In a multigeneration study of minks fed carp from the Saginaw Bay of Lake Huron, no consistent, statistically significant effects on several reproductive performance variables were found, but markedly reduced neonatal survival occurred (Restum et al. 1998). It is unknown if the differences in the rat and mink studies are due to possible species differences in neonatal survival response or differences in contaminant content in the administered diets.

The limited studies of reproductive endpoints in humans and animals exposed to Great Lake fish in the diet do not provide consistent evidence that human consumption of Great Lakes fish present obvious risks for impaired reproduction, but further investigation of the reduced neonatal survival in carp-fed minks may be useful to explore possible relevance to human exposure scenarios.

Three studies that examined physical developmental variables such as birth weight in infants of mothers who consumed Great Lakes fish before and during pregnancy reported variable results (Dar et al. 1992; Fein et al. 1984; Lonky et al. 1996). One study (Fein et al. 1984) reported small, but statistically significant, deficits in birth weight and head circumference in newborns of mothers who consumed Lake Michigan fish, but similar results were not found in studies of Wisconsin (Dar et al. 1992) and New York (Lonky et al. 1996) infants of Great Lakes fish-eating mothers. No standard developmental toxicity studies of animals exposed to Great Lakes fish in the diet were located. However, physical developmental variables were not statistically significantly affected in multigenerational studies of rats

and minks exposed to Great Lakes fish in the diet (Feeley et al. 1998; Restum et al. 1998). In general, the studies of physical developmental endpoints in humans and animals do not provide consistent evidence that consumption of Great Lakes fish may increase risk for adverse effects.

Despite limitations in study design, the Michigan prospective study of neurodevelopment in children of Lake Michigan fisheating mothers suggests an association between frequent consumption of contaminated Great Lakes fish by women and long-lasting, subtle deficits in cognitive behavior and activity in their children (Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a). Due to the likelihood of co-exposure to a number of biopersistent contaminants in Great Lakes fish, the possible contribution of several potentially neurotoxic chemicals, such as PCBs, CDDs, CDFs, methylmercury, DDE, and hexachlorobenzene, is plausible and cannot be ruled out. The first phases of a similar ongoing prospective study of neurodevelopment in children of Lake Ontario fisheating mothers also found evidence for subtle behavioral deficits in newborns of these mothers compared with newborns of non-fisheating women (Lonky et al. 1996; Stewart 1999, 2000b). Both studies reported statistically significant associations between indices of prenatal exposure to PCBs from maternal consumption of fish and deficits in neonatal behavioral development (Jacobson et al. 1984; Lonky et al. 1996; Stewart et al. 1999, 2000b).

In a multigenerational study of rats exposed to diets containing Lake Huron or Lake Ontario salmon at several levels, impairment in a test of working memory was reported for F1 males fed the high-exposure Lake Ontario fish diet and F2 males fed the high-exposure Lake Huron fish diet (Pappas et al. 1998) and decreased concentrations of frontal cortical and striatal dopamine were found in F1, but not F2, rats fed diets containing the highest level of fish (Seegal 1999; Seegal et al. 1998). Other studies (Daly 1991; Hertzler 1990; Stewart et al. 2000a) found changes in behavioral responses in rats fed Lake Ontario fish diets that were not replicated in the multigenerational study (Feeley and Jordan 1998; Feeley et al. 1998; Pappas et al. 1998). Although the neurobehavioral and neurochemical results from the rat studies are somewhat equivocal due to inconsistencies, and the levels of dietary exposure at which effects were observed are expected to be 15- to 60-fold higher than those experienced by most people whose diet included Great Lakes sportfish, the animal results provide some supporting evidence for the plausibility of an association of neurodevelopment deficits in children of mothers who frequently consume contaminated Great Lakes fish.

Assessments of neurological endpoints in adult Great Lakes sportfish consumers are restricted to a study finding statistically significant relationships between small performance deficits in tests of attention and

motor function and an index of fish consumption in a group of Quebec adults whose diet included fish from the St. Lawrence Lakes (Mergler et al. 1998) and another study finding no statistically significant relationship between fine motor performance and an index of fish consumption in a group of elderly (50–90 years of age) adults whose diets included varying amounts of Great Lakes fish (Schantz et al. 1999).

In summary, frequent dietary consumption of contaminated Great Lakes fish by child-bearing-aged women has been associated in two prospective epidemiological studies with changes in the neurological development of their children (Fein et al. 1984; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b; Lonky et al. 1996; Stewart 2000b), but other studies provide no consistent evidence that consumption of Great Lakes fish presents obvious risks for impaired reproduction, impaired immune capabilities, or physical birth defects (Buck et al. 1997; Courval et al. 1999; Feeley and Jordan 1998; Feeley et al. 1998; Hertzler 1990; Kostyniak et al. 1999; Mendola et al. 1995, 1997; Vena et al. 1996). Some support for the plausibility of an association between neurological effects and frequent consumption of Great Lakes sportfish is provided by studies of animals fed very large amounts of Great Lakes fish (Daly 1991; Hertzler 1990; Pappas et al. 1998; Seegal et al. 1998; Stewart et al. 2000a), but the overall weight of the evidence is inadequate to determine causal relationships between fish consumption and health hazards in humans. In addition, the studies cannot determine with certainty which chemicals in the fish may cause the potential effects, or determine modes of joint toxic actions that may occur among the components of the complex chemical mixture. PCBs have been proposed as a toxicant involved in the possible association between maternal fish consumption and altered childhood neurological development based on statistically significant associations between PCB levels in maternal fluids and neurological deficits in children (Fein et al. 1984; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b; Lonky et al. 1996; Stewart et al. 2000b), but other hypotheses have been proposed, including the possible involvement of other persistent chemicals in contaminated fish or synergistic interactions between PCBs and other neurotoxicants in fish (see Bemis and Seegal 1999 or Seegal 1999 for discussion).

Thus, although the studies of Great Lakes fish consumption by humans and animals do not provide conclusive evidence of causation, or of dose-response relationships with all five mixture components, they are useful in identification of endpoints for further consideration as possible endpoints of concern for the mixture, in particular, neurodevelopmental effects.

### 2.1.2 Studies of Health Effects Associated with Consumption of Baltic Sea Fish

There are several studies examining health endpoints in groups of Swedish people whose diet included fatty fish (e.g., herring, salmon, mackerel, or eel) from the Baltic Sea including an examination of immunological and hepatic endpoints (Svensson et al. 1994), a series of studies examining birth weight of infants (Rylander and Hagmar 1995, 1999; Rylander et al. 1996, 1998a, 1998b), and an examination of body height and weight and performance in a psychometric test in 18-year-old males (Rylander and Hagmar 1999). There is also a study of immunological endpoints in groups of captive harbor seals fed herring from either the Baltic Sea or the relatively uncontaminated Atlantic Ocean (Ross et al. 1995). Monitoring studies indicate that fatty fish from the Baltic Sea are contaminated with halogenated aromatic hydrocarbons including CDDs, CDFs, and PCBs, DDT and its metabolites, DDD and DDE, other halogenated pesticides such as hexachlorobenzene, and organic mercury, although there are indications that levels of contaminants in Baltic Sea fatty fish have been declining in the past 20 years (Bignert et al. 1998; Svensson et al. 1994).

Blood levels of DDT and several CDDs, and PCBs, DDT, and erythrocyte levels of methylmercury were significantly elevated in a subset of a group of 23 male Baltic Sea fish consumers compared with 20 male nonconsumers (Svensson et al. 1994; Table 4). Blood samples from all 23 fish eaters and 20 controls were also analyzed for white blood counts, immunoglobulins, numbers of different types of lymphocytes, serum bilirubin levels, and serum liver enzyme levels. The high consumers had lower median proportions and numbers of natural killer cells (lymphocytes carrying the CD56 marker) in peripheral blood than nonconsumers (5.9 versus 9.7% and  $0.1 \times 10^9/L$  versus  $0.2 \times 10^9/L$ ), but the differences were of marginal statistical significance (p values ranged from 0.06 to 0.15). No other significant differences between the groups were found for other immunological endpoints including total neutrophil and leukocyte counts, counts of other subpopulations of lymphocytes, and serum levels of immunoglobulins and C-reactive protein. Levels of serum bilirubin and liver enzyme activities were not significantly different between the groups. Statistically significant correlations were found for decreases in proportions or numbers of natural killer cells and (1) increased fish consumption and (2) increased plasma levels of several PCB congeners or *p,p'*-DDT, but the correlations were not statistically significant for plasma levels of other PCB congeners, total toxic equivalents of CDDs+CDFs, or erythrocyte levels of organic mercury. The small number of subjects included in this study limited its statistical power of detection.

**Table 4. Median Levels of Persistent Chemicals in High Consumers and Nonconsumers of Baltic Sea Fatty Fish (Source: Svensson et al. 1994)<sup>c</sup>**

	High consumers (n=7-9)	Nonconsumers (n=4)
Toxic equivalents of CDDs/CDFs in blood fat (pg/g)	64 <sup>a</sup>	21
Non- <i>ortho</i> -PCBs (i.e., co-planar) in plasma (pg/g)		
3,3',4,4'	0.2 <sup>a</sup>	0.08
3,3',4,4',5	2.8 <sup>a</sup>	1.1
3,3',4,4',5,5'	1.9	0.7
<i>ortho</i> - PCBs in plasma (ng/g)		
2,3',4,4',5	0.5 <sup>a</sup>	0.2
2,2', 4,4', 5,5'	3.6 <sup>a</sup>	1.7
<i>p,p'</i> -DDT in plasma (ng/g) <sup>b</sup>	0.4 <sup>a</sup>	0.06
Organic mercury in erythrocytes (ng/g)	8.4 <sup>a</sup>	1

<sup>a</sup>Significantly ( $p \leq 0.05$ ) higher than nonconsumer level.

<sup>b</sup>Report indicated that only *p,p'*-DDT was quantified, but monitoring studies of contaminants in flesh from several fatty fish species from the Baltic Sea indicated that ratios of sums of DDT, DDE, and DDD concentrations to DDT concentrations ranged from about 2 to 10 (Bignert et al. 1998).

<sup>c</sup>Data in this table are consistent with a study comparing blood levels of organochlorine compounds and mercury in a larger number of Swedish fishermen and non-fishermen matched for age and county of residence (Svensson et al. 1995). Baltic Sea (east coast, n=150) and Atlantic (west coast, n=100) fishermen ate almost 2 times more fatty fish meals per week than non-fishermen referent groups. Blood levels of methylmercury were about 2 times higher in both fishermen groups than levels in referent groups (median levels were about 20 nmol Hg/L vs. 10 nmol Hg/L). Pooled plasma samples from Baltic Sea fishermen showed levels of CDDs, CDFs, and PCBs that were 2-6 times higher than levels in Atlantic fishermen and referent groups (Svensson et al. 1995).

Although the study by Svensson et al. (1994) found no statistically significant changes in immunological endpoints in human males consuming Baltic Sea fish, Ross et al. (1995) reported that a group of 11 captive harbor seals fed Baltic Sea herring for 2.5 years displayed significantly diminished delayed-type hypersensitivity (DTH) responses (skin swelling and antibody titer) to an intradermal ovalbumin skin challenge compared with the responses of a group of 11 seals fed Atlantic Ocean herring. Analysis of blubber biopsies of the seals, taken after 2 years of feeding, showed that Baltic Sea-fed seals had significantly higher concentrations of chlorinated aromatic hydrocarbons than Atlantic Ocean-fed seals. Concentrations (expressed as Ah-receptor toxic equivalents) of mono and diortho PCBs, planar PCBs, and combined CDDs and CDFs for Baltic-fed and Atlantic-fed seals were: 140 and 36 ng toxic equivalent (TEQ)/kg lipid; 51 and 22 ng TEQ/kg lipid; and 18 and 4 ng TEQ/kg lipid, respectively. Biopsy samples were not analyzed for *p,p'*-DDE, hexachlorobenzene, or methylmercury, but it is likely, based on results

from other studies (Bignert et al. 1998; Svensson et al. 1994), that these compounds were present in the Baltic Sea herring.

Results from a series of studies provide limited evidence that women who frequently consume Baltic Sea fish may have an increased risk of having children with low birth weights (Rylander et al. 1995, 1996, 1998a, 1998b). The results from the studies, however, do not establish a causal association between increased risk for low birth weight and consumption of Baltic Sea fish, because the association does not appear to be strong (some of the analyses found associations of only marginal statistical significance, and elevations in ORs that were statistically significant were not large), the magnitude of the possible effect does not appear to be large (median birth weight values for exposed cohorts were generally <5% lower than values in referent groups), and dose-response relationships were not demonstrated (no consistent evidence was found for increasing risk for low birth weight or decreasing birth weight values with increasing indices of fish consumption).

In the first study, birth weights of infants born between 1973 and 1991 were compared between cohorts of Swedish fishermen's wives from the east coast (Baltic Sea) and west coast (Atlantic Ocean) and respective regional populations (Rylander et al. 1995). The east- and west-coast cohorts contained 1,501 and 3,553 children, respectively. Interviews of subsets of 69 women from the fishermen's wives cohorts and a referent group indicated that the east- and west-coast groups ate locally caught fish more than twice as often than the referent group. Using 2,500 or 3,000 g as reference values defining low birth weight, the east-coast cohort showed marginally significantly elevated ORs for frequency of low birth weights (adjusted for year of birth, maternal age, and parity) compared with regional referent values (OR=1.22, 95% confidence interval [CI] 0.98–1.52; and OR=1.13, 95% CI 1.00–1.27); ORs for the west-coast cohort were not elevated. Median birth weight for the east-coast cohort (3,530 g) was slightly, but significantly, lower than that for the west-coast cohort (3,610 g). After excluding subjects without smoking information, with multiple births, or with major malformations, ORs for low birth weight (adjusted for maternal age, parity, marital status, and smoking habits) remained elevated for the east-coast cohort (OR=1.25, 95% CI 0.87–1.77 and OR=1.44, 95% CI 1.18–1.76, for respective reference values of 2,500 and 3,000 g).

The second study (Rylander et al. 1996) was a case-control analysis of 72 cases of low birth weight (1,500–2,750 g) from the east-coast maternal cohort and 154 referent cases with birth weights between 3,250 and 4,500 g, selected from the same cohort and matched to the cases according to gender, parity, and year of birth. Mothers were interviewed regarding dietary and smoking habits and place of living

during childhood and adolescence. The habit of currently eating at least four Baltic Sea fish meals per month was associated with an elevated risk of marginal statistical significance for having an infant with low birth weight (OR=1.9, 95% CI 0.9–3.9; adjusted for maternal age and smoking habits). No evidence of increasing risk for low birth weight with increasing current fish consumption was found, and no statistically significant associations were found between increased risk for low birth weight and consumption of Baltic Sea fish during the period when the infant was born. After adjustment for maternal age and smoking habits during the year of childbirth, a statistically significant association was found between increased risk for having a low birth weight infant and growing up in a fishing village (OR=2.1, 95% CI 1.0–4.3).

A third study examined birth weights of 1,719 and 2,682 infants born between 1973–1993 to 1,030 and 1,537 mothers who were sisters of fishermen from the Swedish east and west coasts, respectively (Rylander et al. 1998a). Using 2,500 or 3,000 g as reference values for low birth weights, east-coast fishermen's sisters had a significantly increased risk for having a low-birth-weight infant compared with the general population. Respective ORs and 95% CIs were 1.6 [1.1–2.3] or 1.3 [1.1–1.5], excluding multiple births and infants with major malformations, but the report did not indicate if adjustments were made for confounding from smoking habits.

The latest study in the series was a case-control analysis of 57 cases of east-coast fishermen's wives who had infants with low birth weights (1,500–2,750 g) and 135 referent cases with birth weights between 3,250 and 4,500 g, selected from the same cohort and matched to the cases according to gender, parity, and year of birth (Rylander et al. 1998b). Plasma levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB 153) were measured in the mothers in 1995. No statistically significant association was found between increased risk for low birth weight and 1995 plasma levels of CB153 >200 ng/g (OR=1.8, 95% CI 0.8–4.0, adjusted for maternal age and smoking habit during year of birth). Plasma concentrations during the year of childbirth were estimated from the 1995 values, using a kinetic model that assumed a 33% reduction of body burden for each lactation period, a 5-year biologic half-life during non-lactating periods, and either a 3 or 5% yearly reduction in levels of CB153 in dietary fish. Associations of marginal statistical significance were found between increased risk of low birth weight and birth-year maternal plasma concentrations of CB153 >300 ng/g under the assumption of a 3% yearly reduction in CB153 levels in fish (OR=2.1, 95% CI 1.0–4.7), or concentrations >400 ng/g with a 5% yearly reduction assumption (OR=2.3, 95% CI 0.9–5.9).

In a related study, body height and weight and performance on a military-service-enrollment



psychometric test were examined in 18-year-old males whose mothers' were married to fishermen of the east coast (n=268) or west coast (n=510) of Sweden (Rylander and Hagmar 1999). No statistically significant differences in distributions of body height or weight or in distribution of scores for the psychometric test were found for either group of fishermen's sons compared with regional expectations.

In summary, results from the studies of Baltic Sea fish consumption include limited evidence that women who frequently eat Baltic Sea fish may have increased risk for having children with low body weight at birth (Rylander et al. 1995, 1996, 1998a, 1998b), no convincing evidence that 18-year-old sons of Baltic Sea fishermen's wives were smaller or performed differently on a psychometric test compared with the general population (Rylander and Hagmar 1999), no statistically significant changes in several immunological endpoints or indices of hepatic damage in men who frequently consumed Baltic Sea fish (Svensson et al. 1994), and significantly diminished delayed-type hypersensitivity responses indicating impaired immunological competence in captive harbor seals fed Baltic Sea herring for 2 years compared with seals fed Atlantic Ocean herring (Ross et al. 1995). Thus, impaired immunological competence and low birth weight are plausible health hazards from dietary exposure to mixtures of chemicals in Baltic Sea fish, but the data are inadequate to establish causal relationships between fish consumption and effects in humans. The studies do not clearly demonstrate dose-response relationships for the potential health hazards, and because of design limitations, they cannot determine with certainty the components that may cause the effects, or determine modes of joint toxic action among the mixture components.

Although the studies of Baltic Sea fish consumption by humans and animals do not provide conclusive evidence of causation, or of dose-response relationships with all five mixture components, they are useful in identification of endpoints for further consideration as possible endpoints of concern for the mixture. These endpoints are immunological and developmental (low-birth weight).

## **2.2 Component Mixtures**

No studies were located that examined health effects in humans or animals exposed to four- or three-membered mixtures of the five components of concern. No PBPK/PD models were found for four-, three-, or two-membered mixtures of these chemicals. The following subsections present evaluations of health effects data and discussions of mechanistic information pertinent to the joint toxic action of each pair of components.

### 2.2.1 2,3,7,8-TCDD and Hexachlorobenzene

No studies were located that compared effects on health endpoints following oral exposure to binary mixtures of 2,3,7,8-TCDD (or other CDDs) and hexachlorobenzene with effects following exposure to the compounds alone.

Hexachlorobenzene competitively inhibited the binding of 2,3,7,8-TCDD to Ah-receptor sites in *in vitro* rat hepatic cytosol preparations (Hahn et al. 1989). The affinity of hexachlorobenzene for the Ah receptor was about 10,000-fold lower than that of 2,3,7,8-TCDD. Rats fed a diet containing 3,000 ppm hexachlorobenzene for 4–7 days showed a decrease in TCDD-binding specific activity in hepatic cytosol preparations compared with activity in preparations from control rats (Hahn et al. 1989). The decrease was principally due to a decrease in the number of Ah-receptor-binding sites in the cytosol (Hahn et al. 1989). Such a decrease in cytosolic Ah-receptor levels has been observed following administration of other Ah receptor ligands (such as TCDD and 3-methylcholanthrene) and has been proposed to be due to movement of ligand-receptor complexes into the nucleus (see Appendix A).

In immature rats given single intraperitoneal doses of hexachlorobenzene (400  $\mu\text{mole/kg}$  in corn oil = 113.9 mg/kg), two days before injection of 10 or 30  $\mu\text{g/kg}$  doses of 2,3,7,8-TCDD in corn oil, decreases in body weight gains and relative thymus weights, compared with controls, were much larger than in rats given 10 or 30  $\mu\text{g/kg}$  2,3,7,8-TCDD (0.031 or 0.093  $\mu\text{mole/kg}$ ) alone (Li et al. 1989). For example, 13 days after injection of 30  $\mu\text{g/kg}$  TCDD, rats with hexachlorobenzene pretreatment had lost an average of about 10 grams of body weight and had an average relative thymus weight <0.01% of body weight (some of the rats in this group were athymic) compared with a body weight gain of about 40 grams and a relative thymus weight of 0.15% in rats given 30  $\mu\text{g/kg}$  2,3,7,8-TCDD alone. During this 13-day period, control rats showed a body weight gain of 80 grams and relative thymus weight of 0.25%. Single doses of hexachlorobenzene alone, as high as 3,000  $\mu\text{mole/kg}$ , had no effect on body weight gain or relative thymus weight.

Because hexachlorobenzene exposure alone showed no effects on body weight gain or thymus weight, the data appear to indicate that hexachlorobenzene pretreatment potentiates these effects of 2,3,7,8-TCDD, which have been proposed to be mediated via the Ah receptor. Three days after injection of 400  $\mu\text{mole/kg}$  hexachlorobenzene, cytosolic levels of the Ah receptor in the thymus, lung, and kidney (but not in the liver) were decreased by about 50% compared with vehicle controls. After 14 days, thymic Ah receptor levels returned to levels similar to control levels, but remained depressed in the lung

and kidney. Li et al. (1989) also observed that sole administration of hexachlorobenzene or TCDD induced hepatic cytochrome P-450 (CYP) enzyme activity levels (arylhydrocarbon hydroxylase [AHH] and ethoxyresorufin O-deethylase [EROD]) that persisted 15 days after dose administration, but enzyme levels induced by hexachlorobenzene at the maximum level tested (3,000  $\mu\text{mole/kg}$ ) were 50% lower than maximum levels induced by 30  $\mu\text{g/kg}$  2,3,7,8-TCDD. Li et al. (1989) noted that the mechanistic significance of hexachlorobenzene-induced changes in Ah receptor levels, CYP enzyme induction patterns, and potentiation of 2,3,7,8-TCDD toxicity was unknown.

One possible expectation of the effect of a hexachlorobenzene pretreatment, or simultaneous exposure to large doses of hexachlorobenzene and small doses of 2,3,7,8-TCDD, is that, by depleting cytosolic levels of the Ah receptor, hexachlorobenzene may inhibit formation of TCDD-Ah receptor complexes and subsequent development of TCDD toxic effects. This is the opposite, however, of what was observed in the Li et al. (1989) rat study. Subsequent studies providing confirming evidence for hexachlorobenzene potentiation of acute TCDD toxicity and a plausible mechanistic explanation were not located. One possible partial explanation of the observation is that the potentiation may involve hexachlorobenzene interacting with some unidentified component, other than the Ah receptor, of the mechanism by which acute exposure to TCDD produces body weight wasting and thymic atrophy.

It is unknown if the apparent potentiation of TCDD toxicity by hexachlorobenzene is dependent on the absolute dose levels of the two agents. TCDD alone at the tested dose levels produced impaired body weight gain and decreased thymus relative weights, whereas hexachlorobenzene alone, at dose levels up to 7- to 8-fold higher than the dose which was potentiating, was without effect on these endpoints. Other animal studies have reported that oral exposure to hexachlorobenzene can produce thymic atrophy and decreased body weight gain that may be similar to the wasting syndrome produced by 2,3,7,8-TCDD (Barnett et al. 1987; Courtney 1979; Smith et al. 1987; Vos 1986), but intraperitoneal doses of hexachlorobenzene alone that were examined in the Li et al. (1989) study were not high enough to cause these effects in the rat strain that was studied. Other issues of uncertainty regarding the observed apparent potentiation include whether it is dependent on:

- the sequence of exposure (will it also occur with simultaneous exposure to TCDD and hexachlorobenzene?);
- the relative dose levels of the two compounds (the potentiating dose of hexachlorobenzene was approximately 4,000- or 13,000-fold greater than the dose levels of TCDD on a  $\mu\text{mole/kg}$

basis—is this a requirement for the apparent potentiation?);

- the duration and route of exposure (will long-term coexposure to oral hexachlorobenzene and TCDD produce the same potentiation as acute, intraperitoneal exposure?); and
- the type of TCDD-induced effect. (For example, although hexachlorobenzene potentiated TCDD-induced thymic atrophy, it is uncertain that hexachlorobenzene will potentiate TCDD immunosuppression. In humans, childhood and adult thymectomy produces no adverse effects on immune function. In addition, thymectomy of adult animals did not modify TCDD-induced suppression of antibody response to sheep red blood cells, and suppression of immune responses occurred at dose levels significantly lower than those required to produce thymic atrophy in adult animals [see Kerkvliet 1994 for review]).

In summary, an acute intraperitoneal administration study of rats found that pretreatment with hexachlorobenzene potentiated 2,3,7,8-TCDD-induced effects on body weight and thymus weight (Li et al. 1989), but the study design has several limitations (e.g., incomplete characterization of the dose-response relationships for the individual compounds and dependence of the apparent potentiation on relative dose levels) that do not allow a full characterization of the possible potentiation of hexachlorobenzene on these TCDD effects. No additional studies were located that further examined or replicated this effect, and a plausible mechanistic explanation of the reported potentiation is not readily apparent.

Studies designed to examine the possible influence of 2,3,7,8-TCDD on hexachlorobenzene toxicity were not located. Oxidative metabolism of hexachlorobenzene is important to the expression of hexachlorobenzene induction of hepatic porphyria and perhaps other hexachlorobenzene-induced effects, but the involvement of the parent material in some effects has also been proposed (e.g., thyroid disruption; see van Raaij et al. 1993 and Appendix B). 2,3,7,8 TCDD is well-known as a potent inducer of CYP1A isozymes (Kohn et al. 1996), but rat experiments with triacetyloleandomycin (TAO), a selective inhibitor of CYP3A4, indicate that CYP3A4 enzyme activities are important for the expression of hexachlorobenzene hepatic porphyria (den Besten et al. 1993). den Besten et al. (1993) postulated that uroporphyrinogen decarboxylase is inhibited by an as yet unidentified reactive intermediate that is formed in the liver during the CYP3A4-catalyzed transformation of hexachlorobenzene to pentachlorophenol, based on observations that repeated exposure of rats to high doses of pentachlorobenzene (which is also metabolized to pentachlorophenol and tetrachlorohydroquinone) did not induce porphyria. Even if TCDD may induce enzymes involved in the metabolism of hexachlorobenzene, capabilities of

downstream enzymes (e.g., Phase II enzymes) may be sufficient, or may also be induced, so that increased concentrations of toxic metabolites may not occur with co-exposure to TCDD relative to hexachlorobenzene alone.

Mechanistic understanding of other hexachlorobenzene-induced health effects (such as neurological effects, decreased circulating levels of thyroid hormones, and disruption of female reproductive organs) is insufficient to clearly indicate whether TCDD induction of CYP or Phase II enzymes involved in hexachlorobenzene metabolism will influence hexachlorobenzene toxicity. Although it is known that hexachlorobenzene can bind to the Ah receptor *in vitro* with an affinity that is 10,000-fold less than 2,3,7,8-TCDD (Hahn et al. 1989), the degree to which the Ah receptor is involved in the expression of hexachlorobenzene toxicity is unknown.

Table 5 provides a brief summary of the toxicological interaction data for 2,3,7,8-TCDD and hexachlorobenzene. These data were discussed in more detail in the text.

**Table 5. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of Hexachlorobenzene and the Influence of Hexachlorobenzene on Toxicity/Carcinogenicity of 2,3,7,8-TCDD by Sequential Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal Exposure (mg/kg/day)						
2,3,7,8-TCDD Influence on Toxicity/Carcinogenicity of Hexachlorobenzene						
Acute	thymus and body weight		0.010 or 0.030 <sup>a</sup> +113.9 (r) <sup>b</sup>		No apparent interaction, but hexachlorobenzene treatment alone at doses tested was without effect on these endpoints.	Li et al. 1989
Hexachlorobenzene Influence on Toxicity/Carcinogenicity of 2,3,7,8-TCDD						
Acute	thymus and body weight	113.9+0.010 or 0.030 (r)			Greater than additive	Li et al. 1989

<sup>a</sup>First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup>Species code: r = rat

## 2.2.2 2,3,7,8-TCDD and *p,p'*-DDE

2,3,7,8-TCDD and *p,p'*-DDE have both been demonstrated to disrupt the development of the male rat reproductive system (i.e., cause antiandrogenic effects) and are thought to disrupt development by different mechanisms (see Table 1 and Appendices A and C). Administration of single doses of 2,3,7,8-TCDD as low as 0.16–1 µg/kg to pregnant rats on gestation day 15 (a treatment that is thought to produce *in utero* and lactational exposure of offspring) produced a host of effects on the reproductive system of male offspring without affecting plasma androgen levels (Bjerke et al. 1994; Gray et al. 1997; Roman et al. 1995, 1998a, 1998b). Observed antiandrogenic effects include decreased accessory sex organ weights including prostate, decreased daily sperm production, and decreased cauda epididymal sperm number; decreased responsiveness of the adult prostate to androgenic stimulation; shortened anogenital distance, and decreased messenger ribonucleic acid (mRNA) levels of prostatic androgen-regulated genes. It has been hypothesized that 2,3,7,8-TCDD, via an initial interaction with the Ah receptor, may indirectly affect androgen signaling by altering growth factor pathways, but specific molecular events have not been determined (Gray et al. 1995; Roman et al. 1998b). Antiandrogenic effects observed in male offspring rats following *in utero* and lactational exposure to *p,p'*-DDE include shortened anogenital distance, increased nipple retention, decreased prostate weight and cauda epididymal sperm number, and delayed attainment of puberty (see Loeffler and Peterson 1999 for review). These effects, like those from 2,3,7,8-TCDD, are observed without changes in circulating androgen levels, but *p,p'*-DDE is a direct competitive inhibitor of ligand binding to the androgen receptor (Kelce et al. 1995, 1997), whereas 2,3,7,8-TCDD is not expected to interfere with androgen receptor-ligand binding (Roman et al. 1998b).

Co-exposure of pregnant rats to oral doses of 2,3,7,8-TCDD and *p,p'*-DDE in a corn oil (95%)/ acetone (5%) vehicle produced a statistically significant greater percentage reduction in the average relative weights of the ventral or dorsolateral prostates of male offspring at weaning (65 and 70% of controls at postnatal day 21, respectively), compared with TCDD exposure alone (81 and 86% of controls) or *p,p'*-DDE exposure alone (83 and 83% of controls) (Loeffler and Peterson 1999). The effects of these compounds alone or in combinations on prostate weight were transient (observed at 21 postnatal days) and were not observed at postnatal days 49 or 63. Pregnant rats were exposed to 0.25 µg 2,3,7,8-TCDD alone/kg on gestation day 15, 100 mg *p,p'*-DDE alone/kg on gestation days 14–18, or a combination of these two protocols. As expected, serum levels of androgens (3-alpha diol, testosterone) in male offspring were not affected (compared with controls) by *in utero* and lactational exposure to either chemical alone or to the mixture. Cauda epididymal sperm numbers at postnatal day 63 in male offspring were

significantly decreased by exposure to 2,3,7,8-TCDD or *p,p'*-DDE alone (decreased by 16.7 or 17.6% compared with controls, respectively), but mixed exposure did not significantly decrease the number further (22%). Patterns of immunostaining with anti-androgen receptor antibody in prostate tissue of male offspring exposed to the mixture showed qualitative characteristics of the effects of both compounds individually. Several other measures of antiandrogenic activity were examined, but no significant exposure-related effects were observed in any of the exposed groups. These endpoints have been demonstrated to be affected in male rat offspring at dosage levels of 2,3,7,8-TCDD or *p,p'*-DDE higher than those used in this study and included anogenital distance, age of puberty, weights of other accessory sex organs (seminal vesicles, epididymides, and testes), daily sperm production at postnatal days 49 or 63, and prostate levels of mRNA for several androgen-regulated genes.

The authors variously referred to the response on prostate weight to the mixture of TCDD and *p,p'*-DDE as augmented, potentiated, and additive, but acknowledged that the design of the study is inadequate to make definitive conclusions regarding the mode of joint action (additive, greater-than-additive, or less-than-additive) (Loeffler and Peterson 1999). To conduct a more rigorous examination, the authors noted that “several doses of each compound would have to be tested in various combinations (i.e., isobolographic design)”. A simple non-statistical analysis of the data provides a rough indication that the organ weight response to the mixture may be explained by additivity, but the reliance on an assumption of a linear dose-response relationship in this analysis and the lack of a statistical test precludes discarding the possibility of greater-than-additive or less-than-additive joint action on this endpoint. In this analysis, the differences in the average 21 day postnatal relative prostate weights for the TCDD alone group and the control group mean (about 0.11 mg/g body weight calculated from data in Figure 3 of Loeffler and Peterson 1999) and the *p,p'*-DDE group mean and the control mean (about 0.12 mg/g body weight) are summed (0.23 mg/g body weight) and compared with the change in relative prostate weight produced by the mixture (about 0.20 mg/g body weight). The predicted “additive” response of the individual components (0.23 mg/g body weight) is similar to the observed response to the mixture (0.20 mg/g body weight), but application of statistical tests (that account for experimental and biological variability) to compare these values is not possible.

The sum of changes in relative prostate weights induced by 2,3,7,8-TCDD and *p,p'*-DDE alone compared with the response to the binary mixture suggests that these compounds may additively affect male rat prostate weight development, but more definitive tests of this hypothesis would require an experimental design with several doses of each compound and a statistical test of additivity. The available results provide no evidence for marked synergistic or antagonistic interactions between concurrent oral exposure



to doses of 2,3,7,8-TCDD and *p,p'*-DDE. Mixed exposure did not affect several other measures of antiandrogenic activity that have been demonstrated to be adversely affected by doses of the individual compounds higher than those used in this study.

Table 6 provides a brief summary of the toxicological interaction data for 2,3,7,8-TCDD and *p,p'*-DDE. These data were discussed in more detail in the text.

**Table 6. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of *p,p'*-DDE and the Influence of *p,p'*-DDE on Toxicity/Carcinogenicity of 2,3,7,8-TCDD by Simultaneous Exposure**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day)						
<i>2,3,7,8</i> -TCDD Influence on Toxicity/Carcinogenicity of <i>p,p'</i> -DDE						
Acute	prostate weights in offspring		0.00025 <sup>a</sup> +100 (r) <sup>b</sup>		Additive action is suggested, but the study design precludes discarding the possibility of greater-than or less-than-additive action.	Loeffler and Peterson 1999
<i>p,p'</i> -DDE Influence on Toxicity/Carcinogenicity of 2,3,7,8-TCDD						
Acute	prostate weights in offspring		100+0.00025 (r)		Additive action is suggested, but the study design precludes discarding the possibility of greater-than or less-than-additive action.	Loeffler and Peterson 1999

<sup>a</sup>First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup>Species code: r = rat

### 2.2.3 Hexachlorobenzene and *p,p'*-DDE

No *in vitro* or *in vivo* studies were located regarding possible interactions between hexachlorobenzene and *p,p'*-DDE in affecting health-related endpoints in humans or animals.

Sensitive shared targets of hexachlorobenzene and *p,p'*-DDE oral toxicity include the liver (hepatomegaly and degenerative histological effects), immune system (suppression of humoral and cell-mediated immunological responses), and pre- and post-natal neurological development (altered neurobehavior) (see Table 1 and Appendices B and C). Both chemicals produce cancer in orally exposed animals.

Hepatic porphyria from repeated exposure to hexachlorobenzene has been postulated to be dependent on CYP3A4- or CYP1A-mediated metabolism and to involve an unidentified reactive intermediate formed during transformation to pentachlorophenol (see Appendix B). DDT, DDE, and DDD have been demonstrated in rats to induce hepatic CYP2B1, and to a lesser degree CYP3A4, but not CYP1A isozymes (see Appendix C). If simultaneous exposure to DDE and hexachlorobenzene cause an increased induction of CYP3A4 enzymes (compared with hexachlorobenzene alone) so that capabilities of Phase II enzymes to control liver concentrations of the reactive hexachlorobenzene metabolite are exceeded, then a potentiation of hexachlorobenzene-induced liver toxicity may occur. No studies were located that investigated hepatic metabolic interactions between hexachlorobenzene and *p,p'*-DDE (or DDT), but this projection is not reliable given that hexachlorobenzene can induce its own metabolism and downstream Phase II enzymes would need to be saturated for any potentiation to occur.

In the absence of relevant interaction data or sufficient mechanistic understanding, an unambiguous projection of interactions between *p,p'*-DDE and hexachlorobenzene cannot be made. Future studies designed to examine possible interactions of hexachlorobenzene and *p,p'*-DDE in affecting neurological, developmental, hepatic, immunological, or cancer endpoints following oral exposure may help to determine if interactions occur.

### 2.2.4 2,3,7,8-TCDD and Methylmercury

2,3,7,8-TCDD, other CDDs, CDFs, and PCBs have been demonstrated to produce immunotoxic effects in animals such as lymphoid tissue depletion and increased susceptibility to infectious agents (Kerkvliet 1994; see Appendix A). Mechanistic studies indicate that immunotoxic effects induced by acute or subacute exposures to 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons are likely to involve

initial mediation by the Ah receptor and multiple sites within the immune system (Kerkvliet 1994). Animal studies with mercury indicate that mercuric salts and methylmercury can cause both an autoimmune stimulation and a suppression of the immune system depending on dose and genetic characteristics (ATSDR 1999b; see Appendix D). For example, 2-week oral exposure to 14.8 mg Hg/kg/day as mercuric chloride decreased thymus weight in mice, 0.7 mg Hg/kg/day as mercuric chloride increased lymphoproliferative responsiveness to T-cell mitogens in another mouse strain known to be sensitive to mercury-induced autoimmunity, and 0.5 mg Hg/kg/day as methylmercury in the diet suppressed natural killer cell activity in another strain of mouse that is resistant to mercury-induced autoimmunity (ATSDR 1999b; Ilback 1991).

Two *in vitro* studies of immunological endpoints in rat cultured lymphocytes found no evidence for interactions between methylmercury and a synthetic mixture of CDDs, CDFs, and PCBs at low concentrations that were reflective of concentrations in flesh of St. Lawrence River fish (Omara et al. 1997, 1998).

In the first experiment (Omara et al. 1997), cell viability, cell proliferation in response to T- and B-cell mitogens, and intracellular calcium concentrations were measured in cultured rat lymphocytes exposed for 72 hours to methylmercury, CDDs+CDFs, PCBs, or a methylmercury/CDD/CDF/PCB mixture. Culture-medium concentrations were reflective of the extremes of the ranges of concentrations found in flesh of fish from the St. Lawrence River: methylmercury (0.1 or 2 µg/mL), a CDD/CDF mixture of 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin, 2,3,7,8-TCDF, and 1,2,3,7,8-pentachlorodibenzofuran in a 20:5:2.5:80:15 weight proportion (0.01 or 0.5 µg/mL), and a PCB mixture of Aroclor 1242, 1254, and 1260 in a 3:4:3 weight proportion (0.01 or 0.5 µg/mL). Mixed exposures included all possible combinations of these concentrations of methylmercury, the CDD/CDF mixture, and the PCB mixture (e.g., low methylmercury+high CDD/CDF+high PCB; low methylmercury+high CDD/CDF+low PCB; etc.; n=8).

No significant effects on lymphocyte cell viability or calcium contents were found following exposure to either concentrations of the CDD/CDF or PCB mixtures alone, or to methylmercury alone at the lower concentration or in combination with the CDD/CDF/PCB mixtures (Omara et al. 1997). Exposure to the CDD/CDF or PCB mixtures alone, at the tested concentrations, did not suppress the lymphocytic response to any of the test mitogens, but stimulated the response of splenic and peripheral blood lymphocytes to one of the tested mitogens, concanavalin A. In contrast, methylmercury, alone at 2 µg/mL, significantly decreased viability of the lymphocytes after 4 or 24 hours of exposure, and, at

0.1 µg/mL, significantly suppressed the responses of the different types of lymphocytes to several T- and B-cell mitogens (Omara et al. 1997). The effects of methylmercury on lymphocyte viability and lymphocyte mitogenic ability were not significantly different in the presence of the CDD/CDF/PCB mixtures.

In a similar design, the second experiment measured rat splenocyte mixed leukocyte reaction, splenic natural killer cell activity, and phagocytic activities of splenic, peritoneal, and peripheral blood lymphocytes after 24- or 72-hour exposure (Omara et al. 1998). The high concentration of methylmercury (2 µg/mL), alone or in combination with the CDD/CDF/PCB mixtures, was cytolethal to rat splenocytes, peritoneal lymphocytes, and peripheral blood lymphocytes. Exposure to the lower methylmercury concentration (0.1 µg/mL), alone or in combination with the CDD/CDF/PCB mixtures, caused no significant suppression of splenocyte mixed leukocyte reaction, splenic natural killer cell-mediated lysis of Yac-1 cells, or phagocytosis of fluorescent beads by splenic, peritoneal, or peripheral blood lymphocytes.

No other *in vitro* or *in vivo* studies were located regarding possible interactions between 2,3,7,8-TCDD (or other CDDs) and methylmercury (or other forms of mercury) in affecting other health-related endpoints in humans or animals.

Results from the Omara et al. (1997, 1998) experiments provide no evidence of interactions or additivity between methylmercury and Ah-receptor-interacting halogenated aromatic hydrocarbons in affecting a number of immunological endpoints in rat lymphocytes under low-level, *in vitro* conditions. The levels of CDDs, CDFs, and PCBs examined in these experiments were below thresholds for immunosuppressive effects. This feature of the study design restricts conclusions that can be made from the data about the modes (additive, greater-than-additive, or less-than-additive) of possible joint action of the components on the endpoints examined. In summary, the data show that at the doses tested, CDDs, CDFs, and PCBs did not significantly change (enhance or antagonize) the *in vitro* immunosuppressive effects (suppression of lymphocyte viability and mitogenic ability) caused by methylmercury alone and did not jointly act with methylmercury to produce effects that were not caused by methylmercury alone. CDDs, CDFs, PCBs, and methylmercury have all been demonstrated to produce various adverse effects on the immune system, but the available results provide no information on how methylmercury may influence the effects of CDDs, CDFs, or PCBs on the evaluated immune system endpoints and thus, only limited information on joint action CDDs and methylmercury on the immune system.

### 2.2.5 Hexachlorobenzene and Methylmercury

No *in vitro* or *in vivo* studies were located regarding possible interactions specifically between hexachlorobenzene and methylmercury, but two published studies have looked for possible acute-exposure interactions between hexachlorobenzene, HCB, and mercuric chloride,  $\text{HgCl}_2$  (Lecavalier et al. 1994; Renner 1980). Given that the health effects from methylmercury are thought to be mediated by the divalent mercuric ion, these studies are evaluated herein with the recognition that they can provide no information of potential interactions with hexachlorobenzene at steps involved in the absorption, distribution, and metabolism of methylmercury, processes that are responsible for the greater potency of methylmercury compared with mercuric salts (ATSDR 1999b; see Appendix D).

In the most extensive study (with respect to endpoints examined), groups of 10 female Sprague-Dawley rats were exposed to single gavage doses of 0, 400, or 600 mg HCB/kg; 0, 10, or 12.5  $\text{HgCl}_2$ /kg; or (400 mg HCB+10 mg  $\text{HgCl}_2$ )/kg, (400 mg HCB+12.5 mg  $\text{HgCl}_2$ )/kg, (600 mg HCB+10 mg  $\text{HgCl}_2$ )/kg, or (600 mg HCB+12.5 mg  $\text{HgCl}_2$ )/kg (Lecavalier et al. 1994). Endpoints included mortality by the end of a 14-day observation period, necropsy examination, histopathological examination of about 30 tissues and organs from surviving animals, hematological variables, serum clinical chemistry variables, several hepatic microsomal CYP enzyme activities (e.g., ethoxyresorufin deethylase), and residues of hexachlorobenzene and mercury in samples of brain, liver, kidney, spleen, serum, and fat.

Deaths occurred in the 600-mg/kg hexachlorobenzene groups, but incidences were not markedly influenced by co-exposure to mercury (1/10, 1/10, and 2/10 deaths occurred in mixed 600-mg HCB/kg groups with 0, 10, or 12.5 mg  $\text{HgCl}_2$ /kg). Liver weights were significantly increased in hexachlorobenzene-exposed groups without significant elevations in hepatic CYP enzymes or serum enzyme activities indicative of liver damage. Histopathological examinations showed mild to moderate hepatic cytoplasmic vacuolation, anisokaryosis, and pyknotic hepatocytes in hexachlorobenzene-exposed groups. Mercury exposure alone did not significantly change liver endpoints compared with controls. Lecavalier et al. (1994) reported that co-administration of mercury did not markedly change the liver endpoints compared with hexachlorobenzene alone. Hexachlorobenzene-exposed groups also showed histopathological changes in the thyroid (reduced follicle size and colloid density and increased epithelial height) and thymus (reduced cortical and medullary volume), whereas mercury-exposed groups showed renal histopathological changes (protein casts, cellular casts, and interstitial sclerosis). The “severity of these changes” was reported to be “additive in nature” for the mixed exposure groups, but the data were not presented in a way that this contention could be quantitatively assessed. Levels of hexachlorobenzene or

mercury in serum, brain, kidney, liver, or fat were not significantly different in the mixed exposure groups compared with the groups exposed to hexachlorobenzene or mercury alone. Although the design and reporting of this study are inadequate to fully characterize the modes of possible joint actions of hexachlorobenzene and mercury on indices of liver damage and histopathological changes in tissues, the results indicate that mercury co-exposure (at doses of 10 or 12.5 mg/kg) did not change the low incidence of lethality in the high-dose (600 mg/kg) hexachlorobenzene groups and that mercury co-exposure did not change liver responses to hexachlorobenzene.

In an earlier study, groups of 10 female Sprague-Dawley rats were given single doses (in a corn oil vehicle) of 0, 400, 600, or 800 mg HCB/kg; and 0, 3.6, 6, 8, 10, 12.5, or 18 mg HgCl<sub>2</sub>/kg (Renner et al. 1980). Other groups were given mixtures of 3.6, 6, 8, 10, 12.5, or 18 mg HgCl<sub>2</sub>/kg with 400 mg HCB/kg or 6, 12.5, or 18 mg HgCl<sub>2</sub>/kg with 600 mg HCB/kg. No mortality occurred in groups exposed to hexachlorobenzene alone. Mortalities occurred in groups exposed to mercuric chloride alone at 12.5 mg/kg (1/10) and 18 mg/kg (5/10). Mortalities occurred in the presence of 400 mg hexachlorobenzene and mercuric chloride at doses of 10 mg/kg (10/10), 12.5 mg/kg (1/10), and 18 mg/kg (10/10). In the presence of 600 mg hexachlorobenzene and mercuric chloride, no rats died with co-exposure to 6 mg HgCl<sub>2</sub>/kg, but all rats died with co-exposure to 12.5 or 18 mg HgCl<sub>2</sub>/kg. The study design is inadequate to evaluate the mode of possible joint action of mercury and hexachlorobenzene in producing lethality in rats (e.g., a dose-response relationship for lethality from hexachlorobenzene was not characterized to any degree), but some evidence is presented that co-exposure to 400 or 600 mg/kg hexachlorobenzene increased mercury-induced lethality at 4 or 5 mercury dose levels of 10 mg/kg or greater.

In summary, Renner (1980) reported that 400 or 600 mg/kg doses of hexachlorobenzene potentiated mercuric chloride acute lethality in rats (given 10, 12.5, or 18 mg HgCl<sub>2</sub>/kg doses), but Lecavalier et al. (1994) did not find that 10 or 12.5 mg HgCl<sub>2</sub>/kg produced lethality in rats in the presence or absence of 400 or 600 mg/kg hexachlorobenzene. Examining other endpoints after acute oral exposure scenarios involving several dose levels of hexachlorobenzene and mercuric chloride (alone or in combination), Lecavalier et al. (1994) reported that no evidence was found in rats that mercuric chloride affected hexachlorobenzene-induced liver, thyroid, or thymus effects or that hexachlorobenzene affected mercury-induced kidney effects, but the results were inadequately reported to allow quantitative assessment. No studies were located regarding potential interactions between these chemicals under repeated exposure scenarios, and no information was found regarding interactions between hexachlorobenzene and methylmercury under any exposure scenario.

Because no data regarding the toxicological interactions of hexachlorobenzene with *methylmercury* were available, Table 7 provides a brief summary of the data for hexachlorobenzene and *mercuric chloride*.

Further discussion of these data and their relevance to the chemicals of concern was provided in the text.



**Table 7. Summary of Available Data on the Influence of Hexachlorobenzene on Toxicity/Carcinogenicity of Mercuric Chloride and the Influence of Mercuric Chloride on Toxicity/Carcinogenicity of Hexachlorobenzene by Simultaneous Exposure<sup>a</sup>**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day)						
Hexachlorobenzene Influence on Toxicity/Carcinogenicity of Mercuric Chloride						
Acute	lethality	400 <sup>b</sup> +10 (r) 600+10 (r)	400+10 (r) 600+10 (r)		Indeterminate due to conflicting results from similar experiments.	Renner 1980 Renner 1980 Lecavalier 1994 Lecavalier 1994
Mercuric Chloride Influence on Toxicity/Carcinogenicity of Hexachlorobenzene						
Acute	hepatocyte vacuolation		10+400 (r) 12.5+400 (r) 10+600 (r) 12.5+600 (r)		Additivity was reported; data were not presented, so conclusion could not be evaluated.	Lecavalier 1994 Lecavalier 1994 Lecavalier 1994 Lecavalier 1994

<sup>a</sup>Data for the pair, *methylmercury* and hexachlorobenzene, were not located, so available data for mercuric chloride and hexachlorobenzene were evaluated as explained in text.

<sup>b</sup>First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>c</sup>Species code: r = rat

### 2.2.6 *p,p'*-DDE and Methylmercury

No *in vitro* or *in vivo* studies were located regarding possible interactions between *p,p'*-DDE and methylmercury (or other forms of mercury) in affecting health-related endpoints in humans or animals.

Oral exposure to either *p,p'*-DDE or methylmercury has been shown to adversely influence pre- and post-natal neurological development, and both compounds are known to produce adverse effects on the fully developed neurological system (see Table 1 and Appendices C and D). Joint actions between these two compounds in producing deficits in neurological development or function are plausible, but modes of possible joint action are unknown and unstudied. Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects on mitosis through impairment of microtubule assembly, inhibition of various enzymes such as protein kinase C, inhibition of transport mechanisms in developing brain cells, and alteration of synaptosomal release of neurotransmitters (e.g., dopamine) that may involve non-specific changes in membrane integrity or disruption of calcium homeostasis and subsequent activation of second messenger system or cell death (Appendix D).

*p,p'*-DDE's actions on the developing and mature neurological system are poorly understood (see Appendix C), but, as with *p,p'*-DDT, interference with sodium channels and potassium gates in neuronal membranes and inhibition of neuronal ATPases may be involved. Obvious cellular or molecular sites of possible interactions between *p,p'*-DDE and methylmercury are not apparent.

### 2.2.7 PCBs and 2,3,7,8-TCDD

Mixtures of PCBs display a wide array of effects that have considerable similarities with the wide array of effects produced by 2,3,7,8-TCDD and other CDDs (see Appendices A and E). Some PCB congeners are thought to produce toxic effects through initial mechanisms of action (e.g., Ah-receptor mediation and induction of hepatic CYP1A1, 1A2, and 1B1, and associated Phase II enzymes) that are shared with 2,3,7,8-TCDD, although 2,3,7,8-TCDD is generally much more potent than PCB mixtures or individual PCB congeners. Thus, the Ah receptor represents a likely molecular site for interactions between PCBs and 2,3,7,8-TCDD that can have toxicological significance. Based on assumptions of a shared initial mechanism involving the Ah receptor and additive joint action, a component-based Toxic Equivalency Factor (TEF) approach to evaluating health hazards from complex mixtures containing CDDs, CDFs, and PCBs has been used to some extent (see Appendices A and E). However, PCB congeners with Ah-receptor affinity are minor components in commercial and environmental PCB mixtures, and there is increasing evidence that PCB congeners can produce toxic effects through both Ah receptor dependent

and independent mechanisms (see Appendix E). In addition, there is some evidence for a few endpoints, reviewed herein, that there may be non-additive interactions between 2,3,7,8-TCDD and PCB mixtures or congeners. Consequently, it has been recommended that the TEF approach should be used cautiously, especially for complex mixtures in which PCBs may predominate (Hansen 1998; Safe 1998a, 1998b). To conduct exposure-based health assessments involving mixtures of CDDs, CDFs, and PCBs, ATSDR (1998) has recommended using the TEF, but also has derived MRLs for PCB mixtures for the same purpose (ATSDR 2000). The PCB MRLs are based on health effects from commercial PCB mixtures (e.g., Aroclor 1254) or synthetic mixtures of PCBs designed to mimic composition of environmental PCB mixtures. They were derived because PCB congeners with Ah-receptor affinity are minor components in environmental mixtures, multiple mechanisms may be involved in the development of PCB-induced health effects, and non-additive joint actions on toxicity targets may exist between specific PCB congeners and between PCB congeners and CDDs (ATSDR 2000).

