

Appendix A: Background Information for CDDs

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 absorption can be influenced by the 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 to 10 years in humans (ATSDR 1998; Aylward et al. 2006), close to a year in monkeys, and 10–100 days in laboratory rodents (ATSDR 1998; Van den Berg et al. 1994). Because of the long half-life of most of the halogenated aromatic hydrocarbons, the chemicals persist in body for relatively long periods of time following single exposures.

A.1 Health Effects

Evidence of endocrine disruption includes alterations in thyroid hormone levels, estrogenic and antiandrogenic reproductive alterations, and impaired development of the reproductive system. Decreases in thyroxine levels have been observed in rats following acute (0.1–0.3 µg/kg) or intermediate (0.05–0.8 µg/kg) exposures to 2,3,7,8-TCDD (ATSDR 1998). In humans, reproductive effects most likely to be associated with endocrine disruption include alterations in sex ratios, primarily from paternal exposure

(ATSDR 1998; Mocarelli et al. 2000; Ryan et al. 2002); increased length of menstrual cycle resulting from pre-pubescent exposure (Eskenazi et al. 2002); increased age of menopause (Eskenazi et al. 2005); decreased serum testosterone levels; increased serum follicle-stimulating hormone; and increased luteinizing hormone in males (ATSDR 1998). Estrogenic effects observed in adults of several animal species exposed to 2,3,7,8-TCDD include reduced fertility, litter size, and uterine weights; endometriosis; suppression of the estrous cycle (10 µg/kg); delayed puberty; and premature reproductive senescence (ATSDR 1998; Franczak et al. 2006; Yang et al. 2000). Antiandrogenic effects have been observed in males of several animal species and include loss of germ cells, degeneration of spermatocytes and spermatozoa, and decreased reproductive capability (ATSDR 1998).

Impaired development of the reproductive system has been observed in male and female offspring of rats exposed to 2,3,7,8-TCDD during gestation and/or lactation. In the female offspring, accelerated onset of constant estrus, shortened reproductive lifespan, and external urogenital malformations (clefting, hypospadias, vaginal thread, and delayed vaginal opening) were observed after a single dose of 1 µg/kg administered on Gd 8 or 15 (ATSDR 1998). Alterations in androgen status (decreased plasma testosterone levels, delay in testes descent, delay in external signs of puberty, and decreased ventral prostate and seminal vesicle weights), testes and cauda epididymis weights, spermatogenesis (decreased daily sperm production, amount of mature sperm in cauda epididymis, and amount of sperm ejaculated), and demasculinization and partial feminization of sexual behavior have been observed in male offspring exposed; effects were observed at 0.064 µg/kg and higher administered on Gd 15 (ATSDR 1998).

Perinatal exposure in animals results in structural malformations, functional alterations, decreased growth, and fetal/newborn mortality; many of the effects occurred at 2,3,7,8-TCDD doses that were not maternally toxic. In addition to the reproductive effects previously discussed, observed developmental effects include fetal/newborn mortality (≥ 0.7 µg/kg) or decreased survival (≥ 0.00064 µg/kg) (ATSDR 1998), decreased fetal and newborn body weight (≥ 0.7 µg/kg) (ATSDR 1998), increased incidence of cleft palate and skeletal anomalies (≥ 0.1 µg/kg) (ATSDR 1998), hydronephrosis (≥ 0.5 µg/kg) (ATSDR 1998), immunotoxicity (thymic atrophy and immunosuppression) (≥ 1.5 µg/kg) (ATSDR 1998), altered learning and memory (≥ 0.1 µg/kg) (Markowski et al. 2002; Seo et al. 1999, 2000), altered brain development (≥ 0.1 µg/kg) (Hojo et al. 2006; Hood et al. 2006; Nishijo et al. 2007; Zareba et al. 2002), and altered social behavior (≥ 0.00012 µg/kg) (ATSDR 1998).

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maternally toxic. In addition to the reproductive effects previously discussed, observed developmental effects include fetal/newborn mortality ($\geq 0.7 \mu\text{g}/\text{kg}$) or decreased survival ($\geq 0.00064 \mu\text{g}/\text{kg}$) (ATSDR 1998), decreased fetal and newborn body weight ($\geq 0.7 \mu\text{g}/\text{kg}$) (ATSDR 1998), increased incidence of cleft palate and skeletal anomalies ($\geq 0.1 \mu\text{g}/\text{kg}$) (ATSDR 1998), hydronephrosis ($\geq 0.5 \mu\text{g}/\text{kg}$) (ATSDR 1998), immunotoxicity (thymic atrophy and immunosuppression) ($\geq 1.5 \mu\text{g}/\text{kg}$) (ATSDR 1998), altered learning and memory ($\geq 0.1 \mu\text{g}/\text{kg}$) (Markowski et al. 2002; Seo et al. 1999, 2000), altered brain development ($\geq 0.1 \mu\text{g}/\text{kg}$) (Hojo et al. 2006; Hood et al. 2006; Zareba et al. 2002), and altered social behavior ($\geq 0.00012 \mu\text{g}/\text{kg}$) (ATSDR 1998).

