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

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ACKNOWLEDGMENT

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, following peer review. This final version of the document, released in 2004, includes changes made in response to public comments. However, no new literature searches were done.

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All reviewers were selected in conformity with the conditions for peer review specified in
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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

Breast-feeding offers the developing infant the benefits of balanced nutrition and passive immunization, but the detection of persistent, environmental chemicals in human breast milk samples from various regions of the world has led to concerns that these chemicals may have detrimental effects on the health and/or development of children. Chlorinated dibenzo-*p*-dioxins (CDDs), hexachlorobenzene, *p,p'*-DDE, methylmercury, and polychlorinated biphenyls (PCBs) were selected as an important subset of persistent chemicals detected in breast milk for the purposes of reviewing data on their joint toxic actions following oral exposure.

Epidemiological studies in Michigan, North Carolina, New York, the Netherlands, and the Faroe Islands found statistically significant associations between increasing concentrations of particular persistent chemicals found in maternal fluid samples (i.e., PCBs, CDDs, *p,p'*-DDE, or mercury in cord serum or breast milk) and deficits in motor and cognitive functions in children. The Netherlands and Faroe Islands studies also demonstrated beneficial effects of breast feeding on neurological development. A study of formula-fed monkeys exposed to a PCB mixture from birth to 20 weeks found evidence that lactational exposure to persistent chemicals may contribute to neurodevelopmental deficits. These studies identify mild neurodevelopmental deficits as a possible health hazard, but the results are suggestive that observed deficits may have been associated with gestational rather than lactational exposure to persistent chemicals. These studies do not establish causal relationships between exposure to persistent chemicals in breast milk and neurological deficits. Furthermore, they are not useful for assessment of health hazards specific to a community or scenarios involving exposures to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs.

To facilitate exposure-based assessments of possible health effects associated with oral exposures to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs in environmental media, food, and/or breast milk, available data on the joint toxic action of mixtures of these breast milk contaminants were reviewed, and the weights of evidence were assessed concerning the mode of joint toxic action of pairs of the five chemicals. Only a limited amount of evidence is available on the existence of greater-than-additive or less-than-additive interactions between a few pairs of the chemicals of concern: (1) hexachlorobenzene potentiation of tetrachlorodibenzo-*p*-dioxin (TCDD) reduction of body and thymus weights (a greater-than-additive interaction); (2) PCB antagonism of TCDD immunotoxicity (less-than-additive interaction); (3) PCB antagonism of TCDD developmental toxicity (less-than-additive

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interaction); and (4) synergism between PCBs and methylmercury in disrupting regulation of brain levels of dopamine that may influence neurological function and development (greater-than-additive interaction). Weight-of-evidence analyses of these data, however, indicate that scientific evidence for these interactions is limited and is inadequate to characterize the possible modes of joint action on these toxicity targets. 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 project potential non-additive interactions. Therefore, it is recommended that additivity be assumed as a public health protective measure in exposure-based assessments of health hazards from exposure to mixtures of these components.

A target-organ toxicity dose (TTD) modification of the hazard index approach is recommended for carrying out exposure-based screening assessments of possible health effects from oral exposure to mixtures of the chemicals. TTDs for the individual chemical components are derived, and application of the approach is described. There are several reasons supporting this recommendation to use a component-based approach. There are no direct data available to characterize health hazards (and dose-response relationships) from mixtures containing all five components. Physiologically-based pharmacokinetic/pharmacodynamic (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. If the screening assessment indicates a potential hazard to public health, further evaluation is needed, using biomedical judgment and community-specific health outcome data, and taking into account community health concerns.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

Ah	arylhydrocarbon		RfD	Reference Dose
AHH	arylhydrocarbon hydroxylase			
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
			TT4	total thyroxine and free thyroxine
CDD	chlorinated dibenzo-*p*-dioxin		TAO	triacetyloleandomycin
CDF	chlorinated dibenzofuran		TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
CI	confidence interval			
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
			$\geq$	greater than or equal to
kg	kilogram		=	equal to
			<	less than
LOAEL	lowest-observed-adverse-effect level		$\leq$	less than or equal to
LSE	Levels of Significant Exposure			
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 pharamacokinetic			
PCB	polycholrinated biphenyl			
ppb	parts per billion			
ppm	parts per million			
ppt	parts per trillion			
RfC	Reference Concentration			

1. Introduction

The primary purpose of this Interaction Profile for chlorinated dibenzo-*p*-dioxins (CDDs), hexachloro-benzene, *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 minimal risk level (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 the Agency for Toxic Substances and Disease Registry (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's Division of Toxicology (DT) 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.

Breast-feeding is widely recognized as offering the developing infant the benefits of balanced nutrition and passive immunization against microbial infections (Pohl and Tylenda 2000), but the detection of persistent environmental pollutants in breast milk samples from general populations in the United States, the Netherlands, Sweden, and elsewhere has led to concerns that these chemicals may have detrimental effects on the health and/or development of breast-fed children. Environmental chemicals that have been detected in samples of human breast milk include mercury and methyl mercury, lead, cadmium, PCBs, CDDs, chlorinated dibenzofurans (CDFs), brominated diphenylethers (BDEs), and persistent forms of organochlorine pesticides such as *p,p'*-dichlorodiphenylether (*p,p'*-DDE), hexachlorobenzene, mirex, and lindane (Abadin et al. 1997; Hooper and McDonald 2000; Pohl and Hibbs 1996; Pohl and Tylenda 2000). Chemicals that are lipophilic and resistant to metabolic degradation have a tendency to increasingly accumulate with increasing levels of the food chain and to distribute to fatty tissue and breast milk within the human body.

Five persistent chemicals or chemical classes detected in human milk (CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs) were selected for the purposes of reviewing available data on their joint actions in producing toxic effects following oral exposure. PCBs, *p,p'*-DDE, and hexachloro-benzene were selected because they have been detected with very high frequency in breast milk in a recent study of women residing in the U.S. Great Lakes region (Kostyniak et al. 1999), in a study of the general population in North Carolina (Rogan et al. 1986a), and in a recent Canadian study of the general population that included the Great Lakes basin (Newsome et al. 1995). It is also expected that breast milk of fish-eating populations in the U.S. Great Lakes region may contain CDDs and methylmercury, because these chemicals have been detected in Great Lakes fish (ATSDR 2001c). Whereas recent U.S. monitoring studies have not focused on the presence of CDDs and methylmercury in breast milk, CDDs have been detected in earlier U.S. studies, as well as in studies in the Netherlands, Canada, Germany, New Zealand, Japan, and Russia (Pohl and Hibbs 1996), and methylmercury has been detected in breast milk samples from Japan, Germany, and Sweden (Abadin et al. 1997). In addition, elevated levels of PCBs and mercury were detected in samples of breast milk from mothers residing in the North Atlantic Faroe Islands where the seafood diet includes pilot whale meat and blubber (Grandjean et al. 1995a).

Another reason for selecting these five chemicals is that there is a fair amount of overlap in the wide range of endpoints or organs that these chemicals affect in humans and/or animals (see Appendices A–E, Table 1). This overlap leads to concern that, following exposure to mixtures of the five chemicals in breast milk or other food sources, 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).

This profile begins with a brief review of recent studies designed to examine whether or not detrimental effects on the health and/or development of breast-fed children may be associated with persistent chemicals detected in breast milk. Available data on the joint toxic actions of the five chemicals of concern are next reviewed. The weight of evidence is assessed concerning whether binary mixtures of these chemicals may be expected to jointly act in additive, less-than-additive, or greater-than-additive manners. Following the review of these data, their relevance to public health concerns associated with exposures to mixtures of these chemicals is discussed, and recommendations are made for exposure-based assessments of joint toxic actions of this mixture of chemicals.

