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 hexachlorobenzene is thought to be poorly absorbed by the respiratory tract (ATSDR 1996). Absorbed hexachlorobenzene is widely distributed in mammalian tissues with preferential distribution to tissues and fluids with high fat content (ATSDR 1996). Hexachlorobenzene is transferred via the placenta to the developing fetus and to suckling neonates via breast milk (ATSDR 1996). In humans, concentrations of hexachlorobenzene tend to increase with age, indicative of bioaccumulation (ATSDR 1996). Absorbed hexachlorobenzene is eliminated from the body predominately as metabolites in the urine, although unchanged hexachlorobenzene has been detected in urine of exposed animals (ATSDR 1996). Data from studies of humans and animals indicate that pentachlorophenol and pentachlorobenzene are the predominant metabolites of hexachlorobenzene, that minor metabolites include tetrachlorobenzene, tetrachlorophenols, chlorinated tetrahydroquinones, trichlorophenol, and derivatives of glutathione conjugates, and that hexachlorobenzene metabolism is slow (ATSDR 1996). Oxidative metabolism of hexachlorobenzene is thought to be catalyzed by cytochrome P450 enzymes (predominately by 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 bio-accumulates. van Birgelen (1998) proposed that a relative potency value of 0.0001 be used for hexa-chlorobenzene 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 extrapolation from animals to humans, 10 for human variability, and 3 for the use of a minimal LOAEL). LOAELs for developmental effects in intermediate and chronic studies ranged from 2 to 64 mg/kg/day (excluding liver effects in multigeneration studies, which were considered hepatic rather than developmental effects). Therefore, the acute oral MRL of 0.008 mg/kg/day should be protective for developmental effects by longer-term exposure as well, and is adopted as the $TTD_{DEVELOP}$ for hexachlorobenzene.

Summary (TTDs for hexachlorobenzene)

$MRL_{(HEPATIC)}$ = 0.00002 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 ($3x10^{-4}$ mg/kg/day)

$MRL_{(NEURODEVELOP)} = 0.0003$ mg Hg/kg/day ($3x10^{-4}$ mg/kg/day; chronic MRL)

$TTD_{REPRO} = 0.0004$ mg Hg/kg/day ($4x10^{-4}$ 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.

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'-tetra-chlorobiphenyl, 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
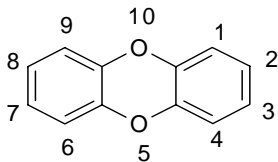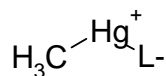

Chlorinated dibenzo-p-dioxins

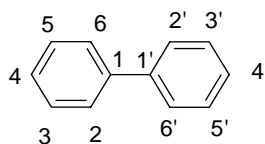Congeners can have chlorine substitutions at positions 1–4 and 6–9.
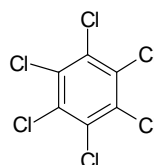
Hexachlorobenzene
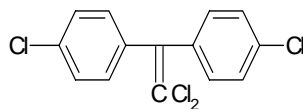
*p,p'*DDE

Methylmercury (L indicates a ligand.)

PCBs

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