Studies of children of mothers with high background levels of CDDs, CDFs, and PCBs have found significant subclinical alterations in neurobehavioral outcomes, thyroid function, immune function and liver enzyme levels (ATSDR 1998); however, the correlation coefficients were low, suggesting that only a small amount of the variance can be attributed to CDDs and related compounds and it is not possible to determine the relative contribution of individual chemicals to the observed effects.

Recently, increased levels of TSH in newborns exposed to TCDD in utero in the Seveso cohort indicated possible problems with regulation of thyroid hormone metabolism (Baccarelli et al. 2008). The authors reported that the mean TCDD levels correlated with TSH levels above or below $5 \mu\text{U}$ per ml serum. The $5 \mu\text{U}/\text{ml}$ standard is significant as it was established by the WHO as an indicator of potential thyroid problems in neonates. The authors noted that higher TCDD exposures across all three zones showed increased TSH concentrations. The group mean of 39 ppt TCDD was associated with TSH levels above the standard.

A.2 Mechanisms of Action

CDDs 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- β 1) and increased expression of EGF receptor, and changes in thyroid hormone metabolism leading to lowered thyroid hormone levels (ATSDR 1998; Devito and Birnbaum 1994; Van den Berg et al. 1994). Many of the toxic and biological responses to CDDs 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

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 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 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- β 1) and increased expression of the EGF receptor, which subsequently lead to altered medial cell proliferation in the developing palatal shelves (Abbott et al. 1994). TCDD-induced disruptions in the development of the kidney and male and female reproductive organs observed in animal experiments are also thought to be associated with TCDD-induced changes in levels of growth factors and receptors (ATSDR 1998). The molecular events between TCDD-induced changes in gene expression and reduced levels of growth factors remain to be elucidated (ATSDR 1998).

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 metabolite and T₄ binding to plasma transport proteins (ATSDR 1998).

A.3 Health Guidelines

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, an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability), and a modifying factor of 0.7 (to adjust for the difference in higher bioavailability of 2,3,7,8-TCDD from an oil gavage vehicle than from food).

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 of 0.00012 µg/kg/day for neurodevelopmental 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. The International Agency for 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.” Subsequently, the U.S. National Toxicology Program (NTP) listed TCDD as known to be a human carcinogen in the January 2001 addendum to the Ninth Report on Carcinogens with the rationale

similar to that of the IARC. 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 U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) does not list a weight-of-evidence classification for 2,3,7,8-TCDD or other CDDs.

A.4 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to 2,3,7,8-TCDD were derived for thyroid, neurodevelopmental, and repro-developmental effects. Chronic oral TTDs for these endpoints are derived below, using the methods described by ATSDR (2001, Section 2.3.2). The derivations are based on data provided in the toxicological profile (ATSDR 1998), and in particular, the oral Levels of Significant Exposure (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.

Developmental Effects on Thyroid

Thyroid effects of 2,3,7,8-TCDD have been well studied, albeit primarily by acute and intermediate exposure (ATSDR 1998). Several studies reported effects in animals (Kociba et al. 1978; NTP 1982; Van Birgelen et al. 1995). On February 17, 2012, EPA released the dioxin health hazard (re)assessment for noncarcinogenic effects (IRIS 2012). The chronic oral RfD was listed as 0.7 pg/kg/day. The RfD is based on two studies using the cohorts exposed in Seveso during the industrial accident. One of them reported increased TSH in neonates exposed in utero (Baccarelli et al. 2008). LOAELs of 0.02 ng/kg/day were modeled from internal doses (blood levels), and an uncertainty factor of 30 was used in the RfD derivation. The RfD of 0.7 pg/kg/day (0.7×10^{-9} mg/kg/day) can be used as a $TTD_{\text{thyroid-develop}}$ for 2,3,7,8-TCDD.

Neurodevelopmental Effects

As described in Section A.4 above, the chronic oral MRL for 2,3,7,8-TCDD (ATSDR 1998) is based on neurodevelopmental effects (changes in social behavior in offspring) in monkeys. Therefore, the $TTD_{\text{neurodevelop}}$ for 2,3,7,8-TCDD is the chronic oral MRL of 0.000001 $\mu\text{g/kg/day}$ (1×10^{-9} mg/kg/day).

Developmental Effects on Reproductive Endpoints

A number of studies have found that gestational exposure to 2,3,7,8-TCDD affects androgen levels, secondary sex organs, spermatogenesis, fertility, and sexual behavior in the offspring (ATSDR 1998). In 2012, EPA released the dioxin health hazard (re)assessment for noncarcinogenic effects (IRIS 2012). The chronic oral RfD was listed as 0.7 pg/kg/day. The RfD is based on two studies using the cohorts exposed in Seveso during the industrial accident (see also the thyroid effects). Decreased sperm count and mobility was found in men exposed to TCDD as boys (Mocarelli et al. 2008). LOAELs of 0.02 ng/kg/day were divided by an uncertainty factor of 30 to derive the RfD.