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[a]				
	2,3,7,8-TCDD	Hexachloro-benzene	*p,p'*-DDE	Methyl-mercury	PCBs
Wasting syndrome	x	x			x
Kidney damage				x	
Liver damage	**X**	**X**[b]	x[b]		x
Immunosuppression	x[b]	x	x	x	x[b]
Thyroid hormone disruption	**X**	**X**			x
Female reproductive organ disruption	x	**X**[b]		x	x
Male reproductive organ disruption	**X**		x	x	x
Neurological impairment	**X**	**X**	**X**	**X**	x
Altered neurological development (pre- and/or post-natal)	x[b]	**X**[b]	x[c]	**X**[b]	**X**[b]
Altered female reproductive organ development	x				x
Altered male reproductive organ development	x		x[b]		x
Other developmental effects (malformations or fetotoxicity)	x	**X**	x	x	x
Cancer[d]	x	x	x	x	x

[a]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.

[b]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).

[c]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).

[d]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).

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 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 ethoxyresorufin *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 1994b). 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 1994b); body weight wasting and thymic atrophy from acute exposure (Hori et al. 1997; Safe 1994b); and porphyria and porphyria cutanea tardea (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 Ganey 1995; Ganey 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 detrimental effects on the health and/or development of breast-fed children may be associated with persistent chemicals detected in breast milk. Review of these studies follows.

Concerns that biopersistent and lipophilic chemicals accumulating in breast milk may present health problems offsetting benefits of breast feeding have led to studies examining possible relationships between adverse effects in breast-fed children and chemicals detected in breast milk, and to studies examining several health endpoints in animals following exposure to mixtures of biopersistent chemicals during gestation and/or lactation. Biopersistent, potentially toxic chemicals that have been detected in breast milk include PCBs, CDDs, CDFs, pesticides or their persistent metabolites such as *p,p'*-DDE and hexachlorobenzene, and metals including cadmium, lead, and mercury (Abadin et al. 1997; DeKoning and Karmaus 2000; Kostyniak et al. 1999; Newsome et al. 1995; Pohl and Hibbs 1996; Pohl and Tylenda 2000; Rogan 1996).

Results from studies examining concentrations of CDDs, *p,p'*-DDE, hexachlorobenzene, mercury, and PCBs in breast milk indicate that mean or median concentrations show a 10- to 100-fold range among studies for each of these chemicals (Table 2). This variation has been ascribed to numerous factors including spatially and temporally related differences in exposure of individuals and groups of individuals, differences in sampling, and differences in analytical techniques across studies (Abadin et al. 1997; DeKoning and Karmaus 2000; Pohl and Hibbs 1996; Pohl and Tylenda 2000; Rogan 1996). Breast milk monitoring studies conducted in Sweden for the past 20–30 years indicate that exposure to certain persistent chemicals may be decreasing during this period, but exposure to others may be increasing. For example, average concentrations of CDDs, CDFs, and PCBs (Hooper and McDonald 2000) and *p,p'*-DDE (Table 2; Pohl and Tylenda 2000) in Swedish breast milk samples have been decreasing, while levels of polybrominated diphenyl ethers have been increasing (Hooper and McDonald 2000).

Table 2. Levels of Chemicals of Concern in Human Breast Milk Samples from General Populations

Chemical	Range of mean or median concentrations (ng/g lipid)	Newborn intake via breast milk[a] (μg/kg/day)	Region	Reference
CDDs and CDFs	0.013–0.028[b]	0.00009–0.00057[c]	United States, Canada, Germany, New Zealand, Japan, Russia	Pohl and Hibbs 1996
CDDs and CDFs	0.162–0.485[b]	0.00115–0.00344[a]	South Vietnam (1970–1973)	Pohl and Hibbs 1996
Mercury (total)	130–793[c]	0.922–5.625	Japan, Germany, Sweden	Abadin et al. 1997
Hexachloro-benzene	5–63	0.035–0.447	New Zealand, Brazil, Arkansas, Australia, Canada, Mexico, Quebec Caucasians	Pohl and Tylenda 2000
Hexachloro-benzene	100–>1,000	0.709–>7.094	France, Spain, Quebec Inuits, Slovak Republic, Czech Republic	Pohl and Tylenda 2000
p,p'-DDE	500 1,200 2,000	3.547 8.513 14.188	Sweden 1989 Sweden 1979 Sweden 1967	Pohl and Tylenda 2000
p,p'-DDE	300–>3,000	2.128–21.281	New Zealand, Brazil, France, Australia, Quebec Caucasians and Inuits, Arkansas, Canada, Slovak Republic, Czech Republic, Germany, North Carolina	Pohl and Tylenda 2000; Rogan et al.1986a
PCBs	167–1,770	1.185–12.556	Japan, Quebec Caucasians and Inuits, New York, Michigan, Netherlands, Poland, Finland, Croatia, North Carolina	DeKoning and Karmaus 2000

[a]Converted from 0.6–3.6 μg Hg/dL, using a conversion factor of 45.4 g lipid/10 dL milk (DeKoning and Karmaus 2000). Organic forms accounted for about 7–50% of total mercury (Abadin et al. 1997).
[b]Measured in 2,3,7,8-TCDD toxic equivalents (TEQs).
[c]Calculated, based on assumptions of 3.2 kg body weight, 45.4 g fat/L milk and 0.5 milk/day (DeKoning and Karmaus 2000), as follows: 5 ng/g fat x 45.4 g fat/L x 0.5 L/day x 1/3.2 kg x 1 μg/1,000 ng=0.035 μg/kg/day.

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. 1994a, 1994b, 1996; Patandin et al. 1998, 1999a, 1999b) of breast-fed children provide some evidence that exposure to mixtures of biopersistent chemicals in human breast milk at exposure levels in the upper range of background levels or exposure during gestation via placental transfer may be associated with mild neuro-developmental delays in some children. Results from these studies are suggestive that the observed delays may have been associated with gestational exposure rather than lactational exposure. Public health agencies concur that the benefits of breast-feeding appear to outweigh the risks for most people (Abadin et al. 1997; Pohl and Hibbs 1996; Pohl and Tylenda 2000; Rogan 1996).

The North Carolina study, started in 1978, measured PCBs and *p,p'*-DDE in breast milk, maternal serum, and cord blood, and followed children to assess whether variability in growth, development, and duration of lactation were associated with levels of these chemicals in breast milk at birth (Gladen and Rogan 1991; Gladen et al. 1988; Rogan et al. 1986a, 1986b, 1987). The maternal participants (n = 880) were volunteers who were planning to deliver at one of three participating hospitals. They were not a random sample of the North Carolina population, but were a cohort expected to have normal exposure (i.e., not elevated relative to the general population) to biopersistent chemicals. Fifty-three percent had a college education, 41% were professionals, 18% smoked, 40% drank alcohol at least once a week, 90% were white with ages ranging from 16 to 41 years, 21% reported eating sport fish at least once during pregnancy, and 88% breast-fed their child, at least to some extent (Rogan et al. 1986a). PCBs and *p,p'*-DDE were detected in about 90 and 99% of breast milk samples, respectively. PCB levels were below detection limits in most samples of cord serum (88%) and placenta (97%), whereas *p,p'*-DDE levels were detected in more than 90% of cord serum and placenta samples. Amounts of PCBs and *p,p'*-DDE in breast milk at birth were used as an index of maternal body burden to examine possible relationships between prenatal exposure to these chemicals and variability in growth, development, and duration of lactation. Median PCB and DDE levels in breast milk at birth were 1.8 and 2.4 ppm (1,770 and 2,400 ng/g fat), respectively. Levels of both chemicals in milk declined by about 20 and 40% over 6 and 18 months of lactation.