### ***Complex PCB mixtures and 2,3,7,8-TCDD***

Studies designed to examine possible binary interactions between complex PCB mixtures and 2,3,7,8-TCDD are restricted to two intraperitoneal-exposure studies examining impaired immune responses to sheep red blood cells in C57BL/6J mice (Bannister et al. 1987; Davis and Safe 1989) and a gavage-exposure study examining cleft palate incidences in offspring of C57BL/6J mice (Haake et al. 1987). Results from this limited database suggest that PCB mixtures antagonize the acute immunotoxicity and the acute developmental toxicity (producing cleft palate) of 2,3,7,8-TCDD, when PCB:TCDD dose ratios were >1,000:1 (see Table 8). Similarly designed studies examining repeated exposure scenarios or other endpoints potentially shared by complex PCB mixtures and 2,3,7,8-TCDD (e.g., cancer or neurodevelopment) do not appear to be available. Whereas the designs of these studies are inadequate to fully characterize the mode of possible joint action of PCB mixtures and 2,3,7,8-TCDD on impairing the immune response to sheep red blood cells and producing cleft palate, they adequately describe conditions under which CDD-effects were antagonized by PCB mixtures and provide some information that the antagonism is dependent on the relative proportions of the PCB mixture dose and the TCDD dose (Davis and Safe 1989).

In response to injection with sheep red blood cells, mice given single intraperitoneal doses of 3.72 or 11.1 nmol 2,3,7,8-TCDD/kg (0.0011 or 0.0036 mg/kg) produced only 156 or 62 plaque-forming cells, respectively, per million viable spleen cells (PFC/10<sup>6</sup> spleen cells), compared with the response of 562 PFC/10<sup>6</sup> spleen cells in non-exposed controls (Bannister et al. 1987). Aroclor 1254 alone displayed

minimal potency in affecting the immune response. Immune responses were not significantly different from control values in mice injected with doses of 5, 15, 75, or 150  $\mu\text{mol}$  Aroclor 1254/kg alone (1.6, 4.9, 24.6, or 49.3 mg/kg), but the high-dose response was marginally impaired (561, 465, 594, or 394 PFC/ $10^6$  spleen cells). Co-injection of 3.72 nmol 2,3,7,8-TCDD/kg with 5, 15, or 75  $\mu\text{mol}$  Aroclor 1254/kg completely restored the immune response to control values (491, 480, or 558 PFC/ $10^6$  spleen cells), whereas co-exposure with 150  $\mu\text{mol}$  Aroclor 1254/kg partially restored the response (372 PFC/ $10^6$  spleen cells). Co-exposure to 11.2 nmol 2,3,7,8-TCDD/kg and 75  $\mu\text{mol}$  Aroclor 1254/kg antagonized the TCDD-induced effect to a limited degree ( $170 \pm 10$  versus  $62 \pm 12$  PFC/ $10^6$  spleen cells for TCDD alone).

In the other intraperitoneal study, mice given 3.7 nmol 2,3,7,8-TCDD/kg (1.2  $\mu\text{g}/\text{kg}$ ) showed a response of 180 PFC/ $10^6$  spleen cells compared with 912 PFC/ $10^6$  spleen cells in non-exposed control mice, and co-exposure with 25 mg/kg of Aroclor 1242, 1248, 1254, or 1260 partially antagonized the TCDD-induced impairment (Davis and Safe 1989). Average respective immune responses in the co-exposed mice were 440, 427, 459, and 459 PFC/ $10^6$  spleen cells; these were significantly higher than the response in mice exposed to TCDD alone. Co-exposure with 25 mg/kg Aroclor 1232 did not significantly change the immune response compared with TCDD exposure alone (244 PFC/ $10^6$  spleen cells). A synthetic mixture of PCB congeners detected in human milk samples also significantly antagonized the immunotoxicity of 3.7 nmol 2,3,7,8-TCDD/kg, at a dose of 50 mg/kg, but not at dose levels of 5 or 25 mg/kg.

Co-treatment of pregnant mice with gavage doses of 20  $\mu\text{g}$  2,3,7,8-TCDD/kg (on gestation day 10) plus 244 mg Aroclor 1254/kg (on gestation day 9) produced 8.2% fetuses with cleft palate per litter compared with 62% per litter in mice treated with TCDD alone (Haake et al. 1987). Exposure to Aroclor 1254 alone or to the corn-oil vehicle produced no fetuses with cleft palate. The same dose level of Aroclor 1254 did not antagonize cleft palate formation induced by 90 mg/kg dexamethasone, a synthetic glucocorticoid hormone analog that is thought to produce developmental effects through an initial binding with glucocorticoid receptors. A lower dose of Aroclor 1254 (250  $\mu\text{mol}/\text{kg}$  or 82 mg/kg) was less effective as a TCDD antagonist, but specific data were not reported (Biegel et al. 1989a).

### ***Less complex PCB mixtures and 2,3,7,8-TCDD***

To examine possible interactions between mixtures of PCBs and 2,3,7,8-TCDD associated with repeated exposure, liver tumor promotion activity in partially hepatectomized rats exposed to a mixture containing 68 ppm 2,3,7,8-TCDD, 223-ppm 1,2,3,7,8-pentachloro-*p*-dioxin, 1,151-ppm 2,3,4,7,8-pentachlorodibenzofuran, 4,130 ppm 3,3',4,4',5-pentachlorobiphenyl, 866,604 ppm 2,3',4,4',5-pentachlorobiphenyl, and 127,824 ppm 2,3,3',4,4',5-hexachlorobiphenyl was compared with predicted tumor promotion activity using TEFs based on tumor promotion activity of the individual components compared to TCDD activity (van der Plas et al. 1999). The mixture composition was reflective of relative concentrations, and accounted for approximately 90% of TCDD toxic equivalents (TEQs), found in Baltic Sea fish samples. Tumor promotion activity was measured in groups of female Sprague-Dawley rats (number and volume of glutathione S-transferase-positive foci in liver) following 20 weekly subcutaneous injections of 0.1 µg 2,3,7,8-TCDD/kg, or 1 µg TEQ/kg of the five-component mixture noted above. Promotion was preceded by initiation with single intraperitoneal injections of 30 mg/kg diethylnitrosamine. Mean foci volume and volume fraction of hepatic foci in the 1-TEQ/kg mixture-exposed groups were about one-half of values for the group promoted with TCDD alone. A six-component mixture was also examined; it contained in addition to the components noted above, 20,000 g 2,2',4,4',5,5'-hexachlorobiphenyl per g of 2,3,7,8-TCDD. Tumor promotion activity for this mixture was greater than that of the five-component mixture, but was still less than that predicted by the TEF method. One possible explanation of the difference between the observed and TEF predicted values is that the components may have interacted in a less-than-additive manner (e.g., less potent PCBs may antagonize tumor promotion by the more potent 2,3,7,8-TCDD), but equally as plausible is the possibility that the TEFs are inaccurate and overestimate tumor promotion potencies.

### ***PCB congeners and 2,3,7,8-TCDD—Acute exposures***

A study of possible binary *in vitro* interactions between 2,3,7,8-TCDD and two PCB congeners in promoting malignant transformation of carcinogen-initiated cultured mouse fibroblasts found that one congener (2,2',4,4',5,5'-hexachlorobiphenyl—a congener without Ah-receptor-agonist activity) antagonized TCDD promotion of transformation, whereas 3,3',4,4',5-pentachlorobiphenyl (which displays Ah-receptor-agonist activity) added to promotion of transformation in the presence of 2,3,7,8-TCDD (Wolfle 1998). These *in vitro* observations of apparent additive joint action of 3,3',4,4',5-pentachlorobiphenyl and 2,3,7,8-TCDD in promoting malignant transformation of mouse fibroblasts concur with observations from subcutaneous-exposure studies observing apparent additive joint action of these

specific congeners in promoting liver tumors in rats (Hemming et al. 1995).

Blockage of ovulation, reduction of ovarian weight gain, and changes in preovulatory hormone levels have been observed in gonadotropin-primed immature female rats given single doses of 2,3,7,8-TCDD and other CDDs alone and in combination (Gao et al. 1999). The slopes of the dose-response relationships for the CDD components were similar to the slope for an equipotent mixture (expressed in total toxic equivalents relative to 2,3,7,8-TCDD [TEQ]), indicating additive joint action by a common mechanism. Comparable similarities in slopes were observed for dose-response relationships for 3,3',4,4',5-pentachlorobiphenyl and an equipotent mixture containing this PCB plus 2,3,4,7,8-pentachlorodibenzofuran, 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (Gao et al. 2000). Another PCB congener (2,2',4,4'-tetrachlorobiphenyl) was found to be inactive in this reproductive toxicity assay, but the effect of its presence in a mixture with the effective components was not examined (Gao et al. 2000).

There are several studies examining possible interactions within binary mixtures of a few PCB congeners and 2,3,7,8-TCDD in suppressing immune responses to sheep red blood cells and producing cleft palate during *in utero* development, using similar acute exposure protocols and the same mouse strain as studies observing antagonism by complex PCB mixtures of TCDD effects on these endpoints (see Table 8). Examined PCB congeners either did not produce these effects or were so much less potent than 2,3,7,8-TCDD that, with co-exposure treatments, TCDD effects predominated. The limited information from these studies indicates that non-additive interactions, both potentiation and antagonism, can occur between PCB congeners and 2,3,7,8-TCDD, depending on the endpoint, the PCB congener (some are antagonistic, one has been identified as synergistic, and some show no effect on TCDD toxicity), the PCB dose, and/or the PCB:TCDD dose ratio. Mechanistic understanding of these non-additive interactions is poor, and extensive studies examining how PCB molecular structure parameters may be associated with these interactions have not been carried out. Nevertheless, the limited number of congeners and endpoints examined indicate the possibility of wide variance in how individual PCB congeners may interact with 2,3,7,8-TCDD in producing these acute effects.

Seven congeners (six hexachlorobiphenyls and one pentachlorobiphenyl) were examined for binary joint action with 2,3,7,8-TCDD in producing immune suppression (Biegel et al. 1989b; Davis and Safe 1990; Smialowicz et al. 1997). Three (2,3,3',4,5,5'- and 2,2',4,4',5,5'-hexachlorobiphenyl, and 2,3,3',4,5'-pentachlorobiphenyl) were partially antagonistic (i.e., they did not completely prevent the immune suppression). Relationships between PCB:TCDD dose ratio and antagonism varied among the

antagonistic congeners. For example, antagonism by 2,2',4,4',5,5'-hexachlorobiphenyl was observed at weight ratios above about 330,000:1, but not at ratios below about 40,000:1 (Biegel et al. 1989b; Smialowicz et al. 1997). In contrast, antagonism by 2,3,3',4,5,5'-hexachlorobiphenyl occurred at ratios above about 15,000:1, but not at 6,000:1 (Davis and Safe 1990). Among the non-antagonistic congeners, some were immunotoxic, showing variable potencies (e.g., the potency of 2,3,3',4,4',5-hexachlorobiphenyl was about 1,000-fold greater than that of 2,2',4,4',5,5'-hexachlorobiphenyl), and others were not immunotoxic (2,2',4,4',6,6'-hexachlorobiphenyl).

The mechanism involved in PCB antagonism of TCDD acute immunosuppression is unknown. Davis and Safe (1990) suggested that it may occur by mechanisms other than through the Ah receptor, as some antagonistic PCB congeners (e.g., 2,2',4,4',5,5'-hexachlorobiphenyl) do not displace TCDD from the Ah receptor. Smialowicz et al. (1997) proposed that the antagonism was a functional antagonism (two chemicals producing opposite effects on the same physiological function) based on observations that oral exposure to 2,2',4,4',5,5'-hexachlorobiphenyl alone enhanced the immune response compared with control values. However, intraperitoneal exposure to 2,2',4,4',5,5'-hexachlorobiphenyl alone did not enhance the immune response to sheep red blood cells in another study (Biegel et al. 1989b).

Three congeners, none of which caused cleft palate by themselves, have been examined for interaction with TCDD-induction of cleft palate and show distinctly different interactions (see Table 8). 2,3,3',4,4',5-Hexachlorobiphenyl markedly potentiated TCDD-induced cleft palate (Birnbaum et al. 1985). For example, gavage doses of 0.003 mg 2,3,7,8-TCDD/kg alone on gestation days 10–13 produced 4.2% of fetuses with cleft palate per litter, but combined exposure with 40 or 80 mg 2,3,3',4,4',5-hexachlorobiphenyl/kg produced 19.9 and 43.1%, respectively. In contrast, 2,2',4,4',5,5'-hexachlorobiphenyl and 2,2',4,4'-tetrachlorobiphenyl were antagonistic (Biegel et al. 1989a, 1989b; Birnbaum et al. 1985; Morrissey et al. 1992). The relationship of antagonism with dose of the 2,2',4,4',5,5'-congener was shown to have an inverted U shape. Percentage of fetuses with cleft palate in groups exposed to 0.015 mg 2,3,7,8-TCDD/kg and 0, 62.5, 125, 250, 500, or 1,000 mg 2,2',4,4',5,5'-hexachlorobiphenyl/kg were 35, 23, 5, 1, 0, and 38% (Morrissey et al. 1992). Results from the earlier studies (Biegel et al. 1989b; Birnbaum et al. 1985) indicated that the examined PCB congeners or TCDD alone induced increased incidence of hydronephrosis in mouse offspring and provided no evidence of non-additive interactions. However, after examining a wider range of 2,2',4,4',5,5'-hexachlorobiphenyl doses in combination with 0.015 mg 2,3,7,8-TCDD/kg, partial antagonism of TCDD-induced hydronephrosis was observed to have a sharp inverted U shaped relationship with PCB dose. Percentages of fetuses per litter with hydronephrosis were 99, 98.1, 97.6, 63.5, and 100% for groups exposed to mixtures of

0.015 mg 2,3,7,8-TCDD plus 0, 62.5, 250, 500, or 1,000 mg 2,2',4,4',5,5'-hexachlorobiphenyl/kg (Morrissey et al. 1992).

The mechanisms of PCB congener antagonism or synergism of TCDD developmental toxicity are unknown. No other data are available regarding how other congeners or other complex PCB mixtures may influence TCDD-induced cleft palate and kidney malformations in mouse offspring.

### ***PCB congeners and 2,3,7,8-TCDD—Intermediate exposures***

A series of studies of female Sprague-Dawley rats exposed to binary mixtures in the diet for 13 weeks examined possible interactions between 2,3,7,8-TCDD and three PCB congeners (one with no ortho chlorines—3,3',4,4',5-pentachlorobiphenyl; one with a single ortho chlorine—2,3,3',4,4',5-hexachlorobiphenyl; and one with two ortho chlorines—2,2',4,4',5,5'-hexachlorobiphenyl) in affecting several endpoints including serum levels of thyroid hormones, body and organ weights, and hepatic levels of porphyrins and retinoids (van Birgelen et al. 1992, 1994a, 1994b, 1996). The PCB:TCDD concentration ratios administered in these studies were chosen to reflect relative concentrations in human milk and fat samples (1.5–450:1, 240–2,400:1, and 2,000–200,000:1 for the 3,3',4,4',5-, 2,3,3',4,4',5-, and 2,2',4,4',5,5'-congeners, respectively).

Results from these studies provide no evidence for synergistic interactions between 3,3',4,4',5-pentachlorobiphenyl and 2,3,7,8-TCDD or 2,3,3',4,4',5-hexachlorobiphenyl and 2,3,7,8-TCDD in decreasing thyroid hormone levels, body weights, thymus weights, or hepatic retinoid levels or in increasing relative liver weights or hepatic levels of porphyrin (see Tables 8 and 9). Some evidence for less-than-additive joint action was found for these endpoints, but these apparent interactions may have been due to near-maximal effects occurring at the dose levels used. For example, hepatic retinol levels after 13 weeks were 14.9, 6.2, 1.5, and 0.6 mg/g liver in rats provided 3,3',4,4',5-pentachlorobiphenyl alone at dietary concentrations of 0, 7, 50, or 180 ppb; whereas in groups whose diet additionally included 0.4 or 5 ppb 2,3,7,8-TCDD, hepatic retinol concentrations were 8.2, 10.7, 4.0, and 4.8 mg/g liver and 2.2, 0.9, 1.8, and 1.4 mg/g liver, respectively (van Birgelen et al. 1994b). Similar results were found for the combined effects of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-TCDD on the same endpoints, with the notable exception of evidence for synergistic action in decreasing thyroid hormone levels (van Birgelen et al. 1992) and increasing hepatic porphyrin levels (van Birgelen et al. 1996). The apparent synergism was especially marked for joint action on hepatic porphyrin levels. Average hepatic levels of porphyrins after 13 weeks on diets containing 0, 10, 30, or 100 ppm 2,2',4,4',5,5'-hexachlorobiphenyl were 1.9, 2.8, 1.4,



and 3.0 µg/g liver (Van Birgelen et al. 1996). In contrast, porphyrin levels were 1.9, 300, 969, and 1,223 µg/g liver and 2.5, 22, 1,527, and 1,094 µg/g liver in rats fed the same respective 2,2',4,4',5,5'-hexachlorobiphenyl concentrations plus 0.5 or 5 ppb 2,3,7,8-TCDD in the diet (van Birgelen et al. 1996).

Tables 8 and 9 summarize the available interactions data for PCBs and 2,3,7,8-TCDD.

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Parenteral Exposure (mg/kg/day) – PCB Mixtures						
Acute	impaired immune response to sheep red blood cells (intraperitoneal)			1.6 <sup>a</sup> +0.0012 (m) <sup>b</sup> 4.9+0.0012 (m) 24.6+0.0012 (m) 49.3+0.0012 (m) 24.6+0.0036 (m)  25+0.0012 (m)  25+0.0012 (m)  5+0.0012 (m) 25+0.0012 (m)	PCB mixtures, with the exception of Aroclor 1232, antagonized the acute immunotoxicity of 2,3,7,8-TCDD in mice, at dose ratios >1,000:1.	Bannister et al. 1987 (Aroclor 1254)  Davis and Safe 1989 (Aroclors 1242, 1248, 1254, 1260)  Davis and Safe 1989 (Aroclor 1232)  Davis and Safe 1989 (PCB mix reflective of human milk)

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Parenteral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl)						
Acute	impaired immune response to sheep red blood cells (intraperitoneal)		0.36–36+0.0012 (m) 7.2+0.0012 (m) 16–32+0.0012 (m) 25–100+0.0012 (m) 36–361+0.0012 (m) 180–361+0.0012(m)  36.1+0.0012 (m)	18–72+0.0012 (m) 65+0.0012 (m)  400–1,000+0.0012 (m)	3/7 Congeners examined partially antagonized the acute immunotoxicity of TCDD; relationships between dose (or dose ratio) and antagonism were different for the antagonistic congeners.	Davis and Safe 1990 (2,3,3',4,4',5-HCB) (2,3,3',4,5,5'-HCB) (2,3,3',4,5'-PeCB) (2,3',4,4',5',6-HCB) (2,2',4,4',5,6'-HCB) (2,2',4,4',6,6'-HCB)  Biegel et al. 1989b (2,2',4,4',5,5'-HCB)
Inter-mediate	liver tumor promotion activity (subcutaneous)		µg/kg/week 1.00+0.100 (r) 3.16+0.316 (r) 10.0+1.00 (r)		Combined exposure with TCDD did not show obvious deviation from additive effect on development of γ-glutamyl transpeptidase altered foci.	Hemming et al. 1995 (3,3',4,4',5-PeCB)

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) – PCB Mixtures						
Acute	increased offspring with cleft palate			244+0.020 (m)	A complex mixture of PCBs completely antagonized TCDD-induced cleft palate formation at examined dose ratio (12,200:1).	Haake et al. 1987 (Aroclor 1254)
Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl)						
Acute	increased offspring with cleft palate	40–80+0.012 (m) 10–20+0.003 (m)	25–50+0.003 (m)  90.2+0.020 (m)  62.5+0.015 (m) 1,000+0.015 (m)	270.7+0.020 (m) 217.5+0.020 (m)  125–500+ 0.015 (m)	1/3 Congeners (2,3,3',4,4',5-) potentiated TCDD-induced cleft palate formation; 2/3 congeners were partially antagonistic (2,2',4,4',5,5'- and 2,2',4,4'-). Relationship between dose and antagonism for 2,2',4,4',5,5'-HCB increased to a maximum then declined to no effect (inverted U shape).	Birnbaum et al. 1985 (2,3,3',4,4',5-HCB) (2,2',4,4',5,5'-HCB)  Biegel et al. 1989a, 1989b (2,2',4,4',5,5'-HCB) (2,2',4,4'-TCB)  Morrissey et al. 1992 (2,2',4,4',5,5'-HCB)

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; TCB = tetrachlorobiphenyl)						
Acute	increased offspring with hydronephrosis		62.5–250+0.015 (m) 1,000+0.015 (m)  90.2+0.020 270.7+0.020	500+0.015 (m)	Relationship between dose and partial antagonism appeared to be a steep inverted U.	Morrissey et al. 1992 (2,2',4,4',5,5'-HCB)  Biegel et al. 1989b (2,2',4,4',5,5'-HCB)
Acute	impaired immune response to sheep red blood cells		3.58+0.001 (m) 35.8+0.001 (m) 3.58–358+0.010 (m)	358+0.001 (m)	Antagonism of low dose of TCDD observed at dose ratio of 358,000:1. No antagonism of high TCDD dose.	Smialowicz et al. 1997 (2,2',4,4',5,5'-HCB)

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl)						
Inter-mediate	decreased thyroid hormone levels (total and free T4)	13 weeks 0.98–9.8+0.00005 (r) 0.98–9.8+0.0005 (r)	4 weeks 0.98–9.8+0.00005 (r) 0.98–9.8+0.0005 (r)	13 weeks 0.08–0.7+0.0003 (r)  13 weeks (µg/kg/day) 0.5–10.1+0.03 (r) 0.4–9.7+0.3 (r)	Joint action of 2,2',4,4',5,5'-HCB and TCDD to decrease T4 levels was synergistic at 13 weeks.  Other two congeners showed less-than-additive joint action with TCDD on thyroid hormone levels.	van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB)  van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)  van Birgelen et al. 1994b (3,3',4,4',5-PeCB)

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl)						
Inter-mediate	decreased body weight and thymus weight; increased relative liver weight		13 weeks 0.98–9.8+0.00005 (r) 0.98–9.8+0.0005 (r)	13 weeks 0.08–0.7+0.0003 (r)  13 weeks (µg/kg/day) 0.5–10.1+0.03 (r) 0.4–9.7+0.3 (r)	2,2',4,4',5-HCB did not influence TCDD effects on body weight and thymus weight, and additively affected relative liver weight.  Other 2 congeners showed less than additive joint action with TCDD on these endpoints.	van der Kolk et al. 1992 (2,2',4,4',5,5'-HCB)  van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)  van Birgelen et al. 1994b (3,3',4,4',5-PeCB)

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) - PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl)						
Inter-mediate	increased hepatic porphyrin levels	13 weeks 0.7–6.4+0.00003 (r) 0.6–5.9+0.0003 (r)	13 weeks 0.08–0.7+0.0003 (r)  13 weeks (µg/kg/day) 0.5–10.4+0.03 (r) 0.4–9.7+0.3 (r)		2,2',4,4',5,5'-HCB + TCDD, at individual non-effective doses, jointly increased liver porphyrin levels by about 11–800 times control levels. Other examined congeners showed no such synergism with TCDD.	van Birgelen et al. 1996 (2,2',4,4',5,5'-HCB)  (2,3,3',4,4',5-HCB)  (3,3',4,4',5-PeCB)



**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) - PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl)						
Inter-mediate	decreased hepatic retinoid levels			13 weeks 0.98–9.8+0.00005 (r) 0.98–9.8+0.0005 (r)	All 3 congeners alone decreased hepatic levels of retinol and retinylpalmitate. In combination with TCDD, less than additive action was indicated, but near maximal decreases occurred at TCDD doses alone.	van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB)
				13 weeks 0.08–0.7+0.0003 (r)		van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)
				13 weeks (µg/kg/day) 0.5–10.1+0.03 (r) 0.4–9.7+0.3 (r)		van Birgelen et al. 1994b (3,3',4,4',5-PeCB)

<sup>a</sup>First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup>Species code: m = mouse; r = rat

**Table 9. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Parenteral Exposure (mg/kg/day) – PCB Mixtures (no data)						
Parenteral Exposure (mg/kg/day) – PCB Congeners (PeCB = pentachlorobiphenyl)						
Inter-mediate	liver tumor promotion activity (subcutaneous)		$\mu\text{g/kg/week}$ 0.100 <sup>a</sup> +1.00 (r) <sup>b</sup> 0.316+3.16 (r) 1.00+10.0 (r)		Combined exposure with TCDD did not show obvious deviation from additive effect on development of $\gamma$ -glutamyl transpeptidase altered foci.	Hemming et al. 1995 (3,3',4,4',5-PeCB)

**Table 9. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) – PCB Mixtures (no data)						
Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl)						
Inter- mediate	decreased thyroid hormone levels (total and free T4)	13 weeks 0.00005+0.98–9.8 (r) 0.0005+0.98–9.8 (r)	4 weeks 0.00005+0.98–9.8 (r) 0.0005+0.98–9.8 (r)	13 weeks 0.0003+0.08–0.7 (r)  13 weeks (µg/kg/day) 0.03+0.5–10.1 (r) 0.3+0.4–9.7 (r)	Joint action of 2,2',4,4',5,5'-HCB and TCDD to decrease T4 levels was synergistic at 13 weeks.  TCDD with other 2 PCB congeners showed less-than-additive joint action on thyroid hormone levels.	van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB)  van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)  van Birgelen et al. 1994b (3,3',4,4',5-PeCB)
Inter- mediate	decreased body weight and thymus weight; increased relative liver weight		13 weeks 0.00005+0.98–9.8 (r) 0.0005+0.98–9.8 (r)	13 weeks 0.0003+0.08–0.7 (r)  13 weeks (µg/kg/day) 0.03+0.5–10.1 (r) 0.3+0.4–9.7 (r)	TCDD with 2,2',4,4',5-HCB additively affected relative liver weight.  TCDD with other 2 congeners showed less than additive joint action on these endpoints.	van der Kolk et al. 1992 (2,2',4,4',5,5'-HCB)  van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)  van Birgelen et al. 1994b (3,3',4,4',5-PeCB)

**Table 9. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) – PCB Mixtures (no data)						
Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl)						
Inter- mediate	increased hepatic porphyrin levels	13 weeks 0.00003+0.7–6.4 (r) 0.0003+0.6–5.9 (r)	13 weeks 0.0003+0.08–0.7 (r)  13 weeks (µg/kg/day) 0.03+0.5–10.4 (r) 0.3+0.4–9.7 (r)		2,2',4,4',5,5'-HCB + TCDD, at individual non-effective doses, jointly increased liver porphyrin levels by about 11–800 times control levels. TCDD with other 2 congeners showed no such synergism.	van Birgelen et al. 1996 (2,2',4,4',5,5'-HCB)  (2,3,3',4,4',5-HCB)  (3,3',4,4',5-PeCB)

**Table 9. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs (continued)**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day) – PCB Mixtures (no data)						
Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl)						
Inter-mediate	decreased hepatic retinoid levels			13 weeks 0.00005+ 0.98–9.8 (r) 0.0005 + 0.98–9.8 (r)  13 weeks 0.0003+0.08–0.7 (r)  13 weeks (µg/kg/day) 0.03+0.5–10.1 (r) 0.3+0.4–9.7 (r)	All 3 congeners or TCDD alone decreased hepatic levels of retinol and retinylpalmitate. In combination, less than additive action was indicated, but near maximal decreases occurred at TCDD doses alone.	van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB)  van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)  van Birgelen et al. 1994b (3,3',4,4',5-PeCB)

<sup>a</sup>First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup>Species code: m = mouse; r = rat

### 2.2.8 PCBs and Hexachlorobenzene

As discussed in Appendices B and E, health effects associated with exposure to PCBs or hexachlorobenzene that are common to both include hepatic porphyria, porphyria cutanea tarda, liver hypertrophy, thyroid hormone homeostasis disruption, immunosuppression, impaired neurological development, and liver cancer. Physiological and molecular processes leading to the development of these effects involve potential sites at which PCBs and hexachlorobenzene may interact in affecting these health endpoints, but no *in vitro* or *in vivo* studies were located that were designed to examine possible interactions between PCBs and hexachlorobenzene. Processes in which potential interactions may occur include: induction of Phase I and II enzymes that may influence porphyria, liver hypertrophy, tissue damage from reactive oxygen species, and liver cancer development; disruption of thyroid hormone homeostasis via binding to thyroid hormone transport proteins, altering thyroid hormone metabolism, or damaging thyroid tissue; and disruption of sex hormone homeostasis via estrogenic, anti-estrogenic, androgenic, or anti-androgenic actions. In general, mechanistic understanding of the processes involved in the development of the common toxicity targets is too incomplete to reliably predict whether mixtures of PCBs and hexachlorobenzene may jointly act in additive, less-than-additive, or greater-than-additive fashions.

### 2.2.9 PCBs and *p,p'*-DDE

Results from animal (and some human) studies identify several sensitive shared targets of PCBs and *p,p'*-DDE oral toxicity including the liver (hepatomegaly, degenerative histological effects, and liver cancer), immune system (suppression of cell-mediated immunological responses), neurological development (altered neurobehavior in offspring exposed *in utero* or during nursing periods), and altered reproductive function or development (see Table 1 and Appendices C and E). A limited amount of *in vitro* and *in vivo* data regarding possible interactions between PCBs and *p,p'*-DDE is available as reviewed below, but the data do not provide information relevant to how PCBs and *p,p'*-DDE may jointly act in affecting shared sensitive targets of public health concern.

Incubation of an estrogen receptor preparation from alligator oviducts with a mixture of *p,p'*-DDE, *p,p'*-DDD, Aroclor 1242, *trans* nonachlor, and *cis* nonachlor or a mixture of these agents plus dieldrin, toxaphene, and chlordane inhibited the binding of tritium-labeled 17 $\beta$ -estradiol to estrogen receptors (Vonier et al. 1996). The individual agents, at the concentrations used in these mixtures, did not inhibit the *in vitro* binding of 17 $\beta$ -estradiol to the estrogen receptors. In the absence of other information, these observations do not provide sufficient evidence of interactions between *p,p'*-DDE and Aroclor 1242 that

may influence reproductive functions or development.

Combined dietary exposure of mallards to 40 ppm *p,p'*-DDE and Aroclor 1254 did not alter DDE-induced egg shell thinning, but appeared to decrease the number of intact eggs that were produced compared with values for control groups or groups exposed to either agent alone (Risebrough and Anderson 1975). Dietary exposure of groups of mallards (4 drakes and 10 hens) to 40 ppm *p,p'*-DDE or 40 ppm *p,p'*-DDE +40 ppm Aroclor 1254 for 5 months caused 17 and 19% reduction in mean egg shell thickness compared with control groups (Risebrough and Anderson 1975). Exposure to 40 ppm Aroclor 1254 alone did not affect egg shell thickness. Combined exposure reduced total egg production over the study period by about 35% compared with controls. Egg production in the first 7 weeks was similar in all groups, but markedly dropped thereafter in the DDE+Aroclor 1254 group. About 25% of the decline in egg production in the combined exposure group was attributed to egg eating. Further information or studies regarding this apparent synergism between *p,p'*-DDE and Aroclor 1254 were not located. The relevance of this apparent synergism to possible alterations of reproductive performance in mammals exposed to mixtures of PCBs and *p,p'*-DDE is unknown.

### **2.2.10 PCBs and Methylmercury**

PCBs and methylmercury are well-known neurotoxicants, each affecting pre- and post-natal neurological development, as well as function of the developed neurological system. Effects on neurodevelopment are among the most sensitive effects produced by both PCBs and methylmercury (see Table 1 and Appendices D and E), and their joint toxic action as neurotoxicants is of public health interest. Each chemical's mechanisms of action are thought to involve disruption of calcium homeostatic mechanisms in neural cells leading to activation of various second messenger systems and subsequent changes in neurotransmitter release (e.g., dopamine), cell damage, or cell death (Bemis and Seegal 1999; Kodavanti and Tilson 1997; Kodavanti et al. 1993, 1996). For example, *in vitro* exposure of rat cerebellar cells to methylmercury (Marty and Atchison 1997) or 2,2'-dichlorobiphenyl or 3,3',4,4',5-pentachlorobiphenyl (Kodavanti et al. 1993) caused elevations in intracellular  $Ca^{+2}$  concentrations. A recent *in vitro* study with rat striatal tissue provides evidence of a synergistic joint action to decrease tissue levels of dopamine (Bemis and Seegal 1999).

Four-hour exposures of punches from freshly excised rat striatal slices to either methylmercury or a 1:1 mixture of Aroclors 1254 and 1260 (Aroclor1254/1260) caused decreased dopamine tissue concentrations, and increased dopamine media concentrations, consistent with proposed actions of these

agents on calcium homeostasis and subsequent effects on neurotransmitter release (Bemis and Seegal 1999). Exposure levels were 1, 4, 10, 14, 20, or 40  $\mu\text{M}$  methylmercury and 10, 20, 40, 100, or 200 ppm Aroclor 1254/1260 (4 or 6 punches per exposure level). These concentrations are higher than PCB (0.84–1.9 ppm) or mercury (0.34 ppm) concentrations reported to occur in Great Lakes fish (Bemis and Seegal 1999). Dopamine tissue concentrations showed statistically significant ( $p < 0.05$ ) decreases, compared with controls, with each incremental increase in Aroclor 1254/1260 level ranging from about 90% of control values at 10 ppm to 60% at 200 ppm. In contrast, statistically significant decreases from methylmercury were only observed at the two highest concentrations. Dopamine levels in tissues exposed to 14, 20, and 40  $\mu\text{M}$  methylmercury were 84 (not significantly different from controls), 20, and 1% of control values, indicating a steep dose-response relationship between 14 and 20  $\mu\text{M}$ . Conversely, media levels of dopamine increased with increasing levels of Aroclor 1254/1260 in the media. Media levels of lactate dehydrogenase, measured as indices of the integrity of plasma membranes and viability of the tissue, were not significantly different from control levels at all Aroclor concentrations, but were significantly elevated by about 20% at 20  $\mu\text{M}$  methylmercury.

Striatal tissue samples were also simultaneously exposed to methylmercury at 4, 10, or 14  $\mu\text{M}$  plus Aroclor 1254/1260 at 10, 20, 40, 100, or 200 ppm (Bemis and Seegal 1999). Data for tissue dopamine levels or media dopamine levels were analyzed by a two-factor analysis of variance at each of the three levels of methylmercury. The analysis assumed a linear response-addition model of two factors and an interaction term (personal communication with R. Seegal, 2/23/01). The analysis of tissue concentrations indicated that the interaction term was statistically significant at 4 ( $p < 0.05$ ), 10 ( $p < 0.001$ ), and 14 ( $p < 0.01$ )  $\mu\text{M}$  methylmercury; similar results were obtained for the media concentration interaction term. This study design and statistical analysis provides qualitative information that interactions occurred. Observed combined-exposure mean tissue dopamine concentrations (expressed as a percentage of control values in Figure 1 of Bemis and Seegal 1999) were compared with predicted values that were a sum of the mean dopamine concentrations of tissues exposed to respective concentrations of the individual agents. The observed values were lower than the predicted values at Aroclor 1254/1260 concentrations  $\geq 40$  ppm in the presence of 10 or 14  $\mu\text{M}$  methylmercury and the predicted values at Aroclor 1254/1260 concentrations  $\geq 100$  ppm in the presence of 4  $\mu\text{M}$  methylmercury. This comparison is suggestive of a synergistic effect. For example, the observed mean dopamine concentration at 14  $\mu\text{M}$  methylmercury plus 200 ppm Aroclor 1254/1260 was about 20% of control values, whereas the predicted value was 40% of control (based on approximate observed responses of 60% to 200 ppm Aroclor 1254/1260 alone and about 80% to 14  $\mu\text{M}$  methylmercury alone). A statistical test of the comparison of the observed and predicted concentrations, however, cannot be constructed because of



limitations in the study design and the reporting of the data in the Bemis and Seegal (1999) report.

In an *in vivo* study of possible interactions between PCBs and methylmercury, groups of pregnant female JCL-ICR mice were fed a normal diet or one containing 500 ppm Kanechlor 500 from gestation day 0 to day 21 after delivery and received gavage doses of methylmercury in corn oil (0, 0.4, or 4 mg Hg/kg/day) from gestation day 15 to day 21 after delivery (Tanimura et al. 1980). An approximate dose of 940 mg/kg/day Kanechlor 500 is estimated based on a calculated food consumption rate of 0.06 kg/day (EPA 1988) and an approximate average body weight of 0.032 kg for the dams. These dose levels are expected to be higher than those experienced by people consuming contaminated fish from the Great Lakes or Baltic Sea. The dose levels were selected based on preliminary studies indicating that dietary exposure of mice to 500 ppm Kanechlor 500 throughout gestation produced no teratogenicity, but some embryoletality and decreased learning ability, and that gavage methylmercury doses of 4 mg Hg/kg/day would cause body weight changes, but no overt pathological changes, in offspring (8 mg Hg/kg/day produced paralysis, gait problems, and lethality in all pregnant mice). At sacrifice on day 21 after delivery, maternal mice exposed to 500 ppm Kanechlor alone or in combination with methylmercury showed similar increased body weights and increased liver weights compared with controls, but no gross pathological changes were detected in autopsies. These effects were not seen in maternal mice exposed to methylmercury alone.

Offspring survival through day 21 after delivery in all treated groups was not significantly different from control group survival, except for the Kanechlor 4 mg Hg/kg group, which showed 82.5% survival at day 21 compared with 98.9% in the control group. Survival of male offspring in all Kanechlor groups showed a marked decline, compared with controls, at about 5 weeks after birth; at 10 weeks after birth, male offspring survival percentages were about 60, 60, and 40% for the groups with Kanechlor plus 0, 0.4, and 4 mg Hg/kg, compared with >90% in the control and methylmercury alone groups. Autopsies of expired offspring revealed no obvious or specific cause of death. Survival data for female offspring were reported to have been similar. At birth, there were no significant exposure-related differences in offspring body weights, but the rate of offspring body weight gain through weaning was decreased in all Kanechlor groups compared with controls. The depression in early offspring body weight gain was most pronounced in the Kanechlor plus 4 mg Hg/kg group.

A battery of developmental tests including negative geotaxis, righting on the surface, cliff avoidance, swimming, auditory startle, and hindlimb support were administered on several preweaning days to all offspring (Tanimura et al. 1980). Statistically significant exposure-related effects were restricted to

decreased hindlimb support test scores in all Kanechlor groups at day 14 and in only the Kanechlor plus 4 mg Hg/kg group at days 17 and 21, and decreased proportion of correct responses in a visual placing test in only the Kanechlor plus 4 mg Hg/kg group at day 21. Tests of general activity (open field), and learning ability (water filled multiple T-maze and conditioned avoidance response) were administered to two male offspring from each litter at several post-weaning intervals. No consistent, statistically significant differences in test performances were found between control and exposed groups, although learning ability in the conditioned avoidance test was reported to have been “slightly inhibited” in methylmercury groups and “tended to be lower, compared to the untreated controls” in the PCB-treated groups.

Reproductive performance and a measure of developmental toxicity were also evaluated in the male and female F1 offspring with a first mating at 10 weeks of age (Tanimura et al. 1980). No statistically significant exposure-related changes were observed in first mating success, live birth index, number of live F2 newborns, F2 survival through day 21 after birth, and F2 offspring body weight through day 21 after birth. Reproductive performance was continuously surveyed in one randomly selected F1 female per litter until 48 weeks of age. No significant exposure-related effects were found on reproductive efficiency, litter sizes, survival rate, growth rate, or prevalence of anomalies in offspring sacrificed on day 21 after birth.

In summary, the mouse study by Tanimura et al. (1980) found no evidence for obvious synergism or additive joint action between Kanechlor 500 and methylmercury in affecting several endpoints evaluated in offspring of female mice exposed during lactation and gestation. Post-natal survival was affected to a greater degree by combined exposure (at a dose level of 4, but not 0.4 mg Hg/kg/day) than exposure to Kanechlor 500 alone; methylmercury alone did not affect post-natal survival. The combined-exposure effect on post-natal survival could be explained by a possible potentiation of Kanechlor 500 lethality by methylmercury, but the possibility of some other mode of joint action cannot be precluded due to design limitations of this study. Other examined endpoints included righting and swimming ability, hindlimb support, general open-field activity and learning ability in offspring at several postnatal periods, reproductive performance in F0 and F1 generations, and prevalence of developmental anomalies. The study did not include doses of the individual agents that influenced most of the examined variables, did not provide any information on dose-response relationships for the individual agents, and provided no information on dose-response relationships for combined exposure and most of the variables. Thus, no meaningful comparisons (statistically based or otherwise) could be made between observed combined-exposure responses and predicted responses based on some concept of joint action. Thus, very limited

information is provided concerning possible modes of joint action of PCBs and methylmercury on post-natal survival, neurobehavior, reproductive performance, and prevalence of developmental anomalies.

Reproductive endpoints, serum thyroid hormone levels (T3 and T4), and histology of brain, kidney, adrenals, pituitary, and thyroid were evaluated in groups of adult ranch-bred mink fed a commercial mink food supplemented with 0 or 1 ppm Aroclor 1254, 1 ppm Hg as methylmercury, 1 ppm Aroclor 1254 +1 ppm methylmercury, or 0.5 ppm Aroclor 1254 +0.5 ppm methylmercury for 8 months that spanned one breeding period (December 1984 through June 1985) (Wren et al. 1987a, 1987b). Exposed groups contained 12 females and 4 males; the control groups had 15 females and 5 males. Food intake and body weight data were not reported, but gross estimates of 0.2 mg/kg/day Aroclor 1254 and 0.2 mg Hg/kg/day are derived for the 1-ppm treatment based on a food intake of 150 g/day and body weight of 0.9 kg for minks (Aulerich et al. 1987). During the third month of exposure, eight females and one male in the 1-ppm methylmercury group, and three females in the 1-ppm Aroclor + methylmercury group died, displaying obvious signs of mercury intoxication (e.g., convulsions, tremors, lethargy). The mortality was attributed to a combination of cold stress and methylmercury poisoning, and surviving minks were fed diets containing 1 ppm methylmercury every other day for the remainder of the study. No exposure-related effects were found on the thyroid, pituitary, adrenal glands, or serum T4 or T3 levels in adult minks that survived the 8-month exposure period. Fertility of adult male mink, percentage of females whelped, or number of offspring born per female were not significantly affected by any of the treatments. The average number of offspring per female at weaning (5 weeks after birth) was significantly ( $p < 0.05$ ) lower in the 1 ppm Aroclor + methylmercury group (2.1 offspring/female) than in the control (4.5), 1 ppm Aroclor (5.0), 1 ppm methylmercury (4.0), or 0.5 ppm Aroclor + 0.5 ppm methylmercury groups (3.6), indicating that post-natal offspring mortalities were increased by combined exposure to the high levels of methylmercury and Aroclor 1254.

Wren et al. (1987b) concluded that these observations showed a synergistic effect of Aroclor 1254 and methylmercury on post-natal survival of mink offspring, but without more information about dose-response relationships on this endpoint, the data do not allow a rigorous conclusion regarding joint action. The data indicate that the two agents, at respective non-effective exposure levels of 1 ppm, added together to induce post-natal mortality, but it is not possible to discern if they added together in a less-than-additive, additive, or greater-than-additive manner. Given that the individual agents were administered at concentrations that did not affect post-natal mortality, demonstration of synergism assuming response addition requires that the response to the 0.5 ppm Aroclor + 0.5 ppm methylmercury treatment would have been significantly greater than the control value; however, post-natal mortality was not changed,

compared with control, by this treatment.

There is evidence to suggest that induction of CYP1A and CYP2B enzymes by PCB mixtures is counteracted by simultaneous exposure to methylmercury in rats and quails (Leonzio et al. 1996a; Takabatake et al. 1980).

In Wistar rats fed a diet containing 50 ppm of a 1:1 mixture of Kanechlors 400:500 for 14 days and subcutaneously exposed to 10 mg Hg/kg/day as methylmercuric chloride on the last 2 days, induction of hepatic levels of CYP and several associated oxygenases (aminopyrine N-demethylase, aniline hydroxylase, p-nitroanisole O-demethylase) was curtailed compared with levels in rats treated with 50 ppm Kanechlor alone (Takabatake et al. 1980). Estimated daily doses of 4.6 mg Kanechlor/kg/day were calculated assuming a food consumption rate of 0.02 kg/day and body weight of 0.217 kg for Wistar rats. Rats fed a normal diet and treated similarly with methylmercuric chloride showed a decrease in hepatic levels of CYP and associated oxygenases compared with rats fed a normal diet (Takabatake et al. 1980).

In quail fed a diet containing 25 ppm methylmercury and 100 ppm Aroclor 1260 for 21 days, induction of hepatic levels of benzoxyresorufin-O-deethylase (BROD, an indicator of CYP2B) and ethoxyresorufin-O-deethylase (EROD, an indicator of CYP1A) was curtailed compared with levels in quail fed a diet containing 100 ppm Aroclor 1260 (Leonzio et al. 1996a). (Information provided in the report was insufficient to calculate estimated doses.) Exposure to 10 ppm Aroclor 1260 induced hepatic levels of these enzymes to a lesser degree than 100 ppm, but this level of induction was not markedly influenced by simultaneous exposure to 2.5 ppm methylmercury in the diet. Levels of BROD and EROD were increased to a small degree, compared with control values, in quail fed a diet containing 2.5 ppm methylmercury alone, but activities were non-significantly decreased in quail fed a diet containing 25 ppm methylmercury alone. Exposure to 25 ppm methylmercury and 100 ppm Aroclor 1260 produced a decrease in serum cholesterol that was associated with a decrease in hepatic levels of Aroclor 1260 compared with exposure to Aroclor 1260 alone (Leonzio et al. 1996a).

A companion study found that a 3-week exposure of quail to 2.5 or 25 ppm methylmercury in the diet or 10 or 100 ppm Aroclor 1260 in the diet increased mean porphyrin levels in liver or excreta and that combined exposure to 2.5 ppm methylmercury +10 ppm Aroclor 1260 or 25 ppm +100 ppm Aroclor 1260 appeared to additively increase porphyrin levels (Leonzio et al. 1996b). For example, differences in total porphyrins in excreta between groups treated with 25 ppm methylmercury alone or 100 ppm

Aroclor 1260 and control values were 3,801 and 2,831 pmol/g of excreta. For the combined exposure group, the difference was 6,070 pmol/g of excreta, which is similar to a predicted value of 6,632 pmol/g based on an additivity assumption.

The results of the studies by Leonzio et al. (1996a) and Takabatake et al. (1980) suggest that simultaneous exposure to methylmercury may counteract the induction of hepatic CYP enzymes by PCBs, but it is unclear if this is due to direct mercury inhibition of enzyme activity, methylmercury inhibition of PCB uptake or distribution, or some other mechanism. Both compounds appear to cause porphyria in quail, and limited data suggest that they may jointly act in an additive manner (Leonzio et al. 1996b).

Tables 10 and 11 summarize the available *in vivo* interactions data for PCBs and methylmercury.

**Table 10. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of Methylmercury**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day)						
Inter-mediate	impaired learning capability in offspring		940 <sup>a</sup> +4 (m) <sup>b</sup>		Data were inadequate for definitive conclusions on joint action.	Tanimura et al. 1980 (Kanechlor 500)
Inter-mediate	decreased neonatal survival in offspring		0.2+0.2 (i)		Data indeterminate to determine joint action; decreased survival not observed with (0.1+0.1) mg/kg/day.	Wren et al. 1987b (Aroclor 1254)
Inter-mediate	increased porphyrins in liver and excreta		2.5 ppm+10 ppm (q) 25 ppm+100 ppm (q)		Results consistent with additivity in producing porphyria.	Leonzio et al. 1996b (Aroclor 1260)

<sup>a</sup>First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup>Species code: i = mink; m = mouse; q = quail; r = rat

**Table 11. Summary of Available Data on the Influence of Methylmercury on Toxicity/Carcinogenicity of PCBs**

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day)						
Acute	induction of hepatic CYP			10 <sup>a</sup> subcutaneous+4.6 (r) <sup>b</sup>	Inhibition of PCB induction of hepatic CYP.	Takabatake et al. 1980 (Kanechlors 400/500)
Inter-mediate	induction of hepatic CYP		2.5 ppm+10 ppm (q)	25 ppm+100 ppm (q)	Inhibition of PCB induction of hepatic CYP at high, but not low, dietary concentrations.	Leonzio et al. 1996a (Aroclor 1260)
Inter-mediate	impaired learning capability in offspring		4+940 (m)		Study design inadequate for definitive conclusions on joint action.	Tanimura et al. 1980 (Kanechlor 500)

**Table 11. Summary of Available Data on the Influence of Methylmercury on Toxicity/Carcinogenicity of PCBs** (*continued*)

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Oral Exposure (mg/kg/day)						
Inter-mediate	decreased neonatal survival in offspring	4+940 (m)	0.4+940 (m)  0.2+0.2 (i)		Methylmercury showed no effect at low dose; possible potentiation of PCB effect at 4 mg/kg, but study designs are inadequate for definitive conclusions on joint action.	Tanimura et al. 1980 (Kanechlor 500)  Wren 1987b (Aroclor 1254)
Oral Exposure (mg/kg/day)						
Inter-mediate	decreased body weight gain in neonates	4+940 (m)	0.4+940 (m)		Methylmercury showed no effect at low dose; possible potentiation at 4 mg/kg, but study design is inadequate for definitive conclusions on joint action.	Tanimura et al. 1980 (Kanechlor 500)
Inter-mediate	porphyria		2.5 ppm+10 ppm (q) 25 ppm+100 ppm (q)		Results consistent with additivity.	Leonzio et al. 1996b (Aroclor 1260)

<sup>a</sup>First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.

<sup>b</sup>Species code: i = mink; m = mouse; q = quail; r = rat



### 2.3 Relevance of the Joint Toxic Action Data and Approaches to Public Health

No studies were located that examined health effects in humans or animals exposed to five-component mixtures containing CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs. Furthermore, PBPK/PD models have not been developed to predict dispositional and toxicological outcomes of joint action of mixtures of these five components.

As discussed in the appendices and illustrated in Table 1, oral exposure to each component of the mixture of concern (2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs) can produce a wide range of health effects that is dependent on dose level, duration of exposure, and genetic, gender, and developmental status of the exposed individual. Table 1 also illustrates that there is a fair amount of overlap in the endpoints or organs that these chemicals affect. This observation is reflected in the endpoints that form the basis of the oral MRLs for these chemicals (Table 12).

**Table 12. Health Effects Forming the Basis of ATSDR Oral MRLs for Chemicals of Concern.**  
(See Appendices A, B, C, D, and E for More Details.)

Duration of exposure	2,3,7,8-TCDD	Hexachlorobenzene	<i>p,p'</i> -DDE <sup>a</sup>	Methylmercury	PCBs
Acute	immuno-suppression in rats	neurobehavioral changes in rat offspring	neurobehavioral changes in mouse offspring	none derived, inadequate data	none derived, inadequate data
Inter-mediate	immuno-suppression in rats	reproductive organ changes in female monkeys	hepatomegaly in rats	None derived, inadequate data	neuro-behavioral changes in monkey offspring
Chronic	neuro-behavioral changes in monkey offspring	liver degeneration and porphyria in rats	none derived, inadequate data	neurobehavioral changes in human offspring	immuno-suppression in monkeys

<sup>a</sup>No MRLs were derived specifically for *p,p'*-DDE, but MRLs for DDT (listed in the DDE column of this table) were based on effects due to *p,p'*-DDT and are expected to be relevant to *p,p'*-DDE.