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

$TTD_{\text{thyroid}} = 0.0000007 \mu\text{g/kg/day}$ ($0.7 \times 10^{-9} \text{ mg/kg/day}$)

$MRL_{\text{neurodevelop}} = 0.000001 \mu\text{g/kg/day}$ ($1 \times 10^{-9} \text{ mg/kg/day}$)

$TTD_{\text{develop}} = 0.0000007 \mu\text{g/kg/day}$ ($0.7 \times 10^{-9} \text{ mg/kg/day}$)

A.6 References

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Appendix B: Background Information for PBDEs

PBDEs are brominated organic compounds used as flame retardant additives in plastics, textiles, and other materials. As additives, they are physically mixed into product applications, rather than chemically bound. Therefore, they have the potential to migrate from the plastic matrix into the environment when conditions are ideal. The structure of PBDEs is provided in Appendix D.

B.1 Toxicokinetics

No information was located regarding absorption of PBDEs in humans following oral exposure. Studies in animals indicate that decaBDE is poorly absorbed, whereas the lower PBDEs are readily absorbed. A study that compared the average tissue concentration following intravenous and oral administration of ^{14}C -labeled decaBDE to rats estimated that oral absorption was $0.33 \pm 0.19\%$ at the highest dietary level tested ($\approx 4,500$ mg/kg/day). Poor absorption was observed over a wide dose range (El Dareer et al. 1987; NTP 1986). A 21-day study with a pentaBDE mixture yielded ranges of 84.3–92.4% absorption for tetra- to hexaBDE congeners (Hakk et al. 2001), whereas a 21-day study with an octaBDE mixture estimated that 84.2–95.1% of the hexaBDEs, 68.5–79.1 of the heptaBDEs, and 55.7–83.3% of octaBDEs were absorbed (Huwe et al. 2002). Inhalation and dermal exposure to PBDEs are of lesser concern than oral exposure (because ingestion of PBDEs in food is thought to be the principal route of exposure for the general population), but limited information from a study in rats indicated that lower PBDEs from a commercial octaBDE aerosol can be absorbed through the lungs (Great Lakes Chemical Corporation 2001a, 2001b).

No studies were located regarding distribution of PBDEs in humans following any route of exposure. Acute-duration oral studies in rats administered ^{14}C -labeled decaBDE found only trace levels of radioactivity in any organ or tissue at any time point (El Dareer et al. 1987; NTP 1986). Analysis of all major organs and tissues in the NTP (1986) study found the highest levels of ^{14}C in the gastrointestinal tract, followed by liver, kidney, lung, skin, and adipose tissue. The results of a study in which synthetic ^{14}C -decaBDE (>98% pure) administered to rats by gavage in a vehicle formulated to enhance solubility and optimize absorption indicated that the highest concentrations of radioactivity were in plasma and blood-rich tissues and that decaBDE did not readily distribute to adipose tissue (Klasson Wehler et al. 2001; Morck and Klasson Wehler 2001; Morck et al. 2003). The investigators speculated that decaBDE does not partition into lipids and is transported through aqueous compartments (e.g., serum and bile) due to binding to transport proteins. Due to their relatively high lipid solubility, lower PBDEs preferentially

partition to lipid-rich tissues (Hakk et al. 1999, 2002) and, in general, the half-life of the congeners (20–120 days) increases with increasing bromination (von Meyerinck et al. 1990).

DecaBDE is poorly absorbed, so generally the analysis of feces of animals administered decaBDE orally reveals mostly unchanged parent compound. However, when given to intact and bile-duct cannulated rats in a vehicle formulated to enhance solubility and optimize absorption, significant metabolism could be demonstrated (Morck and Klasson Wehler 2001; Morck et al. 2003). In intact rats, approximately 90% of a single dose of ^{14}C -decaBDE was excreted in the feces in 3 days. In bile duct-cannulated rats, an average of 9.5% of the dose was excreted via the bile in 3 days, almost all of which represented metabolites. Metabolites were characterized as nonextractable, water-soluble, lipid-bound, phenolic metabolites, and parent compound/neutral metabolites. Another study with decaBDE in rats detected 13 phenolic metabolites in the plasma (Sandholm et al. 2003). The major metabolites were characterized as a hydroxyl-octaBDE, a hydroxyl-nonaBDE, and a hydroxyl-methoxy-hexaBDE. In addition to the debromination reactions, the presence of a methoxy group is suggestive of methylation, and possibly other metabolic processes by bacteria of the gut. A study of the metabolism of ^{14}C -2,2',4,4,5-pentaBDE in intact and bile duct-cannulated rats showed fecal metabolites that were incompletely identified as two mono-OH-pentaBDEs and two mono-OH-tetraBDEs, indicating that some debromination occurred (Hakk et al. 1999, 2002). Metabolites found in the bile included two mono-OH-pentaBDEs, three di-OH-pentaBDEs, and two possible thiol-substituted pentaBDEs. Evidence for reactive intermediates in the feces of normal rats was indicated by high nonextractable fractions ranging from 18 to 52%. A study of ^{14}C -2,2',4,4'-tetraBDE revealed six metabolites in the feces, but these were not precisely identified; the metabolites were tentatively characterized as hydroxylated derivatives (two *ortho*-, one *meta*-, and two *para*-OH-tetraBDEs) and a trace amount of a thiol-tetraBDE (Örn and Klasson-Wehler 1998). Feces from mice treated in the same manner contained the same six metabolites characterized in the rat feces, but the mice metabolized the chemical much more extensively as shown by significantly higher urinary radioactivity.