Birth weight, head circumference, and neonatal jaundice showed no association with PCB and DDE levels in milk at birth, in a multiple regression analysis that included potential cofounding variables such as previous pregnancies, race, sex, age, and sport-fish consumption (Rogan et al. 1986b). Multivariate regression analyses indicated that increasing levels of PCBs and DDE in milk at birth were significantly associated with decreasing scores on two of seven cluster scores on the Brazelton Neonatal Behavioral

Assessment Scale administered to the newborn infants within 3 weeks of delivery (Rogan et al. 1986b). The affected scores were for tonicity involving measures of motor maturity (i.e., general muscle tone, pull-to-sit, activity, and defensive movements) and for reflexes (20 physical reflexes were assessed). Mothers with the highest levels of PCBs or DDE in milk had shorter median durations of lactation (13 or 10 weeks, respectively) than mothers with the lowest levels of these chemicals (26 weeks), but, in multivariate regression analyses, the association between PCB or DDE levels and duration of lactation was not significantly significant (Gladen et al. 1988). No statistically significant associations were found between levels of PCBs or DDE in milk and weight gain or frequency of illness-related physician visits for the children in the first year after birth (Rogan et al. 1987). Children were also assessed with the Bayley Scales of Infant Development at 6, 12, 18, and 24 months (Gladen et al. 1988; Gladen and Rogan 1991). Multivariate regression analyses indicated that decreasing psychomotor development index scores at 6 and 12 months of age, but not mental development index scores, were significantly associated with increasing PCB levels in milk at birth (Gladen et al. 1988). Psychomotor scores at 6 and 12 months were not significantly associated with *p,p'*-DDE levels in milk at birth, or with measures of postnatal PCB or *p,p'*-DDE exposures (Gladen et al. 1988). At 18 and 24 months of age, scores for psychomotor or mental development were not statistically significantly associated with measures of prenatal or postnatal exposure to PCBs or *p,p'*-DDE (Gladen and Rogan 1991).

The Netherlands study, started in 1990, measured PCBs in cord and maternal plasma sampled in the last month of gestation and PCBs and CDDs in breast milk sampled in the second week after delivery and followed children to assess whether variability in birth size, growth, neurological development, and thyroid hormone status could be associated with levels of these chemicals in the biological fluids (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994a, 1994b, 1996; Lanting et al. 1998a, 1998b; Patandin et al. 1998, 1999a, 1999b). The study population included 418 mother/child pairs: 207 pairs from Rotterdam (105 breast-fed and 102 formula-fed) and 211 pairs from Groningen (104 breast-fed and 107 formula-fed). Plasma samples were analyzed for four PCB congeners assessed as non-planar (2,3',4,4',5-pentachlorobiphenyl, 2,2', 3,4,4',5'- and 2,2',4,4',5,5'-hexachlorobiphenyl, and 2,2',3,4,4',5,5'-heptachlorobiphenyl). Milk samples were analyzed for 17 2,3,7,8-substituted CDDs and CDFs, 3 PCBs assessed as planar and 23 PCBs assessed as non-planar (Huisman et al. 1995a).

Within 4 weeks of birth, newborns from the Rotterdam and Groningen groups were examined for neurological deficits and assigned a reflex score (based on 10 reflex measures), a postural tone score (based on 11 muscle tone measures), and a neurological optimality score (based on 60 neurological measures) (Huisman et al. 1995a). Logistic regression analyses that adjusted for potential confounding

variables such as maternal age, smoking, and alcohol consumption indicated that scores for reflex, postural tone, and neurological optimality were not significantly associated with levels of the four non-planar PCBs in maternal or cord plasma (Huisman et al. 1995a). In contrast, significant associations were found for increased incidence of non-optimal neurological scores and increased breast milk levels of 5/7 CDDs, 2/10 CDFs, 1/3 planar PCBs, and 10/23 non-planar PCBs (including 7/17 diortho-substituted PCBs) (Huisman et al. 1995a). Increasing incidence of postural tone scores assessed as hypotonia was significantly associated with increasing breast milk levels of planar PCBs expressed as TCDD toxicity equivalents (i.e., TEQs), but no significant associations were found between reflex scores and breast milk levels of PCBs or other analyzed chemicals (Huisman et al. 1995a). Multivariate regression analysis of the results of a 57-item examination focusing on motor functions of the children at 18 months of age indicated that decreasing neurological optimality scores were significantly associated with increasing levels of PCBs in cord plasma, but no significant associations were found between neurological optimality scores and breast milk levels of PCBs and/or CDDs (Huisman et al. 1995b).

Children from the Rotterdam group were also assessed for mental and psychomotor development using the Dutch version of the Bayley Scales of Infant Development at 3, 7, and 18 months of age (Koopman-Esseboom et al. 1996). Multivariate regression analyses that included potential confounding variables such as maternal education found no significant association at 3, 7, or 18 months between mental development scores and levels of four PCBs in maternal or cord plasma, levels of PCBs in breast milk, or levels of total PCB and dioxin TEQs in breast milk. At 3 months of age, decreasing psychomotor development scores were significantly associated with increasing PCB levels in maternal plasma, but this relationship was not statistically significant at 7 or 18 months. Multivariate regression analyses revealed no significant associations between psychomotor scores at 3, 7, or 18 months and breast milk levels of PCBs or total PCB and dioxin TEQs. At 7 months of age, increasing duration of breast feeding was significantly associated with increasing psychomotor development scores and mental development scores (Koopman-Esseboom et al. 1996).

Neurological status was also assessed in children from the Rotterdam and Groningen groups at 42 months of age (Lanting et al. 1998a, 1998b; Patandin et al. 1999a, 1999b). A neurological optimality score for each child was determined based on a 56-item examination focusing on motor functions (Lanting et al. 1998a, 1998b). Multivariate regression analyses showed no significant associations between neurological optimality score based on motor functions and PCB levels in maternal or cord plasma or levels of dioxins, PCBs, or PCB and dioxin TEQs in breast milk (Lanting et al. 1998a, 1998b). A beneficial effect of breast feeding was found on tests of fluency of movements (Lanting et al. 1998b). Cognitive abilities were also

assessed at 42 months using the Kaufman Assessment Battery for Children (Patandin et al. 1999a). Multivariate regression analyses showed a statistically significant association between decreasing scores on all three scales of the examination and increasing concentrations of PCBs in maternal plasma sampled during gestation, but no statistically significant associations were found between cognitive scores and breast milk levels of non-dioxin-like PCBs or total PCB and dioxin TEQs (Patandin et al. 1999a). The breast-fed group of children at 42 months of age showed a higher median plasma concentration of four sentinel PCBs (0.75 µg/L; range=0.23–5.90) than the median concentration (0.21 µg/L; range=0.08–0.46) of the formula-fed children (Patandin et al. 1999b), but no significant associations were found between cognitive scores and children's plasma PCB levels at 42 month of age (Patandin et al. 1999a). The authors concluded that (1) *in utero* exposure to PCBs was associated with poorer cognitive functioning at 42 months; (2) maternal PCB body burden should be reduced; and (3) breast-feeding should not be discouraged (Patandin et al. 1999a).

Other findings from the Dutch study include:

- statistically significant associations between increasing cord and serum levels and decreasing birth weight or growth rate from birth to 3 months, but no significant association between breast milk levels of PCBs or dioxins (at 2 weeks after birth) and birth weight or 3-month postnatal growth rates (Patandin et al. 1998);

- significant associations between increasing PCB and dioxin TEQ levels in breast milk and decreasing maternal plasma levels of triiodothyronine (T_3) or thyroxine (T_4) or increasing plasma levels of thyroid stimulating hormone (TSH) in infants at 2 weeks and 3 months after birth (Koopman-Esseboom et al. 1994a); and

- no statistically significant associations between PCB levels in maternal or cord plasma or PCB levels in milk and symptoms of respiratory tract infection of the infants in the first 18 months of life or levels of antibodies to mumps, measles, or rubella, but significant associations between changes in T cell subpopulations in the infants at 18 months of age and levels of PCB and dioxin TEQs in maternal or cord plasma (Weisglas-Kuperus et al. 1995).

The neurological effects observed in the North Carolina and Dutch studies were somewhat similar. Neurological deficits associated with motor function in the Bayley Scales of Infant Development were associated with increasing levels of persistent chemicals in cord serum or breast milk samples only at birth or ages <18 months. Assessments at later ages up to 42 months found no significant associations

between decreasing motor function scores and increasing concentrations of persistent chemicals in maternal milk at birth. The Dutch study also assessed cognitive function in the Kaufman Assessment Battery for Children at 42 months; significant associations were found for decreasing scores with increasing indices of prenatal exposures to PCBs, CDDs, and CDFs, but not with increasing indices of post-natal exposure. The Dutch study was also designed to compare breast feeding with formula feeding and found an advantageous effect of breast feeding on fluency of movement at 18 and 42 months. Members of both groups of investigators recommended that breast feeding should not be discouraged, but that maternal exposures to toxic persistent environmental chemicals should be reduced (Patandin et al. 1999b; Rogan 1996).