The observations reflected in Tables 1 and 12 lead to concerns that, following oral exposure to mixtures of these five chemicals: (1) all five chemicals may jointly act to produce altered neurological development, suppression of immune competence, or cancer; (2) four (2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, and PCBs) may jointly act to produce liver damage; (3) four (2,3,7,8-TCDD, hexachlorobenzene, methylmercury, and PCBs) may jointly act to disrupt female reproductive organ function; (4) four (2,3,7,8-TCDD, *p,p'*-DDE, methylmercury, and PCBs) may jointly act to disrupt male reproductive organ function; and (5) three (2,3,7,8-TCDD, *p,p'*-DDE, and PCBs) may jointly act to alter development of male reproductive organs.

Support for altered neurological development as a possible health hazard from frequent oral exposure to the mixture of concern comes from studies of children of women who frequently consumed Great Lakes sportfish. Frequent dietary consumption of contaminated Great Lakes fish by child-bearing-aged women was associated in two prospective epidemiological studies with changes in the neurological development of their children (Fein et al. 1984; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b; Lonky et al. 1996; Stewart 1999, 2000b). Other studies do not provide consistent evidence that consumption of Great Lakes fish presents obvious risks for impaired reproduction, impaired immune capabilities, or physical birth defects (Buck et al. 1997; Courval et al. 1999; Feeley and Jordan 1998; Feeley et al. 1998; Hertzler 1990; Kostyniak et al. 1999; Mendola et al. 1995, 1997; Vena et al. 1996).

There is suggestive evidence for associations between frequent consumption of contaminated Baltic Sea fish and impaired immunological competence (in seals) or low birth weight (in children of mothers who frequently ate Baltic Sea fish), but the data do not clearly demonstrate dose-response relationships (Ross et al. 1995; Rylander and Hagmar 1999; Rylander et al. 1995, 1996, 1998a, 1998b; Svensson et al. 1994).

The weight of the evidence for an association between frequent consumption of contaminated Great Lakes fish and impaired neurological development in children is greater than that for associations between frequent consumption of contaminated Baltic Sea fish and impaired immune capabilities or low birth weight, but none of the weights are sufficient to establish causal relationships between fish consumption and adverse health effects in humans. The Great Lakes fish consumption studies identify subtle impairment of neurological development in children as a possible health hazard of concern, but are unsuitable for developing MRLs for complex mixtures of pollutants in fish flesh because of the likelihood that concentrations of persistent chemicals in fish will be highly variable depending on species and geographical location. Due to exposure data deficiencies, the fish consumption studies cannot determine with certainty which chemicals may cause possible neurodevelopmental deficits. PCBs have been

proposed as toxicants involved in the possible association between maternal fish consumption and altered childhood neurological development based on statistically significant associations between specific PCB levels in maternal fluids and neurological deficits in children (Fein et al. 1984; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b; Lonky et al. 1996; Stewart 2000b), but other hypotheses have been proposed including the possible involvement of other persistent chemicals in contaminated fish or synergistic interactions between PCBs and other neurotoxicants in fish.

Investigations of wildlife species including birds, reptiles, fish, and mammals in the North American Great Lakes region have identified reproductive and developmental problems and cancer that may be related to exposure to mixtures of Great Lakes contaminants (Hicks 1996). For example, teratogenic effects observed in wild bald eagles and other waterbirds in the Great Lakes region are similar to the developmental deformities produced in laboratory studies of white leghorn chickens fed contaminated carp from the Saginaw Bay of Lake Huron and American kestrels fed planar PCBs (Bowerman et al. 2000). The problems observed in wildlife serve to identify potential health problems that may occur in human populations. Along with the studies of fish consumption in human populations, they provide a focus on which to base exposure-based assessments of human health hazards from oral exposures to mixtures of persistent Great Lakes pollutants including CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs.

To carry out exposure-based assessments of possible health hazards associated with oral exposure scenarios involving frequent dietary exposure to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs, component-based approaches that assume additive joint toxic action are recommended, because of the lack of studies that examine relevant endpoints and describe dose-response relationships for oral exposures to mixtures that contain the five components of concern. For noncancer endpoints, a target-organ toxicity dose modification of the hazard index approach is recommended given that a wide range of overlapping toxicity targets can be affected by the components (ATSDR 2001a). For assessment of cancer risks from oral exposure to mixtures of the five components, a components-based approach assuming response additivity is recommended (ATSDR 2001a.).

It is recommended that these approaches treat mixtures of PCB congeners (i.e., total PCBs) as a single entity. This approach is consistent with ATSDR's approach to deriving oral MRLs for PCBs which are based on data linking health effects with exposure to PCB mixtures (Appendix E; ATSDR 2000).

With component-based approaches to assessing health hazards from mixtures, it is important to assess the

joint additive action assumption, and consider the possibility that less-than-additive or greater-than-additive joint actions may occur among the components. The available joint toxic action data for the five components were reviewed in Section 2.2 with this purpose in mind. Tables 14–33 describe binary weight-of-evidence determinations (BINWOEs) for the pairs of the five components of concern using the classification scheme summarized in Table 13 and ATSDR (2001a, 2001b). The numerical scale of this scheme range from -1 for high confidence that a less-than-additive joint action will occur, through 0 for evidence that additive joint action will occur or for indeterministic evidence for the mode of joint action, up to +1 for high confidence that a greater-than-additive interaction will occur. The conclusions presented in these tables were based on the evaluations of the pertinent literature presented in Section 2.2. An overview of the BINWOEs is presented in Table 34. The BINWOEs focus on simultaneous oral exposure, as this is the exposure scenario of most interest for public health concerns for the components of the mixture. A summary discussion of the BINWOEs follows this paragraph and precedes the descriptive tables.

There are no pertinent joint toxic action data, and understanding of mechanisms of action is too incomplete to support projections of joint toxic action for the following pairs of chemicals:

- *p,p'*-DDE and hexachlorobenzene (Tables 18 and 19);
- *p,p'*-DDE and methylmercury (Tables 24 and 25);
- PCBs and hexachlorobenzene (Tables 28 and 29); and
- PCBs and *p,p'*-DDE (Tables 30 and 31).

Lack of joint toxic data, conflicting data, and/or incomplete understanding of mechanisms of action also preclude projecting interactions for the following:

- the effect of 2,3,7,8-TCDD on hexachlorobenzene toxicity (Table 14);
- the effect on methylmercury on 2,3,7,8-TCDD toxicity (Table 21);
- the effect of hexachlorobenzene on methylmercury toxicity (Table 22);
- the effect of PCBs on the following TCDD-induced effects: thyroid hormone disruption, porphyria, impaired reproductive organ function and development, impaired neurological function and development, tumor initiation and promotion (Table 26);

- the effect of 2,3,7,8-TCDD on the following PCB-induced effects: immunosuppression, thyroid hormone disruption, porphyria, developmental toxicity, impaired reproductive organ function and development, impaired neurological function and development, tumor initiation and promotion (Table 27); and
- the effect of methylmercury on the following PCB-induced effects: immunosuppression, thyroid hormone disruption, developmental toxicity not related to neurological deficits or decreased neonatal survival, impaired reproductive performance, tumor initiation and promotion (Table 32).

Evidence of varying quantity and quality is available supporting projections of additive joint action (or no interactive effect) for the following:

- 2,3,7,8-TCDD and *p,p'*-DDE for anti-androgenic effects (Tables 16 and 17);
- 2,3,7,8-TCDD on methylmercury effects on immune system (Table 20);
- methylmercury on hexachlorobenzene hepatotoxicity (Table 23);
- PCBs and 2,3,7,8-TCDD for inducing body and thymus weight changes, hepatomegaly, and decreased liver levels of retinoids (Tables 26 and 27); and
- methylmercury and PCBs for inducing hepatic porphyria (Tables 32 and 33).

Evidence is also available supporting projections of the following possible interactions:

- hexachlorobenzene potentiation (greater-than-additive interaction) of TCDD reduction in body weight and thymus weight (Table 15);
- antagonism (less-than-additive interaction) by PCB mixtures of TCDD induction of immune system suppression and developmental effects (cleft palate and hydronephrosis) (Table 26); and
- synergism (greater-than-additive interaction) between PCBs and methylmercury in disrupting regulation of brain levels of dopamine, a process possibly influencing neurological function and development (Tables 32 and 33).

In summary, there is only a limited amount of evidence that non-additive interactions may exist between a few of the chemical pairs: hexachlorobenzene potentiation of TCDD reduction of body and thymus weights; PCB antagonism of TCDD immunotoxicity and developmental toxicity; and synergism between PCBs and methylmercury in disrupting neurological function and development. For the remaining pairs, additive joint action at shared targets of toxicity is either supported by data (for a few pairs) or is recommended as a public health protective assumption due to lack of interaction data, conflicting interaction data, and/or lack of mechanistic understanding to reliably support projections of modes of joint toxic action.

**Table 13. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions**

Classification		Factor
<b>Direction of Interaction</b>		<b>Direction</b>
=	Additive	0
>	Greater than additive	+1
<	Less than additive	-1
?	Indeterminate	0
<b>Quality of the Data</b>		<b>Weighting</b>
<b>Mechanistic Understanding</b>		
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur is not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
<b>Toxicological Significance</b>		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
<b>Modifiers</b>		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	<i>In vivo</i> data	1.0
b.	<i>In vitro</i> data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

*Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05*

*BINWOE = Direction Factor x Weighting Factor: Ranges from -1 through 0 to +1*

Source: ATSDR 2001a, 2001b

Table 14. Effect of **2,3,7,8-TCDD** on **Hexachlorobenzene**

(see Table 13 for explanation of BINWOE codes)

**BINWOE: ? (0)**

*Direction of Interaction* - The direction of the interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information clearly indicating that possible pharmacokinetic interactions with 2,3,7,8-TCDD will influence hexachlorobenzene toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of the mode of joint action of 2,3,7,8-TCDD and hexachlorobenzene on any toxicity target.

*Mechanistic Understanding* - Hepatic porphyria from repeated exposure to hexachlorobenzene has been proposed to be dependent on CYP1A-mediated metabolism and to involve an unidentified reactive intermediate (that inhibits the heme biosynthetic pathway from uroporphyrinogen) during biotransformation to pentachlorophenol (den Besten et al. 1993). Alternatively, it has been proposed that induction of CYP1A isozymes (via the Ah receptor) leads to a stimulation of the oxidation of uroporphyrinogen to uroporphyrin and an inhibition of heme synthesis from uroporphyrinogen (Sinclair et al. 1997). 2,3,7,8-TCDD is a potent inducer of CYP1A, but does not appear to induce CYP1A isozymes. Both compounds can produce hepatic porphyria, but the mode of their joint action has not been studied, and it is unknown whether they would jointly produce hepatic porphyria in an additive, greater-than-additive, or less-than-additive manner.

Mechanistic understanding of other hexachlorobenzene-induced health effects (such as altered neurological development, decreased circulating levels of thyroid hormones, and disruption of female reproductive organs) is insufficient to clearly indicate whether TCDD induction of CYP or Phase II enzymes will potentiate hexachlorobenzene toxicity (see Appendix B). Although hexachlorobenzene binds to the Ah receptor (with much less affinity than 2,3,7,8-TCDD), the degree to which hexachlorobenzene-induced toxic effects are mediated by the Ah receptor is unknown.

Joint action at several shared target organs (e.g., liver, developing neurological system, reproductive organs, thyroid) is plausible, but whether the action would be additive, greater-than-additive, or less-than-additive is unknown and unstudied.

*Toxicological Significance* - No studies were located that were designed to compare responses of pertinent toxicity targets to mixtures of TCDD and hexachlorobenzene with responses to either compound alone. No studies were located in which pretreatment with TCDD before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity.

*Additional Uncertainties* - If 2,3,7,8-TCDD induces CYP enzymes involved in hexachlorobenzene metabolism, an alternative possibility is that TCDD co-exposure may potentiate hexachlorobenzene-induced porphyria and perhaps other TCDD effects that may be caused by a reactive metabolite. This speculation would lead to a greater-than-additive judgement with very high uncertainty. This alternative judgement does not appear warranted because 2,3,7,8-TCDD appears to induce CYP1A enzymes, rather than CYP1A enzymes, and possible increased rates of formation of the toxic metabolite may not exceed capacity of downstream enzymes to control concentrations in the liver.



Table 15. Effect of **Hexachlorobenzene** on **2,3,7,8-TCDD**

(see Table 13 for explanation of BINWOE codes)

**BINWOE: > IIC1aii** ( $0.32 \times 0.32 \times 1.0 \times 1.0 \times 0.79 = +0.08$ )

for body and thymus weight effects from acute exposure

**BINWOE: > IIC2aii** ( $0.32 \times 0.32 \times 0.79 \times 1.0 \times 0.79 = +0.06$ )

for body and thymus weight effects from non-acute exposure

**BINWOE: ? (0)**

for other effects

*Direction of Interaction* - Greater-than-additive (>) action of hexachlorobenzene on TCDD-induced body and thymus weight effects is based on the observation that pretreatment of rats with single intraperitoneal doses of hexachlorobenzene (400  $\mu$ mole/kg) potentiated effects on body and thymus weights produced by intraperitoneal doses (0.031 or 0.093  $\mu$ mole/kg) of 2,3,7,8-TCDD (Li et al. 1989). The mode of possible joint toxic action with hexachlorobenzene on other TCDD toxicity targets is unknown and unstudied. Mechanistic understanding is inadequate to support reliable projections of the mode of joint action of 2,3,7,8-TCDD and hexachlorobenzene on other toxicity targets.

*Mechanistic Understanding* - The apparent potentiation is opposite to an expectation that pretreatment with hexachlorobenzene, at doses up to 10,000-fold higher (on a mole/kg basis) than 2,3,7,8-TCDD doses, would have inhibited TCDD-induced effects mediated by the Ah receptor, based on the observation that this pretreatment decreased cytosolic levels of the Ah receptor in several tissues. This illustrates that the mechanistic understanding of TCDD toxic effects and the observed interaction are inadequate to provide a reliable explanation or support reliable inferences based on possible biochemical interactions between hexachlorobenzene and 2,3,7,8-TCDD. Thus, the highest uncertainty category (III) was selected for mechanistic understanding. The results suggest that the interaction may occur at some site other than the Ah receptor.

Joint action on several shared target organs is plausible, but mechanistic understanding of toxic actions on other potential shared toxicity targets (e.g., liver, reproductive organs, immune system) is inadequate to support a reliable projection of mode of joint toxic action (additive, greater-than-additive, or less-than-additive).

*Toxicological Significance* - The apparent potentiation by hexachlorobenzene was demonstrated for TCDD effects on body and thymus weight from acute exposure in one study using one species (rat) and a mixed exposure scenario in which doses of hexachlorobenzene were far greater than doses of TCDD. The response appears to be a potentiation since hexachlorobenzene, even at doses 7.5 times higher than the potentiating dose, did not affect body or thymus weights (Li et al. 1989). The highest uncertainty category (C) was selected due to the lack of corroborative results from replicate studies or other studies using other dose levels, other mixture proportions of hexachlorobenzene and TCDD, or other species.

The mode by which hexachlorobenzene may jointly act with TCDD on other more sensitive effects from acute or repeated exposure (immunosuppression, female reproductive organ disruption, or altered neurological development) is unknown and unstudied; thus, the indeterminate (?) direction of interaction category is appropriate.

Table 15. Effect of **Hexachlorobenzene** on **2,3,7,8-TCDD** (*continued*)

*Modifying Factors* - Because of the biopersistence of these chemicals, sequential administration may produce similar interactions as simultaneous exposure. For acute exposure, a "1" is assigned for duration and sequence. For intermediate or chronic exposure, a "2" is assigned, because the interaction was demonstrated for acute exposure. A "ii" is assigned to the oral BINWOEs, because the observed interaction is from intraperitoneal exposure.

*Additional Uncertainties* - The apparent potentiation does not appear to involve an interaction at the Ah receptor, a mechanistic component that is thought also to be involved in the development of several other effects from TCDD. The modes of possible joint toxic actions between TCDD and hexachlorobenzene on these other toxicity targets are unknown. The modifying factors reflect additional uncertainties regarding the applicability of the single, sequential, intraperitoneal dosing protocol to simultaneous oral exposure and durations longer than acute.

Table 16. Effect of **2,3,7,8-TCDD** on ***p,p'*-DDE**  
(see Table 13 for explanation of BINWOE codes)

**BINWOE: = IIC (0)**

for anti-androgenic effects

**BINWOE: ? (0)**

for other effects

*Direction of Interaction* - Support for additive joint toxic action of TCDD and *p,p'*-DDE on male reproductive organ development and function is restricted to an observation that combined exposure to TCDD and *p,p'*-DDE decreased prostate weight in male rat offspring to a greater degree than either compound alone (Loeffler and Peterson 1999). Mechanistic information suggests that they may act on a molecular scale by independent anti-androgenic mechanisms. Modes of possible joint toxic action on several other toxicity targets are unknown and unstudied. Mechanistic understanding is inadequate to support reliable projections of modes of joint actions on other toxicity targets.

*Mechanistic Understanding* - Anti-androgenic effects from *p,p'*-DDE are postulated to involve inhibition of androgen-binding to androgen receptors (Kelce et al. 1995, 1997), whereas TCDD is not expected to interfere with androgen receptor-ligand binding and may indirectly affect androgen signaling by altering growth factor pathways (Roman et al. 1998b). An additive mode of joint toxic action on male reproductive organ function, and development, is plausible on a whole organ level of organization, but is not supported by these hypotheses of *independent* molecular-scale mechanisms of action. Thus, the highest uncertainty category (III) for mechanistic understanding was selected.

Joint actions on several other shared target organs are plausible (e.g., liver, immune system), but mechanistic understanding is inadequate to support a reliable projection of mode of joint toxic action (additive, greater-than-additive, or less-than-additive).

*Toxicological Significance* - Simultaneous administration of oral doses of 0.25 µg/kg 2,3,7,8-TCDD and 100 mg/kg *p,p'*-DDE during gestation transiently decreased prostrate weight in male rat offspring at postnatal day 21 to a greater degree than administration of either compound alone (Loeffler and Peterson 1999). Whereas additive joint action on this endpoint is a plausible explanation of the results (see Section 2.2.2), the study design is inadequate to rule out possible greater-than-additive or less-than-additive joint actions. Due to study design limitations and a lack of corroborative data from other studies, the highest uncertainty category (C) was selected for toxicological significance.

Joint actions at several shared target organs (e.g., liver, immune system) are plausible, but studies designed to characterize modes of possible joint action (additive, greater-than-additive, or less-than-additive) were not located.

*Additional Uncertainties* - Confidence in the additivity projection for anti-androgenic effects would be strengthened with better designed studies that included several dose levels of each compound, alone and in mixture, to more conclusively determine the mode of joint toxic on male reproductive development.

Table 17. Effect of *p,p'*-DDE on 2,3,7,8-TCDD  
(see Table 13 for explanation of BINWOE codes)

**BINWOE: = IIC (0)**  
for anti-androgenic effects  
**BINWOE: ? (0)**  
for other effects

*Direction of Interaction* - Support for additive joint toxic action of *p,p'*-DDE and TCDD on male reproductive organ development and function is restricted to an observation that combined exposure to TCDD and DDE decreased prostate weight in male rat offspring to a greater degree than either compound alone (Loeffler and Peterson 1999). Modes of possible joint toxic action on several other toxicity targets are unknown and unstudied. Mechanistic understanding is inadequate to support reliable projections of modes of joint actions on other toxicity targets.

*Mechanistic Understanding* - Anti-androgenic effects from *p,p'*-DDE are postulated to involve inhibition of androgen-binding to androgen receptors (Kelce et al. 1995, 1997), whereas TCDD is not expected to interfere with androgen receptor-ligand binding and may indirectly affect androgen signaling by altering growth factor pathways (Roman et al. 1998b). An additive mode of joint toxic action on male reproductive organ function, and development, is plausible on a whole organ level of organization, but is not supported by these hypotheses of *independent* molecular-scale mechanisms of action. Thus, the highest uncertainty category (III) for mechanistic understanding was selected.

Joint actions on several other shared target organs are plausible (e.g., liver, immune system), but mechanistic understanding is inadequate to support a reliable projection of mode of joint toxic action (additive, greater-than-additive, or less-than-additive).

*Toxicological Significance* - Simultaneous administration of oral doses of 0.25 µg/kg 2,3,7,8-TCDD and 100 mg/kg *p,p'*-DDE during gestation transiently decreased prostrate weight in male rat offspring at postnatal day 21 to a greater degree than administration of either compound alone (Loeffler and Peterson 1999). Whereas additive joint action on this endpoint is a plausible explanation of the results (see Section 2.2.2), the study design is inadequate to rule out the possibility of greater-than-additive or less-than-additive joint actions. Due to study design limitations and a lack of corroborative data from other studies, the highest uncertainty category (C) was selected for toxicological significance.

Joint actions at several shared target organs (e.g., liver, immune system) are plausible, but studies designed to characterize modes of possible joint action (additive, greater-than-additive, or less-than-additive) were not located.

*Additional Uncertainties* - Confidence in the additivity projection for anti-androgenic effects would be strengthened with better designed studies that included several dose levels of each compound, alone and in mixture, to more conclusively determine the mode of joint toxic on male reproductive development.

Table 18. Effect of *p,p'*-DDE on Hexachlorobenzene

(see Table 13 for explanation of BINWOE codes)

**BINWOE: ? (0)**

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that pharmacokinetic interactions with *p,p'*-DDE will influence hexachlorobenzene toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of the mode of joint toxic action of *p,p'*-DDE and hexachlorobenzene on any toxicity target.

*Mechanistic Understanding* - Joint actions of *p,p'*-DDE and hexachlorobenzene in producing several similar effects (liver damage, immunosuppression, male reproductive organ disruption, altered neurological development, and cancer) are plausible, but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action. Hexachlorobenzene-induced hepatic porphyria is thought to involve an unidentified reactive intermediate produced either by CYP1A3-mediated or CYP1A2-mediated metabolism (den Besten et al. 1993; Sinclair et al. 1997; see Appendix B). DDE induces hepatic CYP1B1 and, to a lesser degree, CYP1A3 in rats. If simultaneous exposure to DDE and hexachlorobenzene cause an increased induction of CYP1A3 enzymes (compared with hexachlorobenzene alone) so that capabilities of downstream Phase II enzymes to control liver concentrations of the reactive hexachlorobenzene metabolite are exceeded, then a potentiation of hexachlorobenzene-induced liver toxicity may occur. No studies were located that investigated hepatic metabolic interactions between hexachlorobenzene and *p,p'*-DDE (or DDT), but this projection is not reliable given that hexachlorobenzene can induce its own metabolism and downstream Phase II enzymes would need to be saturated for the potentiation to occur.

*Toxicological Significance* - No studies were located that were designed to compare responses of pertinent toxicity targets to mixtures of DDE and hexachlorobenzene with responses to either compound alone. No studies were located in which pretreatment with DDE before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., liver, nervous system, immune system, thyroid—see Appendices B and C), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

**Table 19. Effect of Hexachlorobenzene on *p,p'*-DDE**

(see Table 13 for explanation of BINWOE codes)

**BINWOE: ? (0)**

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that possible pharmacokinetic interactions with *p,p'*-DDE will influence hexachlorobenzene toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of the mode of joint toxic action of *p,p'*-DDE and hexachlorobenzene on any toxicity target.

*Mechanistic Understanding* - Joint actions of *p,p'*-DDE and hexachlorobenzene in producing several similar effects (liver damage, immunosuppression, altered neurological development, and cancer) are plausible (see Appendices B and C), but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action. Toxic actions of *p,p'*-DDE are thought to involve the parent compound disrupting functions of membranes in various target organs (e.g., disruption of transport mechanisms in neuronal membranes, disruption of ultrastructure of hepatic mitochondrial membranes; see Appendix C). The possible influence of hexachlorobenzene on DDE molecular mechanisms of toxic action is unstudied.

*Toxicological Significance* - No studies were located that were designed to compare responses of pertinent toxicity targets to mixtures of DDE and hexachlorobenzene with responses to either compound alone. No studies were located in which pretreatment with DDE before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., liver, nervous system, immune system, thyroid; see Appendices B and C), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 20. Effect of **2,3,7,8-TCDD** on **Methylmercury**  
(see Table 13 for explanation of BINWOE codes)

**BINWOE: = IIIBbii (0)**

for immune system suppression

**BINWOE: ? (0)**

for other effects

*Direction of Interaction* - There is *in vitro* evidence that a synthetic mixture of CDDs, CDFs, and PCBs at concentrations that were reflective of concentrations in fish from the St. Lawrence River did not change the effects of methylmercury on rat lymphocyte viability and mitogenic ability (Omara et al. 1997). The additive direction of interaction is selected to reflect a projected lack of effect of CDDs on methylmercury immunotoxicity. For other methylmercury effects, a direction of interaction cannot be reliably projected due to the absence of pertinent joint toxic action data, absence of information that possible pharmacokinetic interactions with TCDD may influence methylmercury toxicity, and inadequate mechanistic understanding supporting a reliable projection of the mode of possible joint toxic action of 2,3,7,8-TCDD and methylmercury on other toxicity targets.

*Mechanistic Understanding* - CDDs are postulated to produce immunotoxic effects such as lymphoid tissue depletion and increased susceptibility to infectious agents via an initial mediation by the Ah receptor and unknown subsequent molecular events within the immune system (Kerkvliet 1994; Appendix A). Mercuric salts and methylmercury have been demonstrated to cause both autoimmune stimulation and a suppression of the immune system, but the mechanisms that may be involved are unknown (Appendix D). Pertinent molecular sites of possible interactions between TCDD and methylmercury are thus unidentified, and the limited mechanistic understanding suggests that CDDs may produce immune effects by different mechanisms than methylmercury (i.e., methylmercury immunotoxicity is not expected to involve Ah receptor mediation). The highest uncertainty category (III) was therefore selected for mechanistic understanding.

Mechanistic understanding of methylmercury-induced critical effects (altered neurological development, immunosuppression, and cancer) is insufficient in itself to project possible interactions with TCDD, although interactions at the Ah receptor do not seem likely.

*Toxicological Significance* - *In vitro* studies of immunological endpoints in rat cultured lymphocytes found no evidence for interactions between methylmercury and a synthetic mixture of CDDs, CDFs, and PCBs at low concentrations reflective of concentrations in St. Lawrence River fish (Omara et al. 1997, 1998), but study design limitations preclude definitive conclusions regarding the mode of possible joint toxic actions on the immune system. No other studies (*in vitro* or *in vivo*) to support or refute the results of this single study were located. A moderate confidence rating for toxicological significance (B) is selected to reflect the lack of supporting data, design limitations of the single available study, and the plausibility that the observed lack of effect of CDDs, CDFs, and PCBs on methylmercury immunotoxicity is relevant to pertinent environmental exposure levels such as fish consumption.

No studies were located that were designed to compare responses of other pertinent toxicity targets to mixtures of TCDD and methylmercury with responses to either compound alone. No studies were located in which pretreatment with TCDD before methylmercury exposure was examined for possible effects on methylmercury toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., nervous system, thyroid; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) for other effects reflects this lack of data.

Table 20. Effect of **2,3,7,8-TCDD** on **Methylmercury** (*continued*)

*Modifying Factors* - The modifying data quality factor of b was selected to reflect that the only available data on joint action is from an *in vitro* study.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.



Table 21. Effect of **Methylmercury** on **2,3,7,8-TCDD**

(see Table 13 for explanation of BINWOE codes)

**BINWOE: = ? (0)**

*Direction of Interaction* - A direction of interaction cannot be reliably projected due to the absence of pertinent joint toxic action data, absence of information that possible pharmacokinetic interactions with methylmercury may influence TCDD toxicity, and inadequate mechanistic understanding supporting a reliable projection of the mode of possible joint toxic action of 2,3,7,8-TCDD and methylmercury on other toxicity targets.

*Mechanistic Understanding* - CDDs are postulated to produce several types of effects via an initial mediation by the Ah receptor and subsequent molecular events within target organs (Kerkvliet 1994; Appendix A). Mercuric salts and methylmercury have been associated with effects that occur in some target organs that overlap with CDD toxicity targets, but molecular mechanisms that may be involved are unknown or poorly understood (Appendix D). Pertinent molecular sites of possible interactions between TCDD and methylmercury are thus unidentified, and the limited mechanistic understanding suggests that CDDs may produce effects by different mechanisms than methylmercury (e.g., methylmercury immunotoxicity is not expected to involve Ah receptor mediation).

Interactions at the Ah receptor do not seem likely, but whether methylmercury may interact with TCDD at other cellular or molecular sites involved in the development of TCDD health effects is unknown.

*Toxicological Significance* - *In vitro* studies of immunological endpoints in rat cultured lymphocytes found no evidence for interactions or additivity between methylmercury and a synthetic mixture of CDDs, CDFs, and PCBs at low concentrations reflective of concentrations in St. Lawrence River fish (Omara et al. 1997, 1998). However, the doses of the mixture used in the study were without effect on the examined endpoints in the absence of methylmercury and the study provides no information on possible effects of methylmercury on CDD-induced effects (see Section 2.2.4).

No studies were located that compared responses of other pertinent toxicity targets to mixtures of methylmercury and TCDD with responses to either compound alone. No studies were located in which pretreatment with methylmercury before TCDD exposure was examined for possible effects on TCDD toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., nervous system, thyroid; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 22. Effect of **Hexachlorobenzene** on **Methylmercury**

(see Table 13 for explanation of BINWOE codes)

**BINWOE: ? (0)**

*Direction of Interaction* - The direction of the interaction cannot be predicted for any toxicity target, because (1) data regarding possible interactions between hexachlorobenzene and methylmercury are restricted to one study (Renner 1980) reporting that hexachlorobenzene potentiated the acute lethality of mercuric chloride in rats, and another study with a similar exposure protocol that did not find similar results (Lecavalier et al. 1994), (2) there is no information indicating that pharmacokinetic interactions with hexachlorobenzene will influence methylmercury toxicity or carcinogenicity, and (3) mechanistic understanding is inadequate to support a reliable projection of interaction.

*Mechanistic Understanding* - Joint actions of methylmercury and hexachlorobenzene in producing effects on common target organs (immune suppression, nervous system impairment including altered neurological development, and cancer; see Appendix B and D) are plausible, but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action.

*Toxicological Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and methylmercury with responses to either compound alone. No studies were located in which pretreatment with hexachlorobenzene before methylmercury exposure was examined for possible effects on methylmercury toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

An acute oral exposure lethality study in rats (Renner 1980) reported that 400 or 600 mg/kg doses of hexachlorobenzene increased incidence of lethality in rats given 10, 12.5, or 18 mgHgCl<sub>2</sub>/kg doses (compared with mercuric chloride alone), but Lecavalier et al. (1994) reported that 10 or 12.5 mg HgCl<sub>2</sub>/kg did not produce lethality in rats in the presence or absence of 400 or 600 mg/kg hexachlorobenzene. The lethality endpoint in these studies is unlikely to be relevant to the development of altered neurological development, the methylmercury-induced health effect of most concern to public health.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 23. Effect of **Methylmercury** on **Hexachlorobenzene**  
(see Table 13 for explanation of BINWOE codes)

**BINWOE: = IIC(0)**  
for liver effects  
**BINWOE: ? (0)**  
for other effects

*Direction of Interaction* - The additive direction of interaction category is selected to reflect a projected lack of effect of methylmercury on hexachlorobenzene hepatotoxicity. Support of this projection is restricted to the observation that co-exposure to acute 10- or 12-mg/kg doses of mercuric chloride did not change liver effects from acute 400- or 600-mg/kg doses of hexachlorobenzene in rats (Lecavalier et al. 1994).

For other hexachlorobenzene effects, the direction of the interaction cannot be predicted in the absence of pertinent joint toxic action data, information clearly indicating that possible pharmacokinetic interactions with methylmercury will influence hexachlorobenzene toxicity or carcinogenicity, and adequate mechanistic understanding supporting a reliable projection of modes of joint action of methylmercury and hexachlorobenzene.

*Mechanistic Understanding* - Joint actions of methylmercury and hexachlorobenzene in producing effects on common target organs (immune suppression, nervous system impairment including altered neurological development, and cancer; see Appendix B and D) are plausible, but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action. Hexachlorobenzene-induced hepatic porphyria is thought to involve an unidentified reactive intermediate produced either by CYP11A-mediated or CYP1A-mediated metabolism (den Besten et al. 1993; Sinclair et al. 1997; see Appendix B), but no information was located indicating that methylmercury or mercury may alter metabolism of hexachlorobenzene. Reliable mechanistic inferences could not be made due to inadequate understanding of the mechanism(s) involved in other hexachlorobenzene-induced critical effects (immunosuppression, female reproductive organ disruption, altered neurological development, and cancer).

*Toxicological Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and methylmercury with responses to either compound alone. No studies were located in which pretreatment with methylmercury before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

A high-dose acute oral study in rats reported that co-exposure to 10 or 12.5 mg/kg doses of mercuric chloride did not change liver effects (increased liver weight and hepatocyte vacuolization) from exposure to 400 or 600 mg/kg hexachlorobenzene (Lecavalier et al. 1994), but the results were inadequately reported to allow quantitative assessment. The limited evidence supports a projection that methylmercury will not affect the hepatotoxicity of hexachlorobenzene and selection of the “additive” direction of interaction category for the effect of methylmercury on hexachlorobenzene. The lowest confidence rating for toxicological significance (“C”) was selected to reflect the absence of data on methylmercury and hexachlorobenzene, the poor reporting of the results of the Lecavalier et al. study, and the lack of corroborative results from other studies or species.

Table 23. Effect of **Methylmercury** on **Hexachlorobenzene** (*continued*)

*Additional Uncertainties* - Additional uncertainties would be associated with the use of the “additive” direction of interaction category for the effect of methylmercury on hexachlorobenzene with repeated exposure scenarios.

Table 24. Effect of *p,p'*-DDE on Methylmercury  
(see Table 13 for explanation of BINWOE codes)  
**BINWOE: ? (0)**

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that pharmacokinetic interactions with *p,p'*-DDE will influence methylmercury toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of interactions between *p,p'*-DDE and methylmercury.

*Mechanistic Understanding* - Oral exposure to either *p,p'*-DDE or methylmercury has been shown to adversely influence pre- and post-natal neurological development, and both compounds are known to produce adverse effects on the fully developed neurological system (see Table 1 and Appendices C and D). Joint actions between these two compounds in producing deficits in neurological developmental or function are plausible, but modes of possible joint action are unknown and unstudied. Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects on mitosis through impairment of microtubule assembly, inhibition of various enzymes such as protein kinase C, inhibition of transport mechanisms in developing brain cells, and alteration of synaptosomal release of neurotransmitters (e.g., dopamine) that may involve non-specific changes in membrane integrity or disruption of calcium homeostasis and subsequent activation of second messenger system or cell death (Appendix D). *p,p'*-DDE's actions on the developing and mature neurological system are poorly understood (see Appendix C), but, as with *p,p'*-DDT, may involve interference with sodium channels and potassium gates in neuronal membranes and inhibition of neuronal ATPases. Obvious cellular or molecular sites of possible interactions between *p,p'*-DDE and methylmercury are not apparent. Reliable mechanistic inferences could not be made due to inadequate understanding of the mechanism(s) involved in methylmercury-induced critical effects (altered neurological development, immunosuppression, and cancer).

*Toxicological Significance* - No studies were located that compared responses to mixtures of *p,p'*-DDE and methylmercury with responses to either compound alone. No studies were located in which pretreatment with *p,p'*-DDE before methylmercury exposure was examined for possible effects on methylmercury toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices C and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 25. Effect of **Methylmercury** on ***p,p'*-DDE**  
(see Table 13 for explanation of BINWOE codes)  
**BINWOE: ? (0)**

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that pharmacokinetic interactions with methylmercury will influence *p,p'*-DDE toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of possible modes of joint toxic actions between *p,p'*-DDE and methylmercury.

*Mechanistic Understanding* - Oral exposure to either *p,p'*-DDE or methylmercury has been shown to adversely influence pre- and post-natal neurological development, and both compounds are known to produce adverse effects on the fully developed neurological system (see Table 1 and Appendices C and D). Joint actions between these two compounds in producing deficits in neurological developmental or function are plausible, but modes of possible joint action are unknown and unstudied. Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects on mitosis through impairment of microtubule assembly, inhibition of various enzymes such as protein kinase C, inhibition of transport mechanisms in developing brain cells, and alteration of synaptosomal release of neurotransmitters (e.g., dopamine) that may involve non-specific changes in membrane integrity or disruption of calcium homeostasis and subsequent activation of second messenger system or cell death (Appendix D). *p,p'*-DDE's actions on the developing and mature neurological system are poorly understood (see Appendix C), but, as with *p,p'*-DDT, may involve interference with sodium channels and potassium gates in neuronal membranes and inhibition of neuronal ATPases. Obvious cellular or molecular sites of possible interactions between *p,p'*-DDE and methylmercury are not apparent. Reliable mechanistic inferences could not be made due to inadequate understanding of the mechanism(s) involved in *p,p'*-DDE-induced critical effects (altered neurological development, liver degeneration, immunosuppression, and cancer).

*Toxicological Significance* - No studies were located that compared responses to mixtures of *p,p'*-DDE and methylmercury with responses to either compound alone. No studies were located in which pretreatment with methylmercury before *p,p'*-DDE exposure was examined for possible effects on *p,p'*-DDE toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices C and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 26. Effect of PCBs on 2,3,7,8-TCDD

(see Table 13 for explanation of BINWOE codes)

**BINWOE: <IIB1aii** ( $0.32 \times 0.79 \times 1 \times 1 \times 0.79 = -0.20$ )

immune effects (suppression of cell-mediated immune response), acute

**BINWOE: <IIB2aii** ( $0.32 \times 0.79 \times 0.79 \times 1 \times 0.79 = -0.16$ )

immune effects (suppression of cell-mediated immune response), non-acute

**BINWOE: <IIC1ai** ( $0.32 \times 0.32 \times 1 \times 1 \times 1 = -0.10$ )

developmental toxicity (cleft palate, hydronephrosis in offspring), acute

**BINWOE: <IIC2ai** ( $0.32 \times 0.32 \times 0.79 \times 1 \times 1 = -0.08$ )

developmental toxicity (cleft palate, hydronephrosis in offspring), non-acute

**BINWOE: =IIC** (0)

body and thymus weight changes, hepatomegaly, decreased hepatic retinoids

**BINWOE: ?** (0)

thyroid hormone disruption, porphyria

**BINWOE: ?** (0)

female reproductive organ development

**BINWOE: ?** (0)

tumor promotion

**BINWOE: ?** (0)

other effects

*Direction of Interaction* - PCB mixtures antagonized TCDD-induced immunosuppression and developmental toxicity in mice. Intermediate-duration dietary exposure of rats to binary mixtures of TCDD plus each of three PCB congeners produced no synergism on changes in body and organ weights and levels of retinoids in liver indicating that PCB mixtures may additively act with 2,3,7,8-TCDD on these endpoints, but one congener (and not the other two) synergistically acted with 2,3,7,8-TCDD to increase hepatic porphyrin levels and deplete serum T4 levels. Available data are inconclusive regarding joint action of PCB mixtures and 2,3,7,8-TCDD in adversely affecting female reproductive organ development and promoting tumors.

*Mechanistic Understanding* - Oral exposures to PCBs or CDDs such as 2,3,7,8-TCDD are associated with wide arrays of health effects that show considerable overlap. Although some PCB congeners have been demonstrated to produce some effects via a common initial mechanistic step with 2,3,7,8-TCDD and other CDDs (binding to the Ah receptor), mechanistic understanding of ensuing processes is too incomplete to provide reliable projections of net physiological responses to joint exposure of PCB mixtures and 2,3,7,8-TCDD. In addition, there is evidence that other PCB congeners produce adverse effects via mechanisms that are independent of Ah receptor mediation, and some PCB congeners counteract effects of other PCB congeners and TCDD. Thus, mechanistic understanding for all directional BINWOEs was assigned a low data quality factor (III) to reflect the inability of available mechanistic understanding to support reliable projections of modes of joint actions between PCB mixtures and TCDD.

*Toxicologic Significance* - PCB mixtures antagonized TCDD-induced immunosuppression (intraperitoneal exposure) and cleft palate formation (oral exposure) in mice (Bannister et al. 1987; Davis and Safe 1989; Haake et al. 1987). There is evidence that individual PCB congeners vary in how they interact with TCDD in affecting these endpoints; some antagonize, some do not, and one was shown to potentiate TCDD-induced cleft palate formation (Biegel et al. 1989a, 1989b; Birnbaum et al. 1985; Morrissey et al. 1992). To reflect uncertainty that the observed antagonisms may occur with

Table 26. Effect of PCBs on 2,3,7,8-TCDD (continued)

*Toxicological Significance (continued)* - environmental PCB mixtures of varying composition and that antagonism will occur on other immune endpoints, a moderate data quality factor (B) was assigned to the BINWOE for immune effects (several PCB mixtures were demonstrated to antagonize TCDD inhibition of cell-mediated immune response, but one [Aroclor 1232] did not), whereas a low data quality factor (C) was assigned for developmental toxicity (the only PCB mixture examined for joint action with TCDD was Aroclor 1254).

A 13-Week dietary exposure rat studies of binary joint action of TCDD with each of three PCB congeners (expected to have various mechanisms of action) found no evidence of synergism on body or organ weight changes or vitamin A depletion in the liver (van Birgelen et al. 1992, 1994a, 1994b, 1996). Evidence for less-than-additive joint action on these endpoints (with each of the three PCB:TCDD binary mixtures examined) was found, but this may have been due to near-maximal effects occurring at the dose levels used. However, evidence was found for synergistic effects between one of the congeners (2,2',4,4',5,5'-hexachlorobiphenyl, but not the others) and TCDD on depletion of serum T4 levels and increased accumulation of porphyrins in liver (van Birgelen et al. 1992, 1996). The mechanistic basis for the apparent synergism between TCDD and this PCB congener without Ah-receptor affinity is unknown. Because of the variability between PCB congeners and the lack of data examining joint action of PCB mixtures and TCDD on T4 depletion and porphyria, the direction of interaction for the BINWOE was judged to be indeterminate.

3,3',4,4',5-Pentachlorobiphenyl appears to affect ovulation, ovarian weight, and circulating hormone levels in immature rats in an additive manner in combination with 2,3,7,8-TCDD and other dioxins (Gao et al. 2000), but the effect of the presence of ineffective PCB congeners, such as 2,2',4,4'-tetrachlorobiphenyl, in influencing these endpoints is unexamined.

The joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors is unexamined with the exception of one study that found evidence for less-than-additive joint action in a mixture of several PCBs with 2,3,7,8-TCDD, 1,2,3,7,8-pentachloro-*p*-dioxin, and 2,3,4,7,8-pentachlorodibenzofuran (van der Plas et al. 1999). There are data suggesting that one PCB congener (3,3',4,4',5-pentachlorobiphenyl) additively promotes tumors with TCDD and another (2,2',4,4',5,5'-hexachlorobiphenyl) antagonizes TCDD-promotion of tumors (Hemming et al. 1995; Wolfle 1998). The available data are inconclusive regarding the joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors.

*Modifying Factors* - The "ii" of the immune effects BINWOEs reflects the intraperitoneal exposure data that is its basis and the expected use for oral exposures; the "2" in the non-acute BINWOE reflects the acute data basis. No modifying factors were used in the BINWOE for body and thymus weight changes, hepatomegaly, and decreased hepatic retinoids because the 13-week dietary exposure data are expected to be directly relevant to oral repeated exposure scenarios.

*Additional Uncertainties* - The BINWOEs were derived to assess how environmental PCB mixtures may influence TCDD toxicity. PCB mixtures are the entity of concern, because humans are exposed to complex PCB mixtures and ATSDR PCB MRLs are based on data for PCB mixtures. There is a large degree of uncertainty in the BINWOEs, given evidence that the composition of environmental PCB mixtures can vary substantially, evidence that PCB congeners can vary in potency, mechanisms of action, and how they interact with TCDD, evidence that interactions between PCBs and TCDD can display complex relationships with dose and dose proportions, and the limited number of studies that have examined how mixtures of PCBs jointly act with TCDD in influencing the wide array of shared toxicity targets.



Table 27. Effect of **2,3,7,8-TCDD** on **PCBs**  
(see Table 13 for explanation of BINWOE codes)

**BINWOE: =IIIC (0)**

body and thymus weight changes, hepatomegaly, decreased hepatic retinoids

**BINWOE: ? (0)**

thyroid hormone disruption, porphyria

**BINWOE: ? (0)**

immune suppression, developmental toxicity

**BINWOE: ? (0)**

female reproductive organ development

**BINWOE: ? (0)**

tumor promotion

**BINWOE: ? (0)**

other effects

*Direction of Interaction* - Intermediate-duration dietary exposure of rats to binary mixtures of TCDD plus each of three PCB congeners produced no synergism on changes in body and organ weights and levels of retinoids in liver indicating that PCB mixtures may additively act with 2,3,7,8-TCDD on these endpoints, but one congener (and not the other two) synergistically acted with 2,3,7,8-TCDD to increase hepatic porphyrin levels and deplete serum T4 levels. Available studies of joint action of PCB mixtures and 2,3,7,8-TCDD on immune suppression and developmental toxicity do not discern how TCDD may influence PCB effects on these endpoints. Available data are inconclusive regarding joint action of PCB mixtures and 2,3,7,8-TCDD in adversely affecting female reproductive organ development and promoting tumors.

*Mechanistic Understanding* - Oral exposures to PCBs or CDDs such as 2,3,7,8-TCDD are associated with wide arrays of health effects that show considerable overlap. Although some PCB congeners have been demonstrated to produce some effects via a common initial mechanistic step with 2,3,7,8-TCDD and other CDDs (binding to the Ah receptor), mechanistic understanding of ensuing processes is too incomplete to provide reliable projections of net physiological responses to joint exposure of PCB mixtures and 2,3,7,8-TCDD. In addition, there is evidence that other PCB congeners produce adverse effects via mechanisms that are independent of Ah receptor mediation, and some PCB congeners counteract effects of other PCB congeners and TCDD. Thus, mechanistic understanding for all directional BINWOEs was assigned a low data quality factor (III) to reflect the inability of available mechanistic understanding to support reliable projections of modes of joint actions between PCB mixtures and TCDD.

*Toxicologic Significance* - A 13-week dietary exposure rat studies of binary joint action of TCDD with each of three PCB congeners (expected to have various mechanisms of action) found no evidence of synergism on body or organ weight changes or vitamin A depletion in the liver (van Birgelen et al. 1992, 1994a, 1994b, 1996). Evidence for less-than-additive joint action on these endpoints (with each of the three PCB:TCDD binary mixtures examined) was found, but this may have been due to near-maximal effects occurring at the dose levels used. However, evidence was found for synergistic effects between one of the congeners (2,2',4,4',5,5'-hexachlorobiphenyl, but not the others) and TCDD on depletion of serum T4 levels and increased accumulation of porphyrins in liver (van Birgelen et al. 1992, 1996). Because of this variability between PCB congeners and the lack of data examining joint action of PCB mixtures and TCDD on T4 depletion and porphyria, the direction of interaction for the BINWOE was judged to be indeterminate (?).

Table 27. Effect of **2,3,7,8-TCDD** on **PCBs** (*continued*)

*Toxicological Significance (continued)* - PCB mixtures antagonized TCDD-induced immunosuppression (intraperitoneal exposure) and cleft palate formation (oral exposure) in mice (Bannister et al. 1987; Davis and Safe 1989; Haake et al. 1987). At the dose levels used in these studies, toxicity of the potent 2,3,7,8-TCDD masked any adverse effects the PCB mixtures may have had on these endpoints. Thus, the influence that TCDD may have on PCB effects on these endpoints is indeterminate from the available data.

3,3',4,4',5-Pentachlorobiphenyl appears to affect ovulation, ovarian weight, and circulating hormone levels in immature rats in an additive manner in combination with 2,3,7,8-TCDD and other dioxins (Gao et al. 2000), but the effect of the presence of ineffective PCB congeners, such as 2,2',4,4'-tetrachlorobiphenyl, in influencing these endpoints is unexamined. No other data are available regarding PCBs and 2,3,7,8-TCDD joint action on these endpoints.

The joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors is unexamined with the exception of one study that found evidence for less-than-additive joint action in a mixture of several PCBs with 2,3,7,8-TCDD, 1,2,3,7,8-pentachloro-*p*-dioxin, and 2,3,4,7,8-pentachlorodibenzofuran (van der Plas et al. 1999). There are data suggesting that one PCB congener (3,3',4,4',5-pentachlorobiphenyl) additively promotes tumors with TCDD and another (2,2,4,4',5,5'-hexachlorobiphenyl) antagonizes TCDD-promotion of tumors (Hemming et al. 1995; Wolfle 1998). The available data are inconclusive regarding the joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors.

*Modifying factors* - No modifying factors were used in the BINWOE for body and thymus weight changes, hepatomegaly, and decreased hepatic retinoids, because the 13-week dietary exposure data are expected to be directly relevant to oral repeated exposure scenarios.

*Additional Uncertainties* - The BINWOEs were derived to assess how 2,3,7,8-TCDD may influence the toxicity of environmental PCB mixtures. PCB mixtures are the entity of concern, because humans are exposed to complex PCB mixtures and ATSDR PCB MRLs are based on data for PCB mixtures. There is a large degree of uncertainty in the BINWOEs, given evidence that the composition of environmental PCB mixtures can vary substantially, evidence that PCB congeners can vary in potency, mechanisms of action, and how they interact with TCDD, evidence that interactions between PCBs and TCDD can display complex relationships with dose and dose proportion, and the limited number of studies that have examined how mixtures of PCBs jointly act with TCDD in influencing the wide array of shared toxicity targets.

Table 28. Effect of PCBs on Hexachlorobenzene  
(see Table 13 for explanation of BINWOE codes)  
**BINWOE: ? (0)**

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no *in vivo* or *in vitro* data examining modes of joint action of PCBs and hexachlorobenzene on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatic porphyria, liver hypertrophy, decreased serum T4 levels, impaired immune response to foreign cells, impaired neurological development, reproductive organ dysfunction, and liver cancer have all been associated with oral exposure to either one of these agents (see Appendices B and E). However, there are no *in vitro* or *in vivo* studies designed to examine how these agents may jointly act to produce these effects. Processes in which potential interactions may occur include: induction of Phase I and II enzymes that may influence porphyria, liver hypertrophy, tissue damage from reactive oxygen species, and liver cancer development; disruption of thyroid hormone homeostasis via binding to thyroid hormone transport proteins, altering thyroid hormone metabolism, or damaging thyroid tissue; and disruption of sex hormone homeostasis via estrogenic, anti-estrogenic, androgenic, or anti-androgenic actions. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater than-additive manners.

*Toxicologic Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and PCBs with responses to hexachlorobenzene or PCBs alone. No studies were located in which pretreatment with PCBs before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible, but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 29. Effect of **Hexachlorobenzene** on **PCBs**

(see Table 13 for explanation of BINWOE codes)

**BINWOE: ? (0)**

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no *in vivo* or *in vitro* data examining modes of joint action of PCBs and hexachlorobenzene on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatic porphyria, liver hypertrophy, decreased serum T4 levels, impaired immune response to foreign cells, impaired neurological development, reproductive organ dysfunction, and liver cancer have all been associated with oral exposure to either one of these agents (see Appendices B and E). However, there are no *in vitro* or *in vivo* studies designed to examine how these agents may jointly act to produce these effects. Processes in which potential interactions may occur include: induction of Phase I and II enzymes that may influence porphyria, liver hypertrophy, tissue damage from reactive oxygen species, and liver cancer development; disruption of thyroid hormone homeostasis via binding to thyroid hormone transport proteins, altering thyroid hormone metabolism, or damaging thyroid tissue; and disruption of sex hormone homeostasis via estrogenic, anti-estrogenic, androgenic, or anti-androgenic actions. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater-than-additive manners.