PBDEs are eliminated mainly in the feces. Studies with decaBDE in animals have reported that $\geq 90\%$ of the administered oral dose is eliminated in the feces within days of dosing (El Dareer et al. 1987; Klasson Wehler et al. 2001; Morck and Klasson Wehler 2001; Morck et al. 2003; NTP 1986). A 21-day dietary study in rats fed a commercial pentaBDE mixture reported that fecal excretion of five tetra- to hexaBDE congeners ranged from 7.6 to 15.8% of the dose (Hakk et al. 2001). A study of an octaBDE mixture reported that fecal excretion ranged from 4.9 to 15.9% of the dose for the hexaBDE congeners, 20.9–

31.5% of the dose for the heptaBDE congeners, and 16.7–44.3% of the dose for the octaBDE congeners (Huwe et al. 2002).

Due to their lipid solubility, PBDEs are excreted into breast milk. The predominant congener identified in milk from U.S. women was 2,2',4,4'-tetraBDE; other congeners detected were 2,2',4-triBDE, 2,4,4'-triBDE, 2,3',4,4'-tetraBDE, 2,2',3,4,4'-pentaBDE, 2,2',3,4,4'-pentaBDE, 2,2',4,4',6-pentaBDE, 2,2',3,4,4',5'-hexaBDE, 2,2',4,4',5,5'-hexaBDE, 2,2',4,4',5,6'-hexaBDE, and 2,2',3,4,4',5',6-heptaBDE (Schechter et al. 2003). DecaBDE was detected in only 7 out of 47 samples.

Estimates of PBDE serum concentrations among electronics-dismantling workers before and after exposure-free vacation (median duration 28 days, range 21–35 days) indicate that the higher brominated congeners have shorter half-lives than lower congeners (Sjödin et al. 1999). Although actual half-lives were not calculated, the data suggest that the half-lives of the lower PBDE congeners were <1 year. Hagmar et al. (2000) estimated a half-life of 96 days for 2,2',3,4,4',5',6-heptaBDE and 6.8 days for decaBDE in occupationally exposed workers.

B.2 Health Effects

The preponderance of health effects data on PBDEs is from studies of orally exposed laboratory animals (ATSDR 2004a). Based on the information summarized below, the animal data indicate that decaBDE is much less likely than lower PBDEs to cause health effects in humans, and that the thyroid and neurobehavioral development are main targets of concern for lower PBDEs in humans, although there is limited information on thyroid effects and no information on neurobehavioral effects in PBDE-exposed humans. Thyroid follicular cell hyperplasia (3,200 mg/kg/day, lowest dose tested) was reported in a 103-week study in mice dosed with a commercial decaBDE mixture (NTP 1986). Intermediate- and chronic-duration oral studies in rats and mice found that penta- and octaBDE commercial mixtures caused effects in the thyroid gland characterized by enlargement and histological alterations (100 mg/kg/day), as well as changes in serum levels of thyroid hormones (≥ 10 mg/kg/day) (WIL Research Laboratories 1984). Exposure to a commercial pentaBDE mixture on gestation day 6 through postnatal day 21 caused serum T₄ reductions at 30 mg/kg/day in maternal rats and ≥ 10 mg/kg/day in their fetuses and neonatal offspring (Zhou et al. 2002). Little information is available on potential neurotoxic effects of PBDEs. PBDEs have not been tested for neurotoxicity using comprehensive test batteries, and most studies used a single dose level of a single congener. Mild impairments in spontaneous motor behavior and learning and memory were found in mice that were exposed to single low doses of 2,2',4,4'-tetraBDE, 2,2',4,4',5-pentaBDE,

2,2',4,4',5,5'-hexaBDE, and/or 2,2',3,3',4,4',5,5',6,6'-decaBDE during perinatal and/or early postnatal periods and tested later in life. Concern for neurodevelopmental toxicity of PBDEs is further raised by the documented effects of lower brominated commercial mixtures on thyroid hormone homeostasis and critical involvement of thyroid hormones in central nervous system development.

Developmental toxicity studies have shown no evidence of teratogenicity of penta- and octaBDEs in rats and rabbits, although fetotoxic effects, including skeletal ossification variations have occurred at maternally toxic doses. No fetotoxic or teratogenic effects were induced in rats exposed to high, but not maternally toxic, doses of commercial decaBDE.

B.3 Mechanisms of Action

Structure-activity studies have shown that some PBDE congeners can bind to the AhR, although binding affinities and induction of AhR-mediated responses are very weak or negligible, particularly for commercial PBDE mixtures and environmentally relevant congeners. This seems to be related to their molecular arrangement in space, as the ether linkage introduces a high barrier to rotation of the aromatic rings and a 120° bend in the alignment of the biphenyl rings. This makes it difficult for PBDEs to assume a planar configuration.