Prospective studies of children whose mothers frequently consumed Lake Michigan (Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a) or Lake Ontario (Lonky et al. 1996; Stewart et al. 1999, 2000b) sport fish contaminated with complex mixtures of persistent chemicals found statistically significant associations between prenatal exposure to PCBs (the only chemicals investigated) 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 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 several study design limitations, the weight of the evidence from these studies is insufficient to establish causal relationships between fish consumption and adverse health effects in humans, but the hypothesis of a possible association between PCB exposure from maternal Great Lakes fish consumption and altered childhood neurological development is plausible based on the findings. 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. (See the ATSDR [2001c] Interaction Profile on Persistent Chemicals Found in Fish for more detailed discussion of these and other studies on possible associations between health effects and consumption of contaminated fish.)

A cohort study of neurobehavioral development in children of mothers residing in the North Atlantic Faroe Islands where pilot whale meat and blubber are components of the diet reported that mild deficits in neuropsychological development of the children at 7 years of age were associated with increasing mercury concentrations in maternal cord blood (Grandjean et al. 1997). In a companion nested matched case-control study, average neuropsychological performance of children whose mothers had a hair-mercury concentration of 10–20 µg/g was compared with that of "control" children whose mothers had hair concentrations below 3 µg/g (Grandjean et al. 1998). The case group showed mild cognitive deficits,

compared with controls, in the domains of motor function, language, and memory (Grandjean et al. 1998). The design of these studies precluded determining the possible contribution of postnatal exposure to neurotoxicants in breast milk or other components of the postnatal diet. More detailed discussion of these studies can be found elsewhere (ATSDR 1999b). In contrast, the early attainment of the ability to sit, creep, and stand in Faroe Island infants through 12 months of age was associated with breast feeding, which was associated with increased hair-mercury concentrations (Grandjean et al. 1995b). In addition, infants who reached these developmental milestones early had significantly higher hair-mercury concentrations than children who attained them later. These results suggest that although breast feeding may have led to higher mercury exposure, the benefits of breast feeding may have offset or masked possible neurodevelopmental effects from mercury.

Although the epidemiological studies point to gestational exposure to persistent toxic chemicals being more important than lactational exposure in affecting neurological development, a study of monkeys found long-term, exposure-related behavioral deficits in formula-fed monkeys exposed to PCBs from birth to 20 weeks (Rice 1997, 1998, 1999a, 1999b; Rice and Haywood 1999). Male monkeys were given oral doses of 0 or 7.5 µg/kg/day of a PCB mixture in a liquid diet formula. The mixture contained 15 congeners representing about 80% of the PCB content of Canadian samples of human breast milk; relative concentrations in the mixture were similar to those in human milk (Arnold et al. 1999; Rice 1997). Monkeys were hand-reared from birth and received only the liquid diet during the first 2 weeks. The liquid diet was supplemented with solid food during the remaining 18 weeks of exposure. Monkeys were assessed between 2.5 and 5 years of age for performance on a series of behavioral tasks. Exposed monkeys showed blood concentrations of PCBs at the end of exposure (1–3 ppb) that were within the range (2–10 ppb) of values for general human populations (Rice 1999a). Daily PCB intakes of the exposed monkeys were within the range of estimates of human newborn PCB intakes from breast milk cited in Table 2. Exposed monkeys showed deficits in a spatial delay alternation task with no indication of a deficit in spatial memory *per se* and performance deficits on a fixed interval schedule of reinforcement. Rice (2000) discussed the parallels between the features of attention deficit hyperactivity disorder (ADHD) in human children and the deficits noted in PCB- and lead-exposed monkeys (impaired discrimination reversal and spatial delayed alternation performance and impaired performance on a fixed interval schedule of reinforcement). Rice (1999a) concluded that "these results have implications for the potential contributions of exposure to PCBs through breast milk to later impairment in cognitive function".

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 μmole/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 P450 (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 μmole/kg) were 50% lower than maximum levels induced by 30 μg/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 hexa-chlorobenzene 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 hexa-chlorobenzene?);

- 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 μmole/kg basis—is this a requirement for the apparent potentiation?);

- the duration and route of exposure (will long-term co-exposure to oral hexachlorobenzene and TCDD produces 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 hexa-chlorobenzene 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 characterizations 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, and mechanistic understanding is inadequate to support a reliable projection of the mode of joint action of CDDs and hexachlorobenzene on any toxicity target. Oxidative metabolism of hexachloro-benzene 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 Raaji 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 studies of the effects of tri-acetyloleandomycin (TAO), a selective inhibitor of CYPIIIA, in rats indicate that CYPIIIA 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 CYPIIIA-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 tetrachlorohydro-quinone) did not induce porphyria. Even if TCDD can induce enzymes involved in the metabolism of hexachlorobenzene, capabilities of downstream enzymes (e.g., Phase II enzymes) might 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 hexa-chlorobenzene metabolism will influence hexachlorobenzene toxicity. Although it is known that hexa-chlorobenzene 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.

A brief summary of the toxicological interaction data for 2,3,7,8-TCDD and hexachlorobenzene is provided in Table 3.

Table 3. 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+113.9[a] (r)[b]		No apparent interaction, but hexa-chlorobenzene 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[a] (r)[b]			Greater than additive	Li et al. 1989

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]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-TCCD 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 prostrate 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., isobolo-graphic 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.

A brief summary of the toxicological interaction data for 2,3,7,8-TCDD and *p,p*'-DDE is provided in Table 4.

Table 4. 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)						
2,3,7,8-TCDD Influence on Toxicity/Carcinogenicity of *p,p'*-DDE						
Acute	prostate weights in offspring		$0.00025+100^{a}$ (r)[b]		Additive action is suggested, but the study design precludes discarding the possibility of greater-than or less-than-additive action.	Loeffler and Peterson 1999
p,p'-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

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]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 CYPIIIA- 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 CYPIIB, and to a lesser degree CYPIIIA, but not CYP1A isozymes (see Appendix C). If simultaneous exposure to DDE and hexachlorobenzene cause an increased induction of CYPIIIA 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 pertinent data on possible mode of joint actions 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 methyl-mercury, 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 methyl-mercury (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 methyl-mercury 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 of studies of Omara et al. (1997, 1998) 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 hexa-chlorobenzene and methylmercury, but two published studies have looked for possible acute-exposure interactions between hexachlorobenzene, HCB, and mercuric chloride, $HgCl_2$ (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 $HgCl_2$/kg; or (400 mg HCB+10 mg $HgCl_2$)/kg, (400 mg HCB+12.5 mg HgCl2)/kg, (600 mg HCB+10 mg $HgCl_2$)/kg, or (600 mg HCB+12.5 mg $HgCl_2$)/kg (Lecavalier et al. 1994). Endpoints included mortality by the end of a 14-day observation period, necropsy examination, histopatholgical 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 hexachloro-benzene 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 $HgCl_2$/kg). Liver weights were significantly increased in hexachloro-benzene-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 histopatho-logical 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 $HgCl_2$/kg (Renner et al. 1980). Other groups were given mixtures of 3.6, 6, 8, 10, 12.5, or 18 mg $HgCl_2$/kg with 400 mg HCB/kg or 6, 12.5, or 18 mg $HgCl_2$/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 $HgCl_2$/kg, but all rats died with co-exposure to 12.5 or 18 mg $HgCl_2$/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$HgCl_2$ /kg doses), but Lecavalier et al. (1994) did not find that 10 or 12.5 mg $HgCl_2$/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 methyl-mercury under any exposure scenario.

Because no data regarding the toxicological interactions of hexachlorobenzene with *methylmercury* were available, Table 5 provides a brief summary of the data for hexachlorobenzene and *mercuric chloride*.