*Toxicologic Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and PCBs with responses to hexachlorobenzene or PCBs alone. No studies were located in which pretreatment with hexachlorobenzene before PCB exposure was examined for possible effects on PCB toxicity at any target organ. Joint actions at several shared target organs are plausible, but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 30. Effect of PCBs on *p,p'*-DDE  
(see Table 13 for explanation of BINWOE codes)

**BINWOE: ? (0)**

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no pertinent *in vivo* or *in vitro* data examining modes of joint action of PCBs and *p,p'*-DDE on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatomegaly, liver cancer, suppression of cell-mediated immune responses, impaired neurological function and development, and impaired reproductive function and development have been associated with oral exposure to PCBs and oral exposure to *p,p'*-DDE (see Appendices C and E). Processes relevant to these shared toxicity targets in which possible interactions may occur include binding to androgen receptors, production of reactive metabolites or metabolic by-products that damage neurological tissue, and disruption of sex hormone homeostasis. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater-than-additive manners.

*Toxicologic Significance* - Interaction data are limited to reports that *p,p'*-DDE and Aroclor 1242, in combination with several pesticides but not by themselves, inhibit *in vitro* binding of 17 $\beta$ -estradiol to alligator estrogen receptors (Vonier et al. 1996) and that 5-month dietary exposure of mallards to both agents simultaneously did not alter *p,p'*-DDE-induced egg shell thinning, but decreased egg production capabilities compared with dietary exposure to either agent alone (Risebrough and Anderson 1975). In the absence of other information, these data are not expected to be directly relevant to DDE- or PCB-induced reproductive effects in humans or other shared toxicity targets mentioned above.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 31. Effect of *p,p'*-DDE on PCBs  
(see Table 13 for explanation of BINWOE codes)

**BINWOE: ? (0)**

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no pertinent *in vivo* or *in vitro* data examining modes of joint action of PCBs and *p,p'*-DDE on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatomegaly, liver cancer, suppression of cell-mediated immune responses, impaired neurological function and development, and impaired reproductive function and development have been associated with oral exposure to PCBs and oral exposure to *p,p'*-DDE. Processes relevant to these shared toxicity targets in which possible interactions may occur include binding to androgen receptors, production of reactive metabolites or metabolic by-products that damage neurological tissue, and disruption of sex hormone homeostasis. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater-than-additive manners.

*Toxicologic Significance* - Interaction data are limited to reports that *p,p'*-DDE and Aroclor 1242, in combination with several pesticides but not by themselves, inhibit *in vitro* binding of 17 $\beta$ -estradiol to alligator estrogen receptors (Vonier et al. 1996) and that 5-month dietary exposure of mallards to both agents simultaneously did not alter *p,p'*-DDE-induced egg shell thinning, but decreased egg production capabilities compared with dietary exposure to either agent alone (Risebrough and Anderson 1975). In the absence of other information, these data are not expected to be directly relevant to DDE- or PCB-induced reproductive effects in humans or other shared toxicity targets mentioned above.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

Table 32. Effect of **PCBs** on **Methylmercury**  
 (see Table 13 for explanation of BINWOE codes)  
**BINWOE: >IICb** ( $0.79 \times 0.32 \times 0.79 = +0.20$ )  
 for impaired neurological function or development  
**BINWOE: ?** (0)  
 for impaired reproductive performance  
**BINWOE: =IIB**(0)  
 for hepatic porphyria  
**BINWOE : ?** (0)  
 for decreased postnatal survival  
**BINWOE : ?** (0)  
 for other effects

*Direction of Interaction* - There is *in vitro* evidence from one study that PCBs and methylmercury may synergistically decrease dopamine levels in rat brain cells presumably via disruption of calcium homeostatic mechanisms (Bemis and Seegal 1999), but obvious synergism or additive joint action in affecting neurobehavioral endpoints was not demonstrated in a mouse *in vivo* study (Tanimura et al. 1980). A greater-than-additive joint action on neurological function or development is projected with a moderate degree of uncertainty. The direction of interaction for impaired reproductive performance is indeterminate (?) due to inadequate data on joint action for this toxicity target (Tanimura et al. 1980). Additive joint action to produce hepatic porphyria is supported by evidence from a study of quails exposed to Aroclor 1260 and methylmercury in the diet (Leonzio 1996b). For decreased postnatal survival from combined exposure to PCBs and methylmercury, data from a mouse study (Tanimura et al. 1980) and a mink study (Wren et al. 1987a, 1987b) are inadequate to support a projection of mode of possible joint action. For other effects, the direction of possible interactions cannot be projected because there are no pertinent *in vivo* or *in vitro* data, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Changes in neurological function or development from PCBs and methylmercury have been proposed to at least partly involve disruption of calcium homeostatic mechanisms in neural cells leading to changes in neurotransmitter release (e.g., dopamine) or cell damage. Combined *in vitro* exposure of rat striatal tissue to a methylmercury and a 1:1 mixture of Aroclor 1254/1260 appeared to synergistically deplete tissue levels of dopamine (Bemis and Seegal 1999). These data suggest a possible synergism between PCB mixtures and methylmercury in affecting neurological dysfunction and development. A moderate uncertainty rating (II; i.e., medium confidence rating) was selected to reflect several areas of uncertainty: (1) mechanistic linkages between changes in dopamine release and the development of PCB- or methylmercury-induced changes in neural function and development are poorly understood; (2) obvious synergism was not observed on *in vivo* endpoints of neurological function in mice exposed to mixtures of methylmercury and PCBs (Tanimura et al. 1980), and (3) the Bemis and Seegal (1999) report had some study design and reporting limitations that prevented a formal statistical characterization of the mode of joint action on dopamine release (see Section 2.2.10).

Mechanistic understanding for other potential shared toxicity targets between PCBs and methylmercury (e.g., impaired reproductive performance, hepatic porphyria) is too incomplete to support reliable projections of modes of joint actions.

Table 32. Effect of PCBs on Methylmercury (*continued*)

*Toxicologic Significance* - In a mouse study involving gestational and lactational exposure to Kanechlor 500 and methylmercury, exposure to either agent alone or in combination did not change several measures of F0- and F1-generation reproductive performance, neurobehavior of offspring, or prevalence of developmental anomalies (Tanimura et al. 1980). The study provides no evidence of obvious synergism or additive joint action between Kanechlor 500 and methylmercury in affecting neurobehavior, reproductive performance, or prevalence of developmental abnormalities. Design limitations of this study preclude more definitive conclusions on mode of joint action on these endpoints (see Section 2.2.10).

The data quality factor for highest toxicologic significance uncertainty (C) is selected due to the design limitations of the Tanimura et al. (1980) and the lack of other better designed studies examining possible joint actions on neurobehavior, reproductive, or developmental endpoints.

Combined gestational and lactational exposure of mice to Kanechlor 500 plus methylmercury, at 4 (but not 0.4) mg Hg/kg/day, decreased postnatal survival to a greater degree than did exposure to Kanechlor 500 alone; methylmercury alone did not affect postnatal survival (Tanimura et al. 1980). In a mink study, dietary concentrations of 1 ppm Aroclor 1254 alone, 1 ppm methylmercury alone, or 0.5 ppm concentrations of each together in the diet, did not affect postnatal survival, but 1 ppm concentrations of each in the diet decreased postnatal survival compared with controls (Wren et al. 1987a, 1987b). The design of these studies preclude comparisons between observed combined-exposure responses and predicted responses based on a presumed mode of joint action (see Section 2.2.10).

Intermediate-duration exposures of quail to methylmercury or Aroclor 1260 in the diet led to accumulation of porphyrins in liver; hepatic porphyrin levels in quail exposed to both agents simultaneously were similar to levels predicted based on additivity of response (Leonzio 1996b). To reflect uncertainty in extrapolating from quails to mammals and the lack of corroborative data, a moderate data quality factor (B) was selected for toxicological significance of the projection of additive joint action to produce hepatic porphyria.

*Modifying factors* - The modifying factor of “b” for the BINWOE for greater-than-additive joint action in impairing neurological function and development reflects uncertainty associated with the *in vitro* basis of the determination.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.



Table 33. Effect of **Methylmercury** on **PCBs**  
 (see Table 13 for explanation of BINWOE codes)  
**BINWOE: >IICb** ( $0.79 \times 0.32 \times 0.79 = +0.20$ )  
 for impaired neurological function or development  
**BINWOE: ?** (0)  
 for impaired reproductive performance  
**BINWOE: =IIB** (0)  
 for hepatic porphyria  
**BINWOE: ?** (0)  
 for decreased postnatal survival  
**BINWOE: ?** (0)  
 for other effects

*Direction of Interaction* - There is *in vitro* evidence from one study that PCBs and methylmercury may synergistically decrease dopamine levels in rat brain cells presumably via disruption of calcium homeostatic mechanisms (Bemis and Seegal 1999), but obvious synergism or additive joint action in affecting neurobehavioral endpoints was not demonstrated in a mouse *in vivo* study (Tanimura et al. 1980). A greater-than-additive joint action on neurological function or development is projected with a moderate degree of uncertainty. The direction of interaction for impaired reproductive performance is indeterminate (?) due to inadequate data on joint action for this toxicity target (Tanimura et al. 1980). Additive joint action to produce hepatic porphyria is supported by evidence from a study of quails exposed to Aroclor 1260 and methylmercury in the diet (Leonzio 1996b). For decreased postnatal survival from combined exposure to PCBs and methylmercury, data from a mouse study (Tanimura et al. 1980) and a mink study (Wren et al. 1987a, 1987b) are inadequate to support a reliable projection of mode of possible joint action. For other effects, the direction of possible interactions cannot be projected because there are no pertinent *in vivo* or *in vitro* data, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Changes in neurological function or development from PCBs and methylmercury have been proposed to at least partly involve disruption of calcium homeostatic mechanisms in neural cells leading to changes in neurotransmitter release (e.g., dopamine) or cell damage. Combined *in vitro* exposure of rat striatal tissue to a methylmercury and a 1:1 mixture of Aroclor 1254/1260 appeared to synergistically deplete tissue levels of dopamine (Bemis and Seegal 1999). These data suggest a possible synergism between PCB mixtures and methylmercury in affecting neurological dysfunction and development. A moderate uncertainty rating (II; i.e., medium confidence rating) was selected to reflect several areas of uncertainty: (1) mechanistic linkages between changes in dopamine release and the development of PCB- or methylmercury-induced changes in neural function and development are poorly understood; (2) obvious synergism was not observed on *in vivo* endpoints of neurological function in mice exposed to mixtures of methylmercury and PCBs (Tanimura et al. 1980), and (3) the Bemis and Seegal (1999) report had some study design and reporting limitations that prevented a formal statistical characterization of the mode of joint action on dopamine release (see Section 2.2.10).

Mechanistic understanding for other potential shared toxicity targets between PCBs and methylmercury (e.g., impaired reproductive performance, hepatic porphyria) is too incomplete to support reliable projections of modes of joint actions. In a mouse study involving gestational and lactational exposure to Kanechlor 500 and methylmercury, exposure to either agent alone or in combination did not change several measures of F0- and F1-generation reproductive performance,

Table 33. Effect of **Methylmercury** on **PCBs** (*continued*)

*Toxicologic Significance (continued)* - neurobehavior of offspring, or prevalence of developmental anomalies (Tanimura et al. 1980). The study provides no evidence of obvious synergism or additive joint action between Kanechlor 500 and methylmercury in affecting neurobehavior, reproductive performance, or prevalence of developmental abnormalities. Design limitations of this study preclude more definitive conclusions on mode of joint action on these endpoints (see Section 2.2.10).

The data quality factor for highest toxicologic significance uncertainty (C) is selected due to the design limitations of the Tanimura et al. (1980) and the lack of other better designed *in vivo* studies examining possible joint actions on neurobehavior, reproductive, or developmental endpoints.

Combined gestational and lactational exposure of mice to Kanechlor 500 plus methylmercury, at 4 (but not 0.4) mg Hg/kg/day, decreased postnatal survival to a greater degree than did exposure to Kanechlor 500 alone; methylmercury alone did not affect postnatal survival (Tanimura et al. 1980). In a mink study, dietary concentrations of 1 ppm Aroclor 1254 alone, 1 ppm methylmercury alone, or 0.5 ppm concentrations of each together in the diet, did not affect postnatal survival, but 1 ppm concentrations of each in the diet decreased postnatal survival compared with controls (Wren et al. 1987a, 1987b). The design of these studies preclude comparisons between observed combined-exposure responses and predicted responses based on a presumed mode of joint action (see Section 2.2.10).

Intermediate-duration exposures of quail to methylmercury or Aroclor 1260 in the diet led to accumulation of porphyrins in liver; hepatic porphyrin levels in quail exposed to both agents simultaneously were similar to levels predicted based on additivity of response (Leonzio 1996b). To reflect uncertainty in extrapolating from quails to mammals and the lack of corroborative data, a moderate data quality factor (B) was selected for toxicological significance of the projection of additive joint action to produce hepatic porphyria.

Combined exposure of rats or quail to commercial PCB mixtures and methylmercury appears to counteract PCB induction of hepatic CYP enzymes (Leonzio et al. 1996a; Takabatake et al. 1980), but the toxicological significance of this interaction is unclear.

*Modifying factors* - The modifying factor of “b” for the BINWOE for greater-than-additive joint action in impairing neurological function and development reflects uncertainty associated with the *in vitro* basis of the determination.

*Additional Uncertainties* - Uncertainties have been addressed in the above discussion of data quality weighting factors.

**Table 34. Matrix of BINWOE Determinations for Repeated Simultaneous Oral Exposure to Chemicals of Concern**

		ON TOXICITY OF				
		2,3,7,8-TCDD	Hexachloro-benzene	<i>p,p'</i> -DDE	Methylmercury	PCBs
E F F E C T  O F	2,3,7,8-TCDD		? (0)	Anti-andro-genic effects =III C(0) Other effects ? (0)	Immune suppression =IIIBbii (0) Other effects ? (0)	Body and organ weight changes, decreased retinoids in liver =III C(0) Other effects ? (0)
	Hexachloro-benzene	Body and thymus weight >IIIA2aii (+0.06) Other effects: ? (0)		? (0)	? (0)	? (0)
	<i>p,p'</i> -DDE	Anti-androgenic effects =III C(0) Other effects ? (0)	? (0)		? (0)	? (0)
	Methyl-mercury	? (0)	Liver effects =III C(0) Other effects ? (0)	? (0)		Neurological effects >IIICb (+0.20) Reproductive performance ? (0) Porphyria =IIIB(0) Other effects ? (0)
	PCBs	Immune suppression <IIIB2aii (-0.16) Developmental <IIIC2ai (-0.08) Body and organ weight changes, decreased retinoids in liver =III C(0) Other effects ? (0)	? (0)	? (0)	Neurological effects >IIICb (+0.20) Reproductive performance ? (0) Porphyria =IIIB(0) Other effects ? (0)	

BINWOE scheme (with numerical weights in parentheses) condensed from ATSDR (2001a):

DIRECTION: = additive (0); > greater than additive (+1); < less than additive (-1); ? indeterminate (0)

**MECHANISTIC UNDERSTANDING:**

- I: direct and unambiguous mechanistic data to support direction of interaction (1.0);  
 II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);  
 III: mechanistic data does not clearly indicate direction of interaction (0.32).

**TOXICOLOGIC SIGNIFICANCE:**

- A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);  
 B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);  
 C: toxicologic significance of interaction is unclear (0.32).

**MODIFYING FACTORS:**

- 1: anticipated exposure duration and sequence (1.0);  
 2: different exposure duration or sequence (0.79);  
 a: *in vivo* data (1.0);  
 b: *in vitro* data (0.79);  
 i: anticipated route of exposure (1.0);  
 ii: different route of exposure (0.79)



### 3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

To conduct exposure-based assessments of possible noncancer or cancer health hazards from oral exposures to mixtures of 2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs, component-based approaches are recommended, because there are no direct data available to characterize health hazards (and dose-response relationships) from exposure to the mixture. In addition, PBPK/PD models have not yet been developed that would predict appropriate target doses of the components. Recommendations focus on oral exposure scenarios (e.g., from fish consumption or other food sources) because these are most pertinent to public health concerns from these biopersistent chemicals. As discussed by ATSDR (1992b, 2001a), the exposure-based assessment of potential health hazard is a screening approach, to be used in conjunction with evaluation of community-specific health outcome data, consideration of community health concerns, and biomedical judgement, to assess the degree of public health hazard presented by mixtures of substances released into the environment. In a component-based approach for noncancer health effects: (1) joint additive actions of the components on shared targets of toxicity are assumed; (2) oral intakes are calculated based on measured concentrations of the components in media of concern (e.g., fish); (3) intakes are divided by MRLs or target-organ toxicity doses (TTDs); and (4) the resulting hazard quotients are summed to arrive at a hazard index. For cancer, a similar approach is taken, but the last two steps involve multiplication of the intakes by EPA cancer slope factors and summation of the resultant risk estimates.

The association reported in two epidemiological studies between frequent dietary consumption of Great Lakes fish by child-bearing-aged women and deficits in the neurological development of their children and between PCB levels in maternal body fluids and degree of neurological deficits (Fein et al. 1984; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b; Lonky et al. 1996; Stewart 1999, 2000b) identifies altered neurological development as a possible health hazard from frequent consumption of fish contaminated with biopersistent chemicals. The observed association, however, does not establish causal relationships and is not directly useful for the purposes of conducting exposure-based assessments of hazards specific to a community or exposure scenario. In contrast, the recommended component-based approaches are useful for this purpose. There is evidence that all five components of the mixture discussed in this profile can act on the developing nervous system, and the approaches allow assessments of the possibility of altered neurological development as well as other health hazards including cancer.

For exposure-based assessments of noncancer hazards from exposure to mixtures containing 2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs, a target-organ toxicity dose (TTD) modification of the hazard index approach as described by ATSDR (2001a) is recommended, because the components can target a wide range of overlapping health endpoints (see Table 1 in Introduction) and the critical effects (i.e., the basis of MRLs) can vary among the components depending on the component and the duration of exposure (see Table 12 in Section 2.3). Table 35 lists the pertinent oral MRLs and TTDs for endpoints of concern (hepatic, endocrine, immunological, neurological, reproductive, and developmental) for each of the components of the mixture. TTDs for chronic oral exposure scenarios have been derived as described in the Appendices, using the methods described by ATSDR (2001a).

For the assessment of the CDDs, concentrations in the media of concern should be converted to TEQs and summed to arrive at exposure levels that can be converted to oral intakes and compared with oral MRLs (or TTDs) for the reference dioxin, 2,3,7,8-TCDD (ATSDR 1998) or, for cancer assessment purposes (see below), used with an oral slope factor for 2,3,7,8-TCDD to estimate risk (EPA 1996; see Appendix A).

For the assessment of PCBs, concentrations of detected congeners in the media of concern should be added and converted to oral intakes (e.g., mg total PCBs/kg/day) for subsequent comparison with oral MRLs (or TTDs) for noncancer effects from PCB mixtures (ATSDR 2000) or, for cancer assessment purposes, with intakes associated with cancer risks ranging from  $1 \times 10^{-4}$  to  $1 \times 10^{-6}$ , calculated using oral slope factors derived by EPA for PCB mixtures (EPA 1996).

In the assessment of noncancer effects, hazard quotients (i.e., the ratio of an exposure estimate to the appropriate MRL) should first be calculated for each of the components (see Figure 2 in *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures*, ATSDR 2001a). If two or more of the individual components have hazard quotients equaling or exceeding ratios of 0.1, then the assessment should proceed. If only one or if none of the components have a hazard quotient that equals or exceeds 0.1, then no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard.

**Table 35. MRLs and TTDs for Repeated Oral Exposure to Chemicals of Concern. (See Appendices A, B, C, D, and E for Details of Derivations.)**

	2,3,7,8-TCDD	Hexachloro- benzene	<i>p,p'</i> -DDE	Methyl- mercury	PCBs
	mg/kg/day				
Hepatic	3x10 <sup>-9</sup>	2x10 <sup>-5</sup> (chronic MRL)	7x10 <sup>-4</sup>	NA	1x10 <sup>-4</sup>
Endocrine	1x10 <sup>-7</sup>	1x10 <sup>-3</sup>	NA	NA	1x10 <sup>-4</sup>
Immunological	2x10 <sup>-8</sup> (intermediate MRL)	4x10 <sup>-4</sup>	2x10 <sup>-3</sup>	3x10 <sup>-4</sup>	2x10 <sup>-5</sup> (chronic MRL)
Neurological	1x10 <sup>-9</sup> (chronic MRL)	8x10 <sup>-4</sup>	6x10 <sup>-2</sup>	3x10 <sup>-4</sup> (chronic MRL)	3x10 <sup>-5</sup> (intermediate MRL)
Reproductive	1x10 <sup>-9</sup>	3x10 <sup>-4</sup> (intermediate MRL)	2x10 <sup>-3</sup>	4x10 <sup>-4</sup>	2x10 <sup>-4</sup>
Developmental	1x10 <sup>-9</sup> (chronic MRL)	8x10 <sup>-3</sup>	2x10 <sup>-3</sup>	3x10 <sup>-4</sup> (chronic MRL)	3x10 <sup>-5</sup> (intermediate MRL)

NA = not applicable

Proceeding with the TTD modification of the hazard index approach involves calculating endpoint-specific hazard indices for each endpoint of concern, as described in ATSDR (2001a, Section 2.3.2 and Figure 2 with accompanying text). For example, a hazard index for developmental effects of this mixture is calculated as follows:

$$HI_{DEV} = \frac{E_{TCDD}}{MRL_{TCDD\ DEV}} + \frac{E_{HCB}}{TTD_{HCB\ DEV}} + \frac{E_{DDE}}{TTD_{DDE\ DEV}} + \frac{E_{MeHg}}{MRL_{MeHg\ DEV}} + \frac{E_{PCB}}{MRL_{PCB\ DEV}}$$

where  $HI_{DEV}$  is the hazard index for developmental toxicity,  $E_{TCDD}$  is the exposure to 2,3,7,8-TCDD (expressed in the same units as the corresponding MRL),  $MRL_{TCDD\ DEV}$  is the MRL for 2,3,7,8-TCDD which is based on developmental toxicity (1x10<sup>-9</sup> mg/kg/day),  $E_{HCB}$  is the exposure to hexachlorobenzene (expressed in the same units as the corresponding TDD),  $TTD_{HCB\ DEV}$  is the TTD for the developmental toxicity of hexachlorobenzene, and so forth. *DDE* and *MeHg* stand for *p,p'*-DDE and methylmercury.

Preliminary evidence that the exposure to the mixture may constitute a hazard is provided when the hazard index for a particular exposure scenario and health endpoint exceeds 1. In practice, concern for the possibility of a health hazard increases with increasing value of the hazard index above 1.

For exposure-based assessments of cancer hazards, cancer risks are estimated by multiplying lifetime oral exposure estimates (i.e., estimated oral intakes in units of mg/kg/day) for each component by the appropriate EPA cancer oral slope factor (in units of risk per mg/kg/day). Oral cancer slope factors are available for 2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, and PCBs (see Appendices A, B, C, and E). If two or more of the components have cancer risks equal to or exceeding  $1 \times 10^{-6}$ , then the component cancer risks are summed to arrive at a cancer risk estimate for the mixture. If only one or if none of the component risks equals or exceeds  $1 \times 10^{-6}$ , then no further assessment of joint toxic action is needed due to the low likelihood that additivity and/or interactions would result in a significant health hazard. Mixture cancer risks equaling or exceeding  $1 \times 10^{-4}$  are taken as an indicator that the mixture may constitute a health hazard.

The addition of hazard quotients (or cancer risks) for a particular exposure scenario assumes that less-than-additive (e.g., antagonistic or inhibitory) or greater-than-additive (e.g., synergistic or potentiating) interactions do not occur among the components of the mixture. A primary objective of this profile is to assess available information on modes of joint toxic actions of 2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs. As discussed in Section 2.3, a weight-of-evidence approach was used to evaluate the possible influence of binary interactions among the components in the overall toxicity of the mixture. Table 34 (at the end of Section 2.3) lists BINWOE determinations that were made for the joint action on various endpoints by the 10 pairs of the components. There is only a limited amount of evidence that non-additive interactions exist for a few of the chemical pairs:

- hexachlorobenzene potentiation of TCDD reduction of body and thymus weights;
- PCB antagonism of TCDD immunotoxicity and developmental toxicity; and
- synergism between PCBs and methylmercury in disrupting neurological function and development.

The low BINWOE numerical scores for these possible interactions (none are higher than 0.2 compared with a maximum score of 1) reflect the quality of the data on which they are based and indicate a fair amount of uncertainty that they will occur (Table 34). For the remaining pairs, additive joint action at shared targets of toxicity is either supported by data (for a few pairs) or is recommended as a public



health protective assumption due to lack of interaction data, conflicting interaction data, and/or lack of mechanistic understanding to reliably support projections of modes of joint toxic action (Table 34). The weight-of-evidence analysis indicates that scientific evidence that greater-than-additive or less-than-additive interactions will occur among the five components is limited and supports the use of the additivity assumption as a public health protective measure in exposure-based screening assessments for potential health hazards from exposure to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs.

When the screening assessment provides preliminary evidence that the mixture may constitute a health hazard (i.e., one or more endpoint-specific hazard indexes exceed one, or the mixture cancer risk equals or exceeds  $1 \times 10^{-4}$ ), additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2001a). The additional evaluation includes biomedical judgment, assessment of community-specific health outcome data, and consideration of community health concerns (ATSDR 1992).



## 4. Conclusions

A component-based approach that assumes additive joint toxic action is recommended for exposure-based screening assessments of possible noncancer or cancer health hazards from oral exposure to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs, for the following reasons. There are no direct data available to characterize health hazards (and dose-response relationships) from mixtures containing all five components. PBPK/PD models have not yet been developed that would predict pertinent target doses of the components under scenarios involving exposure to mixtures of all five components. Finally, available information on toxic actions of the individual components indicates that joint actions of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs on several toxicity targets are plausible, including nervous system development, immune functions, reproductive organ development, and cancer.

Epidemiological studies of possible health hazards associated with consumption of fish likely to have been contaminated with these chemicals identify altered neurological development as a possible health hazard, but do not establish causal relationships and are not directly useful for exposure-based assessments of health hazards specific to a community or an exposure scenario.

Weight-of-evidence analyses of available data on the joint toxic action of mixtures of these components indicate that scientific evidence for greater-than-additive or less-than-additive interactions among these components is limited, and inadequate to characterize the possible modes of joint action on most of the pertinent toxicity targets. Therefore, it is recommended that additivity be assumed as a public health protective measure in exposure-based screening assessments for potential hazards to public health from exposure to mixtures of these components. When the screening assessment indicates a potential hazard, further evaluation is needed, using biomedical judgment and community-specific health outcome data, and taking into account community health concerns.



## 5. List of References

Abbott BD, Birnbaum LS. 1989. Cellular alterations and enhanced induction of cleft palate after coadministration of retinoic acid and TCDD. *Toxicol Appl Pharmacol* 99:287–301.

\*Abbott BD, Perdew GH, Birnbaum LS. 1994. Ah receptor in embryonic mouse palate and effects of TCDD on receptor expression. *Toxicol Appl Pharmacol* 126:16–25.

Adami H-O, Lipworth L, Titus-Ernstoff L, et al. 1995. Organochlorine compounds and estrogen-related cancers in women. *Cancer Causes Control* 6:551–566.

Andersen ME, Birnbaum LS, Barton HA, et al. 1997a. Regional hepatic CYP1A1 and CYP1A2 induction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin evaluated with a multicompartiment geometric model of hepatic zonation. *Toxicol Appl Pharmacol* 144:145–155.

Andersen ME, Eklund CR, Mills JJ, et al. 1997b. A multicompartiment geometric model of the liver in relation to regional induction of cytochrome P450s. *Toxicol Appl Pharmacol* 144:135–144.

Anderson H, Falk C, Fiore B, et al. 1996. Consortium for the health assessment of Great Lakes sportfish consumption. *Toxicol Ind Health* 12(3–4):369–373.

\*Anderson HA, Falk C, Hanrahan L, et al. 1998. Profiles of Great Lakes critical pollutants: A sentinel analysis of human blood and urine. *Environ Health Perspect Suppl* 106(5):279–289.

\*Andersson PL, Blom A, Johannisson A, et al. 1999. Assessment of PCBs and hydroxylated PCBs as potential xenoestrogens: *In vitro* studies based on MCF-7 cell proliferation and induction of vitellogenin in primary culture of rainbow trout hepatocytes. *Arch Environ Contam Toxicol* 37:145–150.

\*Andrews JE, Courtney KD, Stead AG, et al. 1989. Hexachlorobenzene-induced hyperthyroidism and osteosclerosis in rats. *Fundam Appl Toxicol* 12:242–251.

\*Angus WGR, Mousa MA, Vargas VM, et al. 1997. Inhibition of L-aromatic amino acid decarboxylase by polychlorinated biphenyls. *Neurotoxicology* 18:857–868.

\*Arcaro KF, Yi L, Seegal RF, et al. 1999. 2,2',6,6'-Tetrachlorobiphenyl is estrogenic in vitro and in vivo. *J Cell Biochem* 72:94–102.

\*Arnold DL, Bryce F, Karpinski K, et al. 1993b. Toxicological consequences of Aroclor 1254 ingestion by female Rhesus (*macaca mulatta*) monkeys. Part 1B. Prebreeding phase: Clinical and analytical laboratory findings. *Food Chem Toxicol* 31(11):811–824.

\*Arnold DL, Bryce F, McGuire PF, et al. 1995. Toxicological consequences of Aroclor 1254 ingestion by female Rhesus (*macaca mulatta*) monkeys. Part 2. Reproduction and infant findings. *Food Chem Toxicol* 33:457–474.

---

\*Cited in text

\*Arnold DL, Bryce F, Stapley R, et al. 1993a. Toxicological consequences of Aroclor 1254 ingestion by female Rhesus (*macaca mulatta*) monkeys. Part 1A. Prebreeding phase: Clinical health findings. *Food Chem Toxicol* 31(11):799–810.

\*Arnold DL, Nera EA, Stapley R, et al. 1996a. Prevalence of endometriosis in Rhesus (*macaca mulatta*) monkeys ingesting PCB (Aroclor 1254): Review and evaluation. *Fundam Appl Toxicol* 31(1):42–55.

Arnold DL, Stapley R, Bryce F, et al. 1998b. A multigeneration study to ascertain the toxicological effects of Great Lakes salmon fed to rats: Study overview and design. *Reg Toxicol Pharmacol* 27:S1–S7.

Arnold SF, Klotz DM, Collins BM, et al. 1996b. Synergistic activation of estrogen receptor with combinations of environmental chemicals. *Science* 272:1489–1492.

Asplund L, Svensson B, Eriksson U, et al. 1994. Polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT) and 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE) in human plasma related to fish consumption. *Arch Environ Health* 49:477–486.

\*Astroff B, Safe S. 1990. 2,3,7,8-Tetrachlorodibenzo-p-dioxin as an antiestrogen: Effect on rat uterine peroxidase activity. *Biochem Pharmacol* 39:485–488.

ATSDR. 1992a. Toxicological profile for chlorinated dibenzofurans (CDFs). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*ATSDR. 1992b. Public health assessment guidance manual. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*ATSDR. 1996. Toxicological Profile for Hexachlorobenzene. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*ATSDR. 1998. Toxicological Profile for Chlorinated Dibenzo-p-Dioxins: December 1998. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*ATSDR. 1999a. Toxicological Profile for DDT, DDE, DDD: August, 1999: Preliminary draft for Public Comment. Atlanta, GA: Agency for Toxic Substances and Disease Registry, Public Health Service, U.S. Department of Health and Human Services.

\*ATSDR. 1999b. Toxicological Profile for Mercury. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*ATSDR. 2000. Toxicological profile for polychlorinated biphenyls. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*ATSDR. 2001a. Draft guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*ATSDR. 2001b. Draft guidance manual for preparation of an interaction profile. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

ATSDR. 2001c. Interaction profile on Persistent Chemicals Found in Breast Milk: Chlorinated Dibenzop-dioxins, Hexachlorobenzene, *p,p'*-DDE, and Polychlorinated Biphenyls. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

\*Aulerich RJ, Ringer RK. 1977. Current status of PCB toxicity to mink, and effect on their reproduction. *Arch Environ Contam Toxicol* 6:279–292.

\*Aulerich RJ, Bursian SJ, Breslin WJ, et al. 1985. Toxicological manifestations of 2,4,5, 2',4',5'-, 2,3,6,2',3',6'-, and 3,4,5,3',4',5'-hexachlorobiphenyl and Aroclor 1254 in mink. *J Toxicol Environ Health* 15:63–79.

\*Aulerich RJ, Bursian SJ, Evans MG, et al. 1987. Toxicity of 3,4,5,3',4',5'-hexachlorobiphenyl to mink. *Arch Environ Contam Toxicol* 16:53–60.

\*Backlin BM, Bergman A. 1995. Histopathology of postpartum placental sites in mink (*mustela vison*) exposed to polychlorinated biphenyls or fractions thereof. *APMIS* 103(12):843–54.

\*Backlin BM, Madej A, Forsberg M. 1997. Histology of ovaries and uteri and levels of plasma progesterone, oestradiol-17beta and oestrone sulphate during the implantation period in mated and gonadotrophin-releasing hormone-treated mink (*mustela vison*) exposed to polychlorinated biphenyls. *J Appl Toxicol* 17(5):297–306.

\*Backlin BM, Persson E, Jones CJ, et al. 1998. Polychlorinated biphenyl (PCB) exposure produces placental vascular and trophoblastic lesions in the mink (*mustela vison*): a light and electron microscopic study. *APMIS* 106(8):785–99.

\*Bager Y, Kato Y, Kenne K, et al. 1997. The ability to alter the gap junction protein expression outside GST-P positive foci in liver of rats was associated to the tumour promotion potency of different polychlorinated biphenyls. *Chem Biol Interact* 103(3):199–212.

\*Banerjee BD, Ray A, Pasha ST. 1996. A comparative evaluation of immunotoxicity of DDT and its metabolites in rats. *Indian J Exp Biol* 34:517–522.

\*Bannister R, Davis D, Zacherewski T, et al. 1987. Aroclor 1254 as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist: Effects on enzyme induction and immunotoxicity. *Toxicology* 46:29–42.

\*Barnett JB, Barfield L, Walls R, et al. 1987. The effect of in utero exposure to hexachlorobenzene on the developing immune response of BALB/C mice. *Toxicol Lett* 39:263–274.

\*Barsotti DA, Marlar RJ, Allen JR. 1976. Reproductive dysfunction in Rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). *Food Cosmet Toxicol* 14:99–103.

\*Battershill JM. 1994. Review of the safety assessment of polychlorinated biphenyls (PCBs) with particular reference to reproductive toxicity. *Hum Exp Toxicol* 13:581–597.

- \*Bemis JC, Seegal RF. 1999. Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content *in vitro*. *Environ Health Perspect* 107:879–885.
- \*Bergman A, Athanasiadou M, Bergek S, et al. 1992. PCB and PCB methyl sulfones in mink treated with PCB and various PCB fractions. *Ambio* 21(8):570–576.
- \*Biegel L, Harris M, Davis D, et al. 1989b. 2,2',4,4',5,5'-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6J mice. *Toxicol Appl Pharmacol* 97:561–571.
- \*Biegel L, Howie L, Safe S. 1989a. Polychlorinated biphenyl (PCB) congeners as 2,3,7,8-TCDD antagonists: Teratogenicity studies. *Chemosphere* 19(1–6):955–958.
- \*Bignert A, Olsson M, Persson W, et al. 1998. Temporal trends of organochlorines in Northern Europe, 1967–1995. Relation to global fractionation, leakage from sediments and international measures. *Environ Pollut* 99:177–198.
- Birnbaum LS. 1994. The mechanism of dioxin toxicity: Relationship to risk assessment. *Environ Health Perspect Suppl* 102(9):157–167.
- \*Birnbaum LS, Weber H, Harris MW, et al. 1985. Toxic interaction of specific polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Increased incidence of cleft palate in mice. *Toxicol Appl Pharmacol* 77:292–302.
- \*Bitman J, Cecil HC. 1970. Estrogenic activity of DDT analogs and polychlorinated biphenyls. *J Agric Food Chem* 18:1108–1112.
- \*Bjerke DL, Sommer RJ, Moore RW, et al. 1994. Effects of *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure on responsiveness of the male rat reproductive system to testosterone stimulation in adulthood. *Toxicol Appl Pharmacol* 127:250–257.
- \*Bowerman WW, Best DA, Grubb TG, et al. 2000. Assessment of environmental endocrine disruptors in bald eagles of the Great Lakes. *Chemosphere* 1569–1574.
- \*Boyages SC. 2000. The neuromuscular system and brain in hypothyroidism. In: Braverman LE, Utiger RD, eds. *Werner & Ingbar's the thyroid: A fundamental and clinical text*. Eighth edition. Philadelphia, PA: Lippincott Williams & Wilkins, 804–810.
- \*Brandt I, Bergman A. 1987. PCB methyl sulphones and related compounds: Identification of target cells and tissues in different species. *Chemosphere* 8/9:1671–1676.
- \*Brezner E, Terkel J, Perry AS. 1984. The effect of Aroclor 1254 (PCB) on the physiology of reproduction in the female rat-I. *Comp Biochem Physiol* 77:65–70.
- \*Brouwer A, Morse DC, Lans MC, et al. 1998. Interactions of persistent environmental organohalogenes with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. *Toxicol Ind Health* 14(1–2):59–84.
- \*Brown AP, Gamey PE. 1995. Neutrophil degranulation and superoxide production induced by polychlorinated biphenyls are calcium dependent. *Toxicol Appl Pharmacol* 13:198–205.



- Brown NM, Manzillo PA, Zhang J-X, et al. 1998. Prenatal TCDD and predisposition to mammary cancer in the rat. *Carcinogenesis* 19:1623–1629.
- \*Buck GM, Sever LE, Mendola P, et al. 1997. Consumption of contaminated sportfish from Lake Ontario and time-to-pregnancy. *Am J Epidemiol* 146(11):949–954.
- Burbacher TM, Mohamed MK, Mottett NK. 1988. Methylmercury effects on reproduction and offspring size at birth. *Reprod Toxicol* 1(4):267–278.
- \*Byrne JJ, Carbone JP, Hanson EA. 1987. Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polybrominated biphenyl. *Endocrinology* 121:520–527.
- \*Byrne JJ, Carbone JP, Pepe MG. 1988. Suppression of serum adrenal cortex hormones by chronic low-dose polychlorobiphenyl or polybromobiphenyl treatments. *Arch Environ Contam Toxicol* 17:47–53.
- Cantoni C, Comi G. 1997. Changes in the concentrations of pesticide residues in foods and in human tissues between 1960 and 1996. *Outlook On Agriculture* 26(1):47–52.
- Carthew P, Smith AG. 1994. Pathological mechanisms of hepatic tumour formation in rats exposed chronically to dietary hexachlorobenzene. *J Appl Toxicol* 14(6):447–452.
- \*Chauhan KR, Kodavanti PRS, McKinney JD. 2000. Assessing the role of *ortho*-substitution on polychlorinated biphenyl binding to transthyretin, a thyroxine transport protein. *Toxicol Appl Pharmacol* 162:10–21.
- \*Cheek AO, Kow K, Chen J, et al. 1999. Potential mechanisms of thyroid disruption in humans: Interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Environ Health Perspect* 107(4):273–278.
- \*Choksi NY, Kodavanti PRS, Tilson HA, et al. 1997. Effects of polychlorinated biphenyls (PCBs) on brain tyrosine hydroxylase activity and dopamine synthesis in rats. *Fundam Appl Toxicol* 39:76–80.
- \*Chu I, Poon R, Yagminas A, et al. 1998. Subchronic toxicity of PCB 105 (2,3,3',4,4'-pentachlorobiphenyl) in rats. *J Appl Toxicol* 18:285–292.
- \*Chu I, Villeneuve DC, Tagimnas A, et al. 1994. Subchronic toxicity of 3,3',4,4',5-pentachlorobiphenyl in the rat. *Fundam Appl Toxicol* 22:457–468.
- Chu I, Villeneuve DC, Valli VE, et al. 1984. Toxicological response and its reversibility in rats fed Lake Ontario or Pacific coho salmon for 13 weeks. *J Environ Sci Health B* 19(8–9):713–731.
- \*Chu I, Villeneuve DC, Yagminas A, et al. 1995. Toxicity of PCB 77 (3,3',4,4'-tetrachlorobiphenyl) and PCB 118 (2,3',4,4',5-pentachlorobiphenyl) in the rat following subchronic dietary exposure. *Fundam Appl Toxicol* 26:282–292.
- \*Chu I, Villeneuve DC, Yagminas A, et al. 1996a. Toxicity of 2,2',4,4',5,5'-hexachlorobiphenyl in rats: Effects following a 90-day oral exposure. *J Appl Toxicol* 16(2):121–128.

- \*Chu I, Villeneuve DC, Yagminas A, et al. 1996b. Toxicity of 2,4,4'-trichlorobiphenyl in rats following 90-day dietary exposure. *J Toxicol Environ Health* 49(3):301–318.
- \*Cogliano VJ. 1998. Assessing the cancer risk from environmental PCBs. *Environ Health Perspect* 106(6):317–323.
- \*Collins WT, Capen CC. 1980. Fine structural lesions and hormonal alterations in thyroid glands of perinatal rats exposed *in utero* and by the milk to polychlorinated biphenyls. *Am J Pathol* 99:125–142.
- \*Collins WT, Capen CC, Kasza L, et al. 1977. Effect of polychlorinated biphenyl (PCB) on the thyroid gland of rats: Ultrastructural and biochemical investigations. *Am J Pathol* 89:119–136.
- \*Connor K, Ramamoorthy K, Moore M, et al. 1997. Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: Structure-activity relationships. *Toxicol Appl Pharmacol* 145:111–123.
- \*Connor K, Safe S, Jefcoate CR, et al. 1995. Structure-dependent induction of CYP2B by polychlorinated biphenyl congeners in female Sprague-Dawley rats. *Biochem Pharmacol* 50(11):1913–1920.
- \*Cooke PS, Zhao Y-D, Hansen LG. 1996. Neonatal polychlorinated biphenyl treatment increases adult testis size and sperm production in the rat. *Toxicol Appl Pharmacol* 136:112–117.
- \*Corey DA, Juarez de Ku LM, Bingman VP, et al. 1996. Effects of exposure to polychlorinated biphenyl (PCB) from conception on growth, and development of endocrine, neurochemical, and cognitive measures in 60 day old rats. *Growth Dev Aging* 60:131–143.
- \*Courtney KD. 1979. Hexachlorobenzene (HCB): A review. *Environ Res* 20:225–266.
- Courval JM, De Hoog JV, Stein AD, et al. 1997. Sport caught fish consumption and conception failure in Michigan anglers. Montreal, Quebec, Canada: Health Conference 1997 : Great Lakes and St. Lawrence.
- \*Courval JM, DeHoog JV, Stein AD, et al. 1999. Sport-caught fish consumption and conception delay in licensed Michigan anglers. *Environ Res (A)* 80:S183–S188.
- Couture LA, Abbott BD, Birnbaum LS. 1990. A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Recent advances toward understanding the mechanism. *Teratology* 42:619–627.
- Crane JL. 1996. Carcinogenic human health risks associated with consuming contaminated fish from five Great Lakes areas of concern. *J Great Lakes Res* 22(3):653–668.
- Crisp TM, Clegg ED, Cooper RL, et al. 1998. Environmental endocrine disruption: An effects assessment and analysis. *Environ Health Perspect Suppl* 106(1):11–56.
- \*Daly HB. 1991. Reward reductions found more aversive by rats fed environmentally contaminated salmon. *Neurotoxicol Teratol* 13:449–453.

- \*Danse IR, Jaeger RJ, Kroger MK, et al. 1997. Position paper of the American Council on Science and Health: Public health concerns about environmental polychlorinated biphenyls (PCBs). *Ecotoxicol Environ Saf* 38:71–84.
- \*Dar E, Kanarek MS, Anderson HA, et al. 1992. Fish consumption and reproductive outcomes in Green Bay, Wisconsin. *Environ Res* 59:189–201.
- \*Darnerud PO, Morse D, Klasson-Wehler E, et al. 1996. Binding of a 3,3', 4,4'-tetrachlorobiphenyl (CB-77) metabolite to fetal transthyretin and effects on fetal thyroid hormone levels in mice. *Toxicology* 106(1–3):105–114.
- Daston GP, Gooch JW, Breslin WJ, et al. 1997. Environmental estrogens and reproductive health: A discussion of the human and environmental data. *Reprod Toxicol* 11(4):465–481.
- \*Davis D, Safe S. 1989. Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Lett* 48:35–43.
- \*Davis D, Safe S. 1990. Immunosuppressive activities of polychlorinated biphenyls in C57BL/6N mice: Structure-activity relationships as Ah receptor agonists and partial antagonists. *Toxicology* 63:97–111.
- \*DeCaprio AP, McMartin DM, O'Keefe PW, et al. 1986. Subchronic oral toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the guinea pig: Comparisons with a PCB-containing transformer fluid pyrolysate. *Fundam Appl Toxicol* 6:454–463.
- \*De Haan LHJ, Halfwerk S, Hovens SEL, et al. 1996. Inhibition of intercellular communication and induction of ethoxyresorufin-*O*-deethylase activity by polychlorobiphenyls, -dibenzo-*p*-dioxins and -dibenzofurans in mouse hepatic cells. *Environ Toxicol Pharmacol* 1:27–37.
- \*DenBesten C, Bennik MH, Bruggeman I, et al. 1993. The role of oxidative metabolism in hexachlorobenzene-induced porphyria and thyroid hormone homeostasis: A comparison with pentachlorobenzene in a 13-week feeding study. *Toxicol Appl Pharmacol* 119:181–194.
- DenBesten C, Bennik MMH, Van Iersel M, et al. 1994. Comparison of the urinary metabolite profiles of hexachlorobenzene and pentachlorobenzene in the rat. *Chem Biol Interact* 90:121–137.
- \*Den Tonkelaar EM, Verschuuren HG, Bankovska J, et al. 1978. Hexachlorobenzene toxicity in pigs. *Toxicol Appl Pharmacol* 43:137–145.
- DeRosa CT, Johnson BL. 1996. Strategic elements of ATSDR's Great Lakes Human Health Effects Research Program. *Toxicol Ind Health* 12(3–4):315–325.
- \*Desaulniers D, Poon R, Phan W, et al. 1997. Reproductive and thyroid hormone levels in rats following 90-day dietary exposure to PCB 28 (2,4,4'-trichlorobiphenyl) or PCB 77 (3,3',4,4'-tetrachlorobiphenyl). *Toxicol Ind Health* 13(5):627–638.
- de Solla SR, Bishop CA, Van Der Kraak G, et al. 1998. Impact of organochlorine contamination on levels of sex hormones and external morphology of common snapping turtles (*Chelydra serpentina serpentina*) in Ontario, Canada. *Environ Health Perspect* 106(5):253–260.

- \*De Vito MJ, Birnbaum LS. 1994. Toxicology of dioxins and related chemicals. In: Schecter A, ed. Dioxins and health. New York, NY: Plenum Press, 139–162.
- Dickman MD, Leung KMC. 1998. Mercury and organochlorine exposure from fish consumption in Hong Kong. *Chemosphere* 37(5):991–1015.
- \*Durkin P. 1995. Development of mixtures assessment methods: Guidelines for application of the binary weight-of-evidence methodology. Submitted to The Kevric Company, Inc., Silver Spring, MD. SERA TR 95-018-001a.
- Elliott JE, Norstrom RJ, Smith GEJ. 1996. Patterns, trends, and toxicological significance of chlorinated hydrocarbon and mercury contaminants in bald eagle eggs from the Pacific coast of Canada, 1990–1994. *Arch Environ Contam Toxicol* 31:354–367.
- \*EPA. 1988. Recommendations for and documentation of biological values for use in risk assessment. Cincinnati, OH: U.S. Environmental Protection Agency. PB88-179874.
- \*EPA. 1996. PCBs: Cancer dose-response assessment and application to environmental mixtures. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. EPA/600/P-96/001F.
- \*EPA. 1997a. Addition of dioxin and dioxin-like compounds: Modification of polychlorinated biphenyls (PCBs) listing; toxic chemical release reporting; community right-to-know. Washington, DC: U.S. Environmental Protection Agency. Federal Register. 62FR 24887.
- \*EPA. 1997b. Health Effects Assessment Summary Tables: FY 1997 Update. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Emergency and Remedial Response. NTIS PB97-921199.
- \*Eriksson P, Norberg A. 1986. The effects of DDT, DDOH-palmitic acid, and a chlorinated paraffin on muscarinic receptors and the sodium-dependent choline uptake in the central nervous system of immature mice. *Toxicol Appl Pharmacol* 85:121–127.
- \*Eriksson P, Ahlbom J, Frederiksson A. 1992. Exposure to DDT during a defined period in neonatal life induces permanent changes in brain muscarinic receptors and behaviour in adult mice. *Brain Res* 582:277–281.
- \*Eriksson P, Nilsson-Hakansson L, Nordberg A, et al. 1990. Neonatal exposure to DDT and its fatty acid conjugate: Effects on cholinergic and behavioural variables in the adult mouse. *Neurotoxicology* 11:345–354.
- \*Expert Panel. 1994. Interpretive review of the potential adverse effects of chlorinated organic chemicals on human health and the environment: Chapter 5: Polychlorinated biphenyls. *Regul Toxicol Pharmacol* 20(1):S187–S307.
- Fan F, Rozman KK. 1995. Short- and long-term biochemical effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Long-Evans rats. *Toxicol Lett* 75:209–216.

- \*Feeley MM, Jordan SA. 1998. Dietary and tissue residue analysis and contaminant intake estimations in rats consuming diets composed of Great lakes salmon: A multigeneration study. *Regul Toxicol Pharmacol* 27:S8–S17.
- \*Feeley MM, Jordan SA, Gilman AP. 1998. The Health Canada Great Lakes Multigeneration Study—summary and regulatory considerations. *Regul Toxicol Pharmacol* 27:S90–S98.
- \*Fein GG, Jacobson JL, Jacobson SW, et al. 1984. Prenatal exposure to polychlorinated biphenyls: Effects on birth size and gestational age. *J Pediatr* 105(2):315–320.
- \*Fischer LJ, Seegal RF, Gamey PE, et al. 1998. Symposium overview: Toxicity of non-coplanar PCBs. *Toxicol Sci* 41:49–61.
- \*Fitzhugh O. 1948. Use of DDT insecticides on food products. *Ind Eng Chem* 40:704–705.
- Foster WG. 1995. The reproductive toxicology of Great Lakes contaminants. *Environ Health Perspect Suppl* 103(9):63–69.
- \*Franklin MR, Phillips JD, Kushner JP. 1997. Cytochrome P450 induction, uroporphyrinogen decarboxylase depression, porphyrin accumulation and excretion, and gender influence in a 3-week rat model of porphyria cutanea tarda. *Toxicol Appl Pharmacol* 147:289–299.
- \*Gabliks J, Al-Zubaidy T, Askari E. 1975. DDT and immunological responses. *Arch Environ Health* 30:81–84.
- \*Gamey PE, Sirosis JE, Denison M, et al. 1993. Neutrophil function after exposure to polychlorinated biphenyls *in vitro*. *Environ Health Perspect* 101:430–434.
- \*Gao X, Son D-S, Terranova PF, et al. 1999. Toxic equivalency factors of polychlorinated dibenzo-*p*-dioxins in an ovulation model: Validation of the toxic equivalency concept for one aspect of endocrine disruption. *Toxicol Appl Pharmacol* 157:107–116.
- \*Gao X, Terranova PF, Rozman KK. 2000. Effects of polychlorinated dibenzofurans, biphenyls, and their mixture with dibenzo-*p*-dioxins on ovulation in the gonadotropin-primed immature rat: Support for the toxic equivalency concept. *Toxicol Appl Pharmacol* 163:115–124.
- \*Gierthy JF, Acaro KF, Floyd M. 1997. Assessment of PCB estrogenicity in a human breast cancer cell line. *Chemosphere* 34(5–7):1495–1505.
- \*GLWQB. 1985. Report to the International Joint Commission: Report on Great Lakes water quality. Kingston, Ontario: Great Lakes Water Quality Board.
- \*Goldey ES, Crofton KM. 1998. Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. *Toxicol Sci* 45:94–105.
- \*Goldey ES, Kehn LS, Lau C, et al. 1995. Development exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. *Toxicol Appl Pharmacol* 135:77–88.