PBDEs and dioxin-like activity. Tests of dibromo-substituted to heptabromo-substituted BDE in a recombinant H4II rat hepatoma cell line showing AhR-mediated expression of a luciferase reporter gene showed that the tested PBDE congeners were at least 200,000 times less potent than TCDD for inducing AhR-mediated gene expression (Meerts et al. 1998). A study aimed at determining whether PBDE congeners act as AhR agonists or antagonists at sequential stages of the AhR signal transduction pathway leading to CYP1A1 in rat hepatocytes showed that the relative induction potencies of the most active PBDEs toward CYP1A1 were $\approx 10^{-4}$ that of TCDD (similar to some mono-*ortho*-PCBs and two orders of magnitude less than those of coplanar PCBs), and the relative induction potency for the environmentally prominent congeners were essentially zero (Chen and Bunce 2001). Evaluation of the ability of a series of PBDE congeners and commercial mixtures to induce EROD activity in chick and rat hepatocytes, in liver cell lines from rainbow trout, rat, and human, and in a human intestinal cell line showed that congeners which are prominent in the environment were not inducers in any cell line (Chen et al. 2001). For those congeners that had measurable EROD induction activity, their relative potencies were 10^{-3} – 10^{-6} that of 2,3,7,8-TCDD. Limited information on structure-toxicity relationships showed that the potency of a series of PBDEs to inhibit the splenic PFC response to sheep red blood cell (SRBC) antigen paralleled

their potencies as inducers of hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and EROD (Howie et al. 1990). However, the resulting ranking order of potency did not follow the order that would have been expected for a response known to be AhR-mediated. For example, the laterally substituted congeners 3,3',4,4'-tetraCDE and 3,3',4,4',5-pentaCDE were less immunotoxic than their respective monoortho-substituted analogs; this was true also for their enzyme induction potencies. It appeared that increasing *ortho*-substitution was less effective in reducing the “dioxin-like” activity of these compounds. The investigators suggested that the ether bridge in the polyCDE molecules increases the bond length between the two phenyl rings, thus diminishing the effects of *ortho* substituents on the biochemical and toxic potencies of these compounds (Howie et al. 1990).

Following a recent evaluation of data concerning the ability of PBDEs to bind and activate the AhR and induce CYP1A1 activity, the WHO panel of experts concluded that PBDEs had negligible activity, and as such, should not be included in the TEQ scheme for evaluating dioxin-like toxicity (Van den Berg et al. 2006).

Thyroid effects. Studies in rats and mice have shown that the thyroid is a sensitive organ for PBDE toxicity (ATSDR 2004a). The main effects include (1) histological changes in the thyroid indicative of glandular stimulation (e.g., follicular cell hyperplasia similar to that induced by a hypothyroid state) and (2) decreased serum T₄ levels with no accompanying changes in serum thyroid stimulating hormone (TSH). Considering these data and the structural resemblance of PBDEs to T₄, it is hypothesized that, depending on dose, duration, and mixture/congener, PBDEs can disrupt the production, transport, and disposition of thyroid hormones.

The mechanism(s) by which PBDEs decrease serum T₄ levels is unclear. The apparent lack of effect of PBDEs on serum TSH suggests that direct effects on the thyroid leading to inhibition of T₄ synthesis are unlikely. PBDEs are hepatic microsomal enzyme inducers, but there is little evidence that increased enzyme activity leads to greater clearance of thyroid hormones. An indication that increased UDPGT activity may not be the main mechanism for the reduced T₄ levels is provided by a study that found that exposure to pentaBDE for 14 days caused serum T₄ reductions in both mice and rats with no effect on UDPGT activity in the mice, and increased UDPGT in the rats only at higher dose levels (Hallgren et al. 2001). In contrast, the decreases in serum T₄ correlated with the induction of microsomal phase I enzymes (EROD and MROD). As discussed below, increased microsomal enzyme activity could also increase the formation of hydroxylated PBDE metabolites that can bind to T₄ plasma transport proteins.

The possible interaction of PBDEs with T₄ binding to human transthyretin (TTR) was investigated in an *in vitro* competitive binding assay (Meerts et al. 1998, 2000). Tests of 17 congeners ranging from di- to heptaBDE, showed that none of the parent compounds competed with T₄ for binding to human TTR. Incubation of the congeners with rat liver microsomes induced by PB (CYP2B enriched), β-naphthoflavone (CYPIA enriched), or clofibrate (CYP4A3 enriched) indicated that metabolism is necessary to compete with T₄-TTR binding and that potency is likely to be both congener and metabolic enzyme-specific. The CYP2B-enriched liver microsomes were the most potent, causing 9 (diBDEs to pentaBDEs) of the 17 congeners to generate metabolites (not identified) that were effective in displacing T₄ from TTR (60% inhibition). No T₄-TTR inhibition occurred with the higher brominated diphenyl ethers, although it was not verified that these PBDEs were metabolized during the *in vitro* microsomal incubations. Three pure hydroxylated PBDEs, synthesized for their structural resemblance with the thyroid hormones 3,5-diiodothyronine (3,5-T₂), 3,3',5-triiodothyronine (T₃), and 3,3',5,5'-tetraiodothyronine (T₄), were also tested in the T₄-TTR competition binding assay. The relative potencies showed that the T₄-like (2,6-dibromo-4-[2,4,6-tribromophenoxy]phenol) and T₃-like (2-bromo-4-[2,4,6-tribromophenoxy]phenol) hydroxylated PBDEs were 1.42- and 1.22-fold more potent, respectively, than T₄, and the percentage competition at 500 nM exceeded that of the natural ligand. The results of this study suggest an important role for hydroxylation in the mechanism of thyroid toxicity.