Table 5. 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[a]

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[b]+10 (r)[c] 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

[a]Data for the pair, *methylmercury* and hexachlorobenzene, were not located, so available data for mercuric chloride and hexachlorobenzene were evaluated as explained in text.
[b]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[c]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 methyl-mercury (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 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, 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 greater-than-additive and less-than-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 immuno-toxicity 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 6). 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/10^6 spleen cells), compared with the response of

562 PFC/10^6 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 µmol 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 µmol 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 µmol 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 µmol Aroclor 1254/kg antagonized the TCDD-induced effect to a limited degree (170±10 versus 62±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 µg/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 immuno-toxicity 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 µg 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 µmol/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-pentachloro-dibenzofuran, 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-pentachloro-biphenyl 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 [TEQ] relative to 2,3,7,8-TCDD), 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-pentachloro-dibenzofuran, 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 6). 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'-penta-chlorobiphenyl) 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-hexachloro-biphenyl 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 6). 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'-hexa-chlorobiphenyl/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-hexachloro-biphenyl; 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, 1996a). 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-penta-chlorobiphenyl 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. 1996a). 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. 1996a). 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'-hexa-chlorobiphenyl concentrations plus 0.5 or 5 ppb 2,3,7,8-TCDD in the diet (van Birgelen et al. 1996a).

Tables 6 and 7 summarize the available interactions data for PCBs and 2,3,7,8-TCDD.

Table 6. 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 (intra-peritoneal)			1.6^a+0.0012 (m)^b 4.9+0.0012 (m) 24.6+0.0012 (m) 49.3+0.0012 (m) 24.6+0.0036 (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)
				25+0.0012 (m)		Davis and Safe 1989 (Aroclors 1242, 1248, 1254, 1260)
			25+0.0012 (m)			Davis and Safe 1989 (Aroclor 1232)
			5+0.0012 (m) 25+0.0012 (m)	50+0.0012 (m)		Davis and Safe 1989 (PCB mix reflective of human milk)

Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD (*continued*)

Duration	Endpoint	Results: Greater than additive	Results: Additive/no effect	Results: Less than additive	Conclusions	References
		Parenteral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl)				
Acute	impaired immune response to sheep red blood cells (intra-peritoneal)		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 (sub-cutaneous)		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 6. 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)		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)
			90.2+0.020 (m)	270.7+0.020 (m) 217.5+0.020 (m)		
		62.5+0.015 (m) 1,000+0.015 (m)		125–500+ 0.015 (m)		

Table 6. 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)

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 6. 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 6. 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. 1996a (2,2',4,4',5,5'-HCB) (2,3,3',4,4',5-HCB) (3,3',4,4',5-PeCB)

Table 6. 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) 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)	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) van Birgelen et al. 1994a (2,3,3',4,4',5-HCB) van Birgelen et al. 1994b (3,3',4,4',5-PeCB)

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: m = mouse; r = rat

Table 7. 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)		μg/kg/week 0.100[a]+1.00 (r)[b] 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 γ-glutamyl transpeptidase altered foci.	Hemming et al. 1995 (3,3',4,4',5-PeCB)

Table 7. 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 7. 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. 1996a (2,2',4,4',5,5'-HCB) (2,3,3',4,4',5-HCB) (3,3',4,4',5-PeCB)

Table 7. 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)

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]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 hexachloro-benzene that are common to both include hepatic porphyria, porphyria cutanea tarda, liver hypertrophy, disruption of thyroid hormone homeostasis, 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 hexachloro-benzene 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 17β-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 17β-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. This apparent synergism is unlikely to be relevant to possible alterations of reproductive performance in mammals exposed to mixtures of PCBs and *p,p'*-DDE.

2.2.10 PCBs and Methylmercury

PCBs and methylmercury are both 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 neuro-transmitter release (e.g., dopamine), cell damage, or cell death (Bemis and Seegal 1999; Kodavanti and Tilson 1997; Kodavanti et al. 1993, 1996a). 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 µM 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 µM methylmercury were 84 (not significantly different from controls), 20, and 1% of control values, indicating a steep dose-response relationship between 14 and 20 µM. 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 µM methylmercury.

Striatal tissue samples were also simultaneously exposed to methylmercury at 4, 10, or 14 µM 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, 02/23/01). The analysis of tissue concentrations indicated that the interaction term was statistically significant at 4 ($p<0.05$), 10 ($p<0.001$), and 14 ($p<0.01$) µM 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 ≥40 ppm in the presence of 10 or 14 µM methylmercury and the predicted values at Aroclor 1254/1260 concentrations ≥100 ppm in the presence of 4 µM methylmercury. This comparison is suggestive of a synergistic effect. For example, the observed mean dopamine concentration at 14 µM 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 µM 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 considerably 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 embryolethality 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, respectively, 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, and 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 benzoxylresorufin-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 methyl-mercury 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).

A summary of available *in vivo* interactions data for PCBs and methylmercury is presented in Tables 8 and 9.

Table 8. 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[a]+4 (m)[b]		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)

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: i = mink; m = mouse; q = quail; r = rat

Table 9. 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[a] subcutaneous+4.6 (r)[b]	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 9. 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)

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]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. There is a fair amount of overlap in the endpoints or organs that these chemicals affect (Table 1). This observation is reflected in the endpoints that form the basis of the oral MRLs for these chemicals (Table 10).

The observations reflected in Tables 1 and 10 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, hexachloro-benzene, *p,p'*-DDE, and PCBs) may jointly act to produce liver damage; (3) four (2,3,7,8-TCDD, hexa-chlorobenzene, 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.

As discussed in Section 2.1 of this profile, the detection of all five of these, and other, chemicals in breast milk, combined with the knowledge that each is able to alter neurological development, has led to epidemiological studies in Michigan, North Carolina, New York, and the Netherlands examining if there are associations between increasing concentrations of several of these chemicals in maternal cord serum and breast milk (e.g., PCBs, *p,p'*-DDE, and CDDs) and deficits in measures of motor and cognitive function in children. All four studies demonstrated statistically significant associations between concentrations of these persistent chemicals in maternal fluid samples and deficits in neurological development of the children; however, the Netherlands study also was able to demonstrate beneficial effects of breast feeding on neurological development. The results from the human studies are thought to implicate gestational exposure to the persistent chemicals to a greater degree than lactational exposure.

**Table 10. 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	p,p'-DDE[a]	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	neurobehavioral changes in monkey offspring
Chronic	neurobehavioral changes in monkey offspring	liver degeneration and porphyria in rats	none derived, inadequate data	neurobehavioral changes in human offspring	immuno-suppression in monkeys

[a]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.

However, a study of formula-fed monkeys found long-term neurobehavioral deficits in monkeys exposed from birth to 20 weeks to a PCB mixture representative of patterns of congeners found in human breast milk. It is plausible that the monkey study was able to discern PCB-induced effects on postnatal neurological development because they were not masked by potential beneficial effects of breast feeding.

In the absence of studies that examine relevant endpoints and describe dose-response relationships following oral exposures to mixtures that contain these five chemicals (e.g., in food), component-based approaches to assessing their joint action that assume dose additivity for noncancer effects appear to be reasonable for practical public health concerns (e.g., the hazard index approach or the target-organ toxicity dose modification of the hazard index approach). Likewise, a component-based approach assuming response additivity appears reasonable for assessment of cancer risks from oral exposure to mixtures of these five chemicals. Given the overlap in toxicity targets of these chemicals, such approaches are preferable, from a public health protection perspective, to approaches that would assess hazards of the individual components separately.

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

With component-based approaches to assessing health hazards from mixtures of chemicals, 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 of the mixture. With this purpose in mind, the available data on the possible joint actions of pairs of the chemicals of concern were reviewed in Section 2.2. Available data on possible binary interactions among these five chemicals are limited for most of the pairs and PBPK models for pairs of the chemicals are not available. Tables 12–31 describe binary weight-of-evidence determinations (BINWOEs) for the pairs of the five chemicals of concern using the classification scheme summarized in Table 11 and ATSDR 2001a. The numerical scale of this scheme ranges 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 indeteministic evidence for the mode of joint action, up to +1 for high confidence that a greater-than-additive joint action 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 32. The BINWOEs focus on simultaneous oral exposure as this is the exposure scenario of most interest for public health concerns for the subject chemicals and their mixture. A summary discussion of the BINWOES follows this paragraph and precedes the descriptive tables.

There are no pertinent interaction data and understanding of mechanisms of action is too incomplete to make projections of interactions between the following pairs of chemicals:

- *p,p'*-DDE and hexachlorobenzene (Tables 16 and 17);
- *p,p'*-DDE and methylmercury (Tables 22 and 23);

- PCBs and hexachlorobenzene (Tables 26 and 27); and
- PCBs and *p,p'*-DDE (Tables 28 and 29).