- Goldstein JA, Friesen M, Linder RE, et al. 1977. Effects of pentachlorophenol on hepatic drug-metabolizing enzymes and porphyria related to contamination with chlorinated dibenzo-*p*-dioxins and dibenzofurans. *Biochem Pharmacol* 26:1549–1557.
- Goldstein JA, Friesen M, Scotti TM, et al. 1978. Assessment of the contribution of chlorinated dibenzo-*p*-dioxins and dibenzofurans to hexachlorobenzene-induced toxicity, porphyria, changes in mixed function oxygenases, and histopathological changes. *Toxicol Appl Pharmacol* 46:633–649.
- Goldstein JA, Linko P, Hahn ME, et al. 1986. Structure-activity relationships of chlorinated benzenes as inducers of hepatic cytochrome P-450 isozymes in the rat. In: Morris CR, Cabral JRP, ed. Hexachlorobenzene: Proceedings of an international symposium. Lyon, France: International Agency for Research on Cancer, 519–526.
- Gopaldaswamy UV, Nair CKK. 1992. DNA binding and mutagenicity of lindane and its metabolites. *Bull Environ Contam Toxicol* 49:300–305.
- \*Gralla EJ, Fleischman YK, Luthra YK, et al. 1977. Toxic effects of hexachlorobenzene after daily administration to beagle dogs for one year. *Toxicol Appl Pharmacol* 40:227–239.
- Gray LE. 1998. Xenoendocrine disrupters: Laboratory studies on male reproductive effects. *Toxicol Lett* 102-103:331–335.
- Gray LE, Ostby J. 1998. Effects of pesticides and toxic substances on behavioral and morphological reproductive development: Endocrine versus nonendocrine mechanisms. *Toxicol Ind Health* 14:159–183.
- \*Gray LE, Kelce WR, Monosson E, et al. 1995. Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: Reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. *Toxicol Appl Pharmacol* 131:108–118.
- Gray LE, Ostby JS, Kelce WR. 1994. Developmental effects of an environmental antiandrogen: The fungicide vinclozolin alters sex differentiation of the male rat. *Toxicol Appl Pharmacol* 129:46–52.
- \*Gray LE, Ostby JS, Kelce WR. 1997. A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male Long Evans hooded rat offspring. *Toxicol Appl Pharmacol* 146:11–20.
- \*Gray LE Jr, Ostby J, Marshall R, et al. 1993. Reproductive and thyroid effects of low-level polychlorinated biphenyl (Aroclor 1254) exposure. *Fundam Appl Toxicol* 20(3):288–294.
- Gray LE, Ostby J, Wolf C, et al. 1998. The value of mechanistic studies in laboratory animals for the prediction of reproductive effects in wildlife: Endocrine effects on mammalian sexual differentiation. *Environ Toxicol Chem* 17(1):109–118.
- Grimalt JO, Sunyer J, Moreno V, et al. 1994. Risk excess of soft-tissue sarcoma and thyroid cancer in a community exposed to airborne organochlorinated compound mixtures with a high hexachlorobenzene content. *Int J Cancer* 56:200–203.

Grimvall E, Rylander L, Nilsson-Ehle P, et al. 1997. Monitoring of polychlorinated biphenyls in human blood plasma: Methodological developments and influence of age, lactation, and fish consumption. *Arch Environ Contam Toxicol* 32:329–336.

Guttes S, Failing K, Neumann K, et al. 1998. Chlororganic pesticides and polychlorinated biphenyls in breast tissue of women with benign and malignant breast disease. *Arch Environ Contam Toxicol* 35:140–147.

\*Haake JM, Safe S, Mayura K, et al. 1987. Aroclor 1254 as an antagonist of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Lett* 38:299–306.

Hagdoost NR, Newman LM, Johnson EM. 1997. Multiple chemical exposures: Synergism vs. individual exposure levels. *Reprod Toxicol* 11(1):9–27.

\*Hahn ME, Goldstein JA, Linko P, et al. 1989. Interaction of hexachlorobenzene with the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin *in vitro* and *in vivo*. *Arch Biochem Biophys* 270(1):344–355.

\*Hany J, Lilienthal H, Sarasin A, et al. 1999. Developmental exposure of rats to a reconstituted PCB mixture or Aroclor 1254: Effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. *Toxicol Appl Pharmacol* 158:231–243.

\*Hansen H, De Rosa CT, Pohl H, et al. 1998. Public health challenges posed by chemical mixtures. *Environ Health Perspect Suppl* 106(6):1271–1280.

\*Hansen LG. 1998. Stepping backward to improve assessment of PCB congener toxicities. *Environ Health Perspect Suppl* 106(1):171–189.

\*Hansen LG, Li MH, Saeed A, et al. 1995. Environmental polychlorinated biphenyls: Acute toxicity of landfill soil extract to female prepubertal rats. *Arch Environ Contam Toxicol* 29:334–343.

Hardell L, Van Bavel B, Lindstrom G, et al. 1996. Higher concentrations of specific polychlorinated biphenyl congeners in adipose tissue from non-Hodgkin's lymphoma patients compared with controls without a malignant disease. *Int J Oncol* 9:603–608.

\*Harper N, Connor K, Safe S. 1993a. Immunotoxic potencies of polychlorinated biphenyl (PCB), dibenzofuran (PCDF) and dibenzo-*p*-dioxin (PCDD) congeners in C57BL/6 and DBA/2 mice. *Toxicology* 80:217–227.

\*Harper N, Howie L, Connor K, et al. 1993b. Immunosuppressive effects of highly chlorinated biphenyls and diphenyl ethers on T-cell dependent and independent antigens in mice. *Toxicology* 85:123–135.

Harris M, Zacharewski T, Astroff B, et al. 1989. Characterization of 6-methyl-1,3,8-trichlorobenzofuran (MCDF) as a 2,3,7,8-TCDD antagonist in male rats: Induction of monooxygenases. *Chemosphere* 19:769–772.

\*Hayes WJ, Durham WF, Cueto C, et al. 1956. The effect of known repeated oral doses of chlorophenothane (DDT) in man. *J Am Med Assoc* 162(9):890–897.

- \*Hemming H, Bager Y, Flodstrom S, et al. 1995. Liver tumour promoting activity of 3,4,5,3',4'-pentachlorobiphenyl and its interaction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Eur J Pharmacol* 292:241–249.
- \*Hemming H, Flodstrom S, Warngard L, et al. 1993. Relative tumour promoting activity of three polychlorinated biphenyls in rat liver. *Eur J Pharmacol* 248(2):163–174.
- \*Hertzler DR. 1990. Neurotoxic behavioral effects of Lake Ontario salmon diets in rats. *Neurotoxicol Teratol* 12:139–143.
- \*Hicks HE. 1996. The Great Lakes: A Historical Overview. *Toxicol Indus Health* 12:303–313.
- Hicks HE, De Rosa CT, Cibulas W. 1998. U.S. Agency for Toxic Substances and Disease Registry: Great Lakes Human Health Effects Research Program. *J Clean Technol Environ Toxicol Occup Med* 7(4):375–396.
- \*Hirano M, Mitsumori K, Maita k, et al. 1986. Further carcinogenicity study on methylmercury chloride in ICR mice. *Jap J Vet Sci* 48(1)127–135.
- \*Hong R, Taylor K, Abonour R. 1989. Immune abnormalities associated with chronic TCDD exposure in rhesus. *Chemosphere* 18:1785–1797.
- \*Hood A, Hashmi R, Klaassen CD. 1999. Effects of microsomal enzyme inducers on thyroid-follicular cell proliferation, hyperplasia, and hypertrophy. *Toxicol Appl Pharmacol* 160:163–170.
- \*Hori M, Kondo H, Ariyoshi N, et al. 1997. Changes in the hepatic glutathione peroxidase redox system produced by coplanar polychlorinated biphenyls in Ah-responsive and -less-responsive strains of mice: mechanism and implications for toxicity. *Environ Toxicol Pharmacol* 3:267–275.
- \*Huisman M, Koopman-Esseboom C, Fidler V, et al. 1995a. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. *Early Hum Dev* 41:111–127.
- \*Huisman M, Koopman-Essesboom C, Lanting CI, et al. 1995b. Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. *Early Hum Dev* 43:165–176.
- \*Hultman P, Enestrom S. 1992. Dose-response studies in murine mercury-induced autoimmunity and immune-complex disease. *Toxicol Appl Pharmacol* 113:199–208.
- \*Hultman P, Johansson U, Turley SJ, et al. 1994. Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice. *FASEB J* 8:1183–1190.
- \*IARC. 1997. Polychlorinated dibenzo-*para*-dioxins. Lyons, France: International Agency for Research on Cancer. <http://193.51.164.11/htdocs/monographs/vol69/dioxin.html>.
- \*IRIS. 2001. Integrated Risk Information System (IRIS). Cincinnati, OH: National Center for Environmental Assessment. <http://www.epa.gov/iris/>. February 22, 2001.
- \*Ilback N-G. 1991. Effects of methyl mercury exposure on spleen and blood natural killer (NK) cell activity in the mouse. *Toxicology* 67:117–124.



- \*Ilback –G, Sundberg J, Oskarsson A. 1991. Methyl mercury exposure via placenta and milk impairs natural killer (NK) cell function in newborn rats. *Toxicol Lett* 58:149–158.
- Iverson F, Mehta R, Hierlihy L, et al. 1998. Microsomal enzyme activity, glutathione s-transferase-placental form expression, cell proliferation, and vitamin A stores in livers of rats consuming Great Lakes salmon. *Regul Toxicol Pharmacol* 27:S76–S89.
- \*Jacobson JL, Jacobson SW. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med* 335(11):783–789.
- \*Jacobson JL, Jacobson SW, Humphrey HEB. 1990a. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. *J Pediatr* 116(1):38–45.
- \*Jacobson JL, Jacobson SW, Humphrey HEB. 1990b. Effects of exposure to PCBs and related compounds on growth and activity in children. *Neurotoxicol Teratol* 12:319–326.
- \*Jacobson JL, Jacobson SW, Schwartz PM, et al. 1984. Prenatal exposure to an environmental toxin: A test of the multiple effects model. *Dev Psychol* 20(4):523–532.
- \*Jacobson SW, Fein GG, Jacobson JL, et al. 1985. The effect of intrauterine PCB exposure on visual recognition memory. *Child Dev* 56:853–860.
- \*Jansen HT, Cooke PS, Porcelli J, et al. 1993. Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies. *Reprod Toxicol* 7:237–248.
- Johnson BL, Hicks HE, Jones DE, et al. 1998. Public health implications of persistent toxic substances in the Great Lakes and St. Lawrence Basins. *J Great Lakes Res* 24(2):698–722.
- \*Jones CJ, Backlin BM, Stoddart RW, et al. 1997. Environmental pollutants as aetiological agents in female reproductive pathology: Placental glycan expression in normal and polychlorinated biphenyl (PCB) - exposed mink (*Mustela vison*). *Placenta* 18(8):689–699.
- Jordan SA, Feeley MM. 1999. PCB congener patterns in rats consuming diets containing Great Lakes salmon: Analysis of fish, diets, and adipose tissue. *Environ Res A* 80:S207–S212.
- \*Juarez de Ku LM, Sharma-Stokkermans M, Meserve LA. 1994. Thyroxine normalizes polychlorinated biphenyl (PCB) dose-related depression of choline acetyltransferase (ChAT) activity in hippocampus and basal forebrain of 15-day-old rats. *Toxicology* 94:19–30.
- \*Kasza L, Collins WT, Capen CC, et al. 1978. Comparative toxicity of polychlorinated biphenyl and polybrominated biphenyl in the rat thyroid gland: Light and electron microscopic alterations after subacute dietary exposure. *J Environ Pathol Toxicol* 1:587–599.
- Kavlock RJ, Perreault SD. 1994. Multiple chemical exposure and risks of adverse reproductive function and outcome. In: Yang RSH, ed. *Toxicology of chemical mixtures: Case studies, mechanisms, and novel approaches*. San Diego, CA: Academic Press, 245–297.
- \*Kelce WR, Lambright CR, Gray LE, et al. 1997. Vinclozolin and *p,p'*-DDE alter androgen-dependent gene expression: *In vivo* confirmation of an androgen receptor-mediated mechanism. *Toxicol Appl Pharmacol* 142:192–200.

- \*Kelce WR, Stone CR, Laws SC, et al. 1995. Persistent DDT metabolite *p,p*-DDE is a potent androgen receptor antagonist. *Nature* 375:581–585.
- \*Kerkvliet NI. 1994. Immunotoxicology of dioxins and related chemicals. In: Schecter A, ed. *Dioxins and health*. New York, NY: Plenum Press, 199–225.
- \*Kihlstrom JE, Olsson M, Jensen SJ, et al. 1992. Effects of PCB and different fractions of PCB on the reproduction of the mink (*mustela vison*). *Ambio* 21(8):563–569.
- \*Kimbrough RD. 1997. Studies in children: Polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans. *Health Environ Dig* 10(7):61–63.
- \*Kociba RJ, Keyes DG, Beyer JE, et al. 1978. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol Appl Pharmacol* 46:279–303.
- \*Kodavanti PRS, Tilson HA. 1997. Structure-activity relationships of potentially neurotoxic PCB congeners in the rat. *Neurotoxicology* 18(2):425–442.
- \*Kodavanti PRS, Shin D-S, Tilson HA, et al. 1993. Comparative effects of two polychlorinated biphenyl congeners on calcium homeostasis in rat cerebellar granule cells. *Toxicol Appl Pharmacol* 123:97–106.
- \*Kodavanti PRS, Ward TR, McKinney JD, et al. 1995. Increased [<sup>3</sup>H]phorbol ester binding in rat cerebellar granule cells by polychlorinated biphenyl mixtures and congeners: Structure-activity relationships. *Toxicol Appl Pharmacol* 130:140–148.
- \*Kodavanti PRS, Ward TR, McKinney JD, et al. 1996. Increased [<sup>3</sup>H]phorbol ester binding in rat cerebellar granule cells and inhibition of <sup>45</sup>Ca<sup>2+</sup> sequestration in rat cerebellum by polychlorinated diphenyl ether congeners and analogs: Structure-activity relationships. *Toxicol Appl Pharmacol* 138:251–261.
- \*Kohn MC, Sewall CH, Lucier GW, et al. 1996. A mechanistic model of effects of dioxin on thyroid hormones in the rat. *Toxicol Appl Pharmacol* 165:29–48.
- \*Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, et al. 1994. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatr Res* 36(4):468–473.
- \*Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MAJ, et al. 1996. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. *Pediatrics* 97(5):700–706.
- \*Korach KS, Sarver P, Chae K, et al. 1988. Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: Conformationally restricted structural probes. *Mol Pharmacol* 33:120–126.
- \*Kostyniak PJ, Stinson C, Greizerstein HB, et al. 1999. Relation of Lake Ontario fish consumption, lifetime lactation, and parity to breast milk polychlorobiphenyl and pesticide concentrations. *Environ Res* A80:S166–S174.

- \*Kramer VJ, Helferich WG, Bergman A, et al. 1997. Hydroxylated polychlorinated biphenyl metabolites are anti-estrogenic in a stably transfected human breast adenocarcinoma (MCF7) cell line. *Toxicol Appl Pharmacol* 144:363–374.
- \*Krishnan V, Safe S. 1993. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), and dibenzofurans (PCDFs) as antiestrogens in MCF-7 human breast cancer cells: Quantitative structure-activity relationships. *Toxicol Appl Pharmacol* 120(1):55–61.
- \*Larsen BR, Turrio-Baldassarri L, Nilsson T, et al. 1994. Toxic PCB congeners and organochlorine pesticides in Italian human milk. *Ecotoxicol Environ Saf* 28:1–13.
- \*Laug E, Nelson A, Gitzhugh O, et al. 1950. Liver cell alternation and DDT storage in the fat of the rat induced by dietary levels of 1 to 50 ppm DDT. *J Pharmacol Exp Ther* 98:268.
- \*Lecavalier PR, Chu I, Villeneuve D, et al. 1994. Combined effects of mercury and hexachlorobenzene in the rat. *J Environ Sci Health B* 29(5):951–961.
- \*Leonzio C, Fossi MC, Casini S. 1996b. Porphyrins as biomarkers of methylmercury and PCB exposure in experimental quail. *Bull Environ Contam Toxicol* 56:244–250.
- \*Leonzio C, Monaci F, Fossi MC, et al. 1996a. Multiresponse biomarker evaluation of interactions between methylmercury and Arochlor 1260 in quail. *Ecotoxicology* 5:365–376.
- \*Li M-H, Hansen LG. 1996b. Enzyme induction and acute endocrine effects in prepubertal female rats receiving environmental PCB/PCDF/PCDD mixtures. *Environ Health Perspect* 104(7):712–722.
- \*Li M-H, Hansen LG. 1996a. Responses of prepubertal female rats to environmental PCBs with high and low dioxin equivalencies. *Fundam Appl Toxicol* 33:282–293.
- \*Li M-H, Hansen LG. 1997. Consideration of enzyme and endocrine interactions in the risk assessment of PCBs. *Rev Toxicol* 1 :71–156.
- \*Li M-H, Rhine C, Hansen LG. 1998. Hepatic enzyme induction and acute endocrine effects of 2,3,3',4',6'-pentachlorobiphenyl in prepubertal female rats. *Arch Environ Contam Toxicol* 35:97–103.
- \*Li SMA, Denomme MA, Leece B, et al. 1989. Hexachlorobenzene: Biochemical effects and synergistic toxic interactions with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Environ Chem* 22:215–227.
- \*Li X, Rozman KK. 1995. Subchronic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and their reversibility in male Sprague-Dawley rats. *Toxicology* 97:133–140.
- Liljegren G, Hardell L, Lindstrom G, et al. 1998. Case-control study on breast cancer and adipose tissue concentrations of congener specific polychlorinated biphenyls, DDE and hexachlorobenzene. *Eur J Cancer Prev* 7:135–140.
- \*Linder RE, Gaines TB, Kimbrough RD. 1974. The effect of polychlorinated biphenyls on rat reproduction. *Food Cosmet Toxicol* 12:63–77.

- \*Linko P, Yeowell HN, Gasiewicz TA, et al. 1986. Induction of cytochrome P-450 isozymes by hexachlorobenzene in rats and aromatic hydrocarbon (Ah)-responsive mice. *J Biochem Toxicol* 1(2):95–107.
- \*Loeffler IK, Peterson RE. 1999. Interactive effects of TCDD and *p,p'*-DDE on male reproductive tract development in *in utero* and lactationally exposed rats. *Toxicol Appl Pharmacol* 154:28–39.
- \*Longcope C. 2000. The male and female reproductive systems in hypothyroidism. In: Braverman LE, Utiger RD, eds. *Werner & Ingbar's the thyroid: A fundamental and clinical text*. Eighth edition. Philadelphia, PA: Lippincott Williams & Wilkins, 824–827.
- Longnecker MP, Rogan WJ, Lucier G. 1997. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organochlorines in public health. *Ann Rev Public Health* 18:211–244.
- \*Lonky E, Reihman J, Darvill T, et al. 1996. Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. *J Great Lakes Res* 22(2):198–212.
- \*Lundberg C. 1974. Effect of DDT on Cytochrome P-450 and oestrogen-dependent functions in mice. *Environ Physiol Biochem* 4:200–204.
- \*Lundkvist U. 1990. Clinical and reproductive effects of Clophen A50 (PCB) administered during gestation on pregnant guinea pigs and their offspring. *Toxicology* 6:249–257.
- Luster MI, Hong LH, Osborne R, et al. 1986. 1-Amino-3,7,8-trichlorodibenzo-p-dioxin: A specific antagonist for TCDD-induced myelotoxicity. *Biochem Biophys Res Commun* 139(2):747–756.
- MacLuskey NJ, Brown TJ, Schantz S, et al. 1998. Hormonal interactions in the effects of halogenated aromatic hydrocarbons on the developing brain. *Toxicol Ind Health* 14(1–2):185–208.
- \*Mariussen E, Anderson JM, Fonnum F. 1999. The effect of polychlorinated biphenyls on the uptake of dopamine and other neurotransmitters into rat brain synaptic vesicles. *Toxicol Appl Pharmacol* 161:274–282.
- \*Marty MS, Atchison WD. 1997. Pathways mediating  $Ca^{2+}$  entry in rat cerebellar granule cells following *in vitro* exposure to methyl mercury. *Toxicol Appl Pharmacol* 147:319–330.
- \*Mayes BA, McConnell EE, Neal BH, et al. 1998. Comparative carcinogenicity in Sprague-Dawley rats of the polychlorinated biphenyl mixtures Aroclors 1016, 1242, 1254, and 1260. *Toxicol Sci* 41(1):62–76.
- \*Mendola P, Buck GM, Sever LE, et al. 1997. Consumption of PCB-contaminated freshwater fish and shortened menstrual cycle length. *Am J Epidemiol* 146(11):955–960.
- \*Mendola P, Buck GM, Vena JE, et al. 1995. Consumption of PCB-contaminated sportfish and risk of spontaneous fetal death. *Environ Health Perspect* 103(5):498–502.
- Mendoza CE, Shields JB. 1976. Effects of hexachlorobenzene on malathion LD50 and on cholinesterase and carboxylesterase activities in organs of the suckling albino rat. *Toxicol Appl Pharmacol* 35:447–453.

- \*Mergler D, Belanger S, Larribe F, et al. 1998. Preliminary evidence of neurotoxicity associated with eating fish from the upper St. Lawrence River Lakes. *Neurotoxicology* 19(4–5):691–702.
- \*Mitsumori k, Hirano M, Ueda H, et al. 1990. Chronic toxicity and carcinogenicity of methylmercury chloride in B6C3F1 mice. *Fundam Appl Toxicol* 14:179–190.
- \*Mohamed M, Burbacher T, Mottett N. 1987. Effects of methyl mercury on testicular functions in *Macaca fascicularis* monkeys. *Pharmacol Toxicol* 60(1):29–36.
- \*Moore M, Mustain M, Daniel K, et al. 1997. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. *Toxicol Appl Pharmacol* 142:160–168.
- \*Morrissey RE, Harris MW, Diliberto JJ, et al. 1992. Limited PCB antagonism of TCDD-induced malformations in mice. *Toxicol Lett* 60:19–25.
- \*Morse DC, Wehler EK, Wesseling W, et al. 1996. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol* 136:269–279.
- Mumtaz M, Colman J. 1992. The risk assessment of chemical mixtures: Fine tuning the hazard index. In: Dodd DE, Clewell HJ, Mattie DR eds. *Proceedings of the 1992 conference on toxicology: Applications of advances in toxicology to risk assessment*. Wright-Patterson Air Force Base, Ohio: Armstrong Laboratory.
- Mumtaz MM, Durkin PR. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. *Toxicol Ind Health* 8: 377–406.
- Mumtaz MM, De Rosa CT, Durkin PR. 1994. Approaches and challenges in risk assessments of chemical mixtures. In: Yang RSH, ed. *Toxicology of chemical mixtures: Case studies, mechanisms and novel approaches*. New York, NY: Academic Press, 565–597.
- Mumtaz MM, Poirier KA, Colman JT. 1997. Risk assessment for chemical mixtures: Fine-tuning the hazard index approach. *J Clean Technol Environ Toxicol Occup Med* 6(2):189–204.
- \*NCI. 1978. Bioassays of DDT, TDE, and *p,p'*-DDE for possible carcinogenicity. Bethesda, MD: Carcinogenesis Testing Program, National Cancer Institute. Publication No. 78-1386.
- \*Nesaretnam K, Darbre P. 1997. 3,5,3',5'-Tetrachlorobiphenyl is a weak oestrogen agonist *in vitro* and *in vivo*. *J Steroid Biochem Mol Biol* 62(5/6):409–418.
- \*Nesaretnam K, Corcoran D, Dils RR, et al. 1996. 3,4,3',4'-Tetrachlorobiphenyl acts as an estrogen *in vitro* and *in vivo*. *Mol Endocrinol* 923–936.
- \*Ness DK, Schantz SL, Mostagian J, et al. 1993. Perinatal exposure to two polychlorinated biphenyl congeners: Histological effects on the thyroid gland. *Toxicologist* 13(1):358.
- \*NTP. 1982. Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (CAS no. 1746-01-6) in Osborne-Mendel rats and B6C3F1 mice (gavage study). Bethesda, MD: Carcinogenesis Testing Program, National Cancer Institute, National Institute of Health. Research Triangle Park, NC: National Toxicology Program. DHHS publication no. 82-1765.

- \*Okey AB, Riddick DS, Harper PA. 1994. The Ah receptor: Mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. *Toxicol Lett* 70:1–22.
- \*Omara FO, Brochu C, Flipo D, et al. 1997. Immunotoxicity of environmentally relevant mixtures of polychlorinated aromatic hydrocarbons with methyl mercury on rat lymphocytes in vitro. *Environ Toxicol Chem* 16(3):576–581.
- \*Omara FO, Flipo D, Brochu C, et al. 1998. Lack of suppressive effects of mixtures containing low levels of methylmercury (MeHg), polychlorinated dibenzo-*p*-dioxins (PCDDS), polychlorinated dibenzofurans (PCDFS), and aroclor biphenyls (PCBS) on mixed lymphocyte reaction, phagocytic, and natural killer cell activities of rat leukocytes in vitro. *J Toxicol Environ Health A* 54:561–577.
- \*Pappas BA, Murtha SJE, Park GAS, et al. 1998. Neurobehavioral effects of chronic ingestion of Great Lakes chinook salmon. *Regul Toxicol Pharmacol* 27:S55–S67.
- \*Parham FM, Portier CJ. 1998. Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. *Toxicol Appl Pharmacol* 151:110–116.
- Patandin S, Koopman-Esseboom C, De Ridder MAJ, et al. 1998. Effects of environmental exposure to Polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. *Pediatr Res* 44(4):538–545.
- \*Patandin S, Lanting CI, Mulder PGH, et al. 1999. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. *J Pediatr* 134(1):33–41.
- \*Patnode KA, Curtis LR. 1994. 2,2',4,4',5,5'- and 3,3',4,4',5,5'-Hexachlorobiphenyl alteration of uterine progesterone and estrogen receptors coincides with embryotoxicity in mink (*mustela vison*). *Fundam Appl Toxicol* 127:9–18.
- \*Poland A, Knutson JC. 1982. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons. Examinations of the mechanisms of toxicity. *Ann Rev Pharmacol Toxicol* 22:517–554.
- \*Porterfield SP, Hendry LB. 1998. Impact of PCBs on thyroid hormone directed brain development. *Toxicol Ind Health* 14:103–120.
- \*Preston BD, Miller EC, Miller JA. 1985. The activities of 2,2',5,5'-tetrachlorobiphenyl, its 3,4-oxide metabolite, and 2,2',4,4'-tetrachlorobiphenyl in tumor induction and promotion assays. *Carcinogenesis* 6(3):451–453.
- \*Price SC, Ozalp S, Weaver R, et al. 1988. Thyroid hyperactivity caused by hypolipodaemic compounds and polychlorinated biphenyls: The effect of coadministration in the liver and thyroid. *Arch Toxicol Suppl* 12:85–92.
- \*Provost TL, Juarez De Ku LM, Zender C, et al. 1999. Dose- and age-dependent alterations in choline acetyltransferase (ChAT) activity, learning and memory, and thyroid hormones in 15- and 30-day old rats exposed to 1.25 or 12.5 ppm polychlorinated biphenyl (PCB) beginning at conception. *Prog Neuro-Psychopharmacol Biol Psychiat* 23:915–928.

- \*Renner G. 1980. Toxicities of combinations of pentachloronitrobenzene with mercuric chloride or cadmium chloride, and hexachlorobenzene with mercuric chloride administered to rats. *Xenobiotica* 10(7-8):551-556.
- \*Restum JC, Bursian SJ, Giesy JP, et al. 1998. Multigenerational study of the effects of consumption of PCB-contaminated carp from Saginaw Bay, Lake Huron, on mink. 1. Effects on mink reproduction, kit growth and survival, and selected biological parameters. *J Toxicol Environ Health, A* 54:343-375.
- \*Rice DC. 1997. Effect of postnatal exposure to a PCB mixture in monkeys on multiple fixed interval-fixed ratio performance. *Neurotoxicol Teratol* 19(6):429-434.
- \*Rice DC. 1998. Effects of postnatal exposure of monkeys to a PCB mixture on spatial discrimination reversal and DRL performance. *Neurotoxicol Teratol* 20(4):391-400.
- \*Rice DC. 1999. Behavioral impairment produced by low-level postnatal PCB exposure in monkeys. *Environ Res* 80:S113-S121.
- \*Rice DC, Hayward S. 1997. Effects of postnatal exposure to a PCB mixture in monkeys on nonspatial discrimination reversal and delayed alternation performance. *Neurotoxicology* 18(2):479-494.
- \*Rice DC, Hayward S. 1999. Effects of exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on behavior (concurrent random interval-random interval and progressive ratio performance) in rats. *Neurotoxicol Teratol* 21(6):679-687.
- Riddick DS, Huang Y, Harper PA, et al. 1994. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin versus 3-methylcholanthrene: Comparative studies of Ah receptor binding, transformation, and induction of CYP1A1. *J Biol Chem* 269(16):12118-12128.
- \*Rier SE, Martin DC, Bowman RE, et al. 1993. Endometriosis in Rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fundam Appl Toxicol* 21:433-441.
- \*Risebrough RW, Anderson DW. 1975. Some effects of DDE and PCB on mallards and their eggs. *J Wildl Manage* 39(3):508-513.
- \*Robertson LW, Gupta RC. 2000. Metabolism of polychlorinated biphenyls (PCBs) generates electrophiles and reactive oxygen species that damage DNA. In: Williams GM, Aruoma OI, eds. *Molecular drug metabolism and toxicology*. OICA International, 1-19.
- \*Rogan WJ, Gladen BC, McKinney JD, et al. 1986b. Neonatal effects of transplacental exposure to PCBs and DDE. *J Pediatr* 109:335-341.
- \*Rogan WJ, Gladen BC, McKinney JD, et al. 1986a. Polychlorinated biphenyls (PCBs) and dichlorophenyl dichloroethene (DDE) in human milk: Effects of maternal factors and previous lactation. *Am J Public Health* 76:172-177.
- \*Rogan WJ, Gladen BC, McKinney JD, et al. 1987. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: Effects on growth, morbidity, and duration of lactation. *Am J Public Health* 77:1294-1297.

- \*Roman BL, Pollenz RS, Peterson RE. 1998a. Responsiveness of the adult male rat reproductive tract to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure: Ah receptor and ARNT expression, CY1A1 induction, and Ah receptor down-regulation. *Toxicol Appl Pharmacol* 150:228–239.
- \*Roman BL, Sommer RJ, Shinomiya K, et al. 1995. *In utero* and lactational exposure of the male rat to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Impaired prostrate growth and development without inhibited androgen production. *Toxicol Appl Pharmacol* 134:241–250.
- \*Roman BL, Timms BG, Prins GS, et al. 1998b. *In utero* and lactational exposure of the male rat to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin impairs prostrate development: 2. Effects on growth and cytodifferentiation. *Toxicol Appl Pharmacol* 150:254–270.
- \*Ross PS, De Swart RL, Reijnders PJH, et al. 1995. Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. *Environ Health Perspect* 103(2):162–167.
- Rozman K, Mueller W, Coulston F, et al. 1977. Long-term feeding study of hexachlorobenzene in rhesus monkeys: (Preliminary communication). *Chemosphere* 2/3:81–84.
- Rozman K, Rozman T, Greim H. 1981. Enhanced fecal elimination of stored hexachlorobenzene from rats and rhesus monkeys by hexadecane or mineral oil. *Toxicology* 22:33–44.
- \*Rylander L, Hagmar L. 1995. Mortality and cancer incidence among women with a high consumption of fatty fish contaminated with persistent organochlorine compounds. *Scand J Work Environ Health* 21:419–426.
- \*Rylander L, Hagmar L. 1999. Anthropometric and psychometric examinations of conscripts born to mothers with a high intake of fish contaminated with persistent organochlorines. In: *Dioxin 99: International Symposium on halogenated environmental organic pollutants and POPs*. *Organohalogen Compounds* 44:413–416.
- \*Rylander L, Stromberg U, Dyremark E, et al. 1998a. Polychlorinated biphenyls in blood plasma among Swedish female fish consumers in relation to low birth weight. *Am J Epidemiol* 147(5):493–502.
- \*Rylander L, Stromberg U, Hagmar L. 1995. Decreased birthweight among infants born to women with a high dietary intake of fish contaminated with persistent organochlorine compounds. *Scand J Work Environ Health* 21:368–375.
- \*Rylander L, Stromberg U, Hagmar L. 1996. Dietary intake of fish contaminated with persistent organochlorine compounds in relation to low birthweight. *Scand J Work Environ Health* 22:260–266.
- \*Rylander L, Stromberg U, Hagmar L. 1998b. Lowered birthweight among infants born to women with high intake of fish contaminated with persistent organochlorine compounds. In: *Organohalogen Compounds* 38:275–277.
- \*Safe S. 1984. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): Biochemistry, toxicology, and mechanism of action. *CRC Crit Rev Toxicol* 13:319–395.



\*Safe S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit Rev Toxicol* 21:51–88.

Safe SH. 1994. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol* 24(2):87–149.

\*Safe S. 1998a. Limitations of the toxic equivalency factor approach for the risk assessment of TCDD and related compounds. *Teratogenesis Carcinog Mutagen* 17:285–304.

\*Safe S. 1998b. Development validation and problems with the toxic equivalency factor approach for risk assessment of dioxins and related compounds. *J Anim Sci* 76:134–141.

\*Safe SH. 1999. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related environmental antiestrogens: Characterization and mechanism of action. In: Naz RK, ed. *Endocrine disruptors: Effects on male and female reproductive systems*. Boca Raton, FL: CRC Press, 187–221.

Safe SH, Gaido K. 1998. Phytoestrogens and anthropogenic estrogenic compounds. *Environ Toxicol Chem* 17(1):119–126.

\*Sager DB, Girard DM. 1994. Long-term effects on reproductive parameters in female rats after translactational exposure to PCBs. *Environ Res* 66(1):52–76.

\*Sager D, Girard D, Nelson D. 1991. Early postnatal exposure to PCBs: Sperm function in rats. *Environ Toxicol Chem* 10:737–746.

\*Sager DB, Shih-Schroeder W, Girard D. 1987. Effect of early postnatal exposure to polychlorinated biphenyls (PCBs) on fertility in male rats. *Bull Environ Contam Toxicol* 38:946–953.

\*Schantz SL, Gardiner JC, Gasior DM, et al. 1999. Motor function in aging Great Lakes fish eaters. *Environ Res* 80:546–556.

\*Schantz SL, Seo B-W, Wong PW, et al. 1997. Long-term effects of developmental exposure to 2,2',3,5',6-pentachlorobiphenyl (PCB 95) on locomotor activity, spatial learning and memory and brain ryanodine binding. *Neurotoxicology* 18(2):457–468.

\*Schantz SL, Sweeney AM, Gardiner JC, et al. 1996. Neuropsychological assessment of an aging population of Great Lakes fish eaters. *Toxicol Ind Health* 12(3–4):403–417.

Schecter A, Ryan JJ, Papke O. 1998. Decrease in levels and body burden of dioxins, dibenzofurans, PCBs, DDE, and HCB in blood and milk in a mother nursing twins over a thirty-eight month period. *Chemosphere* 37(9–12):1807–1816.

Schielen P, Den Besten C, Vos JG, et al. 1995. Immune effects of hexachlorobenzene in the rat: Role of metabolism in a 13-week feeding study. *Toxicol Appl Pharmacol* 131:37–43.

\*Schoor AG, Bergman A, Brouwer A, et al. 1999. Effects of pentachlorophenol and hydroxylated polychlorinated biphenyls on thyroid hormone conjugation in a rat and a human hepatoma cell line. *Toxicol in Vitro* 13:417–425.

- \*Schoor AG, Cenin PH, van Toor H, et al. 1998a. Effect of Aroclor 1254 on thyroid hormone sulfation in fetal rats. In: Johansson N, Bergman A, Broman D, et al., ed. Organohalogen compounds. Akademitryck, Edsbruk:, Vol. 37, 249–252.
- \*Schoor AG, van Leeuwen-Bol I, Jong WMC, et al. 1998b. *In vitro* inhibition of thyroid hormone sulfation by polychlorobiphenyls: Isozyme specificity and inhibition kinetics. *Toxicol Sci* 45:188–194.
- Schwab BW. 1999. The TEF approach for hexachlorobenzene. *Environ Health Perspect* 107(4):A183–A184.
- Seegal RF. 1996a. Can epidemiological studies discern subtle neurological effects due to perinatal exposure to PCBs? *Neurotoxicol Teratol* 18(3):251–254.
- \*Seegal RF. 1996b. Epidemiological and laboratory evidence of PCB-induced neurotoxicity. *Crit Rev Toxicol* 26(6):709–737.
- \*Seegal RF. 1999. Are PCBs the major neurotoxicant in Great Lakes salmon? *Environ Res* 80:S38–S45.
- \*Seegal RF, Brosch K, Bush, B et al. 1989. Effects of Aroclor 1254 on dopamine and norepinephrine concentrations in pheochromocytoma (PC-12) cells. *Neurotoxicology* 10:757–764.
- \*Seegal RF, Brosch KO, Okoniewski RJ. 1997. Effects of *in utero* and lactational exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'-tetrachlorobiphenyl on dopamine function. *Toxicol Appl Pharmacol* 146:95–103.
- \*Seegal RF, Bush B, Shain W. 1990. Lightly chlorinated *ortho*-substituted PCB congeners decrease dopamine in nonhuman primate brain and in tissue culture. *Toxicol Appl Pharmacol* 106:136–144.
- \*Seegal RF, Pappas BA, Park GAS. 1998. Neurochemical effects of consumption of Great Lakes salmon by rats. *Regul Toxicol Pharmacol* 27:S68–S75.
- \*Seiler P, Fischer B, Lindenau A, et al. 1994. Effects of persistent chlorinated hydrocarbons on fertility and embryonic development in the rabbit. *Human Reprod* 9:1920–1926.
- \*Seo BW, Meserve LA. 1995. Effects of maternal ingestion of Aroclor 1254 (PCB) on the developmental pattern of oxygen consumption and body temperature in neonatal rats. *Bull Environ Contam Toxicol* 55:22–28.
- \*Shain W, Bush B, Seegal R. 1991. Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. *Toxicol Appl Pharmacol* 111:33–42.
- \*Shenker BJ, Berthold P, Rooney C, et al. 1993. Immunotoxic effects of mercuric compounds on human lymphocytes and monocytes. III. Alterations in B-cell function and viability. *Immunopharmacol Immunotoxicol* 15(1):87–112.
- \*Silberhorn EM, Glauert HP, Robertson LW. 1990. Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. *Crit Rev Toxicol* 20:440–496.

\*Silkworth JB, Grabstein EM. 1982. Polychlorinated biphenyl immunotoxicity: Dependence on isomer planarity and the Ah gene complex. *Toxicol Appl Pharmacol* 65:109–115.

Sinclair PR, Gorman N, Sinclair JF, et al. 1995. Ascorbic acid inhibits chemically induced uroporphyrin in ascorbate-requiring rats. *Hepatology* 22(2):565–572.

\*Sinclair PR, Walton HS, Gorman N, et al. 1997. Multiple roles of polyhalogenated biphenyls in causing increases in cytochrome P450 and uroporphyrin accumulation in cultured hepatocytes. *Toxicol Appl Pharmacol* 147:171–179.

\*Smialowicz RJ, DeVito MJ, Riddle MM, et al. 1997. Opposite effects of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin on the antibody response to sheep erythrocytes in mice. *Fundam Appl Toxicol* 37:141–149.

Smith AG, Carthew P, Francis JE, et al. 1993. Enhancement by iron of hepatic neoplasia in rats caused by hexachlorobenzene. *Carcinogenesis* 14(7):1381–1387.

\*Smith AG, Dinsdale D, Cabral JRP, et al. 1987. Goitre and wasting induced in hamsters by hexachlorobenzene. *Arch Toxicol* 60:343–349.

\*Smith AG, Francis JE, Carthew P. 1990a. Iron as a synergist for hepatocellular carcinoma induced by polychlorinated biphenyls in *Ah*-responsive C57BL/10ScSn mice. *Carcinogenesis* 11(3):437–444.

\*Smith AG, Francis JE, Green JA, et al. 1990b. Sex-linked hepatic uroporphyrin and the induction of cytochromes P450IA in rats caused by hexachlorobenzene and polyhalogenated biphenyls. *Biochem Pharmacol* 40(9):2059–2068.

Sonnenschein C, Soto AM. 1998. An updated review of environmental estrogen and androgen mimics and antagonists. *J Steroid Biochem Mol Biol* 65:143–150.

\*Stack AS, Altman-Hamamdizic S, Morris PJ, et al. 1999. Polychlorinated biphenyl mixtures (Aroclors) inhibit LPS-induced murine splenocyte proliferation in vitro. *Toxicology* 139:137–154.

\*Stahl BU, Kettup A, Rozman K. 1992. Comparative toxicity of four chlorinated dibenzo-*p*-dioxins (CDDs) and their mixture: Part I: Acute toxicity and toxic equivalency factors (TEFs). *Arch Toxicol* 66:471–477.

\*Stewart P, Darvill T, Lonky E, et al. 1999. Assessment of prenatal exposure to PCBs from maternal consumption of Great Lakes fish: An analysis of PCB pattern and concentration. *Environ Res* 80:S87–S96.

\*Stewart P, Pagano J, Sargent D, et al. 2000a. Effects of Great Lakes fish consumption on brain PCB pattern, concentration, and progressive-ratio performance. *Environ Res* 82:18–32.

\*Stewart P, Reihman J, Lonky E, et al. 2000b. Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance. *Neurotoxicol Teratol* 22:21–29.

\*Sufit RL, Hodach R, Arends R, et al. 1986. Decreased conduction velocity and pseudomyotonia in hexachlorobenzene-fed rats. In: *Hexachlorobenzene: Proceedings of an international symposium*. IARC Sci Publ 77:361–362..

- \*Svensson B-G, Hallberg T, Nilsson A, et al. 1994. Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. *Int Arch Occup Environ Health* 65:351–358.
- Svensson B-G, Nilsson A, Hansson M, et al. 1991. Exposure to dioxins and dibenzofurans through the consumption of fish. *N Engl J Med* 324:8–12.
- \*Svensson B-G, Nilsson A, Jonsson E, et al. 1995. Fish consumption and exposure to persistent organochlorine compounds, mercury, selenium and methylamines among Swedish fishermen. *Scand J Work Environ Health* 21:96–105.
- Swain WR. 1991. Effects of organochlorine chemicals on the reproductive outcome of humans who consumed contaminated Great Lakes fish: An epidemiologic consideration. *J Toxicol Environ Health* 33:587–639.
- \*Swanson GM, Ratcliffe HE, Fischer LJ. 1995. Human exposure to polychlorinated biphenyls (PCBs): A critical assessment of the evidence for adverse health effects. *Regul Toxicol Pharmacol* 21:136–150.
- \*Takabatake E, Fujita M, Sawa Y. 1980. Combined effects of polychlorinated biphenyls and methylmercury on hepatic microsomal monooxygenases and the hepatotoxic action of bromobenzene. *J Pharmacobiodyn* 3:463–469.
- \*Tanimura T, Ema M, Kihara T. 1980. Effects of combined treatment with methylmercury and polychlorinated biphenyls (PCBs) on the development of mouse offspring. In: Persaud TVN, ed. *Neural and behavioral teratology*. Baltimore, MD: University Park Press, 163–198.
- \*Tilson HA, Kodavanti PRS. 1997. Neurochemical effects of polychlorinated biphenyls: An overview and identification of research needs. *Neurotoxicology* 18(3):727–744.
- \*Tilson HA, Kodavanti PRS. 1998. The neurotoxicity of polychlorinated biphenyls. *Neurotoxicology* 19(4–5):517–526.
- \*Tilson HA, Kodavanti PRS, Mundy WR, et al. 1998. Neurotoxicity of environmental chemicals and their mechanism of action. *Toxicol Lett* 102–103:631–635.
- \*Tithof PK, Contreras ML, Gamey PE. 1995. Aroclor 1242 stimulates the production of inositol phosphates in polymorphonuclear neutrophils. *Toxicol Appl Pharmacol* 131:136–143.
- Toraason M. 1999. 8-Hydroxydeoxyguanosine as a biomarker of workplace exposures. *Biomarkers* 4(1):3–26.
- Tryphonas H. 1995. Immunotoxicity of PCBs (Aroclors) in relation to Great Lakes. *Environ Health Perspect Suppl* 103(9):35–46.
- \*Tryphonas L, Arnold DL, Zawadzka Z, et al. 1986a. A pilot study in adult Rhesus monkeys (*m.mullata*) treated with Aroclor 1254 for two years. *Toxicol Pathol* 14:1–10.
- \*Tryphonas L, Charbonneau S, Tryphonas H, et al. 1986b. Comparative aspects of Aroclor 1254 toxicity in adult *Cynomolgus* and Rhesus monkeys: A pilot study. *Arch Environ Contam Toxicol* 15:159–169.

- Tryphonas H, Fournier M, Lacroix F, et al. 1998a. Effects of Great Lakes fish consumption on the immune system of Sprague-Dawley rats investigated during a two-generation reproductive study: II. Quantitative and functional aspects. *Regul Toxicol Pharmacol* 27:S40–S54.
- \*Tryphonas H, Hayward S, O'Grady L, et al. 1989. Immunotoxicity studies of PCB (Aroclor 1254) in the adult Rhesus (*macaca mulatta*) monkey-preliminary report. *Int J Immunopharmacol* 11(2):199–206.
- Tryphonas H, Luster MI, Schiffman G, et al. 1991. Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the Rhesus (*macaca mulatta*) monkey. *Fundam Appl Toxicol* 16:773–786.
- \*Tryphonas H, McGuire P, Fernie S, et al. 1998b. Effects of Great Lakes fish consumption on the immune system of Sprague-Dawley rats investigated during a two-generation reproductive study: I. Body and organ weights, food consumption, and hematological parameters. *Regul Toxicol Pharmacol* 27:S28–S39.
- Unkila M, Pohjanvirta R, MacDonald E, et al. 1994. Dose response and time course of alterations in tryptophan metabolism by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the most TCDD-susceptible and the most TCDD-resistant rat strain: Relationship with TCDD lethality. *Toxicol Appl Pharmacol* 128:280–292.
- \*van Birgelen APJM. 1998. Hexachlorobenzene as a possible major contributor to the dioxin activity of human milk. *Environ Health Perspect* 106(11):683–688.
- \*van Birgelen APJM, Fase KM, van der Kolk J, et al. 1996. Synergistic effect of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic porphyrin levels in the rat. *Environ Health Perspect* 104(5):550–557.
- \*Van Birgelen APJM, Van Der Kolk J, Fase KM, et al. 1994a. Toxic potency of 2,3,3',4,4',5-hexachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. *Toxicol Appl Pharmacol* 126:202–213.
- \*Van Birgelen APJM, Van Der Kolk J, Fase KM, et al. 1994b. Toxic potency of 3,3',4,4',5-pentachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. *Toxicol Appl Pharmacol* 127:209–221.
- \*Van Birgelen APJM, Van der Kolk J, Fase KM, et al. 1995. Subchronic dose-response study of 3,4,7,8-tetrachlorodibenzo-*p*-dioxin in female Sprague-Dawley rats. *Toxicol Appl Pharmacol* 132:1–13.
- \*van Birgelen APJM, van der Kolk J, Poiger H, et al. 1992. Interactive effects of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on thyroid hormone, Vitamin A, and Vitamin K metabolism in the rat. *Chemosphere* 25:7–10.
- \*Van den Berg M, Birnbaum L, Bosveld ATC, et al. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106(12):775–792.
- \*Van den Berg M, De Jongh J, Poiger H, et al. 1994. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Crit Rev Toxicol* 24(1):1–74.

Van den Berg M, de Vroom E, Olie K, et al. 1986a. Bioavailability of PCDDs and PCDFs on fly ash after semi-chronic oral ingestion by guinea pig and Syrian golden hamster. *Chemosphere* 15(4):519–533.

Van den Berg M, Van Greevenbroek M, Olie X, et al. 1986b. Bio-availability of PCDDs and PCDFs on fly ash after semi-chronic oral ingestion by the rat. *Chemosphere* 13(4):509–513.

\*van der Burght ASAM, Clijsters PJ, Horbach GJ, et al. 1999. Structure-dependent induction of CYP1A by polychlorinated biphenyls in hepatocytes of Cynomolgus monkeys (*Macaca fascicularis*). *Toxicol Appl Pharmacol* 155:13–23.

\*van der Kolk J, van Birgelen APJM, Poiger H, et al. 1992. Interactions of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin in a subchronic feeding study in the rat. *Chemosphere* 25(12):2023–2027.

\*van der Plas SA, Haag-Gronlund M, Scheu G, et al. 1999. Induction of altered hepatic foci by a mixture of dioxin-like compounds with and without 2,2',4,4',5,5'-hexachlorobiphenyl in female Sprague-Dawley rats. *Toxicol Appl Pharmacol* 156:30–39.

\*VanRaaij JAGM, Frijters CMG, Van Den Berg KJ. 1993. Hexachlorobenzene-induced hypothyroidism: Involvement of different mechanisms by parent compound and metabolite. *Biochem Pharmacol* 46(8):1385–1391.

\*Vena JE, Buck GM, Kostyniak P, et al. 1996. The New York angler cohort study: Exposure characterization and reproductive and developmental health. *Toxicol Ind Health* 12:327–334.

\*Verschuuren HG, Kroes R, Den Tonkelaar EM, et al. 1976. Toxicity of methylmercury chloride in rats. III. Long-term toxicity study. *Toxicology* 6:107–123.

Villeneuve DC, Valli VE, Norstrom RJ, et al. 1981. Toxicological response of rats fed Lake Ontario or Pacific coho salmon for 28 days. *J Environ Sci Health B* 16(6):649–689.

\*Viluksela M, Stahl BU, Birnbaum LS, et al. 1998a. Subchronic/chronic toxicity of a mixture of four chlorinated dibenzo-*p*-dioxins in rats. I. Design, general observations, hematology, and liver concentrations. *Toxicol Appl Pharmacol* 151:57–69.

\*Viluksela M, Stahl BU, Birnbaum LS, et al. 1998b. Subchronic/chronic toxicity of a mixture of four chlorinated dibenzo-*p*-dioxins in rats. II. Biochemical effects. *Toxicol Appl Pharmacol* 151:70–78.

\*Vonier PM, Crain DA, McLachlan JA, et al. 1996. Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. *Environ Health Perspect* 104(12):1318–1322.

\*Vos JG. 1986. Immunotoxicity of hexachlorobenzene. In: Morris CR, Cabral JRP, eds. *Hexachlorobenzene: Proceedings of an international symposium*. Lyon, France: International Agency for Research on Cancer, 347–356.

Vos RME, Snoek MC, Van Berkel WJH, et al. 1988. Differential induction of rat hepatic glutathione *s*-transferase isoenzymes by hexachlorobenzene and benzyl isothiocyanate: Comparison with induction by phenobarbital and 3-methylcholanthrene. *Biochem Pharmacol* 37(6):1077–1082.

Weber H, Harris MW, Haseman JK, et al. 1985. Teratogenic potency of TCDD and TCDD-TCDF combinations in C57BL/6N mice. *Toxicol Lett* 26:159–167.

Weber LWD, Lebofsky M, Stahl BU, et al. 1992a. Comparative toxicity of four chlorinated dibenzo-*p*-dioxins (CDDs) and their mixture: Part II: Structure-activity relationships with inhibition of hepatic phosphoenolpyruvate carboxykinase, pyruvate carboxylase, and  $\gamma$ -glutamyl transpeptidase activities. *Arch Toxicol* 66:478–483.

Weber LWD, Lebofsky M, Stahl BU, et al. 1992b. Comparative toxicity of four chlorinated dibenzo-*p*-dioxins (CDDs) and their mixture: Part III: Structure-activity relationship with increased plasma tryptophan levels, but no relationship to hepatic ethoxyresorufin *o*-deethylase activity. *Arch Toxicol* 66:484–488.

\*Weber LWD, Lebofsky M, Stahl BU, et al. 1995. Correlation between toxicity and effects on intermediary metabolism in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated male C257BL/6J and DBA/2J mice. *Toxicol Appl Pharmacol* 131:155–162.

\*Welsch F. 1985. Effects of acute or chronic polychlorinated biphenyl ingestion on maternal metabolic homeostasis and on the manifestations of embryotoxicity caused by cyclophosphamide in mice. *Arch Toxicol* 57:104–113.