Three hydroxylated PBDEs, the 4'-hydroxyl derivatives of 1,3,5-triBDE, 1,3,3',5'-tetraBDE, and 1,3,3',5,5'-pentaBDE, were tested for affinity to the human thyroid hormone receptor proteins THR-α and THR-β *in vitro* (Marsh et al. 1998). These congeners were tested because they theoretically show the highest structural similarity to T₄ and T₃. None of the hydroxylated derivatives effectively competed with the thyroid hormones for binding to either receptor (affinities were 4→1,000 times less than for T₄ and T₃). Because the tested congeners were the most likely to have affinity for the thyroid hormone receptor, it was speculated that other hydroxylated PBDE congeners will have even lower potential for receptor binding. DecaBDE (not hydroxylated) had no effect on thyroid hormone receptor-mediated transcriptional activation by T₃ in HeLaTRDR4-luc human cells; no other congeners were tested in this assay (Sakai et al. 2003).

Estrogenic and antiestrogenic activity. Endocrine disruption can also be caused by effects on the estrogen receptor. The estrogenic and antiestrogenic activities of several PBDE congeners and three hydroxylated PBDEs were assessed *in vitro* using human breast cell line assays based on ER-dependent luciferase reporter gene expression (Meerts et al. 2001). Eleven of 17 PBDE congeners showed estrogenic activity (dose-dependent luciferase induction) in the ER-CALUX assay with T47D.Luc cells,

although the most potent PBDE congeners had EC₅₀ values that were 250,000–390,000 times less potent than 17β-estradiol (E₂). In contrast, the hydroxylated PBDEs that had bromine substitution patterns similar to those of the thyroid hormones T₂ (3,5-diiodothyronine), T₃ (3,3',5-triiodothyronine) showed estrogenic potencies exceeding that of E₂ (no estrogenic activity was induced by the T₄-like hydroxylated PBDE). In the assay for antiestrogenic activity, only 2,2',4,4',5,5'-hexaBDE, 2,3,4,4',5,6-hexaBDE, and 2,3,3',4,4',5,6-heptaBDE were positive. Assays in ERα-specific and ERβ-specific human embryonic kidney cell lines showed that pure and hydroxylated congeners of PBDEs can be agonists of both ERα and ERβ receptors and that metabolism of PBDEs may produce more potent pseudoestrogens. The common structural features among the estrogenic PBDEs in this study are two *ortho* (2,6)-bromine atoms on one phenyl ring, at least one *para*-bromine atom (preferably on the same phenyl ring as the *ortho* bromines), and nonbrominated *ortho-meta* or *meta* carbons on the other phenyl ring (Meerts et al. 2001).

Neurodevelopmental effects. Neurodevelopmental alterations have been reported in mice that were neonatally or perinatally exposed to individual PBDE congeners, including 2,2',4,4'-tetraBDE, 2,2',4,4',5-pentaBDE, 2,2',4,4',5,5'-hexaBDE, and decaBDE (ATSDR 2004a). However, it should be noted that in these studies, the PBDE congeners were administered in a 20% fat emulsion vehicle, which would have greatly and artificially increased absorption, rendering the studies of questionable relevance to environmental exposures to PBDEs, especially with regard to decaBDE which is poorly absorbed under normal conditions. The main effects were observed at adulthood and included reduced spontaneous motor activity, impaired habituation capability, and learning impairment in a maze task. The mechanisms for these behavioral and cognitive effects have not been elucidated, but could include thyroid hormone disruption. Numerous studies of models of maternal hypothyroidism, hypothyroxinemia and congenital hypothyroidism suggest that the timing and severity of thyroid hormone insufficiency predicts the type and severity of neurological deficits (see Zoeller and Rovet 2004 for review).

PBDEs could also be altering neurotransmitter systems in the brain. For example, neonatal exposure to a single oral dose of 2,2',4,4',5-pentaBDE on postnatal 10 altered the behavioral response to nicotine, a cholinergic agent, in adult mice (Viberg et al. 2002a). Neonatal exposure to nicotine and adult exposure of the same animals to 2,2',4,4',5-pentaBDE also affected behavior in mice; the change was not seen in mice exposed only to 2,2',4,4',5-pentaBDE as adults or mice only exposed to nicotine as neonates (Ankarberg et al. 2001). Adult mice that were exposed to a single dose of 2,2',4,4',5,5'-hexaBDE on postnatal day 10 had a decrease in specific α-Bungarotoxin binding sites (cholinergic nicotinic receptors) in the brain hippocampus (Viberg et al. 2001, 2002b).

Disruption of second messenger communication is also a possibility (ATSDR 2004a). *In vitro* exposure to a commercial pentaBDE mixture or to 2,2',4,4'-tetraBDE stimulated arachidonic acid release in rat cerebellar granule neurons; this effect was not seen with a commercial octaBDE product. The release of arachidonic acid appeared to be mediated by the activation of both Ca⁺²-dependent and Ca⁺²-independent cytosolic phospholipase A₂. *In vitro* exposure to a penta mixture and to 2,2',4,4'-tetraBDE also caused translocation of protein kinase C, as indicated by increased phorbol ester binding; an octaBDE mixture did not induce this effect. Other effects of the penta mixture and 2,2',4,4'-tetraBDE included decreases in intracellular calcium buffering by microsomes and mitochondria. The tetra congener was generally more potent than the pentaBDE mixture (mainly comprised of tetra and penta congeners) in these tests.