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

- the effect of 2,3,78-TCDD on hexachlorobenzene toxicity (Table 12);
- the effect of hexachlorobenzene on methylmercury toxicity (Table 20);
- 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 24);
- the effect of 2,3,7,8-TCDD on the following PCB-induced effects: immunosuppression, thyroid hormone disruption, porphyria, developmental toxicity (cleft palate formation and hydronephrosis), impaired reproductive organ function and development, impaired neurological function and development, tumor initiation and promotion (Table 25); 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 31).

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 noncancer effects and cancer (Tables 14 and 15);
- methylmercury and 2,3,7,8-TCDD for noncancer effects and cancer (Tables 18 and 19);
- methylmercury on hexachlorobenzene toxicity (Table 21);
- PCBs and 2,3,7,8-TCDD for inducing body and thymus weight changes, hepatomegaly, and decreased liver levels of retinoids (Tables 24 and 25); and
- methylmercury and PCBs for inducing hepatic porphyria (Tables 30 and 31).

Evidence is also available supporting the following possible interactions:

- hexachlorobenzene potentiation (greater-than-additive interaction) of TCDD reduction in body weight and thymus weight (Table 13);
- antagonism (less-than-additive interaction) by PCB mixtures of TCDD induction of immune system suppression and developmental effects (cleft palate and hydronephrosis) (Table 24); and
- synergism (greater-than-additive interaction) between PCBs and methylmercury in disrupting regulation of brain levels of dopamine, a process influencing neurological function and development (Tables 30 and 31).

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 project potential non-additive interactions.

Table 11. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

Classification	Factor
Direction of Interaction	**Direction**
= Additive	0
> Greater than additive	+1
< Less than additive	−1
? Indeterminate	0
Quality of the Data	**Weighting**
Mechanistic Understanding	
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
Toxicological Significance	
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
Modifiers	
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

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

Table 12. Effect of **2,3,7,8-TCDD** on **Hexachlorobenzene**
(see Table 11 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 CYPIIIA-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 CYPIIIA 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 hexachloro-benzene-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 hexachloro-benzene 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 CYPIIIA 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 13. Effect of **Hexachlorobenzene** on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
BINWOE: > IIIC1aii (0.32 x 0.32 x 1.0 x 1.0 x 0.79 = +0.08)
for body and thymus weight effects from acute exposure
BINWOE: > IIIC2aii (0.32 x 0.32 x 0.79 x 1.0 x 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 μmole/kg) potentiated effects on body and thymus weights produced by intraperitoneal doses (0.031 or 0.093 μmole/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 13. 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 14. Effect of **2,3,7,8-TCDD** on ***p,p'*-DDE**
(see Table 11 for explanation of BINWOE codes)
BINWOE: =IIIC (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 15. Effect of *p,p'*-DDE on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
BINWOE: =IIIC (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 16. Effect of *p,p'*-DDE on **Hexachlorobenzene**
(see Table 11 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 CYPIIIA-mediated or CYP1A-mediated metabolism (den Besten et al. 1993; Sinclair et al. 1997; see Appendix B). DDE induces hepatic CYPIIB and, to a lesser degree, CYPIIIA in rats. If simultaneous exposure to DDE and hexachlorobenzene cause an increased induction of CYPIIIA 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 17. Effect of **Hexachlorobenzene** on *p,p'*-**DDE**
(see Table 11 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 18. Effect of **2,3,7,8-TCDD** on **Methylmercury**
(see Table 11 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

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Table 18. Effect of **2,3,7,8-TCDD** on **Methylmercury** *(continued)*

Toxicological Significance (continued) - 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.

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 19. Effect of **Methylmercury** on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
BINWOE: = ?

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 20. Effect of **Hexachlorobenzene** on **Methylmercury**
(see Table 11 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 hexa-chlorobenzene 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 mg$HgCl_2$ /kg doses (compared with mercuric chloride alone), but Lecavalier et al. (1994) reported that 10 or 12.5 mg $HgCl_2$/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 21. Effect of **Methylmercury** on **Hexachlorobenzene**
(see Table 11 for explanation of BINWOE codes)
BINWOE: =IIIC (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 and D) are plausible, but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action. Hexachloro-benzene-induced hepatic porphyria is thought to involve an unidentified reactive intermediate produced either by CYPIIIA-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 hexa-chlorobenzene 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 22. Effect of *p,p'*-DDE on **Methylmercury**
(see Table 11 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 23. Effect of **Methylmercury** on *p,p'*-**DDE**
(see Table 11 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 24. Effect of **PCBs** on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
BINWOE: <IIIB1aii (0.32 x 0.79 x 1 x 1 x 0.79 = -0.20)
immune effects (suppression of cell-mediated immune response), acute
BINWOE: <IIIB2aii (0.32 x 0.79 x 0.79 x 1 x 0.79 = -0.16)
immune effects (suppression of cell-mediated immune response), non-acute
BINWOE: <IIIC1ai (0.32 x 0.32 x 1 x 1 x 1 = -0.10)
developmental toxicity (cleft palate, hydronephrosis in offspring), acute
BINWOE: <IIIC2ai (0.32 x 0.32 x 0.79 x 1 x 1 = -0.08)
developmental toxicity (cleft palate, hydronephrosis in offspring), non-acute
BINWOE: =IIIC (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 (intra-peritoneal 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 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

Table 24. Effect of **PCBs** on **2,3,7,8-TCDD** (*continued*)

Toxicologic Significance (continued) - (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, 1996a). 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, 1996a). 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'-tetra-chlorobiphenyl, 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-pentachlorobi-phenyl) 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 25. Effect of **2,3,7,8-TCDD** on **PCBs**
(see Table 11 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, 1996a). 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, 1996a). 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 25. Effect of **2,3,7,8-TCDD** on **PCBs** (*continued*)

Toxicologic Significance (continued) - 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-pentachlorobi-phenyl) 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 26. Effect of **PCBs** on **Hexachlorobenzene**
(see Table 11 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 hexachloro-benzene 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 27. Effect of **Hexachlorobenzene** on **PCBs**
(see Table 11 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 hexachloro-benzene 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 28. Effect of **PCBs** on *p,p'*-**DDE**
(see Table 11 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 byproducts 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 17β-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 no 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 29. Effect of *p,p'*-**DDE** on **PCBs**
(see Table 11 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 byproducts 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 17β-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 no 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 30. Effect of **PCBs** on **Methylmercury**
(see Table 11 for explanation of BINWOE codes)
BINWOE: >IICb (0.79 x 0.32 x 0.79 = +0.20)
for impaired neurological function or development
BINWOE: ? (0)
for impaired reproductive performance
BINWOE: =IIIB(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 30. Effect of **PCBs** on **Methylmercury** *(continued)*

Toxicologic Significance - 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 31. Effect of **Methylmercury** on **PCBs**
(see Table 11 for explanation of BINWOE codes)
BINWOE: >IICb (0.79 x 0.32 x 0.79 = +0.20)
for impaired neurological function or development
BINWOE: ? (0)
for impaired reproductive performance
BINWOE: =IIIB (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.