Wildhaber ML, Schmitt CJ. 1996. Estimating aquatic toxicity as determined through laboratory tests of Great Lakes sediments containing complex mixtures of environmental contaminants. *Environ Monit Assess* 41:255–289.

\*Wolfe D. 1998. Interactions between 2,3,7,8-TCDD and PCBs as tumor promoters: Limitations of TEFs. *Teratogen Carcinog Mutagen* 17:217–224.

\*Wong PW, Pessah IN. 1996. *Ortho*-substituted polychlorinated biphenyls alter calcium regulation by a ryanodine receptor-mediated mechanism: Structural specificity toward skeletal- and cardiac-type microsomal calcium release channels. *Mol Pharmacol* 49:740–751.

\*Wong PW, Pessah IN. 1997. Noncoplanar PCB 95 alters microsomal calcium transport by an immunophilin FKBP 12-dependent mechanism. *Mol Pharmacol* 51:693–702.

\*Wong PW, Joy RM, Albertson TE, et al. 1997. *Ortho*-substituted 2,2',3,5',6-pentachlorobiphenyl (PCB 95) alters rat hippocampal ryanodine receptors and neuroplasticity *in vitro*: Evidence for altered hippocampal function. *Neurotoxicology* 18(2):443–456.

\*Wren CD, Hunter DB, Leatherland JF, et al. 1987a. The effects of polychlorinated biphenyls and methylmercury, singly and in combination, on mink. I: Uptake and toxic responses. *Arch Environ Contam Toxicol* 16:441–447.

\*Wren CD, Hunter DB, Leatherland JF, et al. 1987b. The effects of polychlorinated biphenyls and methylmercury, singly and in combination on mink. II: Reproduction and kit development. *Arch Environ Contam Toxicol* 16:449–454.

\*Zoeller RT, Dowling ALS, Vas AA. 2000. Developmental exposure to polychlorinated biphenyls exerts thyroid hormone-like effects on the expression of RC3/neurogranin and myelin basic protein messenger ribonucleic acids in the developing rat brain. *Endocrinology* 141(1):181–189.





## Appendix A: Background Information for Chlorinated Dibenzo-*p*-Dioxins (CDDs)

### A.1 Toxicokinetics

Results from studies of humans and animals given oral doses of 2,3,7,8-TCDD and other CDDs indicate that ingested CDDs can be well absorbed, that the efficiency of gastrointestinal can be influenced by vehicle (i.e., absorption efficiencies are less for CDDs ingested with soil compared with CDDs ingested with an oil vehicle), and that CDDs with higher chlorination (e.g., octa-CDDs) are poorly absorbed compared with less chlorinated CDDs such as tetra-CDDs (ATSDR 1998). Inhalation and dermal exposure to CDDs are of lesser concern than oral exposure (because ingestion of CDDs in food is thought to be the principal route of exposure for the general population), but limited information from exposed human and animal studies indicate that CDDs can be absorbed by these routes. Information from studies of exposed humans and laboratory animals indicates that absorbed CDDs are distributed preferentially to fatty tissues and to a lesser extent, the liver (ATSDR 1998). CDDs can be transferred to the fetus across the placenta and to nursing infants via breast milk. CDDs are slowly metabolized in mammalian tissues via oxidation and reductive dechlorination reactions catalyzed by cytochrome P450 enzymes, followed by conjugation to more polar molecules such as glutathione and glucuronic acid (ATSDR 1998). The metabolism of 2,3,7,8-TCDD and related compounds is required for urinary and biliary excretion, and the rate of metabolism is thought to play a major role in regulating the rate of elimination (and detoxification) of these compounds (Van den Berg et al. 1994). The major routes of excretion of CDDs are via the bile and feces, whereas smaller amounts are excreted via the urine (ATSDR 1998). Monitoring of nursing mothers indicates that lactation can be a significant route of elimination of CDDs (ATSDR 1998). Results from studies of animals and humans exposed to 2,3,7,8-TCDD and related compounds indicate that CDDs and CDFs are slowly eliminated from the body; reported half-lives ranged from about 1–9 years in humans, close to a year in monkeys, and 10–100 days in laboratory rodents (ATSDR 1998; Van den Berg et al. 1994). Because of the long-half life of most of the halogenated aromatic hydrocarbons, animals or humans will be exposed for relatively long periods of time following single exposures.

## A.2 Health Effects

Exposure to CDDs such as 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons have been associated with a range of toxic effects that include (ATSDR 1999b; Devito and Birnbaum 1994):

1. a wasting syndrome that develops slowly in animals following acute lethal doses and body weight decreases following sublethal doses;
2. thymic atrophy in adult and developing animals following administration of nonlethal doses (the developing thymus is affected at lower doses than the adult thymus, and affected juvenile animals also show immune suppression);
3. chloracne in humans and animals following dermal or systemic exposure;
4. hepatic hyperplasia and hepatomegaly accompanied with degenerative lesions (including uroporphyrin accumulation) in certain animal species following repeated exposure and liver dysfunction in some humans immediately following acute high-dose exposures;
5. decreased nerve conduction velocity and histological signs of nerve degeneration in rats exposed to intraperitoneal doses of 2,3,7,8-TCDD (2.2–8.8  $\mu\text{g}/\text{kg}$ );
6. suppression of humoral and cell-mediated immunity with accompanying increased susceptibility to infectious agents in mice following administration of low doses (as low as a single 2,3,7,8-TCDD dose of 10  $\text{ng}/\text{kg}$ );
7. decreased serum or plasma levels of the thyroid hormone, thyroxin (T4), in rodents following acute (0.1–0.3  $\mu\text{g}/\text{kg}$ ) or subchronic (0.05–0.8  $\mu\text{g}/\text{kg}$ ) oral exposure to 2,3,7,8-TCDD presumably involving TCDD-induction of UDP-glucuronyltransferase which catalyzes the metabolism of T4;
8. estrogenic effects (from 2,3,7,8-TCDD) including reduced fertility, litter size, and uterine weights, and suppression of the estrous cycle in adults of several animal species (these effects occur at doses that alter body weights in some species, and at doses without overt toxicity in other species);
9. altered development of female reproductive tissues following perinatal exposure of mice;
10. antiandrogenic effects including loss of germ cells, degeneration of spermatocytes and spermatozoa, and decreased reproductive capability in males of several animal species following exposure to doses producing overt toxicity such as decreased food intake and body weight, and decreased serum testosterone levels, increased serum follicle-stimulating

hormone, and increased luteinizing hormone in male workers exposed to 2,3,7,8-TCDD during manufacture of 2,4,5-trichlorophenol;

11. impaired development of male reproductive tissues and impaired sexual behavior in male adult rats who experienced *in utero* exposure from single doses of 2,3,7,8-TCDD as low as 0.064 µg/kg given on gestation day 15;
12. cleft palate and hydronephrosis in mice following *in utero* exposure to doses that did not produce other fetal or maternal toxic effects, and other malformations in other animal species at doses that produced maternal toxicity;
13. ectodermal dysplasia, neurobehavioral abnormalities, and delays in developmental milestones in offspring of women exposed to mixtures of CDFs and PCBs including dioxin-like and non-dioxin-like PCBs;
14. neurobehavioral effects in offspring of monkeys exposed during the mating period, gestation, and lactation; and
15. cancer, at multiple sites (e.g., liver, thyroid, lung) in a number of animal species following oral exposure to 2,3,7,8-TCDD.

### A.3 Mechanisms of Action

CDDs, CDFs, and dioxin-like PCBs produce a wide spectrum of biochemical effects in mammals that include induction of phase I enzymes (most notably CYP1A1 and CYP1A2) and phase II enzymes (e.g., UDP-glucuronosyl transferase and glutathione-S-transferase), reduction of levels of several growth factors (epidermal growth factor [EGF], transforming growth factor [TGF]- $\alpha$ , and TGF- $\beta$ 1) and increased expression of EGF receptor, and changes in thyroid hormone metabolism leading to lowered thyroid hormone levels (ATSDR 1998; Devito and Birnbaum 1994; Van den Berg et al. 1994). Many of the toxic and biological responses to CDDs, CDFs, and dioxin-like PCBs are thought to be initially mediated through the binding of the parent compounds to a soluble intracellular protein, the Ah receptor (ATSDR 1998; Devito and Birnbaum 1994; Van den Berg et al. 1994). The ligand-receptor complex is thought to be transported to the nucleus where it interacts with deoxyribonucleic acid (DNA) and alters gene expression. For example, the induction of CYP1A1 by 2,3,7,8-TCDD is thought to be due to the interaction of the TCDD-Ah receptor complex with nuclear genetic material leading to increased expression of the CYP1A1 gene (ATSDR 1998).

Alterations in gene expression have been linked to the development of some of the specific toxic responses to 2,3,7,8-TCDD and related compounds. General evidence that the Ah receptor mediates the toxicity of CDDs, CDFs, and related PCBs comes from demonstrations that the toxicity of specific congeners is related to the affinity with which the compounds bind to the Ah receptor and demonstrations that genetic differences in Ah receptor binding affinity between strains of rodents are related to genetic differences in responsiveness to the toxic or biochemical effects of TCDD (Okey et al. 1994).

A working hypothesis of the molecular mechanism by which 2,3,7,8-TCDD induces cleft palate formation in rodents has received considerable research attention and illustrates how toxic effects may be mediated by CDDs, CDFs, and PCBs through the Ah receptor. In this mechanistic scheme, the TCDD-Ah receptor's transcriptional regulation of gene expression is thought to indirectly lead to reduced levels of several growth factors (EGF, TGF- $\alpha$ , and TGF- $\beta$ 1) and increased expression of the EGF receptor, which subsequently lead to altered medial cell proliferation in the developing palatal shelves (Abbott et al. 1994). TCDD-induced disruptions in the development of the kidney and male and female reproductive organs, observed in animal experiments, are also thought to be associated with TCDD-induced changes in levels of growth factors and receptors (ATSDR 1998). The molecular events between TCDD-induced changes in gene expression and reduced levels of growth factors remain to be elucidated (ATSDR 1998).

A proposed mechanistic hypothesis for hepatic porphyria and liver damage induced by a number of halogenated aromatic hydrocarbons provides another illustration of how the Ah receptor is thought to mediate toxicity (Sinclair et al. 1997). In this hypothesis, the ligand-Ah receptor complex initially increases the expression of the CYP1A1 gene. The increased levels of CYP1A1 enzymic activity are thought to stimulate uroporphyrin oxidation from uroporphyrinogen by producing a specific, as yet unidentified, inhibitor of uroporphyrinogen decarboxylase (which is the initial step in heme synthesis from uroporphyrinogen) and by depleting levels of uroporphyrinogen, the substrate of uroporphyrinogen decarboxylase. Increased levels of reactive products of CYP1A1-catalyzed oxidation of xenobiotics may also be involved in the production of the liver damage observed after exposure to halogenated aromatic hydrocarbons.

Results from studies of animals exposed for acute or subacute periods indicate that CDDs, such as 2,3,7,8-TCDD, and related halogenated aromatic hydrocarbons suppress immunological responses to various substances (e.g., tuberculin, sheep red blood cells) or resistance to bacterial, parasitic, viral, or neoplastic disease at doses well below those that cause lymphoid tissue depletion (e.g., atrophy of the thymus) (Kerkvliet 1994). Involvement of the Ah receptor in halogenated aromatic hydrocarbon

immunotoxicity has been established by results from studies with CDDs, CDFs, and PCB congeners with differing binding affinity to the Ah receptor and studies using mice strains that differ genetically at the Ah locus, but the critical target cells and tissues and the molecular events involved in the immunosuppressive effects of 2,3,7,8-TCDD and other halogenated aromatic hydrocarbons remain to be elucidated (see Kerkvliet 1994 for review).

Changes in serum T4 levels from acute exposure to 2,3,7,8-TCDD has been postulated to involve TCDD induction of UDP-glucuronyltransferase, through the Ah receptor, and subsequently increased metabolism and clearance of T4 (ATSDR 1998; Weber et al. 1995). From a comparison of responses to acute exposure to 2,3,7,8-TCDD among rats and different genetic strains of mice, however, Weber et al. (1995) have proposed that the Ah receptor may not be the sole mediator of the toxicity of 2,3,7,8-TCDD. It has been postulated that some aspects of TCDD-disruption of thyroid hormone homeostasis may involve actions not related to Ah receptor mediation, such as interference, by TCDD metabolites, of T4 binding to plasma transport proteins (ATSDR 1998).

Carcinogenic responses to 2,3,7,8-TCDD are thought to not involve direct damage to DNA by TCDD or its metabolites, but have been proposed to involve Ah-receptor-mediated increased formation of DNA-damaging oxidative species from other exogenous and endogenous agents and Ah-receptor-mediated disruption of cellular differentiation and/or division (ATSDR 1998). Mechanistic details at the cellular and molecular level are poorly understood.

#### **A.4 Health Guidelines**

Because humans are exposed to complex and varying mixtures of CDDs and other halogenated aromatic hydrocarbons such as CDFs and PCBs and because there are limited toxicological data for these complex mixtures and most of their components, a Toxic Equivalency Factor (TEF) approach has been adopted to assess the health risks from exposure to these complex mixtures (ATSDR 1998). This approach assumes that components of concern (i.e., CDDs and CDD-like congeners) induce toxic effects through a common initial mechanism mediated by interaction with the Ah receptor and that the doses are additive (i.e., there are no synergistic or antagonistic interactions among the components of the mixture that interact with the Ah receptor). Carcinogenic, immunotoxic, developmental, and reproductive effects from CDDs and CDD-like compounds are generally thought to be mediated through the Ah receptor (ATSDR 1998). The TEF approach compares the relative potency of individual congeners, based on *in vitro* or acute *in vivo* data, with that of 2,3,7,8-TCDD, the best-studied member of this chemical class, so that the TEF for

2,3,7,8-TCDD is 1. The concentration or dose of each active component in the mixture of concern is multiplied by its TEF to arrive at a TEQ, and the TEQs are added to give the total toxic equivalency of the mixture. The hazard or risk presented by exposure to the mixture can be assessed by comparing the mixture total TEQ (in units of mg/kg/day) with an MRL for 2,3,7,8-TCDD or by multiplying the TEQ (in appropriate units) by a cancer slope factor for 2,3,7,8-TCDD (ATSDR 1999a). TEFs have recently been recommended by the World Health Organization for 7 CDD, 10 CDF, and 12 PCB congeners (Van den Berg et al. 1998).

ATSDR (1998) adopted a policy to use MRLs derived for 2,3,7,8-TCDD for other dioxin-like compounds, expressed in total TEQs.

ATSDR (1998) has derived MRLs for acute-, intermediate-, and chronic-duration oral exposure to 2,3,7,8-TCDD.

The acute MRL of 0.0002  $\mu\text{g}/\text{kg}/\text{day}$  was based on a no-observed-adverse-effect-level (NOAEL) of 0.005  $\mu\text{g}/\text{kg}/\text{day}$  and a lowest-observed-adverse-effect level (LOAEL) of 0.01  $\mu\text{g}/\text{kg}$  for immunological effects (increased susceptibility to influenza A-induced mortality) in mice given single gavage doses of 2,3,7,8-TCDD and an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

The intermediate MRL of 0.00002  $\mu\text{g}/\text{kg}/\text{day}$  was based on a NOAEL of 0.0007  $\mu\text{g}/\text{kg}/\text{day}$  and a LOAEL of 0.005  $\mu\text{g}/\text{kg}/\text{day}$  for immunological effects (decreased thymus weight) in guinea pigs fed 2,3,7,8-TCDD in the diet for 90 days and an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

The chronic MRL of 0.000001  $\mu\text{g}/\text{kg}/\text{day}$  was based on a LOAEL for neurobehavioral effects (changes in social behavior in offspring) following the exposure of female monkeys to 2,3,7,8-TCDD in the diet throughout the mating period, gestation, and lactation and an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

ATSDR (1998) concluded that the results of epidemiology and animal studies indicate that 2,3,7,8-TCDD may be a human carcinogen. International Agency Research on Cancer (IARC) (1997) classified 2,3,7,8-TCDD as a Group 1 compound - human carcinogen, based on limited evidence in humans and sufficient evidence in animals for the carcinogenicity of 2,3,7,8-TCDD. IARC (1997) considered the

following in making this evaluation: “(i) 2,3,7,8-TCDD is a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor; (ii) this receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals; (iii) tissue concentrations are similar both in heavily exposed human populations in which an increased overall cancer risk was observed and in rats exposed to carcinogenic dosage regimens in bioassays”. IARC (1997) also concluded that “other polychlorinated dibenzo-*p*-dioxins *are not classifiable as to their carcinogenicity to humans (Group 3)*”, based on inadequate evidence in humans and animals. The EPA Integrated Risk Information System (IRIS) does not list a weight-of-evidence classification for 2,3,7,8-TCDD or other CDDs, but EPA (1997a) concluded, in a proposed rule to add “dioxin and dioxin-like compound” to the list of chemicals subject to release reporting requirements, that “based on the EPA weight of evidence classification criteria, there is sufficient evidence to conclude that 2,3,7,8-TCDD is a probable human carcinogen”. Quantitative cancer risk estimates for CDDs were not derived by EPA in the proposed ruling (EPA 1997a) and are not available on IRIS (IRIS 2001). However, the EPA (1997b) Health Effects Assessment Summary Table lists an oral slope factor of 150,000 per mg/kg/day for 2,3,7,8-TCDD that was based on respiratory and liver tumor incidence for rats exposed to 2,3,7,8-TCDD in the diet.

## **A.5 Derivation of Target-Organ Toxicity Dose (TTD) Values**

TTDs for chronic oral exposure to 2,3,7,8-TCDD were derived for endpoints affected by 2,3,7,8-TCDD and one or more of the other chemicals in the CDD-hexachlorobenzene-DDE-methylmercury mixture that is the subject of this Interaction Profile. The relevant endpoints for 2,3,7,8-TCDD in this mixture include hepatic, endocrine, immunological, neurological, reproductive, and developmental effects. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (1999a, Section 2.3.2). The derivations are based on data provided in ATSDR (1998), and in particular, the oral Levels of Significant Exposure (LSE) table.

### **Hepatic Effects**

Numerous studies have observed liver effects in laboratory animals exposed to 2,3,7,8-TCDD for acute, intermediate, and chronic durations (ATSDR 1998). The lowest LOAEL for hepatic effects in a chronic study was reported by Kociba et al. (1978). Sprague-Dawley rats exposed daily to 2,3,7,8-TCDD in the feed for 2 years were found to have liver lesions, including cytoplasmic vacuolation, hyperplasia, hepatocellular degeneration, and liver necrosis, at doses as low as 0.001 µg/kg/day in females and

0.01 µg/kg/day in males. Hepatic necrosis was reported to be severe and extensive at 0.01 µg/kg/day. A NOAEL was not identified.

Chronic studies by NTP (1982) in Osborne-Mendel rats and B6C3F<sub>1</sub> mice generally support the findings of Kociba et al. (1978). The studies reported toxic hepatitis (liposis and hydropic degeneration of hepatocytes with proliferation of bile ductules and mild fibrosis) in both species with chronic 2-year exposure. For both species, the LOAEL was 0.07 µg/kg/day and the NOAEL was 0.007 µg/kg/day. The NOAEL of 0.007 µg/kg/day in the NTP study is not consistent with the LOAEL of 0.001 µg/kg/day in the Kociba study, but this may be an artifact of the dosing schedule in the NTP study, in which the animals were given 2,3,7,8-TCDD twice a week by gavage (rather than daily in the feed). The intermediate duration studies also support the Kociba et al. (1978) results; the lowest intermediate hepatic LOAEL was 0.005 µg/kg/day for 90 day dietary exposure in guinea pigs (DeCaprio et al. 1986).

A TTD<sub>HEPATIC</sub> can be derived for 2,3,7,8-TCDD by applying an uncertainty factor (UF) of 300 (10 for use of a LOAEL, 3 for extrapolation from rats to humans, and 10 to protect sensitive individuals) to the chronic hepatic LOAEL of 0.001 µg/kg/day from the Kociba et al. (1978) study. This yields a TTD<sub>HEPATIC</sub> of 0.000003 µg/kg/day. The use of an uncertainty factor of 3 rather than 10 for extrapolation from rats to humans follows the MRL derivations in ATSDR (1998) and is based on a comparison of sensitivity to 2,3,7,8-TCDD among animal species.

## Endocrine Effects

Thyroid effects of 2,3,7,8-TCDD have been well studied, albeit primarily by acute and intermediate exposure (ATSDR 1998). Chronic studies reported no effects on the thyroid at doses up to 0.07–0.1 µg/kg/day in rats and 0.3 µg/kg/day in mice (Kociba et al. 1978; NTP 1982). However, these studies included only limited evaluation of thyroid effects (histopathology). Intermediate-duration studies that have looked at thyroid hormone levels have reported effects at lower levels. The lowest LOAEL was reported by Li and Rozman (1995), who found a 50% decrease in serum T4 in male Sprague-Dawley rats treated with 0.03 µg/kg/day of 2,3,7,8-TCDD by gavage in oil once per week for 10 weeks, with a NOAEL of 0.003 µg/kg/day. Applying an uncertainty factor of 30 (3 for extrapolation from rats to humans and 10 to protect sensitive individuals) to the NOAEL of 0.003 µg/kg/day yields a TTD<sub>ENDOCRINE</sub> of 0.0001 µg/kg/day. A 13-week feeding study in rats identified a NOAEL of 0.026 µg/kg/day and a LOAEL of 0.047 µg/kg/day for reduced serum T4 (Van Birgelen et al. 1995). Although chronic studies have not adequately studied the thyroid, the result in the 13-week feeding study suggests that a TTD



based on Li and Rozman (1995) may be adequately protective for thyroid effects by long-term dietary exposure.

### **Immunological Effects**

The immune system is a sensitive target for 2,3,7,8-TCDD in animal studies. Both the acute oral MRL of 0.0002  $\mu\text{g}/\text{kg}/\text{day}$  (immunosuppression) and the intermediate oral MRL of 0.00002  $\mu\text{g}/\text{kg}/\text{day}$  (thymic atrophy) for 2,3,7,8-TCDD are based on immunological effects (ATSDR 1998). The lowest LOAEL for immunological effects in a chronic study was 0.002  $\mu\text{g}/\text{kg}/\text{day}$  for bone marrow and lymphoid tissue degeneration in Rhesus monkeys exposed to 2,3,7,8-TCDD daily in the diet for up to 33 months (Hong et al. 1989). Monkeys exposed to 0.001  $\mu\text{g}/\text{kg}/\text{day}$  in the same experiment did not have immune effects (Hong et al. 1989). Applying an uncertainty factor of 30 (3 for extrapolation from monkeys to humans and 10 for protection of sensitive individuals) to the NOAEL of 0.001  $\mu\text{g}/\text{kg}/\text{day}$  would lead to a  $\text{TTD}_{\text{IMMUNO}}$  of 0.00003  $\mu\text{g}/\text{kg}/\text{day}$ , which is slightly higher than the intermediate oral MRL (0.00002  $\mu\text{g}/\text{kg}/\text{day}$ ) based on immunological effects. This occurs because the intermediate oral MRL is based on a LOAEL of 0.005  $\mu\text{g}/\text{kg}/\text{day}$  for thymic atrophy in a 90-day feeding study in guinea pigs (DeCaprio et al. 1986), which is consistent with the chronic data, but a NOAEL of 0.0007  $\mu\text{g}/\text{kg}/\text{day}$ , which is slightly lower than the chronic NOAEL. Because the intermediate NOAEL on which the intermediate oral MRL is based (0.0007  $\mu\text{g}/\text{kg}/\text{day}$ ) is well below the chronic LOAEL (0.002  $\mu\text{g}/\text{kg}/\text{day}$ ) and also below the chronic NOAEL (0.001  $\mu\text{g}/\text{kg}/\text{day}$ ), the intermediate oral MRL also should be protective for immunological effects by chronic exposure.

### **Neurological Effects**

The chronic MRL of 0.000001  $\mu\text{g}/\text{kg}/\text{day}$  was based on a LOAEL for neurobehavioral effects (changes in social behavior in the offspring) following the exposure of female monkeys to 2,3,7,8-TCDD in the diet throughout the mating period, gestation, and lactation and an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability). Because this MRL is based on neurological effects in a sensitive population (developing fetuses and neonates), it should be protective for neurological effects in other individuals as well.

## Reproductive Effects

Reproductive effects have been widely reported in animals exposed to 2,3,7,8-TCDD (ATSDR 1998). The lowest LOAEL for reproductive effects was for the development of endometriosis in Rhesus monkeys 10 years after the end of a 4-year exposure period during which the monkeys received 2,3,7,8-TCDD in the feed daily. The incidence and severity of the effect were dose-related, with a LOAEL of 0.00012  $\mu\text{g}/\text{kg}/\text{day}$  (Rier et al. 1993). These are the same monkeys that were used in the developmental study upon which the chronic oral MRL is based. ATSDR (1998) considered using the reproductive LOAEL of 0.00012  $\mu\text{g}/\text{kg}/\text{day}$  as the basis for the oral MRL. An uncertainty factor of one for extrapolation from monkeys to humans was proposed by ATSDR (1998) because monkeys appear to be more sensitive to endometriosis than humans (30% background incidence in monkeys and 10% background incidence in humans), along with uncertainty factors of 10 for use of a LOAEL and 10 to protect sensitive individuals. Applying these uncertainty factors (total UF = 100) to the LOAEL of 0.00012  $\mu\text{g}/\text{kg}/\text{day}$  yields a  $\text{TTD}_{\text{REPRO}}$  of 0.000001  $\mu\text{g}/\text{kg}/\text{day}$  for 2,3,7,8-TCDD, which is the same as the chronic oral MRL.

## Developmental Effects

As described under neurological effects above, the chronic oral MRL for 2,3,7,8-TCDD (ATSDR 1998) is based on neurodevelopmental effects (changes in social behavior in offspring of monkeys exposed during the mating period, gestation, and lactation). Thus, the chronic oral MRL of 0.000001  $\mu\text{g}/\text{kg}/\text{day}$  for 2,3,7,8-TCDD is suitable to assess the potential for developmental effects.

## Summary (TTDs for 2,3,7,8-TCDD)

$\text{TTD}_{\text{HEPATIC}} = 0.000003 \mu\text{g}/\text{kg}/\text{day}$  ( $3 \times 10^{-6} \mu\text{g}/\text{kg}/\text{day}$ ;  $3 \times 10^{-9} \text{mg}/\text{kg}/\text{day}$ )

$\text{TTD}_{\text{ENDOCRINE}} = 0.0001 \mu\text{g}/\text{kg}/\text{day}$  ( $1 \times 10^{-4} \mu\text{g}/\text{kg}/\text{day}$ ;  $1 \times 10^{-7} \text{mg}/\text{kg}/\text{day}$ )

$\text{TTD}_{\text{REPRO}} = 0.000001 \mu\text{g}/\text{kg}/\text{day}$  ( $1 \times 10^{-6} \mu\text{g}/\text{kg}/\text{day}$ ;  $1 \times 10^{-9} \text{mg}/\text{kg}/\text{day}$ )

$\text{MRL}_{\text{(IMMUNO)}} = 0.00002 \mu\text{g}/\text{kg}/\text{day}$  ( $2 \times 10^{-5} \mu\text{g}/\text{kg}/\text{day}$ ;  $2 \times 10^{-8} \text{mg}/\text{kg}/\text{day}$ ; intermediate MRL)

$\text{MRL}_{\text{(NEURODEVELOP)}} = 0.000001 \mu\text{g}/\text{kg}/\text{day}$  ( $1 \times 10^{-6} \mu\text{g}/\text{kg}/\text{day}$ ;  $1 \times 10^{-9} \text{mg}/\text{kg}/\text{day}$ ; chronic MRL)

## Appendix B: Background Information for Hexachlorobenzene

### B.1 Toxicokinetics

Data from animal studies indicate that absorption of ingested hexachlorobenzene from the gastrointestinal tract is variable, depending on the solvent vehicle used for administration, ranging from about 6% in an aqueous vehicle to up to 82% in oil-based vehicles (ATSDR 1996; Courtney 1979). Inhaled hexachlorobenzene is thought to be poorly absorbed by the respiratory tract (ATSDR 1996). Absorbed hexachlorobenzene is widely distributed in mammalian tissues with preferential distribution to tissues and fluids with high fat content (ATSDR 1996). Hexachlorobenzene is transferred via the placenta to the developing fetus and to suckling neonates via breast milk (ATSDR 1996). In humans, concentrations of hexachlorobenzene tend to increase with age, indicative of bioaccumulation (ATSDR 1996). Absorbed hexachlorobenzene is eliminated from the body predominately as metabolites in the urine, although unchanged hexachlorobenzene has been detected in urine of exposed animals (ATSDR 1996). Data from studies of humans and animals indicate that pentachlorophenol and pentachlorobenzene are the predominant metabolites of hexachlorobenzene, that minor metabolites include tetrachlorobenzene, tetrachlorophenols, chlorinated tetrahydroquinones, trichlorophenol, and derivatives of glutathione conjugates, and that hexachlorobenzene metabolism is slow (ATSDR 1996). Oxidative metabolism of hexachlorobenzene is thought to be catalyzed by cytochrome P450 enzymes (predominately by CYP1A1/2), epoxyhydratase, and glutathione transferases; reductive dechlorination of hexachlorobenzene also occurs (ATSDR 1996; den Besten et al. 1993). Hexachlorobenzene induces a wide range of hepatic cytochrome P-450 enzymes including those in the CYP1A family (“phenobarbital-inducible”) and the CYP1A1/2 family (“3-methylcholanthrene-inducible”) (Li et al. 1989; Linko et al. 1986).

### B.2 Health Effects

Health effects identified in several studies of people who ingested hexachlorobenzene-contaminated bread in Turkey from 1955 to 1959 include hepatic porphyria, skin lesions (porphyria cutanea tarda) associated with altered heme biosynthesis and accumulation of porphyrins, neurological effects (including muscle weakness and paresthesiae), possible increased risk of fetal toxicity or decreased maternal fertility, and increased frequencies of offspring with dermal scarring and fragile skin, neurological symptoms (paresthesia and weakness), small hands and short stature, and enlarged thyroids (ATSDR 1996; Courtney 1979).

Health effects in animal studies following oral exposure to hexachlorobenzene include hepatomegaly, hepatic degeneration, and hepatic accumulation of porphyrins in rodents; induction of hepatic cytochrome P450 isozymes including CYP1A1/2, CYP1A2, CYP1B1, and CYP2B1, and other hepatic enzyme systems; decreased serum T4 levels, increased serum thyroid stimulating hormone, and enlarged thyroids in rodents; decreased serum levels of corticosterone and cortisol in rodents; increased spleen and lymph node weights and increased susceptibility to certain infectious agents in rodents; electrophysiological changes in the central nervous system of dogs; tremors and muscular weakness in Rhesus monkeys and rodents; alteration of menstrual cycle and degenerative changes of the ovarian follicle in monkeys; altered cellular and humoral immunity capabilities in rodent offspring exposed *in utero* and during lactation; neurobehavioral deficits in rodents exposed during gestation; decreased survival of monkey and rodent offspring exposed during lactation; and cancer at multiple sites (liver tumors, hemangioendotheliomas and thyroid tumors) in hamsters, rats, and mice (ATSDR 1996; Courtney 1979; Li et al. 1989; Smith et al. 1987).

### **B.3 Mechanisms of Action**

It has been hypothesized that oxidative metabolism of hexachlorobenzene is required for the development of hexachlorobenzene-induced hepatic porphyria and that the accumulation of uroporphyrin in liver originates from an irreversible inactivation of uroporphyrinogen decarboxylase, a key enzyme in heme synthesis (ATSDR 1996; den Besten et al. 1993). Cotreatment of rats with hexachlorobenzene and TAO, an inhibitor of cytochrome P450III A1/2, inhibited urinary porphyrin excretion and hepatic porphyrin accumulation compared with rats exposed to hexachlorobenzene alone (den Besten et al. 1993). den Besten et al. (1993) postulated that uroporphyrinogen decarboxylase is inhibited by an as yet unidentified reactive intermediate that is formed in the liver during the CYP1A1/2-catalyzed transformation of hexachlorobenzene to pentachlorophenol. Support for this hypothesis was provided by observations that repeated exposure of rats to high doses of pentachlorobenzene (which is also metabolized to pentachlorophenol and tetrachlorohydroquinone) did not induce porphyria, but increased urinary levels of these metabolites to comparable levels observed in rats treated with porphyria-inducing doses of hexachlorobenzene (den Besten et al. 1993). None of the known hexachlorobenzene metabolites (including pentachlorophenol) is directly porphyrinogenic when administered *in vivo*. The principal metabolites, pentachlorophenol and tetrachlorohydroquinone (TCHQ), can directly inhibit uroporphyrinogen decarboxylase *in vitro*, but only at concentrations well above those expected to be reached in liver cells (den Besten et al. 1993).

Sinclair et al. (1997) proposed that the development of hepatic uroporphyrinemia by a number of planar polyhalogenated aromatic hydrocarbons, including CDDs, CDFs, coplanar PCBs, and hexachlorobenzene proceeds by a mechanism that involves the initial induction of CYP1A1 (mediated by interaction with the Ah receptor) which catalyzes the oxidation of uroporphyrinogen to uroporphyrin. The induced increased levels of CYP1A1 are proposed to stimulate uroporphyrin production at the expense of heme production by depleting levels of uroporphyrinogen, the substrate of uroporphyrinogen decarboxylase, and by producing a specific, as yet unidentified, inhibitor of uroporphyrinogen decarboxylase.

It has also been hypothesized that hexachlorobenzene-induced liver tumors develop by a nongenotoxic mechanism involving compensatory hyperplastic responses to hepatocellular damage (see ATSDR 1996).

Hexachlorobenzene-induced disturbances of thyroid homeostasis have been hypothesized to involve changes in thyroid hormone metabolism resulting in increased elimination from the body and/or a mechanism involving interference of plasma thyroxine transport protein by hydroxylated metabolites of hexachlorobenzene such as pentachlorophenol (see den Besten et al. 1993 and van Raaji et al. 1993 for review). Exposure of animals to hexachlorobenzene, PCBs, or dioxins has been shown to decrease total thyroxine and free thyroxine (TT4) levels without affecting total triiodothyronine (TT3) levels (van Raaji et al. 1993). van Raaji et al. (1993) proposed that decreased serum levels of thyroid hormones produced by oral exposure to hexachlorobenzene may be caused via induction, by hexachlorobenzene itself, of enzymes involved in thyroxine catabolism (especially at low exposure levels), and via competitive inhibition of binding of thyroxine to thyroxine-binding serum proteins by hydroxylated metabolites of hexachlorobenzene (e.g., pentachlorophenol).

Information concerning molecular or cellular details of mechanisms whereby hexachlorobenzene causes neurological effects, effects on neurological development, or reproductive effects were not located (ATSDR 1996; Courtney et al. 1979; den Besten et al. 1993; van Raaji et al. 1993).

Although there is an overlap in the types of effects produced by hexachlorobenzene and 2,3,7,8-TCDD (Li et al. 1989; Smith et al. 1987; van Birgelen 1998), the evidence that hexachlorobenzene toxicity is mediated via the Ah receptor is limited and inconclusive. Hexachlorobenzene produced patterns of hepatic CYP induction in Ah-responsive and nonresponsive mouse strains that were consistent with action through the Ah receptor, but the affinity of hexachlorobenzene to bind to the Ah receptor *in vitro* was 10,000-fold less than that of 2,3,7,8-TCDD (Hahn et al. 1989; Linko et al. 1986).

## B.4 Health Guidelines

ATSDR (1996) did not derive inhalation MRLs for hexachlorobenzene due to inadequate data.

ATSDR (1996) derived an acute oral MRL of 0.008 mg/kg/day based on a LOAEL of 2.5 mg/kg/day for neurobehavioral changes (increased exploratory behavior or slight hyperactivity) in offspring of female rats exposed for 4 days prior to mating with unexposed males and an uncertainty factor of 300 (10 for extrapolation from animals to humans, 10 for human variability and 3 for the use of a minimal LOAEL).

ATSDR (1996) derived an intermediate oral MRL of 0.0003 mg/kg/day based on a LOAEL of 0.1 mg/kg/day for reproductive effects (e.g., ultrastructural changes in ovarian epithelial cells indicative of cellular degeneration) in monkeys exposed for 90 days and an uncertainty factor of 300 (10 for extrapolation from animals to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

ATSDR (1996) derived a chronic oral MRL of 0.00002 mg/kg/day based on a LOAEL of 0.016 mg/kg/day for hepatic effects (peribiliary lymphocytosis and peliosis and fibrosis of the liver) in F0 and F1 rats exposed for up to 130 weeks and an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for human variability, and 10 for use of a LOAEL).

van Birgelen (1998) recently proposed that hexachlorobenzene should be classified as a dioxin-like compound because it binds (competitively with 2,3,7,8-TCDD, albeit with much less affinity) to the Ah receptor, it causes a range of effects in mammals similar to those of other dioxin-like compounds (induction of CYP1A1/2, hepatic porphyria and degenerative effects, changes in thyroid hormone levels and metabolism, reproductive effects, developmental effects, and immunological effects), and it bioaccumulates. van Birgelen (1998) proposed that a relative potency value of 0.0001 be used for hexachlorobenzene based on *in vitro* observations that hexachlorobenzene had a binding affinity to the rat Ah receptor that was about 10,000-fold less than 2,3,7,8-TCDD's affinity (Hahn et al. 1989) and had an ability to induce ethoxyresorufin *O*-deethylase (a CYP1A activity) in chicken hepatocytes that was 5,000- to 10,000-fold less than the induction activity of 2,3,7,8-TCDD (Sinclair et al. 1997). Systemic and cancer health effects from hexachlorobenzene, however, can be evaluated separately without having to include it in the TEF approach. It may be preferable to evaluate hexachlorobenzene health effects outside of the TEF approach, because the health effect data on hexachlorobenzene are sufficient for the derivation of a slope factor and MRLs (as noted above), and support for inclusion of hexachlorobenzene in the TEF approach is limited to the *in vitro* data showing that it binds to the Ah receptor with an affinity that is

10,000-fold less than TCDD.

EPA (IRIS 2001) derived a chronic oral Reference Dose (RfD) of 0.0008 mg/kg/day based on a NOAEL of 0.08 mg/kg/day for liver effects in F0 and F1 rats exposed to hexachlorobenzene in the diet for up to 130 weeks (the LOAEL was 0.29 mg/kg/day) and an uncertainty factor of 100 (10 for interspecies variability and 10 for intraspecies variability). EPA (IRIS 2001) noted that data were inadequate for Reference Concentration (RfC) derivation.

EPA (IRIS 2001) classified hexachlorobenzene as a Group B2 compound - probable human carcinogen, based on inadequate evidence of cancer in studies of hexachlorobenzene-exposed humans and sufficient evidence of cancer in studies of rats, hamsters, and mice exposed to hexachlorobenzene in the diet. EPA (IRIS 2001) noted that the animal data indicate that the liver appears to be the primary target organ for hexachlorobenzene-induced cancer, and that neoplasms of the thyroid and kidney also have been observed. An oral slope factor of 1.6 per (mg/kg)/day was derived based on incidence of hepatocellular carcinomas in female rats exposed to hexachlorobenzene in the diet and a linearized multistage extrapolation method (IRIS 2001). An inhalation unit risk of  $4.6 \times 10^{-4}$  per  $\mu\text{g}/\text{m}^3$  was calculated based on the female rat liver tumor data from the dietary study.

## **B.5 Derivation of Target-Organ Toxicity Dose (TTD) Values**

TTDs for oral exposure to hexachlorobenzene were derived for endpoints affected by hexachlorobenzene and one or more of the other chemicals in the CDD-hexachlorobenzene-DDE-methylmercury mixture that is the subject of this Interaction Profile. The relevant endpoints for hexachlorobenzene in this mixture include hepatic, endocrine, immunological, neurological, reproductive, and developmental effects. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (1999a, Section 2.3.2). The derivations are based on data provided in ATSDR (1996), and in particular, the oral LSE table.

### **Hepatic Effects**

ATSDR (1996) derived a chronic oral MRL of 0.00002 mg/kg/day for hexachlorobenzene based on a LOAEL of 0.016 mg/kg/day for hepatic effects (peribiliary lymphocytosis and peliosis and fibrosis of the liver) in F0 and F1 rats exposed for up to 130 weeks and an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for human variability, and 10 for use of a LOAEL).

## Endocrine Effects

Endocrine effects were not reported in chronic studies. However, intermediate studies reported a number of endocrine effects, including effects on the thyroid, parathyroid, and adrenal glands, primarily at doses of 10–1000 mg/kg/day (ATSDR 1996). The lowest endocrine LOAELs were 5 mg/kg/day (with a NOAEL of 0.5 mg/kg/day) for increased thyroid weight in a 90-day feeding study in pigs (Den Tonkelaar et al. 1978) and 1 mg/kg/day (with a NOAEL of 0.1 mg/kg/day) for increased serum levels of parathyroid hormone in rats treated with hexachlorobenzene by daily gavage for 15 weeks (Andrews et al. 1989). Applying an uncertainty factor of 100 (10 for extrapolation from rats to humans and 10 to protect sensitive individuals) to the NOAEL of 0.1 mg/kg/day for serum parathyroid hormone changes yields a  $TTD_{\text{ENDOCRINE}}$  of 0.001 mg/kg/day for hexachlorobenzene. Although based on an intermediate study, this TTD may be protective for chronic exposure as well due to the sensitive nature of the endpoint tested.

## Immunological Effects

Data regarding the immune effects of hexachlorobenzene are reported by ATSDR (1996). The only chronic study reported a LOAEL of 0.12 mg/kg/day for hyperplasia of lymphoid tissue in the stomach in dogs exposed for one year (Gralla et al. 1977). Acute and intermediate studies reported effects (increased susceptibility to infection, reduced antibody production, increased lymphocyte count, and thymic atrophy) at doses of 8 mg/kg/day or more. A  $TTD_{\text{IMMUNO}}$  of 0.0004 mg/kg/day can be derived by applying an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from dogs to humans, and 10 to protect sensitive individuals) to the chronic LOAEL of 0.12 mg/kg/day.

## Neurological Effects

Overt neurological effects (e.g., lethargy, ataxia, tremors, convulsions and paralysis) occurred at 16 mg/kg/day in rats chronically exposed to hexachlorobenzene and at doses of 32 mg/kg/day and above in intermediate-duration studies (ATSDR 1996). Electrophysiological changes (e.g., reduced conduction velocity) were reported at 3.75 mg/kg/day in a 2-year rat study (Sufit et al. 1986), but this study was apparently not considered reliable by ATSDR (1996). The lowest LOAEL for a neurological effect was 0.4 mg/kg/day for an increase in brain weight in rats in a 90-day feeding study. The NOAEL for this effect was 0.08 mg/kg/day. Although not of chronic duration, this NOAEL may be suitable for TTD derivation because it is based on a sensitive endpoint, and is well below the NOAELs reported in chronic studies (5–120 mg/kg/day). A  $TTD_{\text{NEURO}}$  of 0.0008 mg/kg/day can be derived by applying an uncertainty



factor of 100 (10 for extrapolation from rats to humans and 10 to protect sensitive individuals) to the NOAEL of 0.08 mg/kg/day.

### Reproductive Effects

ATSDR (1996) derived an intermediate oral MRL for hexachlorobenzene of 0.0003 mg/kg/day based on a LOAEL of 0.1 mg/kg/day for reproductive effects (e.g., ultrastructural changes in ovarian epithelial cells indicative of cellular degeneration) in monkeys exposed for 90 days and an uncertainty factor of 300 (10 for extrapolation from animals to humans, 10 for human variability, and 3 for use of a minimal LOAEL). No chronic studies were located that investigated reproductive endpoints. In the absence of chronic data, the intermediate MRL of 0.0003 mg/kg/day, which is based on a sensitive endpoint, is suitable.

### Developmental Effects

ATSDR (1996) derived an acute oral MRL of 0.008 mg/kg/day for hexachlorobenzene based on a LOAEL of 2.5 mg/kg/day for developmental neurotoxicity and an uncertainty factor of 300 (10 for extrapolation from animals to humans, 10 for human variability, and 3 for the use of a minimal LOAEL). LOAELs for developmental effects in intermediate and chronic studies ranged from 2 to 64 mg/kg/day (excluding liver effects in multigeneration studies, which were considered hepatic rather than developmental effects). Therefore, the acute oral MRL of 0.008 mg/kg/day should be protective for developmental effects by longer-term exposure as well, and is adopted as the  $TTD_{DEVELOP}$  for hexachlorobenzene.

### Summary (TTDs for hexachlorobenzene)

$MRL_{(HEPATIC)} = 0.00002 \text{ mg/kg/day}$  ( $2 \times 10^{-5} \text{ mg/kg/day}$ ; chronic MRL)

$TTD_{ENDOCRINE} = 0.001 \text{ mg/kg/day}$  ( $1 \times 10^{-3} \text{ mg/kg/day}$ )

$TTD_{IMMUNO} = 0.0004 \text{ mg/kg/day}$  ( $4 \times 10^{-4} \text{ mg/kg/day}$ )

$TTD_{NEURO} = 0.0008 \text{ mg/kg/day}$  ( $8 \times 10^{-4} \text{ mg/kg/day}$ )

$MRL_{(REPRO)} = 0.0003 \text{ mg/kg/day}$  ( $3 \times 10^{-4} \text{ mg/kg/day}$ ; intermediate MRL)

$TTD_{DEVELOP} = 0.008 \text{ mg/kg/day}$  ( $8 \times 10^{-3} \text{ mg/kg/day}$ )



## Appendix C: Background Information for *p,p'*-DDE

The *p,p'*-isomers are the principal forms of DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) and its derivatives found in the environment because technical grade DDT typically has contained 65–80% *p,p'*-DDT, 15–21% *o,p'*-DDT, traces of *o,o'*-DDT, and up to 4% of *p,p'*-DDD (ATSDR 1999a). *p,p'*-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) is the major degradation and metabolic product of *p,p'*-DDT found in the environment principally because it is metabolized very slowly and accumulates in fatty tissue. In the ensuing discussion, DDT, DDE, or DDD refer to the *p,p'*-isomers, unless specified otherwise.

### C.1 Toxicokinetics

As reviewed by ATSDR (1999a), results from studies of humans and animals indicate that DDT, DDE, and DDD are absorbed following inhalation, oral, and dermal exposures. Animal studies indicate that gastrointestinal absorption of these compounds is enhanced when they are dissolved in digestible oils and that absorption occurs predominately via the intestinal lymphatic system with only a minor portion of absorption occurring into the portal blood. DDT, DDE, and DDD are lipid soluble compounds that once absorbed are readily distributed via the lymph and blood to all tissues and are stored in proportion to the lipid content of the tissue (ATSDR 1999a). DDT and DDE selectively partition into fatty tissue and human breast milk. Studies of female rats exposed to oral doses of DDE indicate that developing offspring are exposed to DDE via transplacental transfer and through maternal milk and that lactational transfer is the major route of transfer. Studies of metabolism in humans and animals indicate that ingested DDT is reductively dechlorinated to DDD, which is further degraded through several intermediates to DDA (2,2-bis(*p*-chlorophenyl)acetic acid), the major urinary metabolite. DDT is converted to DDE by dehydrodechlorination at a slower rate, and further metabolism of DDE (eventually to DDA) is slow, leading to accumulation of DDE in fatty tissue. Metabolites are predominately excreted in the urine after conjugation with glycine, serine, aspartic acid, or glucuronic acid. DDT, DDE, and DDD have been shown to be inducers of cytochrome P450 IIB proteins and, to a lesser extent, cytochrome P450 IIIA proteins, but not cytochrome P450 1A proteins. Results from human and animal studies indicate that DDT metabolites are primarily excreted as conjugates in the urine, but some excretion also occurs via the feces (by biliary excretion) and breast milk.

## C.2 Health Effects

Acute, high-dose oral exposure to DDT is well known to primarily affect the nervous system producing symptoms such as paresthesia of the mouth and face, hyperexcitability to stimuli, tremor, headache, nausea, and convulsions (ATSDR 1999a). Studies in animals indicate that repeated oral exposure to *p,p'*-DDT or its principal accumulating metabolite, *p,p'*-DDE, can also have adverse effects on the liver, immunological system, nervous system, and male reproductive organs (ATSDR 1999a). Chronic-duration oral exposure to *p,p'*-DDE produced increased incidence of hepatic fatty metamorphosis in rats exposed to about 31 mg *p,p'*-DDE/kg/day and hepatic focal necrosis in hamsters exposed to about 48 mg *p,p'*-DDE/kg/day (ATSDR 1999a). Clinical signs of neurological impairment (hunched appearance) were observed in mice and rats after 8 and 22 weeks exposure to oral doses of 59 and 27 mg *p,p'*-DDE/kg/day, respectively (ATSDR 1999a). Immunosuppression from repeated exposure to *p,p'*-DDE or *p,p'*-DDT is also likely as evidenced by suppressed humoral and cell-mediated immunological responses (to ovalbumin) in rats after dietary exposure to 200 ppm *p,p'*-DDT or *p,p'*-DDE (about 20 mg/kg/day) for 6 weeks (Banerjee et al. 1996). Studies in rats show that *p,p'*-DDE is a competitive inhibitor of androgen binding to the androgen receptor and inhibitor of subsequent expression of genes important in the development of male reproductive organs and in maintenance of their function in adulthood (Kelce et al. 1995; 1997; Loeffler and Peterson 1999). Exposure of rats to several oral doses of 100 mg *p,p'*-DDE/kg/day during gestation, prepubescence, or adulthood produced anti-androgenic effects including decreased anogenital distance at birth, delayed onset of puberty, and decreased seminal vesicle and ventral prostate weights accompanied by inhibition of androgen-dependent gene expression in the ventral prostate (Kelce et al. 1995). Estrogenic effects from exposure to technical-grade DDT are thought to be due to the *o,p'*-isomer of DDT which binds the estrogen receptor and induces estrogenic effects such as increased ovarian weight; *p,p'*-DDT and *p,p'*-DDE are ineffective at binding to the estrogen receptor (ATSDR 1999a; Kelce et al. 1995). Multigenerational reproductive studies of animals orally exposed to *p,p'*-DDE have not been conducted, but no significant changes in reproductive endpoints have been observed in several multigenerational studies in mice, rats, or dogs exposed to technical-grade DDT or *p,p'*-DDT at low doses (generally <10 mg/kg/day) that did not produce overt neurological symptoms such as tremors (ATSDR 1999a). Studies examining the effects of *p,p'*-DDE on neurological development were not located, but mice given single oral doses of 0.5 mg *p,p'*-DDT/kg at the peak of neonatal brain development (10 days after birth) showed changes in motor activity in response to being placed in a new environment when they were adults, indicating impaired development of nonassociative learning or control of motor activity capabilities (ATSDR 1999a; Eriksson et al. 1990, 1992).

Studies of agricultural and pest control workers exposed to DDT have not found consistent evidence of DDT carcinogenicity (ATSDR 1999a). A possible association between breast cancer and levels of DDT, DDE, and/or DDD in tissues has been suggested by the results from some case-control studies, but not by results from others (ATSDR 1999a). Chronic dietary exposure to *p,p'*-DDE produced increased incidence of liver tumors in mice and hamsters and increased incidence of thyroid tumors in female rats (IRIS 2001). Chronic dietary exposure to technical-grade DDT produced increased incidence of liver tumors in mice and rats (IRIS 2001).

### C.3 Mechanisms of Action

There is evidence to indicate that DDT acts on the central nervous system by interfering with sodium ion channels and potassium ion gates in neuronal membranes and by inhibiting a specific neuronal ATPase (ATSDR 1999a). At sufficient doses, these effects can cause repetitive firing of action potentials along the neuron. Studies regarding the mode of action of *p,p'*-DDE on nervous system function apparently are not available (ATSDR 1999a), but the close structural similarity to *p,p'*-DDT suggests that similar modes of actions may operate.

Results from *in vitro* and *in vivo* studies of rats exposed to *p,p'*-DDE as adults or *in utero* and during lactation suggest that the effects of DDT on the male reproductive system may be mediated by *p,p'*-DDE inhibition of binding of androgens to the androgen receptor (ATSDR 1999a). Results from *in vivo* and *in vitro* tests for estrogenic activity indicate that *o,p'*-DDT and *o,p'*-DDE are more estrogenically potent (by mimicking natural estrogens) than are *p,p'*-DDT and *p,p'*-DDE (ATSDR 1999a), but the *o,p'*-isomers are present in the environment at much lower concentrations than the *p,p'*-isomers.