B.4 Health Guidelines

ATSDR (2004a) derived an intermediate-duration inhalation MRL of 0.006 mg/m³ for lower PBDEs based on a NOAEL of 1.1 mg/m³ for changes in thyroid hormones in rats intermittently exposed to a commercial octaBDE product for 13 weeks. The MRL of 0.006 mg/m³ was derived by dividing the NOAEL_{HEC} of 0.53 mg/m³ by an uncertainty factor of 30 (3 for species to species extrapolation with dosimetric adjustments and 10 for human variability) and a modifying factor of 3 (for an incomplete database reflecting a single study in one species).

ATSDR (2004a) derived an acute-duration oral MRL of 0.03 mg/kg/day for lower PBDEs based on a NOAEL of 1 mg/kg/day for reduced serum levels of thyroid T₄ hormone in fetal rats that were exposed by gavage to a technical pentaBDE mixture on days 4–20 of gestation. The MRL was estimated by dividing the NOAEL by an uncertainty factor of 30 (10 for animal to human extrapolation and 3 for human variability). A factor of 10 was not used for human variability because the MRL is based on effects observed in a sensitive subgroup.

ATSDR (2004a) derived an intermediate-duration oral MRL of 0.007 mg/kg/day for lower PBDEs based on a LOAEL of 2 mg/kg/day for minimal liver effects (hepatocytomegaly, hepatocyte vacuolation) in rats exposed in the diet to a technical pentaBDE mixture for 90 days. The MRL was estimated by dividing the LOAEL by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for animal to human extrapolation, and 10 for human variability).

ATSDR (2004a) derived an acute-duration oral MRL of 10 mg/kg/day for decaBDE based on a NOAEL of 1,000 mg/kg/day for developmental toxicity in rats exposed by gavage to a commercial decaBDE

mixture for 19 days during gestation. The MRL was estimated by dividing the NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

EPA (IRIS 2007) derived an RfD of 0.01 mg/kg/day for decaBDE based on a NOEL of 1 mg/kg/day in a 2-year dietary study in rats. An uncertainty factor of 100 (10 each for intra and interspecies variability) was used.

EPA (IRIS 2007) derived an oral reference dose of 0.002 mg/kg/day for a commercial pentaBDE mixture based on a NOAEL of 1.77 mg/kg/day for hepatic enzyme induction in rats in a 90-day gavage study. An uncertainty factor of 1,000 (10 each for intra- and interspecies variability and 10 for subchronic to chronic extrapolation) was used.

EPA (IRIS 2007) derived an RfD of 0.003 mg/kg/day for a commercial octaBDE mixture based on a NOAEL of 3.13 mg/kg/day for hepatic enzyme induction and liver histopathology in rats in a 90-day gavage study. An uncertainty factor of 1,000 (10 each for intra- and interspecies variability and 10 for subchronic to chronic extrapolation) was used.

NTP (2004) and IARC (2006) do not include PBDEs in their listings of carcinogens. The EPA (IRIS 2007) has classified decaBDE in Group C, *possible human carcinogen*, based on no human data and limited evidence of carcinogenicity in animals. Based on updated guidelines for carcinogen risk assessment (EPA 2005), decaBDE is classified as a chemical for which there is *suggestive evidence of carcinogenic potential*. Quantitative estimates of carcinogenic risk are not available. The EPA (IRIS 2007) has classified nonaBDE, octaBDE, hexaBDE, pentaBDE, tetraBDE, triBDE, *p,p'*-diBDE, and *p*-BDE in Group D, *not classifiable as to human carcinogenicity*, based on no human data or animal data. Based on updated guidelines for carcinogen risk assessment (EPA 2005), these compounds are classified as chemicals for which there is *inadequate information to assess carcinogenic potential*.

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

The endpoints of concern for PBDEs in this mixture are thyroid, neurobehavioral, and developmental. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2004b). The derivations are based primarily on data provided in ATSDR (2004a), and in particular the LSE tables. As done in the toxicological profile for PBDEs (ATSDR 2004a), TTDs are derived separately for decaBDE and lower PBDEs.

Thyroid Effects (Adults)

DecaBDE. Dose-related increases in thyroid hyperplasia were reported for male Sprague-Dawley rats exposed orally to 80 and 800 mg/kg/day for 30 days (Norris et al. 1973, 1975b), but not in rats exposed to ≤ 90 mg/kg/day for 90 days, rats exposed to $\leq 8,000$ mg/kg/day for 13 weeks, or mice exposed to $\leq 9,500$ mg/kg/day for 13 weeks (IRDC 1976; NTP 1986). The occurrence of thyroid hyperplasia in the rats exposed to ≥ 80 mg/kg/day for 30 days could be related to the low purity composition of the older commercial decaBDE mixture tested by Norris et al. (1973, 1975a, 1975b) (i.e., 77.4% decaBDE, 21.8% nonaBDE, and 0.8% octaBDE, compared to the $\geq 94\%$ decaBDE composition used in the NTP studies. In chronic-duration studies, thyroid follicular cell hyperplasia was increased at $\geq 3,200$ mg/kg/day in male B6C3F₁ mice that were exposed to $\geq 94\%$ pure commercial decaBDE for 103 weeks (NTP 1986), making the 3,200 mg/kg/day dose level possibly a LOAEL for thyroid effects. Because none of these studies examined thyroid function, the true biological significance of the thyroid hyperplasia is difficult to ascertain. Therefore, it is inappropriate to derive a TTD_{THYROID} for decaBDE based on these data.