Table 31. Effect of **Methylmercury** on **PCBs** *(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 *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 32. Matrix of BINWOE Determinations for Repeated Simultaneous Oral Exposure to Chemicals of Concern

EFFECT OF		ON TOXICITY OF				
		2,3,7,8-TCDD	Hexachloro-benzene	*p,p'*-DDE	Methylmercury	PCBs
	2,3,7,8-TCDD	(shaded)	**? (0)**	Anti-andro-genic effects **=IIIC(0)** Other effects **? (0)**	Immune suppression **=IIIBbii (0)** Other effects ? (0)	Body and organ weight changes, decreased retinoids in liver **=IIIC(0)** Other effects **? (0)**
	Hexachloro-benzene	Body and thymus weight **>IIIA2aii (+0.06)** Other effects: **? (0)**	(shaded)	**? (0)**	**? (0)**	**? (0)**
	p,p'-DDE	Anti-androgenic effects **=IIIC(0)** Other effects **? (0)**	**? (0)**	(shaded)	**? (0)**	**? (0)**
	Methylmercury	**? (0)**	Liver effects **=IIIC(0)** Other effects **? (0)**	**? (0)**	(shaded)	Neurological effects **>IICb (+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 **=IIIC(0)** Other effects **? (0)**	**? (0)**	**?(0)**	Neurological effects **>IICb (+0.20)** Reproductive performance **? (0)** Porphyria **=IIIB(0)** Other effects **? (0)**	(shaded)

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 breast milk intake or other food sources) because these are most pertinent to public health concerns from these biopersistent chemicals. As discussed by ATSDR (1992, 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., breast milk); (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 detection of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, PCBs, and other potential neurotoxicants in samples of human breast milk and maternal placental cord serum has led to epidemiological studies of possible neurological deficits in children exposed to persistent chemicals *in utero* and during nursing. 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. Studies in North Carolina (Gladen et al. 1988; Rogan et al. 1986b), the Netherlands (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1996), and the Faroe Islands (Grandjean et al. 1997; 1998) have all reported associations between mild neurological deficits in children and increasing concentrations of persistent chemicals (PCBs or mercury) in maternal cord serum or breast milk samples. The observed associations, however,

do not establish causal relationships between fish consumption or breast feeding and deficits in neurological development. In contrast, there is evidence from the Dutch and Faroe Islands studies that beneficial effects of breast feeding outweigh detrimental effects that may be associated with increased exposure to biopersistent chemicals. For example, the Dutch study found an advantageous effect of breast feeding, compared with formula feeding, on fluency of movement at 18 and 42 months (Lanting et al. 1998b), and the Faroe Islands study found that early attainment of the ability to sit, creep, and stand in Faroe Island infants through 12 months of age was associated with breast feeding, which was associated with increased hair-mercury concentrations (Grandjean et al. 1995b).

Although the epidemiological studies of possible health hazards associated with exposure to biopersistent chemicals in breast milk or fish identify mild neurodevelopmental deficits as a possible health hazard, they are not directly useful for the purposes of conducting exposure-based assessments of hazards specific to a community or scenarios involving exposure to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs. 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 10 in Section 2.3). Table 33 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).

Table 33. 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	*p,p'*-DDE	Methyl mercury	PCBs
	Target Organ Toxicity Dose (TTD) in mg/kg/day				
Hepatic	3×10^{-9}	2×10^{-5} (chronic MRL)	7×10^{-4}	NA	1×10^{-4}
Endocrine	1×10^{-7}	1×10^{-3}	NA	NA	1×10^{-4}
Immunological	2×10^{-8}	4×10^{-4}	2×10^{-3}	3×10^{-4}	2×10^{-5} (chronic MRL)
Neurological	1×10^{-9} (chronic MRL)	8×10^{-4}	6×10^{-2}	3×10^{-4}	3×10^{-5} (intermediate MRL)
Reproductive	1×10^{-9}	3×10^{-4}	2×10^{-3}	4×10^{-4}	2×10^{-4}
Developmental	1×10^{-9} (chronic MRL)	8×10^{-3}	2×10^{-3}	3×10^{-4} (chronic MRL)	3×10^{-5} (intermediate MRL)

NA = not applicable

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 1×10^{-4} to 1×10^{-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.

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 (1×10^{-9} 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 one. 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 1×10^{-6}, then the component cancer risks are summed to derive a cancer risk estimate for the mixture. If only one or if none of the component risks equals or exceeds 1×10^{-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 1×10^{-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 32 (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 32). 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 32). 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, methyl-mercury, 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 1×10^{-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

There are several reasons supporting the recommendation to use component-based approaches that assume additive joint toxic action in exposure-based assessments of possible noncancer or cancer health hazards from oral exposure to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs. 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.

The detection of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, PCBs, and other potential neurotoxicants in samples of human breast milk has led to epidemiological studies of possible neurological deficits in children exposed *in utero* and during nursing to persistent chemicals in breast milk. Mild neurodevelopmental deficits have been identified as a possible health hazard in these studies, but the results are suggestive that observed deficits may have been associated with gestational rather than lactational exposure to persistent chemicals. These studies do not establish causal relationships and are not directly useful for assessment of health hazards specific to a community or scenarios involving exposures to mixtures of CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs.

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.

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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 hydro-carbons, 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 1998; 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 µg/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 ng/kg);
7. decreased serum or plasma levels of the thyroid hormone, thyroxin (T4), in rodents following acute (0.1–0.3 µg/kg) or subchronic (0.05–0.8 µg/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 folloicle-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; and

14. 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]-α, and TGF-β1) 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-α, and TGF-β1) 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 immunosuppresive

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 μg/kg/day was based on a no-observed-adverse-effect-level (NOAEL) of 0.005 μg/kg/day and a lowest-observed-adverse-effect level (LOAEL) of 0.01 μg/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 μg/kg/day was based on a NOAEL of 0.0007 μg/kg/day and a LOAEL of 0.005 μg/kg/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 μg/kg/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 2001a). 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, hepato-cellular degeneration, and liver necrosis, at doses as low as 0.001 µg/kg/day in females and 0.01 µ/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$_1$ mice generally support the findings of Kociba et al. (1978). The studies reported toxic hepatitis (liposis and hydropic degeneration of hepato-cytes 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$_{HEPATIC}$ 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$_{HEPATIC}$ 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$_{ENDOCRINE}$ 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 µ/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 µg/kg/day (immunosuppression) and the intermediate oral MRL of 0.00002 µg/kg/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 µg/kg/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 µg/kg/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 µg/kg/day would lead to a TTD_{IMMUNO} of 0.00003 µg/kg/day, which is slightly higher than the intermediate oral MRL (0.00002 µg/kg/day) based on immunological effects. This occurs because the intermediate oral MRL is based on a LOAEL of 0.005 µg/kg/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 µg/kg/day, which is slightly lower than the chronic NOAEL. Because the intermediate NOAEL on which the intermediate oral MRL is based (0.0007 µg/kg/day) is well below the chronic LOAEL (0.002 µg/kg/day) and also below the chronic NOAEL (0.001 µg/kg/day), the intermediate oral MRL should be protective for immunological effects by chronic exposure as well. Therefore, the intermediate oral MRL of 0.00002 µg/kg/day is adopted as the TTD_{IMMUNO} for 2,3,7,8-TCDD.

Neurological Effects

The chronic MRL of 0.000001 µg/kg/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 µg/kg/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 µg/kg/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 µg/kg/day yields a TTD_{REPRO} of 0.000001 µg/kg/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 µg/kg/day for 2,3,7,8-TCDD is suitable to assess the potential for developmental effects.

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

$TTD_{HEPATIC}$ = 0.000003 µg/kg/day ($3x10^{-6}$ µg/kg/day; $3x10^{-9}$ mg/kg/day)

$TTD_{ENDOCRINE}$ = 0.0001 µg/kg/day ($1x10^{-4}$ µg/kg/day; $1x10^{-7}$ mg/kg/day)

TTD_{REPRO} = 0.000001 µg/kg/day ($1x10^{-6}$ µg/kg/day; $1x10^{-9}$ mg/kg/day)

$MRL_{(IMMUNO)}$ = 0.00002 µg/kg/day ($2x10^{-5}$ µg/kg/day; $2x10^{-8}$ mg/kg/day; intermediate MRL)

$MRL_{(NEURODEVELOP)}$ = 0.000001 µg/kg/day ($1x10^{-6}$ µg/kg/day; $1x10^{-9}$ mg/kg/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 hexachloro-benzene is thought to be poorly absorbed by the respiratory tract (ATSDR 1996). Absorbed hexachloro-benzene 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 hexachloro-benzene tend to increase with age, indicative of bioaccumulation (ATSDR 1996). Absorbed hexachloro-benzene 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 CYPIIIA1/2), 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 P450 enzymes including those in the CYPIIIA 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, CYPIIIA, 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 P450IIIA1/2, 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 CYPIIIA1/2-catalyzed transformation of hexa-chlorobenzene 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 pentachloro-phenol and tetrachlorohydroquinone) did not induce porphyria, but increased urinary levels of these metabolites to comparable levels observed in rats treated with porhyria-inducing doses of hexachloro-benzene (den Besten et al. 1993). None of the known hexachlorobenzene metabolites (including penta-chlorophenol) is directly porphyrinogenic when administered *in vivo*. The principal metabolites, penta-chlorophenol 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 uroporphyria by a number of planar poly-halogenated 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 thyroxin 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 thryroxin to thyroxin-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 2001d) 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 2001d) noted that data were inadequate for Reference Concentration (RfC) derivation.