It has been hypothesized that DDT and DDE produce degenerative effects in liver tissue through disruption of the ultrastructure of mitochondrial membranes and that subsequent regenerative processes may contribute to the promotion of liver tumors (ATSDR 1999a). Other mechanistic hypotheses by which DDT and DDE may promote the development of initiated liver cells include the inhibition of apoptosis (i.e., programmed cell death) and the reduction of gap junctional intercellular communication (ATSDR 1999a).

## C.4 Health Guidelines

ATSDR (1999a) did not derive any MRLs for DDE isomers and did not discuss whether or not the MRLs for DDT (see next paragraph) were applicable to DDE or were restricted to the *p,p'*-isomers.

ATSDR (1999a) derived an acute oral MRL of 0.002 mg/kg/day and an intermediate oral MRL of 0.007 mg/kg/day for DDT based on a LOAEL of 0.5 mg/kg/day for neurodevelopmental effects in mice exposed to single gavage doses of DDT and a NOAEL of 0.07 for liver effects in rats exposed to DDT in the diet for 15–27 weeks, respectively. ATSDR (1999a) did not derive a chronic oral MRL for DDT due to the inadequacy of available data to describe dose-response relationships for chronic exposures at low dose levels.

IRIS (2001) does not list an RfD or RfC for *p,p'*-DDE, but lists an RfD of 0.0005 mg/kg/day for *p,p'*-DDT that was based on a NOAEL of 0.05 mg/kg/day for liver hepatomegaly in rats exposed to 1 ppm commercial DDT in the diet (81% *p,p'*-isomer and 19% *o,p'*-isomer). EPA (IRIS 2001) classified *p,p'*-DDE as a Group B2 compound - probable human carcinogen based on no data on the potential carcinogenicity of *p,p'*-DDE in humans and sufficient evidence of carcinogenicity from animal studies. Dietary exposure to *p,p'*-DDE produced increased incidence of liver tumors in two strains of mice and in hamsters and increased incidence of thyroid tumors in female rats. EPA (IRIS 2001) derived an oral slope factor of 0.34 per mg/kg/day based on incidence data for liver tumors in mice and hamsters exposed to *p,p'*-DDE in the diet, but did not derive a cancer inhalation unit risk estimate for *p,p'*-DDE.

## C.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for oral exposure to *p,p'*-DDE were derived for endpoints affected by *p,p'*-DDE and one or more of the other chemicals in the CDD-hexachlorobenzene-DDE-methylmercury mixture that is the subject of this Interaction Profile. The relevant endpoints for *p,p'*-DDE in this mixture include hepatic, immunological, neurological, reproductive, and developmental effects. Chronic oral TTDs for these endpoints are derived below, using the methods described by ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (1999a), and in particular, the oral LSE table.

Toxicity data for *p,p'*-DDE are limited (ATSDR 1999a). Far more data are available for the parent *p,p'*-DDT and for technical DDT, which is 65–80% *p,p'*-DDT (*p,p'*-DDE is the primary metabolite and degradation product of *p,p'*-DDT). Therefore, MRL values derived by ATSDR (1999a) based on studies

of *p,p'*-DDT and technical DDT are expected to be relevant to *p,p'*-DDE, as are TTD values based on these data.

### Hepatic Effects

ATSDR (1999a) derived an intermediate oral MRL of 0.0007 mg/kg/day for DDT based on a NOAEL of 0.07 and LOAEL of 0.4 mg/kg/day for hepatic effects (cellular hypertrophy, cytoplasmic oxyphilia) in Osborne-Mendel rats fed commercial DDT in the diet for 15–27 weeks (Laug et al. 1950). An uncertainty factor of 100 (10 for extrapolation from rats to humans and 10 to protect sensitive individuals) was applied to the NOAEL to derive the MRL. EPA (IRIS 2001) based the chronic oral RfD for *p,p'*-DDT (0.0005 mg/kg/day) on the same study. In its derivation, EPA did not use an additional uncertainty factor for extrapolation from subchronic to chronic duration because of a corroborating chronic study by Fitzhugh (1948). This was a 2-year feeding study in rats that reported a LOAEL for liver lesions of 0.5 mg/kg/day (the lowest dose tested). EPA noted that an RfD derived based on this study (0.5 mg/kg/day divided by an uncertainty factor of 1,000) would be identical to that derived by EPA based on the Laug et al. (1950) study, which supports the RfD derivation based on the intermediate-duration study. EPA concluded that the Laug et al. (1950) study was of sufficient duration to observe toxic effects. By the same reasoning, the intermediate oral MRL should be protective for hepatic effects by chronic exposure as well. Therefore, the intermediate oral MRL for DDT of 0.0007 mg/kg/day is adopted as the  $TTD_{\text{HEPATIC}}$  for *p,p'*-DDE.

### Immunological Effects

Immune system effects of DDT/DDE have been studied primarily by intermediate exposure (ATSDR 1999a). Chronic studies reported no effects on the thymus, spleen, or lymph nodes at *p,p'*-DDE doses up to 59 mg/kg/day in rats and 49 mg/kg/day in mice (NCI 1978). However, these studies included only limited evaluation of immune effects (histopathology). Intermediate-duration studies of DDT that have looked at more subtle measures of immune response have reported effects at lower levels. The lowest reliable intermediate LOAEL was reported by Gabliks et al. (1975), who found decreased mast cells and reduced severity of anaphylactic response following challenge with diphtheria toxoid in rats immunized with diphtheria toxoid and fed a diet containing 1.9 mg/kg/day of DDT for 31 days. A NOAEL was not identified. Applying an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from rats to humans, and 10 to protect sensitive individuals) to the LOAEL of 1.9 mg/kg/day yields a  $TTD_{\text{IMMUNO}}$  of 0.002 mg/kg/day. Due to the sensitivity of the endpoint tested and the large uncertainty

factor applied, this TTD may be sufficiently protective to use for chronic exposure.

### Neurological Effects

The nervous system is a well known target for DDT. Doses in excess of 8 mg/kg/day have been reported to produce ataxia, tremors, convulsions, and reduced brain lipid levels in humans and laboratory animals by acute, intermediate, and chronic exposure (ATSDR 1999a). A chronic human NOAEL of 0.61 mg/kg/day for neurological effects was identified in a study by Hayes et al. (1956), in which volunteers were treated with technical DDT at doses up to 0.61 mg/kg/day for 12–18 months. The subjects were observed for tremors and coordination problems, and were given a battery of tests designed to detect peripheral and central nervous system dysfunction. No deficits were identified. A  $TTD_{NEURO}$  of 0.06 mg/kg/day can be derived from this chronic human NOAEL by applying an uncertainty factor of 10 to protect sensitive individuals. This TTD is higher, however, than the acute oral MRL of 0.002 mg/kg/day for DDT based on neurodevelopmental effects in mice exposed neonatally (see the section below on developmental effects). Neonatal organisms are considered a sensitive population. Because developmental effects are influenced by timing of dose as well as dose level, it is not clear that a longer exposure period would contribute additionally to the effects. Therefore, it appears reasonable to adopt the acute oral MRL for DDT of 0.002 mg/kg/day as the  $TTD_{NEURO}$  for *p,p'*-DDE.

### Reproductive Effects

The reproductive effects of DDT are reviewed by ATSDR (1999a). The lowest LOAEL identified was for decreased number of implanted ova, lengthening of the estrus cycle, decreased corpora lutea, and decreased implants in female mice exposed to 1.67 mg/kg/day of *p,p'*-DDT for up to 12 weeks, including pre mating and gestation (Lundberg 1974). This study did not identify a NOAEL. With the exception of decreased fertility reported at 13 mg/kg/day in one study, chronic multigeneration reproduction studies with DDT (mostly the technical mixture) were negative and reported NOAELs of 0.15–10 mg/kg/day. A  $TTD_{REPRO}$  of 0.002 mg/kg/day for *p,p'*-DDE can be derived from the LOAEL of 1.67 mg/kg/day of *p,p'*-DDT by applying an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from mice to humans, and 10 to protect sensitive individuals). Because the Lundberg (1974) study included pre mating and gestational exposure, and because the estimated NOAEL (LOAEL/10) is consistent with the NOAELs reported in multigeneration studies, this TTD is expected to be protective for chronic exposure.



## Developmental Effects

ATSDR (1999a) derived an acute oral MRL of 0.002 mg/kg/day based on developmental effects (increased motor activity) in 10-day-old NMRI mice given a single dose of 0.5 mg/kg/day of technical DDT by gavage (Eriksson and Nordberg 1986). An uncertainty factor of 300 was used (10 for use of a LOAEL, 10 for extrapolation from mice to humans, and 3 to protect sensitive individuals). An uncertainty factor of 3 rather than 10 was used for sensitive individuals because the neonatal mouse was considered to be a sensitive subject. Developmental effects occurred with LOAELs of 16.5–41.3 mg/kg/day and NOAELs of 1.9–8.3 mg/kg/day in chronic studies and with LOAELs of 16.8–84 mg/kg/day and NOAELs of 1.7–16.8 mg/kg/day in intermediate studies. This suggests that the developmental endpoint in the Eriksson and Nordberg (1986) study was more sensitive than those studied by other researchers. Because developmental effects are influenced by timing of dose as well as dose level, it is not clear that a longer exposure period would contribute additionally to the effects observed. Therefore, it appears reasonable to adopt the acute oral MRL for DDT of 0.002 mg/kg/day as the  $TTD_{\text{DEVELOP}}$  for *p,p'*-DDE.

### Summary (TTDs for *p,p'*-DDE)

$MRL_{\text{(HEPATO)}} = 0.0007 \text{ mg/kg/day } (7 \times 10^{-4} \text{ mg/kg/day})$

$TTD_{\text{IMMUNO}} = 0.002 \text{ mg/kg/day } (2 \times 10^{-3} \text{ mg/kg/day})$

$TTD_{\text{NEURO}} = 0.002 \text{ mg/kg/day } (2 \times 10^{-3} \text{ mg/kg/day})$

$TTD_{\text{REPRO}} = 0.002 \text{ mg/kg/day } (2 \times 10^{-3} \text{ mg/kg/day})$

$TTD_{\text{DEVEL}} = 0.002 \text{ mg/kg/day } (2 \times 10^{-3} \text{ mg/kg/day})$



## Appendix D: Background Information for Methylmercury

### D.1 Toxicokinetics

Results from studies with humans and laboratory animals indicate that methylmercury and its salts (e.g., methylmercuric chloride and methylmercuric nitrate) are readily and completely absorbed by the gastrointestinal tract, but quantitative information on absorption of methylmercury by the respiratory tract is not available (ATSDR 1999b). Absorbed methylmercury is widely distributed among tissues with the kidney showing the highest accumulation of mercury. Mercury from methylmercury can also accumulate in the brain and fetus due to methylmercury's abilities to penetrate the blood-brain and placental barriers and its conversion in the brain and fetus to the inorganic divalent cation (ATSDR 1999b). Excretion of methylmercury and other organic forms of mercury is thought to occur predominately in the feces through biliary excretion. Studies with animals indicate that methylmercury, but not inorganic mercury, can be reabsorbed from the gall bladder and the intestine, resulting in a biliary-hepatic cycle that contributes to longer clearance half-times for methylmercury compared with inorganic mercury (ATSDR 1999b). Intestinal flora and various mammalian tissues can produce the divalent mercury ion from methylmercury presumably via hydroxyl radicals produced by cytochrome P450 reductase (ATSDR 1999b). Inorganic mercury enters an oxidation-reduction equilibrium between itself, mercurous mercury ( $\text{Hg}^+$ ), and metallic mercury ( $\text{Hg}^0$ ) (ATSDR 1999b).

### D.2 Health Effects

The nervous system is one of the primary sites of toxicity in humans and animals following exposure to elemental mercury, methylmercury, or inorganic salts of mercury (ATSDR 1999b). Neurological and behavioral disorders (including hand tremors, emotional lability, and performance deficits in tests of cognitive and motor function) have been observed in humans following inhalation of metallic mercury vapor, ingestion or dermal application of medicinal products containing inorganic mercurous salts, or ingestion of seafood contaminated with methylmercury. A single case study of lethal ingestion of mercuric chloride reported neurological symptoms and brain lesions. Animal studies have demonstrated changes in neurobehavioral function, morphology of neurological tissues, and brain neurochemistry following inhalation exposure to metallic mercury or oral exposure to methylmercury. Data for neurological effects of inorganic mercuric mercury salts are limited, and whether these effects were associated with oral dosing is uncertain. Effects on neurological development ranging from delays in

motor and verbal development to severe brain damage have been observed in children of human mothers orally exposed to organic forms of mercury, including methylmercury (ATSDR 1999b). Animal studies provide confirmatory evidence that neurological development of the fetus can be impaired by exposure of the dams by inhalation to elemental mercury or orally to methylmercury (ASTDR 1999b). Effects on neurological development appear to occur at much lower doses of methylmercury than those producing other effects discussed below (ATSDR 1999b).

The kidney is another major site of mercury toxicity. Degeneration or necrosis of the proximal convoluted tubules has been observed in humans and animals exposed to elemental mercury, inorganic mercury, or methylmercury (ATSDR 1999b). In the absence of renal tubular degeneration, exposure to inorganic mercury has been associated in several human cases and certain genetically disposed animals (New Zealand rabbits and certain strains of mice) with a toxic glomerular response (proteinuria, deposition of immune material in the renal mesangium and glomerular blood vessels, and minimal glomerular cell hyperplasia) that is thought to involve mercury-induced autoimmunity through a stimulation of the humoral and cellular immune systems and systemic autoimmunity (ATSDR 1999b; Hultman and Enestrom 1992; Hultman et al. 1994). Studies demonstrating an association of this type of autoimmune response with exposure to methylmercury were not located (ATSDR 1999b).

Immunosuppressive effects have also been associated with mercury exposure including decreased T-cell reactivity and decreased B cell levels in peripheral blood of mercury-exposed humans, increased susceptibility of mercury-exposed animals to infectious agents, and decreased natural killer cell activity in the spleen and blood of methylmercury-exposed rats (ATSDR 1999b; Hultman and Enestrom 1992; Ilback 1991; Ilback et al. 1991).

Effects on male and female reproductive organs or functions associated with mercury exposure include decreased sperm motility in male monkeys orally exposed to methylmercury, decreased spermatogenesis and degeneration of seminiferous tubules in male mice after prolonged oral exposure to methylmercury, impaired spermatogenesis and infertility in male rats and mice following parenteral administration of methylmercury, and increased abortions, increased resorptions, or decreased implantations in female monkeys, guinea pigs, and mice orally exposed to methylmercury (ATSDR 1999b).

### D.3 Mechanisms of Action

The high-affinity binding activity of divalent mercuric ion to thiol compounds or sulfhydryl groups of proteins is thought to be a central molecular mode involved in the various toxic actions of inorganic mercury and methylmercury (see ATSDR 1999b for review). The greater potency of methylmercury in producing toxic effects, relative to mercuric salts, is thought to be due to differences in dispositional processes, including gastrointestinal absorption and hepato-biliary recycling, leading to longer retention times and higher doses of the mercuric ion at sites of toxicity.

Mercury-induced damage to neurological or renal tissues has been postulated to involve oxidative stress damage from mercury-induced depletion of reduced glutathione levels, depolarization of mitochondrial inner membranes leading to hydrogen peroxide formation, and depleted levels of reduced pyridine nucleotides (ATSDR 1999b). It has been further postulated that neurons are particularly sensitive to mercury because of their low endogenous glutathione content or their inefficient glutathione reduction activity (ATSDR 1999b).

Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects of methylmercury on mitosis through impairment of microtubule assembly, methylmercury and inorganic mercury inhibition of enzymes such as protein kinase C, and inhibition of transport mechanisms in developing brain cells (ATSDR 1999b).

Molecular and cellular events underlying the immunosuppressive effects of mercury such as increased susceptibility to infectious agents are unclear, but Shenker et al. (1993) showed that methylmercury or mercuric chloride inhibited the mitogenic responses of cultured human T or B cells at concentrations that were about 10-fold lower than those that caused cytotoxicity, and that methylmercury was more potent than mercuric chloride. These authors postulated that immunosuppression involves inhibition by mercury of early stages in the response of these cells to mitogens. The genetically-controlled autoimmunity response to mercury that leads to glomerulonephropathy has been proposed to involve mercury disruption of the balance of helper and suppressor cells within the immunoregulatory network, but the molecular and cellular events that lead to glomerular immune-complex deposits have not been elucidated (ATSDR 1999b). Hultman et al. (1994) showed that, in a genetically susceptible mouse strain, prolonged exposure to inorganic mercury caused glomerular immune-complex deposits as well as stimulation of humoral immunity (increased levels of IgM and IgG1), cellular immunity (increased expression of class II molecules and increased mitogen-induced proliferation of T and B cells), and systemic autoimmunity

(increased autoantibodies against the nucleolus).

#### **D.4 Health Guidelines**

ATSDR (1999b) derived a chronic oral MRL of 0.0003 mg Hg/kg/day for methylmercury based on observations of no adverse effects in a 66-month evaluation of neurobehavioral development in children who were conceived, born, and resided on the Seychelles Islands and were members of an isolated population that consumed a high quantity and variety of ocean fish containing methylmercury. A NOAEL of 0.0013 mg methylmercury/kg/day was calculated based on an average level of mercury in maternal hair, 15.3 ppm, from a group (n=95) of the most highly exposed mothers. The NOAEL was divided by a factor of 4.5 to arrive at the MRL. The factor of 4.5 was the sum of an uncertainty factor of 3 (1.5 to address variability in hair-to-blood ratios among women and fetuses in the U.S. population plus 1.5 to address any additional sources of human variability in response to methylmercury) and a modifying factor of 1.5 to address uncertainty regarding the sensitivity of the neurobehavioral tests used in the available report of the Seychelles Islands cohort study.

ATSDR (1999b) did not derive acute or intermediate-duration oral MRLs or any inhalation MRLs for methylmercury due to the absence of data or the lack of sufficient information regarding exposure levels associated with observed effects. ATSDR (1999b) derived acute and intermediate duration oral MRLs for inorganic mercury based on NOAELs for renal effects in rats exposed to mercuric chloride, but did not derive a chronic oral MRL for inorganic mercury due to inadequate data.

EPA (IRIS 2001) derived a chronic oral RfD of 0.0001 mg/kg/day for methylmercury based on an estimated NOAEL of 1.1 µg/kg/day for delayed onset of walking and talking, low clinical neurological scores (<3), mental symptoms, or seizures in infants born to Iraqi mothers who ate contaminated bread during pregnancy. The NOAEL was estimated by a benchmark dose approach as the 95% lower confidence limit for a daily dietary intake associated with 10% incidence for the above neurological effects. The NOAEL was divided by an uncertainty factor of 10 to derive the RfD (3 for human variability including variability in biological half-life of methylmercury and hair: blood ratios and 3 for database deficiencies including the lack of a two-generation reproductive study and lack of data on the effect of exposure duration on developmental neurological effects and adult paresthesia).

EPA (IRIS 2001) classified methylmercury in Group C - possible human carcinogen, based on inadequate data in humans and limited evidence of carcinogenicity in animals. The animal evidence was judged to

be limited because: methylmercury-induced tumors (kidney tumors) were observed at a single site, in a single species and in a single sex; the tumors were observed only in the presence of profound nephrotoxicity; several nonpositive cancer bioassays have also been reported; and the evidence that methylmercury is genotoxic is equivocal. Quantitative estimates of cancer risk from oral or inhalation exposure were not derived based on evidence that methylmercury exerts its carcinogenic effects only at high doses above a maximum tolerated dose and that systemic noncancer effects on the nervous system would be seen at methylmercury exposure levels lower than those required to produce kidney damage and subsequent kidney tumor development.

## **D.5 Derivation of Target-Organ Toxicity Dose (TTD) Values**

TTDs for oral exposure to methylmercury were derived for endpoints affected by methylmercury and one or more of the other chemicals in the CDD-hexachlorobenzene-DDE-methylmercury mixture that is the subject of this Interaction Profile, using the methods described in ATSDR (1999a, Section 2.3.2). The relevant endpoints for methylmercury in this mixture include immunological, neurological, reproductive, and developmental effects. Chronic oral TTDs for these endpoints are derived below. The derivations are based on data provided in ATSDR (1999b), and in particular, the oral LSE table. Where the data were inadequate to derive a chronic oral TTD for a given endpoint, the chronic oral MRL is recommended as a conservative alternative that is protective of human health.

### **Immunological Effects**

Although immunosuppression is a known toxic endpoint for mercury, quantitative dose-response information for methylmercury is limited. Ilback (1991) reported a LOAEL of 0.5 mg Hg/kg/day for decreased thymus weight and cell number and reduced natural killer cell activity in mice fed methylmercury in the diet for 12 weeks. No other data were located regarding oral exposure to organic mercury (ATSDR 1999b). Because a free standing intermediate LOAEL with no supporting data is not a suitable basis for TTD derivation, the chronic oral MRL of 0.0003 mg Hg/kg/day is adopted as TTD<sub>IMMUNO</sub> for methylmercury. Using the chronic oral MRL as the TTD is protective of human health.

## Neurological Effects

The neurological effects of methylmercury are well known in humans and animals (ATSDR 1999b). Effect levels in humans have not been identified, but animal studies have reported degenerative lesions in the central nervous system and functional deficits at doses as low as 0.015 mg Hg/kg/day with intermediate and chronic exposure. As described in Section D.4 above, the chronic oral MRL developed by ATSDR (1999b) is based on a NOAEL for neurological effects in a sensitive population (developing children). Therefore, the chronic oral MRL should be protective for neurological effects in other individuals as well.

## Reproductive Effects

Studies of the reproductive effects of methylmercury are described in ATSDR (1999b). The lowest reliable LOAEL for reproductive effects was 0.06 mg Hg/kg/day, which produced an increased rate of reproductive failure due to decreased conceptions and increased early abortions and stillbirths in female monkeys treated with methylmercury for 4 months (Burbacher et al. 1988). The NOAEL in this study was 0.04 mg Hg/kg/day. Although there was a report of sperm effects in male monkeys exposed to 0.025 or 0.035 mg Hg/kg/day by gavage for 20 weeks (Mohamed et al. 1987), this study was not considered reliable by ATSDR (1999b). Chronic studies in rodents reported testicular lesions (tubular atrophy) and decreased spermatogenesis at approximately 0.7 mg Hg/kg/day, with NOAEL values of roughly 0.1 mg Hg/kg/day in both sexes (Hirano et al. 1986; Mitsumori et al. 1990; Verschuuren et al. 1976). The 4-month monkey study is a suitable basis for a TTD. Application of an uncertainty factor of 100 (10 for extrapolation from monkeys to humans and 10 to protect sensitive individuals) to the NOAEL of 0.04 mg Hg/kg/day yields a  $TTD_{\text{REPRO}}$  of 0.0004 mg Hg/kg/day, which is only slightly higher than the chronic oral MRL of 0.0003 mg Hg/kg/day.

## Developmental Effects

As described in Section D.4 above, the chronic oral MRL for methylmercury (ATSDR 1999b) is based on a NOAEL for developmental effects (neurobehavioral development) in children. Therefore, the chronic oral MRL of 0.0003 mg Hg/kg/day is suitable for use to assess the developmental effects of methylmercury exposure.



**Summary (TTDs for methylmercury)**

$TTD_{\text{IMMUNO}} = 0.0003 \text{ mg Hg/kg/day}$  ( $3 \times 10^{-4} \text{ mg/kg/day}$ )

$MRL_{\text{(NEURODEVELOP)}} = 0.0003 \text{ mg Hg/kg/day}$  ( $3 \times 10^{-4} \text{ mg/kg/day}$ ; chronic MRL)

$TTD_{\text{REPRO}} = 0.0004 \text{ mg Hg/kg/day}$  ( $4 \times 10^{-4} \text{ mg/kg/day}$ )



## Appendix E: Background Information for PCBs

PCBs were manufactured in the United States between about 1930 and 1977, predominately for use as coolants and lubricants in electrical equipment, such as transformers and capacitors, due to their general inertness (they resist degradation by acids or alkali) and heat stability (ATSDR 2000). The manufacture of PCBs in the United States was stopped due to the evidence that they accumulate and persist in the environment and can cause toxic effects. Due to their biostability and lipophilicity, PCBs accumulate and concentrate in food chains; PCB concentrations in fatty tissue increase with increasing order of species in a food chain. There are 209 possible congeners of chlorinated biphenyls. PCBs were manufactured as complex mixtures of chlorinated biphenyls that varied in the degree of chlorination. For example, the commercial product Aroclor 1242, was a mixture of mono- through hepta-chlorinated biphenyls with an average chlorine content of 42%. Once released into the environment, commercial mixtures of PCBs undergo slow changes (predominately volatilization and biotransformation), so that patterns of PCBs in samples of food, human milk, or other environmental biota do not resemble any one particular commercial mixture (ATSDR 2000; Brouwer et al. 1998).

PCB congeners that have chlorines at the meta positions (3, 3', 5, or 5' carbons in the phenyl rings) or the para positions (4 or 4' carbons) can assume a co-planar geometry (i.e., the 2 rings can exist in the same plane), which is important in determining binding to the Ah receptor, a mediator of some of the toxic effects of PCBs. Increasing degrees of chlorination at the ortho positions (2, 2', 6, and 6' carbons) leads to increasing steric hinderance that prevents a co-planar geometry. In general, PCBs with no or only a single chlorine at an ortho position are co-planar, whereas congeners with two or more ortho chlorines are non-co-planar. PCBs without ortho chlorines generally account for only minor percentages of total PCBs in commercial PCB mixtures or samples of environmental biota (ATSDR 2000).

### E.1 Toxicokinetics

PCBs can be absorbed via the inhalation, oral, and dermal routes of exposure, and are expected to be absorbed by passive diffusion across cell membranes (ATSDR 2000). Data on absorption of inhaled PCBs are insufficient to estimate rates of absorption, but studies of humans and animals exposed to airborne PCBs provide qualitative information that inhaled PCBs can be absorbed (ATSDR 2000). Ingested PCBs appear to be efficiently absorbed based on studies of infants consuming PCBs in their mothers' breast milk and studies of animals indicating retention percentages ranging from 60 to 100% of

ingested doses. Studies of animals dermally exposed to doses of radiolabeled PCBs for 24 hours reported absorption efficiencies ranging from about 15 to 60% of administered doses based on monitoring of urine for several weeks post-dosing.

Once absorbed, PCBs tend to accumulate in lipid-rich tissues, but PCBs have been detected in other tissues as well (ATSDR 2000). For example, in rats given gavage doses of Aroclors 1254 or 1260, the highest concentrations of PCBs were found in fat tissue, followed by concentrations in kidney, liver, and brain; plasma and muscle tissue showed the lowest concentrations. PCB concentrations in human milk can be high relative to other tissue due to high fat content in breast milk, and PCBs are efficiently transferred to children through breast-feeding. Results from animal studies support the importance of breast-feeding transfer to infants, and further indicate that PCBs can cross the placental barrier and enter the fetus. The amount of PCBs transferred to offspring is expected to be higher during lactation than during gestation. For example, in female rats administered PCBs before gestation, an average of 0.003% of the administered dose was transferred to the fetus, whereas 5% was transferred to sucklings (ATSDR 2000).

Rat studies indicate that different PCB congeners can accumulate to different degrees in different tissues. In rats given gavage doses of Aroclor 1254 (comprised of 2.1% mono-, di-, and tri-chlorinated PCB congeners, 19.1% tetra-, 49.6% penta-, 25.9% hexa-, 2.9% hepta-, and 0.5% octa- and nona-chlorinated PCB congeners), heavily chlorinated congeners (with 6–9 chlorines) accounted for greater percentages of total PCBs in analyzed tissues than in Aroclor 1254 itself (Kodavanti et al. 1998). Most PCBs in Aroclor 1254 have at least one ortho chlorine; PCBs without ortho chlorines account for <3% of PCBs in Aroclor 1254. Hexa- through nona-chlorinated congeners accounted for 29.3% of PCBs in Aroclor 1254, and, in contrast, 70, 66, and 49% of total PCBs in frontal cortical brain, liver, and fat tissues, respectively. Observations that lower chlorinated congeners or congeners with two adjacent unsubstituted carbons (i.e., at the meta and para positions; 3,4 or 3',4' positions) are metabolized more quickly than higher chlorinated congeners or congeners without adjacent unsubstituted carbons (ATSDR 2000; Parham and Portier 1998; Safe 1994) may provide at least a partial explanation of this differential tissue accumulation among PCB congeners.

Hydroxylated PCBs (i.e., phenolic PCBs) are the major metabolites of PCBs in humans and animals, and are formed either by direct catalysis or via arene oxide intermediates by several CYP oxygenase isozymes (ATSDR 2000; Expert Panel 1994; Safe 1994). Phenolic PCBs can be further hydroxylated to form dihydrodiols and catechols, or conjugated with glucuronides or sulfates, which facilitates excretion in bile

or urine. Glutathione conjugates are formed from arene oxide intermediates by glutathione S-transferase catalysis and transported to the intestine in the bile (Safe 1994). In the intestine, cleavage of the carbon-sulfur bond by microbes leads to the formation of thiol intermediates, which can be methylated and reabsorbed. Following reabsorption, the methylated thiols can be further oxidized to form methylsulfonyl-PCBs, which have been proposed to be involved in respiratory toxic effects from PCB exposure (Bergman et al. 1992; Brandt and Bergman 1987). Non-ortho-substituted PCBs appear to be preferentially metabolized initially by CYP isozymes that are induced by 3-methylcholanthrene (e.g., CYP1A1 and 1A2), whereas PCBs with multiple ortho substitutions appear to be preferentially metabolized by phenobarbital-inducible isozymes (e.g., CYP2B2, 2B1, and 3A) (ATSDR 2000; Expert Panel 1994). Congeners with mono-ortho substitution appear to be metabolized by both types of CYP isozymes.

Comparison of congener concentrations in commercial PCB mixtures with concentrations in adipose tissue from exposed workers indicates that some PCB congeners are more readily transformed by metabolism than others (ATSDR 2000). For example, both 2,2',4,4',5,5'-hexachlorobiphenyl and 2,2',4,4',6,6'-hexachlorobiphenyl are found in commercial PCB mixtures and in environmental samples, but 2,2',4,4',5,5'-hexachlorobiphenyl was detected in the workers' adipose tissue and 2,2',4,4',6,6'-hexachlorobiphenyl was not (ATSDR 2000). Results from rat studies indicate that the rate of metabolism decreases as the degree of chlorination on both phenyl rings increase and is dependent on the position of chlorine atoms on the phenyl ring (ATSDR 2000; Parham and Portier 1998; Safe 1984). Higher rates of hydroxylation are expected with PCBs that have two adjacent unsubstituted carbons in a phenyl ring at the 3,4 or 3',4' positions (i.e., meta-para unsubstituted carbons). For example, in humans exposed to PCBs, hexa- and hepta-chlorinated congeners were more slowly cleared from the blood than tetra- and penta-chlorinated congeners, and, among tetra- and penta-chlorinated congeners, those without adjacent unsubstituted carbons were more slowly cleared than those with adjacent unsubstituted carbons. In mice administered one of five tetrachlorobiphenyls, elimination half-lives for the congeners increased in the following order: 2,6,2',6' = 2,3,2',3' < 2,3,5,6 << 3,4,3',4' = 3,5,3',5, consistent with decreasing rate of metabolism in this sequence.

Different PCBs induce different spectrums of CYP isozymes (Connor et al. 1995; Hansen 1998). Commercial mixtures such as Aroclor 1254 and 1242 induce both types of CYP isozymes. Co-planar PCBs without ortho substitution (e.g., the 3,3',4,4'-, 3,3',4,4',5-, and 3,3',4,4',5,5'-congeners) are among the most potent PCB inducers of CYP1A1/1A2 and have the greatest affinity for the Ah receptor. Mono-ortho PCBs with lateral substitutions (e.g., the 2,3,3',4,4'-, 2,3,4,4',5-, 2',3',4,4',5-, 2',3,4,4',5-,

2,3,3',4,4',5-, 2,3,3',4,4',5'-, 2,3',4,4',5,5'-, and 2,3,3',4,4',5,5'-congeners) induce both CYP1A1/1A2 and CYP2B1/2B2 isozymes and have less affinity for the Ah receptor than the non-ortho PCBs. Some di-ortho PCBs induce both types of CYP isozymes and have less affinity for the Ah receptor than the mono-ortho congeners (e.g., the 2,2',3,3',4,4'-, 2,2',3,4,4',5'-, and 2,2',3,3',4,4',5-congeners). In contrast, most congeners with multiple ortho chlorines and one or two para chlorines (e.g., 2,2',4,4'-, 2,2',4,4',5-, 2,2',4,5,5'-, 2,3,3',4',6-, 2,2',4,4',5,5'-, 2,3,3', 4',5,6-, 2,2',3,4,4',5',6-, 2,2',3,4',5,5',6-, 2,2',3,3',4,4',5,5'-, and 2,2',3,3',4,5,5',6'-congeners) induce only the CYP2B1/2B2 and 3A isozymes and essentially do not bind to the Ah receptor.

In general, PCB congeners display a wide range of elimination rates that have been demonstrated in several cases to be associated with the rates at which they are metabolized (i.e., more rapidly metabolized PCBs are more rapidly excreted) (ATSDR 2000). Studies with animals given parenteral or oral doses of PCB mixtures or individual PCBs indicate that excretion of PCBs and their metabolites occurs via feces and urine with much greater amounts excreted in the feces (ATSDR 2000). For example, within 42 days of administration of an intravenous dose of radiolabeled 3,3',5,5'-tetrachlorobiphenyl (a PCB that is more rapidly metabolized than other more highly chlorinated PCBs) to rats, 80% of the dose was excreted in the feces and 6.1% was excreted in the urine. Less than 10% of radioactivity in bile, feces, and urine was parent compound. Within 40 weeks of administration of an intravenous dose of a poorly metabolized PCB (2,2',4,4',5,5'-hexachlorobiphenyl), rats excreted 16% of the dose in feces and 0.8% in the urine. Another significant route of elimination is breast milk; it has been estimated that an infant in an industrialized country may accumulate about 7% of its lifetime PCB body burden during 6 months of breast feeding (ATSDR 2000).

## E.2 Health Effects

Associations have been noted between occupational exposure to commercial mixtures of PCBs and several health effects, including chloracne and other skin changes; various hepatic effects including increased serum levels of liver enzymes and lipids, induction of drug-metabolizing enzymes, and hepatomegaly; decreased birth weight in offspring (of occupationally exposed mothers); and eye irritation (Safe 1994; Swanson et al. 1995).

Studies of cancer mortality in occupationally-exposed workers have not found consistent or strong evidence of carcinogenicity, but findings of increased incidence of liver tumors in studies of rats exposed to commercial PCB mixtures suggest that PCBs are probable human carcinogens (ATSDR 2000; Safe

1994). IARC (1987) classified the human evidence as limited, whereas EPA (2000f) classified the human evidence as inadequate, but suggestive. Some cohort mortality studies of workers exposed during capacitor manufacturing and repair found increased risk for liver, biliary tract, gall bladder, and/or intestinal cancers, but statistically significant increases were not observed in all studies, and clear demonstrations of increasing risk with increasing exposure indices were not found (ATSDR 2000). Most case-control studies examining possible associations between breast cancer in women and concentrations of PCBs in breast tissue or blood found no statistically significant association (ATSDR 2000; Swanson et al. 1995).

Two incidences of consumption of PCB-contaminated cooking oil, one in Japan (the “Yusho” incident) and the other in Taiwan (the “Yucheng” incident), were associated with acne and skin pigmentation in adults and abnormalities in offspring including dark pigmentation of the skin, lower birth weight, and slower development (ATSDR 2000; Safe 1994; Swanson et al. 1995). These incidents are usually cited in discussion of the health effects of PCBs, but it is generally thought that the health effects were due primarily to PCDFs rather than PCBs (ATSDR 2000; Expert Panel 1994; Safe 1994; Swanson et al. 1995).

Studies of people and animals with diets containing Great Lakes fish (contaminated with PCBs and other biopersistent chemicals) provide suggestive evidence that frequent dietary consumption of contaminated fish by child-bearing-aged women may be associated with subtle neurobehavioral effects in their children, but no consistent evidence for associations with impaired reproduction, immune capabilities, or physical birth defects (Buck et al. 1997; Courval et al. 1999; Daly 1991; Dar et al. 1992; Feeley and Jordan 1998; Feeley et al. 1998; Fein et al. 1984; Hertzler 1990; Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a, 1990b; Kostyniak et al. 1999; Lonky et al. 1996; Mendola et al. 1995, 1997; Mergler et al. 1998; Restum et al. 1998; Schantz et al. 1999; Stewart et al. 1999, 2000b; Vena et al. 1996). In one prospective study, limited evidence was presented relating maternal PCB exposure levels and deficits in neonatal behavioral development, short-term memory during infancy, and general intellectual ability in early school years (Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a). Statistically significant relationships between maternal PCB exposure levels (cord blood concentrations of PCBs with 7–9 chlorines) and deficits in neonatal behavioral development also were found in another more recent prospective study (Lonky et al. 1996; Stewart et al. 1999, 2000b). Studies of people and animals with diets containing contaminated Baltic Sea fish provide suggestive evidence that contaminated fish consumption may be associated with impaired immunological competence or low birth weight, but do not clearly demonstrate dose-response relationships for the potential health hazards (Ross et al. 1995;

Rylander and Hagmar 1999; Rylander et al. 1995, 1996, 1998a, 1998b; Svensson et al. 1994). Results from a North Carolina study (Gladen and Rogan 1991; Gladen et al. 1988; Rogan et al. 1986a, 1986b, 1987) and a Dutch study (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994, 1996; Patandin et al. 1998, 1998b, 1999) of breast-fed children provide some evidence that exposure to PCBs in human breast milk at exposure levels in the upper range of background levels or exposure to PCBs *in utero* may result in mild neurodevelopmental delays in some children. It is plausible that exposure to PCBs may have contributed to these associations, but these studies of possible health effects from environmental exposure to PCB-containing complex mixtures cannot determine with certainty which chemicals may cause the effects or determine possible interactions that may occur among the components.

Oral exposure to commercial mixtures of PCBs has been demonstrated to produce a wide array of toxic effects in animals including:

1. inhibition of body weight gain or body weight loss in rats, rabbits, monkeys, or minks after acute, intermediate, or chronic exposure (ATSDR 2000; Safe 1994);
2. increased porphyrin levels in liver, urine, or kidneys in rats after intermediate exposure (ATSDR 2000; Safe 1994);
3. dermal effects including acne, alopecia, or finger- and toenail loss in monkeys or rats exposed for intermediate or chronic exposure (ATSDR 2000);
4. induction of hepatic levels of Phase I (CYP oxygenases) and Phase II (e.g., UDP-glucuronyltransferases) enzymes (ATSDR 2000; Safe 1994);
5. increased liver weight, increased serum cholesterol, or degenerative liver changes (e.g., fatty changes, necrosis) in rats after acute exposure, in monkeys, rats, or mice after intermediate exposure, and in monkeys or rats after chronic exposure (ATSDR 2000);
6. altered thyroid hormone levels (e.g.,  $T_4$ ), histology, or weight in adult rats after acute exposure, in rats or mice after acute *in utero* exposure, and in adult rats after intermediate exposure (ATSDR 2000; Safe 1994);
7. fetal toxicity and decreased fetal survival in rats and hydronephrosis in mice exposed for acute durations *in utero*, and fetal toxicity and decreased survival in monkeys, rats, mice, rabbits, guinea pigs, or minks exposed for intermediate durations, or in monkeys exposed for chronic durations (ATSDR 2000);
8. altered neurobehavior and/or brain chemistry in adult rats after acute exposure or adult monkeys or rats after intermediate exposure (ATSDR 2000);
9. altered neurobehavior in rats or mice after acute *in utero* exposure, in offspring of rats or mice



- exposed for intermediate durations, or in offspring of monkeys exposed for chronic durations (ATSDR 2000);
10. impaired reproductive function or altered reproductive organ weight or structure in adult monkeys, rats, mice, or mink after intermediate exposure, or in adult monkeys after chronic exposure (ATSDR 2000);
  11. altered reproductive function or reproductive organ weight or structure in rats after acute *in utero* exposure (ATSDR 2000);
  12. decreased immunological responsiveness (e.g., increased mortality from microbial infection or decreased antibody production in response to foreign blood cells) and/or altered organ weights or histopathology of thymus or spleen in monkeys, rats, mice, rabbits, or guinea pigs exposed for intermediate durations and in monkeys exposed for chronic durations (ATSDR 2000);
  13. increased incidence of liver tumors in rats exposed for chronic durations, and promotion (but not initiation) of preneoplastic lesions and tumors in the liver and lung of rats and mice following initiation by other carcinogens such as N-nitrosodiethylamine (ATSDR 2000).

### **E.3 Mechanisms of Action**

Mechanisms by which the broad array of toxic effects observed in animals orally exposed to PCB mixtures develop are incompletely understood, but there is evidence to suggest that PCB congeners differ qualitatively and quantitatively in biological activities and that multiple and diverse mechanisms are involved in responses to PCB mixtures. Research in the 1970s and 1980s focused on mechanistic similarities between PCBs and CDDs involving initial mediation of effects by the Ah receptor (Poland and Knutson 1982; Safe 1990, 1994), but research through the 1990s has found increasing evidence for the involvement of alternative mechanisms for several PCB-induced effects (Chauhan et al. 2000; Cheek et al. 1999; Fischer et al. 1998; Hansen et al. 1998; Harper et al. 1993a, 1993b; Safe 1994; Tilson and Kodavanti 1998). An in-depth and all-inclusive review of the many recent and ongoing research efforts regarding PCB mechanisms of action is outside of the scope of this profile; rather, an overview of this large body of research is presented with the intent of providing information relevant to public health issues.

## ***PCB Effects Involving Ah-receptor Dependent Mechanisms***

### INDUCTION OF HEPATIC CYP1A OXYGENASES AND PHASE II ENZYMES

PCBs induce hepatic Phase I enzymes (CYP oxygenases) and Phase II enzymes (e.g., UDP glucuronyltransferases, epoxide hydrolase, or glutathione transferase) to varying degrees and specificities (Connor et al. 1995; Hansen et al. 1998; Safe 1994). Demonstration of relationships between PCB molecular structure and induction of CYP isozymes has provided a framework within which much mechanistic research has been conducted. In general, commercial mixtures of PCBs induce both 3-methylcholanthrene-type (CYP1A1 and 1A2) and phenobarbital-type (CYP2B1, 2B2, and 3A) CYPs. Strong structure-activity relationships have been demonstrated between CYP1A1/1A2 induction in rodents and non-ortho and mono-ortho PCBs, which can assume a coplanar molecular configuration and bind to the Ah receptor (Connor et al. 1995; Hansen et al. 1998; Safe 1994). In structure-activity studies of CYP1A induction in hepatocytes from cynomolgus monkeys by 20 PCBs varying in degree and pattern of chlorine substitution (4–7 chlorines), the most potent inducers were without ortho chlorines (van der Burght et al. 1999). Many PCBs with ortho chlorines (mono-, di-, tri-, and tetra-ortho congeners) displayed no CYP1A induction activity, but a few mono-ortho and multiple-ortho congeners displayed activities that were about 1,000- and 10,000-fold less than the most potent non-ortho congeners, respectively (van der Burght et al. 1999). A working mechanistic hypothesis involves initial binding of coplanar PCBs to the Ah receptor in the cytosol of target cells, transport of the ligand-receptor complex to the nucleus, and subsequent changes in gene expression (e.g., induction of CYP1A1/1A2) leading to toxic responses via subsequent molecular mechanisms that are largely unexplored. Support for this hypothesis comes from the similarity in the array of PCB effects compared with the array produced by 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons via initial Ah-receptor mediation, results from *in vitro* binding studies, and results from congener-specific *in vivo* studies of specific endpoints (e.g., enzyme induction and down regulation, body weight, and immunological responses to sheep red blood cells) in mouse strains and rat genders differing in responsiveness to Ah-receptor mediation (Hori et al. 1997; Safe 1990, 1994).

The complexity of Ah-receptor mediated effects on hepatic enzyme levels is illustrated by results from a study with mouse strains differing in Ah-receptor responsiveness and three PCB congeners (Hori et al. 1997). Ah responsive (C57BL/6) and Ah non-responsive (DBA/2 mice) were given single intraperitoneal doses of 3,3',4,4',5-pentachlorobiphenyl, a congener with high Ah receptor affinity, 3,3',4,4'-tetrachlorobiphenyl, a congener with lesser affinity, and 2,2',5,5'-tetrachlorobiphenyl, a low-affinity ligand.

Only the high-affinity 3,3',4,4',5-congener produced body weight wasting in the dose range tested (up to 50 mg/kg) in Ah-responsive C57BL/6 mice, and this effect was accompanied by a decrease in selenium-dependent glutathione peroxidase and an increase in  $\theta$  glutathione *S*-transferase. The effect on levels of these Phase II enzymes was not produced by the other congeners in C57BL/6 mice, and did not occur in DBA/2 mice exposed to any of the congeners, indicating the involvement of Ah-receptor mediation. These Phase II enzymes both play protective roles in scavenging intracellularly generated peroxides and the balance of their activities is likely to influence a cell's ability to withstand damage from peroxides.

#### BODY WEIGHT WASTING, THYMIC ATROPHY, AND PORPHYRIA

In addition to induction of hepatic levels of CYP1A1/1A2/1B1 and induction or repression of some Phase II enzymes, PCB-induced effects that appear to predominately involve Ah-receptor initiated mechanisms include body weight wasting and thymic atrophy from acute exposure (Hori et al. 1997; Safe 1994) and porphyria and porphyria cutanea tardea (Franklin et al. 1997; Smith et al. 1990a, 1990b). For example, single intraperitoneal doses of 5 mg/kg 3,3',4,4',5-pentachlorobiphenyl, a potent inducer of CYP1A1 and a high-affinity Ah-receptor agonist (relative to other PCBs), produced marked body weight wasting in Ah-responsive C57BL/6 mice, but not in DBA/2 mice, which have a low-affinity Ah-receptor (Hori et al. 1997). Showing a link between Ah-receptor responsiveness and development of uroporphyria, female F344 rats had significantly higher hepatic levels of porphyrins and ethoxyresorufin deethylase activity (an indicator of CYP1A1) in response to exposure to 0.005% Aroclor 1254 in the diet for 15 weeks than did male rats (Smith et al. 1990b). A similar gender-specific correlation between porphyrinogenic response and CYP1A induction was observed in iron-loaded F344 rats exposed to single intraperitoneal doses of 63 mg Aroclor 1254/kg (Franklin et al. 1997). In mice of the Ah-responsive C57BL/6 strain, a single dose of iron-dextran (600 mg Fe/kg), followed by feeding of a diet containing 0.01% Aroclor 1254 for up to 12 months, produced markedly increased hepatic levels of porphyrins and liver enlargement, but this response to iron and Aroclor 1254 was not observed in similarly treated DBA/2 mice (Smith et al. 1990a). Exposure to iron-dextran alone caused a moderate porphyria in C57BL/6 mice, but not in DBA/2 mice, lending support to a postulate that there are constitutive genetic differences between these strains that influence porphyria development and do not involve Ah-receptor mediation (Smith et al. 1990a). One mechanistic hypothesis proposes that induction of CYP1A2 by the Ah-receptor-PCB complex leads to generation of a competitive inhibitor of uroporphyrinogen decarboxylase in the liver and subsequent accumulation of porphyrins (see Franklin et al. 1997).

## Ah RECEPTOR TEF APPROACH TO HEALTH HAZARD ASSESSMENT

A TEF approach to evaluating health hazards from exposure to complex environmental mixtures containing PCBs, CDDs, and CDFs has been developed and is used to some extent to guide public health decisions because humans are exposed to complex and varying mixtures of these halogenated aromatic hydrocarbons and there are limited toxicological data for these complex mixtures and many of their components (ATSDR 1998; Safe 1990, 1994; van den Berg et al. 1998). PCBs were included in this component-based approach because (1) the spectrum of effects in animals exposed to some PCB mixtures and congeners is similar to the spectrum produced by 2,3,7,8-TCDD (via Ah receptor initial mediation) and (2) coplanar PCBs display Ah receptor binding affinities that were related to their potency in producing health effects in rodents such as body weight wasting and inhibition of immunological responses to sheep red blood cells (Safe 1990, 1994). The TEF approach compares the relative potency of individual congeners, based on *in vitro* or acute *in vivo* data, with that of 2,3,7,8-TCDD, the best-studied member of this chemical class, so that the TEF for 2,3,7,8-TCDD is 1. The concentration or dose of each active component in a mixture of concern is multiplied by its TEF to arrive at a TEQ, and the TEQs are added to give the total toxic equivalency of the mixture, which are compared with reference exposure levels for 2,3,7,8-TCDD expected to be without significant risk for producing health hazards. TEFs have recently been recommended by the World Health Organization for 7 CDD, 10 CDF, and 12 PCB congeners (Van den Berg et al. 1998).

Limitations in using the TEF approach for assessing health hazards from PCB-containing environmental media revolve around the inherent assumptions that the components jointly act in an additive manner through a common Ah-receptor initial mechanism and the evidence that Ah-receptor-binding congeners in PCB-containing environmental mixtures are minor components (Hansen 1998; Safe 1998a, 1998b). Several studies have provided evidence of non-additive interactions between specific PCB congeners and between some PCB congeners and 2,3,7,8-TCDD (Safe 1998a, 1998b), and there is evidence, discussed below, that several Ah-receptor-independent mechanisms may make contributions to toxic effects from PCB mixtures.

## ***PCB Effects Involving Ah-receptor Independent Mechanisms***

### **INDUCTION OF HEPATIC CYP2B OXYGENASES**

In contrast to the distinct relationships between CYP1A1/1A2 induction, PCB molecular structures, and Ah-receptor initiation of toxic effects, relationships between potency in inducing CYPs 2B1/2B2/3A, PCB structural properties, and toxic effects are less clear (Connor et al. 1995). For example, some PCBs with two ortho chlorines and lateral chlorines induce both types of CYPs and display a very small affinity for the Ah receptor, whereas other di-ortho PCBs with one or two para chlorines predominately induce CYP2B1/2B2/3A and have no measurable affinity for the Ah receptor (Connor et al. 1995; Hansen 1998). Nevertheless, it is clear that PCB induction of phenobarbital-type CYPs is independent of the Ah receptor and that the most potent inducers of CYP have at least two ortho chlorines and one or two para chlorines.

Other PCB-induced effects involving Ah-receptor independent mechanisms include: neurological and neurodevelopmental effects involving changes in brain dopamine levels (Seegal 1996b, 1998; Seegal et al. 1989, 1990; Shain et al. 1991), inhibition of dopamine vesicular uptake (Mariussen et al. 1999), and/or changes in brain cell intracellular calcium homeostasis and related signal transduction processes (Kodavanti and Tilson 1997; Tilson and Kodavanti 1997, 1998; Tilson et al. 1998; Wong and Pessah 1996, 1997; Wong et al. 1997); and tissue injury related to activation of neutrophils (Brown and Gamey 1995; Gamey et al. 1993; Tithof et al. 1995).

### **BRAIN DOPAMINE LEVELS AND NEUROLOGICAL EFFECTS**

Aroclor 1254 decreased cellular levels of dopamine in cultured pheochromocytoma cells, which synthesize, store, release, and metabolize dopamine in a manner similar to the intact mammalian central nervous system (Seegal et al. 1989). Daily oral exposure of adult nonhuman primates (*Macaca nemestrina*) to Aroclor 1016, a commercial mixture of lightly chlorinated PCB congeners, for 20 weeks, likewise, produced decreased dopamine concentrations in brain regions including the caudate, putamen, substantia nigra, and hypothalamus (Seegal et al. 1990). In these brain regions, only three PCB congeners were detected (2,4,4'-trichlorobiphenyl and 2,2',4,4'- and 2,2',5,5'-tetrachlorobiphenyl), suggesting that nonplanar PCBs, which are poor Ah receptor agonists, may have been responsible for the effect. Structure-activity studies of 50 PCB congeners in the pheochromocytoma *in vitro* system found that the most active congeners had two ortho chlorines (e.g., 2,2',4,6-, 2,2',5,5'-, and 2,2',4,5-tetrachlorobiphenyl) and that congeners that were relatively strong Ah receptor agonists (e.g., 3,3',4,4'-tetrachlorobiphenyl,

3,3',4,4',5-pentachlorobiphenyl) were inactive or had minimal effects on dopamine levels (Shain et al. 1991). However, ortho substitution was not the sole determinant of activity in this system; for example, a congener with four ortho chlorines (2,2',6,6'-tetrachlorobiphenyl) had no effect on dopamine levels in pheochromocytoma cells (Shain et al. 1991). The effect on dopamine levels has been postulated to involve decreased dopamine synthesis via direct or indirect PCB inhibition of tyrosine hydroxylase (Choksi et al. 1997; Seegal 1996b) or L-aromatic amino acid decarboxylase (Angus et al. 1997) and/or decreased uptake of dopamine into vesicles (Mariussen et al. 1999). For example, several congeners that were inactive in causing dopamine level changes in pheochromocytoma cells (e.g., 2,2',6,6'- and 3,3',4,4'-tetrachlorobiphenyl) were much less active in inhibiting vesicular uptake of dopamine than other more active congeners (e.g., 2,2',4,6- and 2,2',4,5'-tetrachlorobiphenyl) (Mariussen et al. 1999).

#### DISRUPTION OF $Ca^{+2}$ HOMEOSTASIS AND NEUROLOGICAL EFFECTS

Neurological and/or neurodevelopmental effects from exposure to PCBs also have been hypothesized to involve interference with calcium homeostatic mechanisms and intracellular second messenger systems by PCB congeners that are not effective Ah receptor agonists (see reviews by Kodavanti and Tilson 1997; Tilson and Kodavanti 1998; Tilson et al. 1998). In agreement with structure-activity relationships observed for PCB effects on dopamine levels in pheochromocytoma cells (Shain et al. 1991), 2,2'-dichlorobiphenyl altered intracellular calcium homeostasis in cultured rat cerebellar granule cells (increased free calcium levels and inhibited calcium buffering systems) at non-cytotoxic exposure concentrations (higher concentrations were cytotoxic) (Kodavanti et al. 1993). In contrast, 3,3',4,4',5'-pentachlorobiphenyl, one of the most effective Ah receptor agonists among tested PCBs (Safe 1994), was not cytotoxic in the tested concentration range and did not alter calcium homeostasis to as great an extent as 2,2'-dichlorobiphenyl (Kodavanti et al. 1993). Using phorbol ester binding in rat cerebellar granule cells as a measure of protein kinase C translocation (which is thought to play key roles in cellular signal transduction in neurons and be regulated by several intracellular factors including intracellular levels of free calcium), commercial mixtures of PCBs (Aroclors 1016, 1254, and 1260) were shown to increase protein kinase C translocation in a concentration-dependent manner with varying potencies (Kodavanti et al. 1995). Aroclors 1016 and 1254 were more potent than Aroclor 1260. Examination of 24 PCB congeners showed that the most potent congeners (e.g., 2,2'-dichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, and 2,2',4,6,6'-pentachlorobiphenyl) had multiple ortho chlorines, whereas congeners without ortho chlorines tended to have either no or lower activities (Kodavanti et al. 1995). Similar results were found in structure-activity studies of 24 PCB congeners and their effects on *in vitro*  $Ca^{+2}$  sequestration by microsomes and mitochondria from freshly isolated rat cerebellar cells (Kodavanti

et al. 1996). Structure activity relationships for PCB congeners and protein kinase C translocation in rat cerebellar granule cells and  $\text{Ca}^{+2}$  sequestration were similar to relationships for PCB congener-induced changes in dopamine levels in pheochromocytoma cells. For example, 2,2',5,5'- and 2,2',4,6-tetrachlorobiphenyl were among the most potent congeners and 2,2',6,6'- and 3,3',4,4'-tetrachlorobiphenyl were inactive in all three systems (Kodavanti et al. 1995, 1996; Shain et al. 1991).

One proposed molecular target for PCB disruption of calcium homeostasis that may be involved in neurological and neurodevelopmental effects is ryanodine-sensitive  $\text{Ca}^{+2}$  channels. Commercial PCB mixtures with intermediate to high degrees of chlorination (Aroclors 1248, 1254, 1260) enhanced ryanodine binding to calcium release channels in sarcoplasmic reticulum membranes from skeletal or cardiac rabbit muscles, and mixtures with lower (Aroclors 1221, 1232) or higher chlorination (Aroclor 1268) showed little enhancement (Wong and Pessah 1996). Examination of selected pentachlorobiphenyls indicated that ortho substitution favored activity; 2,2',3,5',6-pentachlorobiphenyl induced the greatest enhancement of ryanodine binding, whereas the 3,3',4,4',5-isomer did not enhance binding (Wong and Pessah 1996). The 2,2',4,6,6'-isomer with full substitution at the ortho positions produced less enhancement than the 2,2',3,5',6-isomer, indicating that some degree of rotation about the biphenyl bond may be important for full activity. Results from studies with hippocampal slices from freshly dissected rat brains indicated that perfusion with a triortho congener (2,2',3,5',6-pentachlorobiphenyl) enhanced ryanodine binding and inhibited electrophysiological responses to electrical pulse stimulations, but a mono-ortho congener (2,3',4,4'-tetrachlorobiphenyl) showed no enhancement of ryanodine binding and no inhibition of electrophysiological responses to stimulation (Wong et al. 1997). Offspring of rats exposed to gavage doses of 8 or 32 mg/kg/day 2,2',3,5',6-pentachlorobiphenyl on gestation days 10–16 displayed neurobehavioral changes as adults (depressed open field locomotor activity, faster acquisition on a working memory task, and no changes in a delayed spatial alternation task), and changes in ryanodine binding to calcium channels in specific regions of the brain (e.g., decreased in hippocampus and increased in cerebral cortex) (Schantz et al. 1997). Although it is not understood how these changes in ryanodine binding are specifically related to the observed neurobehavioral changes, the results from this series of studies emphasize the potential importance of Ah receptor independent mechanisms in PCB-induced neurological and neurodevelopmental effects.

## NEUTROPHIL FUNCTION AND IMMUNOLOGICAL EFFECTS AND TISSUE DAMAGE

PCB-induced functional changes in neutrophils may be involved in impaired immune defenses against pathogens or enhanced inflammatory responses (e.g., production of reactive oxygen species and cytolytic enzymes) leading to tissue injury. Incubation of quiescent cultured rat peritoneal neutrophils with Aroclor 1242 stimulated neutrophil production of superoxide anion and induced degranulation in a concentration-dependent manner without producing cytotoxicity (Gamey et al. 1993). In neutrophils that were activated for these functions, Aroclor 1242 produced further increases in superoxide anion production, but inhibited the activated degranulation process. Similar effects were observed when neutrophils were incubated with 2,2',4,4'-tetrachlorobiphenyl, a congener that has little affinity for the Ah receptor and induces phenobarbital-type CYPs, but 3,3',4,4'-tetrachlorobiphenyl, an Ah receptor agonist and inducer of 3-methylcholanthrene-type CYPs, did not affect neutrophil function (Gamey et al. 1993). The effects of 2,2',4,4'-tetrachlorobiphenyl on *in vitro* production of superoxide anion by neutrophils were inhibited when neutrophils were incubated in the absence of extracellular calcium or in the presence of TMB-8, an antagonist of the intracellular mobilization of calcium (Brown and Gamey 1995). In addition, neutrophil degranulation induced by 2,2',4,4'-tetrachlorobiphenyl was enhanced by coexposure with the calcium ionophore A23187 (Brown and Gamey 1995). A mono-ortho congener, 2,3,4,5-tetrachlorobiphenyl, displayed somewhat different effects on neutrophil functions than those from the 2,2',4,4'-congener; it stimulated degranulation in quiescent and activated neutrophils, but only increased superoxide anion production in activated neutrophils, not in quiescent cells. The results from the neutrophil studies suggest the involvement of an Ah-receptor independent mechanism that involves PCB-induced increases in intracellular calcium or PCB effects on a signal transduction pathway that is dependent on calcium availability (Brown and Gamey 1995).

### ***PCB Effects Involving Ah-receptor Dependent and Independent Mechanisms***

PCB-induced effects that may involve both Ah-receptor dependent and independent mechanisms include liver hypertrophy (Hori et al. 1997); neurodevelopmental effects or reproduction effects involving changes in steroid hormone homeostasis (Arcaro et al. 1999; Connor et al. 1997; Gierthy et al. 1997; Fischer et al. 1998; Li and Hansen 1997; Nesaretnam and Darbre 1997; Nesaretnam et al. 1996; Seegal et al. 1997) and/or thyroid hormone disruption (Brouwer et al. 1998; Hansen 1998; Li and Hansen 1996a,



1996b, 1997); immunological effects (Harper et al. 1993a, 1993b; Silkworth and Grabstein 1982; Stack et al. 1999); and cancer through non-genotoxic mechanisms involving promotion of oncogenic cells (Cogliano 1998; Safe 1994) and/or genotoxic mechanisms (Robertson and Gupta 2000).

## LIVER HYPERTROPHY

Liver hypertrophy in animals is produced by oral exposure to commercial PCB mixtures and appears to involve both Ah-receptor dependent and independent mechanisms. An illustration of this phenomenon is the observation that single intraperitoneal doses of any one of three PCB congeners varying in affinity for the Ah receptor produced liver hypertrophy in Ah responsive (C57BL/6) and Ah non-responsive (DBA/2) mice (Hori et al. 1997). The studied congeners were 3,3',4,4',5-pentachlorobiphenyl, a congener with high Ah receptor affinity, 3,3',4,4'-tetrachlorobiphenyl, a congener with lesser affinity, and 2,2',5,5'-tetrachlorobiphenyl, a low-affinity Ah-receptor ligand.

## REPRODUCTIVE EFFECTS

There are several studies examining female reproductive function variables in rats (Brezner et al. 1984; Hany et al. 1999; Linder et al. 1974; Sager and Girard 1994), mice (Welsch 1985), rabbits (Seiler et al. 1994), minks (Aulerich and Ringer 1977; Backlin and Bergman 1995; Kihlstrom et al. 1992), and monkeys (Arnold et al. 1995, 1996a; Barsotti et al. 1976) repeatedly exposed orally to commercial PCB mixtures, predominately Aroclor 1254. In general, results from these studies identify minks and monkeys as sensitive species.

In minks, repeated exposure to low doses of Aroclor 1254 or Clophen A-50 (0.4–1.8 mg/kg/day) caused reproductive failure that has been associated with fetal death following embryo implantation (Aulerich and Ringer 1977; Backlin and Bergman 1995; Backlin et al. 1997; Kihlstrom et al. 1992). This effect may predominately involve Ah-receptor mediation, as evidenced by observations that only 1/10 mink exposed to 2.5 ppm Aroclor 1254 in the diet from 1 month prior to breeding through parturition produced offspring, whereas exposure by a similar protocol to 2,2',4,4',5,5'-hexachlorobiphenyl or 2,2',3,3',6,6'-hexachlorobiphenyl at concentrations up to 5 ppm did not influence reproductive performance (Aulerich et al. 1985). In contrast, exposure to dietary concentrations as low as 0.1 ppm 3,3',4,4',5,5'-hexachlorobiphenyl in this study (Aulerich et al. 1985) and 0.05 ppm in another study (Aulerich et al., 1987), caused mortality and prevented the minks from reproducing. Dietary exposure of minks to a fraction of Aroclor 1254, containing only congeners with no ortho-chlorines or a single ortho-

chlorine and representing <20% of the total weight of Aroclor 1254, reduced litter size and fetal survival and increased incidence of interrupted pregnancies to a similar degree as doses of the complete Aroclor 1254 mixture (1.3 mg/kg/day) containing the same amount of these congeners (Kihlstrom et al. 1992). These results suggest the importance of Ah-receptor mediation of PCB-induced reproductive impairment in minks.

Another mink study comparing reproductive effects from intraperitoneal doses of 2,2',4,4',5,5'- and 3,3',4,4',5,5'-hexachlorobiphenyl not only reinforces the idea that congeners with high Ah-receptor affinity are more potent than congeners with low Ah-receptor affinity, but also provides evidence that Ah-receptor independent mechanisms may be involved (Patnode and Curtis 1994). Administration of single 20-mg/kg doses of the 2,2',4,4',5,5'-isomer (a poor Ah-receptor agonist that has been detected in wild mink tissues at concentrations 50-fold greater than the 3,3',4,4',5,5'-isomer) to pregnant minks on the approximate date of implantation did not affect the number of implantation sites (assayed 14 days after dose administration), but significantly decreased the number of embryos and embryonic weight, crown-to-rump length, and head length. The 3,3',4,4',5,5'-isomer (at lower dose levels of 0.4 or 0.8 mg/kg) also did not affect the number of implantation sites, but produced more severe effects on embryo survival as well as the weight, crown-to-rump length, and head length of surviving embryos (Patnode and Curtis 1994).

The mechanisms involved in PCB-induced reproductive impairment in minks are unknown, but examination of mid- to late-gestation placentae from minks exposed to Clophen A50 by light and electron microscopy revealed degenerative lesions in maternal (endothelial detachment and thrombosis in maternal vessels) and fetal (trophoblastic disintegration and loss of fetal capillary integrity) tissues (Backlin et al. 1998). Jones et al. (1997) postulated that the mechanisms are likely to be multifactorial given the possibility of direct and/or indirect tissue damaging actions of PCBs and the wide range of reported effects of PCBs on steroid hormone synthesis and functions including PCB regulation of CYP oxygenases that activate or deactivate different endogenous steroid hormones, estrogenic and antiestrogenic effects of PCBs, and PCB regulation of estrogen and progesterone receptor levels (see Battershill 1994; Li and Hansen 1997; Patnode and Curtis 1994).

Impaired ability to conceive and decreased fetal survival have been observed following repeated exposure of female Rhesus monkeys to commercial PCB mixtures. Exposure to dietary levels of 2.5 or 5 ppm Aroclor 1248 (approximately 0.1 or 0.2 mg/kg/day) for 16–19 months (including a 7-month period before breeding with non-exposed males) produced resorptions or abortions in 3/8 and 4/6 impregnated female

Rhesus monkeys, respectively, compared with 0/12 in a control group (Barsotti et al. 1976). In this study, 12/12, 8/8, and 6/8 females became impregnated in the 0-, 2.5-, and 5-ppm groups, respectively. Another study fed encapsulated Aroclor 1254 at dose levels of 0, 0.005, 0.02, 0.04, or 0.08 mg/kg/day to female rhesus monkeys for 37 months before breeding with non-exposed males and continued dosing through mating and gestation (Arnold et al. 1995). Incidences of abortions, resorptions, or stillbirths were 2/11, 5/10, 3/4, 2/6, and 4/5 in impregnated monkeys in the control through high-dose groups, respectively; respective incidences for impregnation success were 11/16, 10/16, 4/15, 6/14, and 5/15 (Arnold et al. 1995). Mechanisms for these effects in monkeys are unknown, but microscopic examination of tissues from control and exposed monkeys in the second monkey study found no evidence for an association with endometriosis (Arnold et al. 1996a).

The plausibility that PCB effects on reproductive function (and other functions such as neurobehavior and immunological competence) may involve PCB effects on endocrine functions has led to investigations of the estrogenic and anti-estrogenic activities of PCB mixtures and individual congeners, and the effects of PCBs or related halogenated aromatic compounds on steroid hormone metabolism via induction of Phase I or Phase II enzymes. How these PCB effects are specifically related to PCB effects on reproductive function is unknown, but the results of these investigations provide further evidence that reproductive effects from PCB mixtures may not be restricted to Ah-receptor mediation alone and are likely to involve multiple mechanisms that have yet to be elucidated.

The estrogenic and anti-estrogenic activities of some commercial PCB mixtures, PCB congeners, and hydroxylated derivatives of PCB congeners have been assayed by examining uterine variables in immature or ovariectomized female rodents, cell proliferation or gene expression variables in cultured cells including human breast cancer or HeLa cells, and *in vitro* binding to estrogen receptor preparations (see Andersson et al. 1999; Arcaro et al. 1999; Battershill 1994; Connor et al. 1997; Gierthy et al. 1997; Hansen 1998; Kramer et al. 1997; Krishnan and Safe 1993; Li and Hansen 1997; Moore et al. 1997; Safe 1999; Safe et al. 1998b for reviews). In general, PCB-induced estrogenic activities have been characterized as weak compared to the endogenous hormone, 17 $\beta$ -estradiol, a wide variability of responses has been observed across types of PCBs and assays indicating the involvement of multiple mechanisms (e.g., direct binding to the estrogen receptor is not the only way that estrogenic or anti-estrogenic physiological effects may be mediated), anti-estrogenic activities have been most strongly associated with PCBs that are Ah receptor agonists, and hydroxylated metabolites of PCBs are postulated to be at least partly responsible for physiological responses to PCBs that may involve changes in estrogen receptor-dependent physiological processes.

Early studies showed that subcutaneous administration of 8 mg of Aroclors 1221, 1232, 1242, or 1248 increased uterine weight and glycogen content in rats, but similar exposure to Aroclors 1254, 1260, 1262, or 1268 did not produce this estrogenic effect (Bitman and Cecil 1970). More recent studies have provided further evidence that PCB mixtures can produce estrogenic responses (albeit weak) and that PCB congeners with multiple ortho chlorines (or their hydroxylated metabolites) may be at least partly responsible for these responses. Intraperitoneal doses of Aroclor 1242 (8 mg/rat on day 20 or 0.08 or 0.32 mg/rat on days 20 and 21) significantly increased uterine wet weight in immature female rats to about 40% of the increase produced by 0.001 mg 17 $\beta$ -estradiol (Jansen et al. 1993). Similar increases in uterine wet weight were produced by exposure to di-ortho congeners or hydroxylated derivatives (0.640 mg 2,2',5,5'-tetrachlorobiphenyl or 0.250 mg 2,4,6-trichloro-4'-hydroxy-biphenyl on days 20 and 21), but not by exposure to a coplanar congener without ortho chlorines (0.160 mg 3,3',4,4'-tetrachlorobiphenyl). In another study, the tetra-ortho congener, 2,2',6,6'-tetrachlorobiphenyl, displayed similarly weak estrogenic responses in an *in vitro* human breast cancer cell assay and an *in vivo* immature female rat assay (Arcaro et al. 1999). This congener did not competitively bind *in vitro* to recombinant human estrogen receptors  $\alpha$  and  $\beta$ , but a hydroxylated metabolite, 2,2',6,6'-tetrachloro-4'-hydroxy-biphenyl, competitively bound to estrogen receptor  $\alpha$  and produced proliferative responses in the breast cancer assay at concentrations about 10-fold lower than effective concentrations of the parent molecule (Arcaro et al. 1999).

Combined exposure of immature rats to 0.32 mg Aroclor 1242 and 0.001 mg 17 $\beta$ -estradiol produced a response similar to estradiol alone, indicating no obvious anti-estrogenic activity, but combined exposure to 0.001 mg estradiol and 0.160 mg 3,3',4,4'-tetrachlorobiphenyl markedly diminished the estradiol-induced increase in uterine wet weight (Jansen et al. 1993). Anti-estrogenic effects similar to those from 3,3',4,4'-tetrachlorobiphenyl were observed in rodent uterine tissue (Astroff and Safe 1990) and human breast cancer cells (Krishnan and Safe 1993) by other congeners with no or single ortho chlorines (e.g., 3,3',4,4',5-pentachlorobiphenyl, 2',3,3',4,4',5-hexachlorobiphenyl), but commercial PCB mixtures were not anti-estrogenic in the breast cancer cell assay. Whereas the data collected by Krishnan and Safe (1993) suggest that anti-estrogenic activities of PCBs may be related to Ah receptor binding affinity, anti-estrogenic activities of hydroxylated PCB congeners with multiple ortho chlorines have been observed in several assay systems (Connor et al. 1997; Moore et al. 1997; Safe et al. 1998b).

Structure-activity relationships for estrogenic activities of PCB congeners or their metabolites are less clear. Some hydroxylated PCBs (2,4,6-trichloro-4'-hydroxy-biphenyl and 2,3,4,5-tetrachloro-4'-hydroxy-biphenyl) have been demonstrated to competitively bind to mouse estrogen receptor preparations and to

increase uterine weight and glycogen in immature mice (Korach et al. 1988). In other estrogenic assays, 2,2',4,4',6-tetrachlorobiphenyl, 2,4,4',6-tetrachloro-4-hydroxy-biphenyl, and 2,4,6-trichloro-4'-hydroxy-biphenyl were equally effective in stimulating proliferation of human breast cancer cells, but only 2,4,6-trichloro-4'-hydroxy-biphenyl caused significant induction of vitellogenin in cultured brown trout hepatocytes (Andersson et al. 1999). A structure-activity study of eight hydroxylated PCBs in a series of *in vivo* and *in vitro* estrogenic assays found that structure activity relationships were complex and differed from one assay to the next (Connor et al. 1997; Safe et al. 1998b). For example, all but one of the compounds displayed competitive binding to rat and mouse cytosolic estrogen receptors (affinities ranged from about  $10^{-3}$  to  $10^{-5}$  of  $17\beta$ -estradiol's affinity), but no estrogenic activities (wet weight, peroxidase activity, progesterone receptor level) were produced in the uteri of immature rats and mice exposed to three consecutive daily doses of the individual hydroxylated PCB congeners at levels of 25, 50, or 100 mg/kg. In contrast, four of the hydroxylated congeners produced estrogenic effects in cultured human breast cells and HeLa cells (Connor et al. 1997; Safe et al. 1998b).

Complex effects on male reproductive organs and functions have been observed in animals exposed to commercial PCB mixtures including reduced testes weight in adult male offspring of guinea pigs exposed during gestation to Clophen A50 (Lundkvist 1990), reduced testes weight in adult male offspring of female rats exposed from 50 days prior to mating through birth of offspring to 4 mg/kg/day Aroclor 1254 or a mixture of PCBs reflective of the composition of human milk samples (Hany et al. 1999), reduced fertility (without changes in reproductive organ weights, sperm production, or sperm morphology) in adult male offspring of female rats exposed to doses of 8 mg/kg Aroclor 1254 and higher on lactation days 1, 3, 5, 7, and 9 (Sager et al. 1987, 1991), and elevated testes weight and increased sperm production in adult rats exposed to subcutaneous doses of Aroclor 1242 or 1254 (0.4–3.2 mg/day) on postnatal days 0–25 (Cooke et al. 1996). Mechanisms involved in these effects on male reproductive organ development are unknown, but have been postulated to involve developmentally specific periods of responsiveness such as long-lasting elevation of testosterone-metabolizing enzymes from *in utero* exposure leading to reduced testes weight (Hany et al. 1999) and continued depression of thyroid hormone levels during the neonatal period leading to Sertoli cell proliferation and increased testes weight (Cooke et al. 1996). Whether or not PCB estrogenic and anti-estrogenic effects may be involved in any of these effects is unknown, but decreases in adult testis size and sperm production following early developmental exposure to other estrogenic compounds, such as 2,3,7,8-TCDD, is well documented (Gray et al. 1995).

## DISRUPTION OF THYROID HORMONE HOMEOSTASIS

Concern that the thyroid hormone system may be important in PCB mechanisms of toxicity stems from mainly two important types of observations (Brouwer et al. 1998; Porterfield and Hendry 1998):

(1) extensively corroborated findings in experimental animals that exposure to PCBs *in utero* and/or during early development (e.g., through breast milk) can deplete levels of circulating thyroid hormone in the fetus or neonate, which may give rise to a hypothyroid state during development (Collins and Capen 1980; Cooke et al. 1996; Corey et al. 1996; Darnerud et al. 1996; Goldey et al. 1995; Juarez de Ku et al. 1994; Li et al. 1998; Morse et al. 1996; Provost et al. 1999; Rice 1999; Schuur et al. 1998a; Seo and Meserve 1995; Zoeller et al. 2000); and (2) the recognition of the importance of thyroid hormones in normal development of the brain, as evident from neurodevelopmental disorders and deficits associated with hypothyroidism (Boyages 2000). The latter are typified by iodine deficiency (e.g., endemic cretinism), which can produce a wide range of neurodevelopmental deficits, including auditory, motor, and intellectual deficits. These outcomes suggest an importance of thyroid hormones in the normal development of the fetal cochlea, basal ganglia, and cerebral cortex, which begin to develop in humans during the second trimester of gestation. This is also the time in which the fetal thyroid gland becomes functional.

Evidence for a potential thyroid hormone involvement in PCB toxicity rests largely on observations made in experimental animals, including rodents and nonhuman primates. Although the studies differ in design, the emerging picture from these studies is that, depending on dose and duration, PCBs can disrupt the production and disposition of thyroid hormones at a variety of levels. The major findings include: (1) histological changes in the thyroid gland indicative of both stimulation of the gland (e.g., similar to that induced by thyroid stimulating hormone (TSH) or a hypothyroid state) and a disruption of the processing of follicular colloid needed for normal production and secretion of thyroid hormone (Chu et al. 1994, 1995, 1996a, 1996b, 1998; Collins and Capen 1980; Collins et al. 1977; Hansen et al. 1995; Tryphonas et al. 1986b); (2) depression of serum  $T_4$  and  $T_3$  levels, which may effectively create a hypothyroid state (Byrne et al. 1987; Collins and Capen 1980; Cooke et al. 1996; Corey et al. 1996; Darnerud et al. 1996; Desauliniers et al. 1997; Goldey et al. 1995; Gray et al. 1993; Hansen et al. 1995; Hood et al. 1999; Juarez de Ku et al. 1994; Kasza et al. 1978; Li et al. 1998; Morse et al. 1996; Price et al. 1988; Provost et al. 1999; Rice 1999; Schuur et al. 1998a; Seo and Meserve 1995; Van Birgelen et al. 1995; Zoeller et al. 2000); (3) increased rates of elimination of  $T_4$  and  $T_3$  from serum (Goldey and Crofton 1998); (4) increased activities of  $T_4$ -UDP-GT in liver (Chu et al. 1995; Desauliniers et al. 1997; Morse et al. 1996; Schuur et al. 1998a; Van Birgelen et al. 1995), which is an important metabolic elimination

pathway for  $T_4$  and  $T_3$ ; (5) decreased activity of iodothyronine sulfotransferases in the liver, which are also important in the metabolic elimination of iodothyronines (Schoor et al. 1998a, 1998b, 1999); (6) decreased activity of iodothyronine deiodinases including brain Type-2 deiodinase, which provide the major pathways for the production of the active thyroid hormone,  $T_3$  (Morse et al. 1996; Schoor et al. 1998a); and (7) decreased binding of  $T_4$  to transthyretin an important transport protein for both  $T_4$  and  $T_3$  (Cheek et al. 1999; Darnerud et al. 1996).

The above observations suggest that PCBs can disrupt the production of thyroid hormones, both in the thyroid and in peripheral tissues, can interfere with their transport to peripheral tissues, and can accelerate the metabolic clearance of thyroid hormones. The most convincing evidence that PCBs can exert toxicity by disrupting the thyroid hormone system derives from two studies in rats. In one study, neurobehavioral deficits in pups that experienced exposures to Aroclor 1254 *in utero* and during nursing, were significantly attenuated by subcutaneous injections of  $T_4$  that increased serum  $T_4$  and  $T_3$  concentrations that were otherwise depressed in the PCB-exposed animals (Goldey and Crofton 1998). While this study examined relatively high doses of Aroclor 1254 ( $\geq 1$  mg/kg/day), it nevertheless demonstrated neurodevelopmental effects that are directly relevant to observations made in epidemiological studies and to neurological sequelae of fetal hypothyroidism, including motor disturbances and hearing.

In the second study, increased testes weight and sperm production in rats that were administered Aroclor 1254 on postnatal days 1–25 were attenuated by injections of  $T_4$  on postnatal days 1–25, which also prevented the depression in serum  $T_4$  concentrations (Cooke et al. 1996). Here again, although produced by relatively large doses of Aroclor 1254 ( $\geq 40$  mg/kg/day, subcutaneous), similar effects can be produced by other hypothyroid-inducing agents, including 6-propyl-2-thiouracil (PTU). Furthermore, the effects observed may reflect a disruption of the normal sexual maturation process, which is known to be associated with neonatal hypothyroidism in humans (Longcope 2000).

The effects PCBs on thyroid hormone status appear to involve Ah-receptor mediated actions as well as actions that appear to be independent of the Ah receptor. Depressed levels of serum  $T_4$  have been observed in rats given oral doses of coplanar PCB congeners (Desaulniers et al. 1997; Van Birgelen et al. 1994b) or di-ortho-substituted congeners that have relatively low affinity for the Ah receptor (Ness et al. 1993; Van Birgelen et al. 1992). At least one potential Ah-receptor mediated mechanism for this effect is the induction of UDP-GT, which catalyzes the metabolic elimination of  $T_4$  to the  $T_4$ -glucuronide conjugate (Desaulniers et al. 1997; Van Birgelen et al. 1995). However, the UDP-GT mechanism does not appear to be important in the depression of  $T_4$  levels produced by non-coplanar PCBs. Li and Hansen

(1996b) observed depressed serum T<sub>4</sub> levels in rats administered a PCB mixture extracted from soil. Treatment of the mixture with activated charcoal greatly reduced the content of co-planar PCBs in the mixture, substantially decreased the potency of the mixture for inducing UDG-GT and EROD, but had little effect on the potency for depressing T<sub>4</sub> levels. This suggests that an Ah-independent mechanism may exist that is not related to UDP-GT induction.

PCBs, including poly-ortho-substituted PCBs, which have a very low affinity for the Ah receptor, inhibit the binding of T<sub>4</sub> to transthyretin, an important transport protein for both T<sub>4</sub> and T<sub>3</sub> (Chauhan et al. 2000; Cheek et al. 1999; Darnerud et al. 1996). Inhibition of binding of thyroid hormones to transthyretin could alter hormone delivery to target tissues, including the brain, and could also result in depressed levels of serum total TT<sub>4</sub> or TT<sub>3</sub> (Brouwer et al. 1998).

## IMMUNOLOGICAL EFFECTS

Studies with inbred mice strains differing in Ah-receptor responsiveness indicate that immunosuppression from PCB mixtures involves Ah-receptor mediation (e.g., Harper et al. 1993a; Silkworth and Grabstein 1982), but there is evidence that other mechanisms also may contribute to PCB-induced immunological effects (Harper et al. 1993a, 1993b; Stack et al. 1999). Illustrating the importance of Ah-receptor mediation for some PCB congeners, Ah-responsive C57BL/6 mice given single intraperitoneal doses of 100 mg/kg 3,3',4,4'-tetrachlorobiphenyl showed marked decreases in the number of splenic plaque-forming cells (PFCs) formed in response to immunization with sheep red blood cells (sheep red blood cells (SRBC), which are T-cell dependent antigens) compared with similarly treated Ah-non-responsive DBA/2 mice (Silkworth and Grabstein 1982). In addition, ED<sub>50</sub> values for 2,3,7,8-TCDD, three CDFs, and two PCBs without ortho substitution (3,3',4,4',5-pentachlorobiphenyl and 3,3',4,4',5,5'-hexachlorobiphenyl) in this immunotoxicity assay were lower in C57BL/6 mice than in DBA/2 mice, and the order of immunotoxic potency of these six compounds was the same as that for potency in inducing CYP1A1 (Harper et al. 1993a). In another study, a series of four hexachlorinated biphenyls with differing chlorine substitution patterns displayed varying ED<sub>50</sub> values in the same immunotoxicity assay as follows: 2, >1,000, 120, and >1,000 μmol/kg for a mono-ortho (2,3,3',4,4',5'-), a di-ortho- (2,2',4,4',5,5'-), a tri-ortho- (2,2',4,4',5',6'-), and a tetra-ortho-isomer (2,2',4,4',6,6'-), respectively (Harper et al. 1993b). Harper et al. (1993b) concluded that immunotoxic potency decreases (i.e., ED<sub>50</sub>s increase) with increasing ortho-chlorine substitution of PCBs, but, as shown above, the decrease was not monotonic with increasing degree of chlorination. Furthermore, this relationship did not apply to more highly chlorinated PCBs with three or four ortho chlorines that are inactive as Ah-receptor agonists and only minimally induce CYP1A1



(Harper et al. 1993b). Three nonachlorobiphenyls (2,2',3,3',4,4',5,5',6-, 2,2',3,3',4,4',5,6,6'-, and 2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl) and decachlorobiphenyl displayed ED50s for inhibition of the splenic PFC response to SRBC in C57BL/6 mice that were less than those for hexachlorobiphenyl isomers with multiple ortho chlorines reported above: 15, 7, 17, and 35  $\mu\text{mol/kg}$ , respectively. These results are consistent with the hypothesis that some PCBs induce immunotoxicity via Ah-receptor *independent* mechanisms. In an *in vitro* assay of cell proliferation in response to lipopolysaccharide (a T-cell independent antigen), Aroclors 1221, 1242, 1254, or 1260 inhibited the proliferative response similarly in splenocytes from either C57BL/6 or DBA/2 mice (Stack et al. 1999). Two non-ortho and two mono-ortho PCBs that have been demonstrated to be effective Ah-receptor agonists and CYP1A1 inducers did not inhibit the *in vitro* proliferative response to lipopolysaccharide, but two di-ortho congeners (2,2',3,4,4',5- and 2,2',4,4',5,5'-hexachlorobiphenyl) significantly inhibited the response. These *in vitro* results provide supporting evidence for the existence of mechanisms of PCB immunotoxic actions that are independent of the Ah receptor.

## CANCER

Lifetime oral exposure to any one of four commercial PCB mixtures (Aroclors 1016, 1242, 1254, and 1260) has been demonstrated to produce liver tumors in female rats; Aroclor 1260 also induced liver tumors in male rats (Mayes et al. 1998). Mixtures with high chlorination content (e.g., Aroclor 1254) were generally more potent than mixtures with low chlorine content (e.g., Aroclor 1016) (Mayes et al. 1998). Tumor promotion by commercial PCB mixtures following initiation by a variety of chemical agents also has been investigated in a number of animal systems including rat liver, rat kidney, mouse skin, and newborn mouse liver and lung (see Silberhorn et al. 1990 for review). The tumor promoting effect of extended exposure to PCB mixtures was demonstrated principally in the liver of rats; there is some evidence that PCB mixtures also can promote tumors in mouse lung and mouse skin, but not in rat kidneys. The mechanism of PCB-induced cancer is poorly understood, but there is evidence to suggest that both Ah-receptor dependent and independent mechanisms may be involved.

PCB promotion of tumors does not appear to be solely an Ah-receptor mediated process, since individual congeners that are not Ah receptor agonists have tumor promotion capabilities in animal systems. For example, 2,2',5,5'-tetrachlorobiphenyl, 2,2',4,4'-tetrachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl were shown to promote liver tumors in female Sprague-Dawley rats (Hemming et al. 1993; Preston et al. 1985). In addition, 2,2',5,5'-tetrachlorobiphenyl, 2,2',3,3',4,4'-hexachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl were potent inhibitors of *in vitro* gap junctional cellular communication, an assay that is

indicative of tumor promotion capacity (Bager et al. 1997; De Haan et al. 1996). A general working mechanistic hypothesis for PCB promotion of liver tumors involves indirect stimulation of cell proliferation following cell or tissue injury by reactive metabolites of PCBs (Silberhorn et al. 1990). Alternatively, the cell injury could be caused by increased intracellular concentrations of other reactive species (e.g., superoxide anion or other reactive oxygen species) caused by an overall imbalance from PCB-induced perturbations of cellular biochemical processes, including induction of CYP oxygenases and glutathione S-transferases, repression of selenium-dependent glutathione peroxidases, and/or disruption of calcium homeostatic processes and signal transduction pathways (Silberhorn et al. 1990).

PCB mixtures have not shown consistent tumor initiating activity in animal initiation-promotion protocols (Silberhorn et al. 1990), but demonstration that chronic oral exposure to commercial PCB mixtures induced liver tumors in female rats (Mayes et al. 1998) suggests that PCBs may have both tumor initiating and promoting activities. Although PCB mixtures generally have been found to be inactive as mutagens in *Salmonella typhimurium* strains and in several other tests of genotoxicity that may be predictive of tumor initiation capability (see Silberhorn et al. 1990 for review), *in vitro* studies with rat microsomes have indicated that metabolism of lower chlorinated PCBs (e.g., 4-chlorobiphenyl, 3,4-dichlorobiphenyl, and 3,4,5-trichlorobiphenyl) can lead to covalently modified macromolecules including proteins and DNA (see Robertson and Gupta 2000 for review). Studies demonstrating the Ah-receptor dependence or independence of this potential genotoxic effect from PCBs were not located. The available data indicate that PCBs are not potent genotoxicants, but the possible involvement of genotoxic mechanisms (involving covalent modification of proteins and/or DNA) in the development of PCB-induced cancer is not without some experimental support.

The relative contribution that Ah-receptor dependent and independent mechanisms may make to carcinogenic responses to PCB mixtures is unknown. Safe (1994) compared carcinogenic responses of female rats to 2,3,7,8-TCDD in the diet with responses of female rats of the same strain to Aroclor 1260 in the diet using the TEF approach. TCDD at a TEQ feed concentration of 2,100 ppt induced hepatic adenocarcinomas in 11/50 (22%) rats, whereas a TEQ of only 1,040 ppt from Aroclor 1260 induced adenocarcinomas in 24/47 (51%) rats. For this situation, the TEF approach markedly underestimated the carcinogenic response to Aroclor 1260. A possible explanation is that PCB congeners that are not Ah receptor agonists and are abundant in Aroclor 1260 make significant contributions to the mixture's carcinogenicity. Although this comparison suggests that the TEF approach may underestimate cancer responses to complex PCB mixtures, another study of the tumor promotion activity of a simpler mixture of two CDDs, one CDF, and three PCBs in female rats found that the TEF approach overestimated the

observed response by a factor of about 2 (van der Plas et al. 1999). The mixture contained 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, 3,3',4,4',5- and 2,3',4,4',4-pentachlorobiphenyl, and 2,3,3',4,4',5-hexachlorobiphenyl at relative levels found in Baltic Sea herring. The rats were initiated with an injection of diethylnitrosoamine, 24 hours after a partial hepatectomy, and were administered weekly subcutaneous injections of the mixture for 20 weeks starting 6 weeks after initiation. The volume and volume fraction of glutathione *S*-transferase-positive altered hepatic foci were taken as indicators of tumor promotion activity in this study (van der Plas et al. 1999). Although the composition of this mixture reflected relative concentrations and accounted for >90% of total TEQs in Baltic Sea herring, it did not contain PCBs with multiple ortho chlorines which comprise the predominant bulk of PCB weight in most commercial and environmental mixtures. For example, non-, mono-, and di-ortho congeners accounted for <1, 18, and 82% of PCB weight per gram of fat in human milk samples from Italy (Larsen et al. 1994). Another group of rats was similarly treated with the same synthetic mixture plus a di-ortho PCB congener (2,2',4,4',5,5'-hexachlorobiphenyl), which is one of the predominant PCB congeners in environmental mixtures and has minimal Ah receptor agonist activity (van der Plas et al. 1999). Mean foci volume and foci volume fraction were increased in rats treated with the supplemented mixture compared with the mixture without the di-ortho congener, but the observed responses were still less than that predicted by the TEF approach. Better understanding of the relative contributions of Ah receptor dependent and independent mechanisms to the carcinogenicity of PCB mixtures awaits further research.

#### **E.4 Health Guidelines**

ATSDR (2000) derived an intermediate oral MRL for PCB mixtures of 0.03 µg/kg/day based on a LOAEL of 0.0075 mg/kg/day for neurobehavioral alterations in infant monkeys that were exposed to a PCB congener mixture representing 80% of the congeners typically found in human breast milk (Rice 1997, 1998, 1999; Rice and Hayward 1997, 1999). The infant monkeys were given oral doses of 0 or 0.0075 mg/kg/day from birth to 20 weeks of age. The dose level was selected to be equivalent to an approximate daily intake of a nursing human infant whose mother's milk contains 50 ppb PCBs. Treated monkeys showed decreases and variable increases in response latencies across three tasks of nonspatial discrimination reversal, retarded acquisition of a delayed alternation task, increased errors at short delay task responses, and alterations in fixed-interval and fixed-ratio performance tasks. The findings were interpreted to suggest that post-natal PCB exposure resulted in impaired learning, impaired perseverative behavior, and/or inability to inhibit inappropriate responding. To derive the MRL, the LOAEL was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to

humans, and 10 for human variability).

ATSDR (2000) derived an chronic oral MRL for PCB mixtures of 0.02 µg/kg/day based on a LOAEL of 0.005 mg/kg/day for decreased antibody response to sheep red blood cells in Rhesus monkeys exposed to self-ingested capsules of Aroclor 1254 in a glycerol/corn oil mixture (Tryphonas et al. 1989, 1991). The LOAEL was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

Because consensus has emerged on the inappropriateness of assessing environmental PCBs as if they were Aroclors, EPA has developed an approach for assessing cancer risk from environmental PCBs by considering both toxicity and environmental processes (Cogliano 1998; EPA 1996; IRIS 2001). This approach uses animal studies of commercial PCB mixtures to develop a range of human cancer potency estimates and then considers the effect of environmental processes to determine appropriate values for representative classes of environmental mixtures. Guidance is provided for assessing cancer risks from different exposure pathways, less-than-lifetime and early-life exposures, and mixtures containing dioxin-like constituents.

## **E.5 Derivation of Target-Organ Toxicity Dose (TTD) Values**

TTDs for chronic oral exposure to PCB mixtures were derived for endpoints affected by PCBs and one or more of the other chemicals in the CDD-hexachlorobenzene-DDE-methylmercury-PCB mixture that is the subject of this Interaction Profile. The relevant endpoints for PCBs in this mixture include hepatic, endocrine, immunological, neurological, reproductive, and developmental effects. Chronic oral TTDs for these endpoints are derived below, using the methods described in ATSDR (2001a, Section 2.3.2). The derivations are based on data provided in ATSDR (2000), and in particular, the oral LSE table.

### **Hepatic Effects**

Several studies of groups of humans exposed to PCBs have reported associations between exposure and changes in indices of hepatic damage (e.g., increased serum levels of aspartate aminotransferase), but limitations in study design, such as lack of appropriate controls or adjustment of potential confounding variables, preclude establishing a causal relationship from the human data (ATSDR 2000). In contrast, studies of orally exposed animals have reported a broad spectrum of PCB-induced hepatic effects including hepatic enzyme induction, liver enlargement, hepatic porphyria, and histopathologic changes in

liver tissue ranging from hepatocellular hypertrophy and vacuolization to fatty degeneration, hepatocellular necrosis, bile duct hyperplasia, and liver tumors (ATSDR 2000). The lowest exposure levels associated with liver changes in available animal studies are 0.04 mg/kg/day (no NOAEL was identified) for decreased serum cholesterol in Rhesus monkeys exposed to Aroclor 1254 for 37 months (Arnold et al. 1993a, 1993b), 0.08 mg/kg/day (with a NOAEL of 0.04 mg/kg/day) for increased relative liver weight in Rhesus monkeys exposed to Aroclor 1254 for 72 months (Arnold et al. 1997), 0.2 mg/kg/day (no NOAEL was identified) for hepatocyte necrosis and biliary tract hypertrophy in Rhesus monkeys exposed to Aroclor 1254 for 12 or 28 months (Tryphonas et al. 1986a, 1986b), and 1 mg/kg/day (no NOAEL was identified) for hepatocellular hypertrophy and increased levels of serum enzymes in male rats exposed to Aroclor 1254 or 1260 for 24 months (Mayes et al. 1998). Applying an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from monkeys to humans, and 10 for human variability) to the LOAEL of 0.04 mg/kg/day for decreased serum cholesterol in Rhesus monkeys (Arnold et al. 1993a, 1993b) yields a  $TTD_{\text{HEPATIC}}$  of 0.1  $\mu\text{g}/\text{kg}/\text{day}$  for PCB mixtures.

## Endocrine Effects

Studies examining possible associations between PCB exposure and disruption of thyroid hormone status in humans have not found consistent evidence for this type of PCB endocrine effect, but animal studies firmly establish causal relationships between PCB exposures and several types of endocrine effects including disruption of thyroid structural integrity, disruption of thyroid hormone homeostasis, and impaired reproductive function and development that may involve disruption of steroid hormone homeostasis (ATSDR 2000). The lowest oral exposure levels associated with adverse endocrine effects in animals include 0.09 mg/kg/day (no NOAEL was identified) for decreased serum thyroid hormone levels in rats exposed to Aroclor 1254 for 5 months (Byrne et al. 1987); 0.25 mg/kg/day (with a NOAEL of 0.05 mg/kg/day) for decreased serum levels of adrenal hormones in rats exposed to Aroclor 1254 for 5–7 months (Byrne et al. 1988); 0.1 mg/kg/day (no NOAEL was identified) for decreased serum thyroxin levels in rats exposed to Aroclor 1254 for 15 weeks (Gray et al. 1993); 0.1 mg/kg/day (no NOAEL was identified) for decreased serum levels of T3 and T4 in offspring of rats exposed to Aroclor 1254 from gestation day 1 through post-partum day 31 (Provost et al. 1999); and 0.2 mg/kg/day (no NOAEL was identified) for thyroid desquamation in monkeys exposed to Aroclor 1254 for 28 months (Tryphonas et al. 1986b). Dividing the rat LOAEL of 0.09 mg/kg/day for decreased serum thyroid hormone levels produced by intermediate-duration exposure (Byrne et al. 1987) by an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolating from rats to humans, and 10 for human variability) yields a  $TTD_{\text{ENDOCRINE}}$  of 0.1  $\mu\text{g}/\text{kg}/\text{day}$ . This value is expected to be protective of chronic-duration exposure

because of the large uncertainty factor.

### **Immunological Effects**

Studies of PCB-exposed groups of humans have reported immune system effects such as increased susceptibility to respiratory tract infections and decreased serum levels of IgA and IgM antibodies, but provide insufficient evidence to conclusively establish a causal relationship between immune system impairment and exposure (ATSDR 2000). In contrast, studies of rats, mice, guinea pigs, and rabbits exposed to commercial mixtures of PCBs have clearly shown PCB-induced immune system effects such as splenic and thymic atrophy, reduced antibody production against foreign antigens, and increased susceptibility to bacterial infection (ATSDR 2000). Monkeys appear to be particularly susceptible to PCB immunotoxicity. The lowest exposure level associated with immune effects in animals orally exposed to PCB mixtures is 0.005 mg/kg/day for decreased IgM and IgG antibody responses to sheep red blood cells in female Rhesus monkeys exposed to Aroclor 1254 for 23 months (Tryphonas et al. 1989). No NOAEL was identified in this study. As described in Section E.4 above, this LOAEL serves as the basis of the chronic oral MRL for PCBs of 0.02 µg/kg/day.

### **Neurological Effects**

Subtle neurobehavioral changes have been observed in studies of children of mothers consuming large amounts of Great Lakes fish contaminated with PCBs and other biopersistent pollutants (ATSDR 2000). Deficits in measures of neurological development have been associated with increasing indices of PCB exposure, but precise and accurate adjustment for possible confounding variables has not always been possible in these studies. Studies in animals support the human data. Neurobehavioral changes have been observed in rats and monkeys following pre- and/or postnatal exposure to commercial Aroclor mixtures, experimental mixtures of PCBs similar to those found in human breast milk, single PCB congeners, and contaminated fish from the U.S. Great Lakes (ATSDR 2000). As described in Section E.4 above, ATSDR (2000) derived the intermediate oral MRL of 0.03 µg/kg/day for PCB mixtures based on a LOAEL of 0.0075 mg/kg/day (no NOAEL was identified) for neurobehavioral changes in infant monkeys that were orally exposed from birth to 20 weeks of age to a synthetic mixture of PCBs representing 80% of the PCB congeners found in samples of human breast milk and an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolating from monkeys to humans, and 10 for human variability). The intermediate-duration oral MRL is only slightly above the chronic oral MRL of 0.02 µg/kg/day (based on immunological effects in adult monkeys), and is expected to provide protection against possible

neurological and neurodevelopmental effects from chronic exposure.

## Reproductive Effects

Some studies examining reproductive endpoints in PCB-exposed humans found suggestive indications that exposure to PCBs may be associated with menstrual disturbances in women and effects on sperm in men, but no consistent or sufficient evidence for obvious reproductive impairment (ATSDR 2000). In contrast, the reproductive toxicity of oral exposure to PCBs is well established in animals. Effects in female animals include: prolonged estrus, decreased sexual receptivity, and reduced implantation rate in adult rats and/or their offspring exposed before sexual maturation; decreased conception rate in mice; partial or total inhibition of reproductive capability in minks; and prolonged menstruation and decreased fertility in monkeys (ATSDR 2000). Effects in male animals include altered reproductive organ morphology, impaired sperm production, and impaired fertility in male rats and mice exposed before sexual maturation (ATSDR 2000). The lowest oral exposure levels associated with adverse reproductive effects include 0.9 mg/kg/day (with a NOAEL of 0.2 mg/kg/day) for decreased reproduction rates and litter size in minks exposed to Aroclor 1254 for 21 weeks (Aulerich and Ringer 1977); 0.1 mg/kg/day (no NOAEL was identified) for increased menstrual length in Rhesus monkeys exposed to Aroclor 1248 for 7 months (Barsotti et al. 1976); 0.1 mg/kg/day (no NOAEL was identified) for decreased spermatogenesis and libido in male Rhesus monkeys exposed to Aroclor 1248 for 17 months (Allen and Norback 1976); and 0.02 mg/kg/day (with a NOAEL of 0.005 mg/kg/day) for reduced conception rate in female Rhesus monkeys exposed to Aroclor 1254 for 37 months (Arnold et al. 1995). Applying an uncertainty factor of 30 (3 for extrapolation from monkeys to humans and 10 for human variability) to the NOAEL of 0.005 mg/kg/day for reduced conception rate in monkeys (Arnold et al. 1995) yields a  $TTD_{\text{REPROD}}$  of 0.2  $\mu\text{g}/\text{kg}/\text{day}$ .

## Developmental Effects

The development of the neurological system appears to be a target of critical public health concern associated with pre- and/or post-natal exposure to PCB mixtures (ATSDR 2000). Subtle neurobehavioral effects suggesting impaired learning or perseverative behavior have been observed in monkeys exposed from birth to 20 weeks to oral doses as low as 0.0075 mg/kg/day (Rice 1997, 1998, 1999; Rice and Hayward 1997, 1999). This dose was estimated to correspond to PCB levels in human breast milk of 50 ppb. As discussed in Section E.4 above, these findings serve as the basis of the intermediate oral MRL of 0.03  $\mu\text{g}/\text{kg}/\text{day}$ . This value is only slightly above the chronic oral MRL of 0.02  $\mu\text{g}/\text{kg}/\text{day}$  based on

impaired immune response in adult monkeys and is expected to be protective of neurodevelopmental effects from chronic oral exposure to PCBs.

### **Summary (TTDs for PCBs)**

$TTD_{\text{HEPATIC}} = 0.1 \mu\text{g/kg/day}$  ( $1 \times 10^{-4}$  mg/kg/day)

$TTD_{\text{ENDOCRINE}} = 0.1 \mu\text{g/kg/day}$  ( $1 \times 10^{-4}$  mg/kg/day)

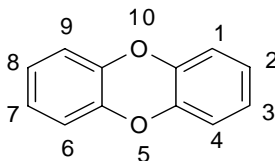
$MRL_{\text{(IMMUNO)}} = 0.02 \mu\text{g/kg/day}$  ( $2 \times 10^{-5}$  mg/kg/day; chronic MRL)

$MRL_{\text{(NEURODEVELOP)}} = 0.03 \mu\text{g/kg/day}$  ( $3 \times 10^{-5}$  mg/kg/day; intermediate MRL)

$TTD_{\text{REPRO}} = 0.2 \mu\text{g/kg/day}$  ( $2 \times 10^{-4}$  mg/kg/day)

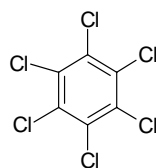


## Appendix F: Chemical Structures of Mixture Components

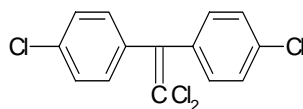


### Chlorinated dibenzo-p-dioxins

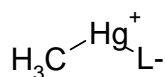
Congeners can have chlorine substitutions at positions 1–4 and 6–9.



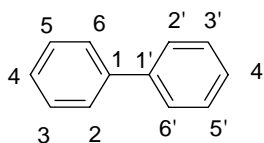
### Hexachlorobenzene



### p,p'-DDE



Methylmercury (L indicates a ligand.)



### PCBs

Congeners can have chlorine substitutions at positions 2–6 and 2'–6'.