EPA (IRIS 2001d) classified hexachlorobenzene as a Group B2 compound—probable human carcinogen, based on inadequate evidence of cancer in studies of hexchlorobenzene-exposed humans and sufficient evidence of cancer in studies of rats, hamsters, and mice exposed to hexachlorobenzene in the diet. EPA (IRIS 2001d) 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 extra-polation method (IRIS 2001d). An inhalation unit risk of 4.6×10^{-4} per $\mu g/m^3$ 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 $TTD_{ENDOCRINE}$ 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_{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_{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 extra-polation 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 hexachloro-benzene.

Summary (TTDs for hexachlorobenzene)

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

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

TTD_{IMMUNO} = 0.0004 mg/kg/day (4×10^{-4} mg/kg/day)

TTD_{NEURO} = 0.0008 mg/kg/day (8×10^{-4} mg/kg/day)

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

$TTD_{DEVELOP}$ = 0.008 mg/kg/day (8×10^{-3} 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 vescicle 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 2001b). Chronic dietary exposure to technical-grade DDT produced increased incidence of liver tumors in mice and rats (IRIS 2001c).

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 (2001b, 2001c) 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 2001b) 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 2001b) 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, immuno-logical, neurological, reproductive, and developmental effects. Chronic oral TTDs for these endpoints are derived below, using the methods described by ATSDR (1999a, 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 2001c) 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 $TTD_{HEPATIC}$ 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_{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 premating 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 can be derived from the LOAEL of 1.67 mg/kg/day 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 premating 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 $TTD_{DEVELOP}$ for *p,p'*-DDE.

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

$MRL_{HEPATO} = 0.0007$ mg/kg/day (7×10^{-4} mg/kg/day)
$TTD_{IMMUNO} = 0.002$ mg/kg/day (2×10^{-3} mg/kg/day)
$TTD_{NEURO} = 0.002$ mg/kg/day (2×10^{-3} mg/kg/day)
$TTD_{REPRO} = 0.002$ mg/kg/day (2×10^{-3} mg/kg/day)
$TTD_{DEVEL} = 0.002$ mg/kg/day (2×10^{-3} 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 gastro-intestinal 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 methyl-mercury 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 (Hg^+), and metallic mercury (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, methyl-mercury 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 necleolus).

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 2001e) 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 2001e) 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 that 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 methyl-mercury 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_{IMMUNO} 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_{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 methyl-mercury exposure.

Summary (TTDs for methylmercury)

TTD_{IMMUNO} = 0.0003 mg Hg/kg/day (3×10^{-4} mg/kg/day)

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

TTD_{REPRO} = 0.0004 mg Hg/kg/day (4×10^{-4} 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. 1998b).

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 hindrance 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 1994b) 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 1994b). 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 1994b). 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'-hexa-chlorobiphenyl 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 1994b; 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

1994b). 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 1994b; 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 1994b; 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. 1994a, 1996; Patandin et al. 1998, 1999a, 1999b) 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 1994b);

2. increased porphyrin levels in liver, urine, or kidneys in rats after intermediate exposure (ATSDR 2000; Safe 1994b);

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 1994b);

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 1994b);

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); and

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 qualitatitively 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, 1994b), 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 1994b; 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.

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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 1994b). 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 1994b). 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, 1994b).

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'-tetra-chlorobiphenyl, 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 θ 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 1994b) and porphyria and porphyria cutanea tardea (Franklin et al. 1997; Smith et al. 1990a, 1990b). For example, single intraperitioneal 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, 1994b; 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, 1994b). 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 Ganey 1995; Ganey 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 1994b), 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. 1996a). Structure activity relationships for PCB congeners and protein kinase C translocation in rat cerebellar granule cells and 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-tetrachloro-biphenyl 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, 1996a; 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 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-pentachloro-biphenyl) 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. 1997b). 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 (Ganey 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 (Ganey et al. 1993). The effects of 2,2',4,4'-tetrachlorbiphenyl 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 Ganey 1995). In addition, neutrophil degranulation induced by 2,2',4,4'-tetrachlorobiphenyl was enhanced by co-exposure with the calcium ionophore A23187 (Brown and Ganey 1995). A mono-ortho congener, 2,3,4,5-tetrachloro-biphenyl, 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 Ganey 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; Fischer et al. 1998; Gierthy et al. 1997; Li and Hansen 1997; Nesaretnam and Darbre 1997; Nesaretnam et al.1996; Seegal et al. 1997) and/or thyroid hormone disruption (Brouwer et al. 1998b; 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 1994b) 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. 1999b; 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 (Auerlich 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. 1998b). 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, 17β-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 17β-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'-tetra-chlorobiphenyl). 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 α and β, but a hydroxylated metabolite, 2,2',6,6'-tetrachloro-4'-hydroxy-biphenyl, competitively bound to estrogen receptor α 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 17β-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 17β-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. 1999b), 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. 1999b) 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. 1998b; 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 hyopothyroid 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 1999a; 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 1999a; 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 (Schuur 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; Schuur 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 (≥ 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 (≥ 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 (Desauliniers 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 (Desauliniers 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_4 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_4 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_4 to transthyretin, an important transport protein for both T_4 and T_3 (Chauhan et al. 2000; Cheek et al. 1999; Darnerud et al. 1996). Inhibition of binding of thyroid hormones to transthryetin could alter hormone delivery to target tissues, including the brain, and could also result in depressed levels of serum total TT_4 or TT_3 (Brouwer et al. 1998b).

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, ED50 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'-hexachloro-biphenyl) 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 ED50 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., ED50s 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 µmol/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 (1994b) 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, 1999a, 1999b; 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 perserverative 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 2001f). 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 (2000b, 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, hepato-cellular 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 $TTD_{HEPATIC}$ of 0.1 μg/kg/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 desquammation 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 $TTD_{ENDOCRINE}$ of 0.1 μg/kg/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 Ig M 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_{REPROD} of 0.2 µg/kg/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 perserverative 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, 1999a, 1999b; 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 µg/kg/day. This value is only slightly above the chronic oral MRL of 0.02 µg/kg/day based on impaired immune response in adult monkeys and is expected to be protective of neurological neurodevelopmental effects from chronic oral exposure to PCBs.

Summary (TTDs for PCBs)

$TTD_{HEPATIC} = 0.1$ μg/kg/day ($1x10^{-4}$ mg/kg/day)

$TTD_{ENDOCRINE} = 0.1$ μg/kg/day ($1x10^{-4}$ mg/kg/day)

$MRL_{(IMMUNO)} = 0.02$ μg/kg/day ($2x10^{-5}$ mg/kg/day; chronic MRL)

$MRL_{(NEURODEVELOP)} = 0.03$ μg/kg/day ($3x10^{-5}$ mg/kg/day; intermediate MRL)

$TTD_{REPRO} = 0.2$ μg/kg/day ($2x10^{-4}$ mg/kg/day)

Appendix F: Chemical Structures of Mixture Components
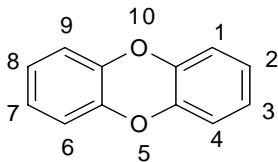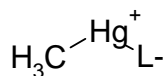

Chlorinated dibenzo-p-dioxins

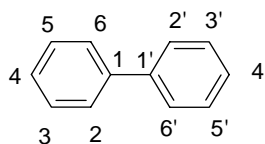Congeners can have chlorine substitutions at positions 1–4 and 6–9.
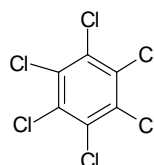
Hexachlorobenzene
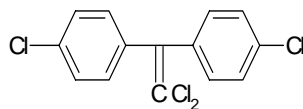
*p,p'*DDE

Methylmercury (L indicates a ligand.)

PCBs

Congeners can have chlorine substitutions at positions 2–6 and 2'–6'.