Lower PBDEs. No chronic-duration oral studies are available for lower PBDEs. Thyroid function (serum T₄ and T₃ levels, but not TSH) and thyroid gland morphology were assessed in a comprehensive 90-day feeding study of a commercial pentaBDE (DE-71) mixture in male and female Sprague-Dawley rats (WIL Research Laboratories 1984). Effects observed in both sexes included significantly reduced plasma T₄ levels at ≥ 10 mg/kg/day and increased follicular cell hyperplasia at 100 mg/kg/day; no significant thyroid-related changes occurred at 2 mg/kg/day. The thyroid hyperplasia was mild and transient as it was characterized as very slight in severity at all doses and was no longer observed at 24 weeks postexposure in any animals. This study was used to derive an intermediate-duration oral MRL of 0.007 mg/kg/day for pentaBDE based on a LOAEL of 2 mg/kg/day for liver effects. The decrease in T₄ at 10 mg/kg/day is a LOAEL for thyroid effects and the NOAEL is 2 mg/kg/day. Applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to the NOAEL results in a TTD_{THYROID} of 0.02 mg/kg/day for lower PBDEs.

Developmental Endocrine Effects

DecaDBE. The existing intermediate-duration oral MRL for decaBDE is based on a developmental study in rats exposed during pregnancy (Hardy et al. 2002). However, the highest dose tested in that study, 1,000 mg/kg/day, caused no significant alterations in the endpoints evaluated, which included

maternal clinical observations, maternal body weight/weight gain and food consumption, maternal gravid uterine and liver weights, maternal gross lesions, total number of corpora lutea, uterine implantations, early and late resorptions, viable and nonviable fetuses, and fetal weight and sex. In addition, fetuses were examined grossly (all fetuses) and evaluated for skeletal/cartilaginous malformations and ossification variations (approximately half of each litter), and evaluated for visceral malformations (remaining fetuses). Since a LOAEL was not identified, it is not appropriate to derive a $TTD_{\text{DEVELOPMENTAL}}$ for decaBDE based on these data.

Lower PBDEs. The existing acute-duration oral MRL for lower PBDEs is based on a developmental study in rats (Zhou et al. 2002) and can be used as $TTD_{\text{DEVELOPMENTAL}}$ for this mixture. In the study, female Long-Evans rats were administered 0, 1, 10, or 30 mg/kg/day of a technical pentaBDE mixture by gavage from gestation day 6 through postnatal day 21. Dams were sacrificed on gestation day 20 and postnatal day 22, and offspring were sacrificed on gestation day 20 and postnatal days 4, 14, 36, and 90. There were no exposure-related effects on maternal body weight gain, litter size, sex ratio, or offspring viability and growth as assessed by numbers of pups at birth and on postnatal days 4–21; body weight of pups on postnatal days 4–90; and eye opening status on postnatal days 11–18. Serum measurements of thyroid T_3 and T_4 hormone levels showed that serum T_4 was significantly reduced in the rat dams at 30 mg/kg/day (gestation day 20 and postnatal day 22) and offspring at ≥ 10 mg/kg/day (gestation day 20 and postnatal days 4 and 14). The MRL is based on a NOAEL of 1 mg/kg/day for alterations in T_4 in serum from gestation day 20 fetuses and was calculated by applying an uncertainty factor of 30 (10 for animal to human extrapolation and 3 for human variability) to the NOAEL. A factor of 10 was not used for human variability because the MRL is based on effects observed in a sensitive subgroup. The MRL, and $TTD_{\text{DEVELOPMENTAL}}$ for lower PBDEs is 0.03 mg/kg/day.

Neurobehavioral Effects

DecaBDE. No relevant information was located regarding neurobehavioral effects of commercial decaBDE mixtures in human or in animals. Therefore, a $TTD_{\text{NEUROBEHAVIORAL}}$ for a decaBDE mixture cannot be derived.

Lower PBDEs. The only relevant information regarding neurobehavioral effects of lower PBDEs is that available in three abstracts summarized in ATSDR (2004a). In these studies (MacPhail et al. 2003; Taylor et al. 2002, 2003), pregnant rats were exposed to a pentaBDE commercial mixture (DE-71) and neurobehavioral endpoints were evaluated in the offspring at various ages. The highest dose tested was

100 mg/kg/day. No treatment-related significant alterations in motor and sensory behavior were reported. It should be noted that these results, described without much detail in the abstracts, have not been published in full as to date. Therefore, it is inappropriate to use these data for TTD derivation. ATSDR (2004a) also summarizes a series of studies in which neurobehavioral tests were administered to adult mice that were exposed to single gavage doses of PBDE congeners as neonates. However, these studies used a high-fat solvent to dissolve PBDE congeners for administration, rendering these studies non-useful for predicting the effects associated with exposures under normal conditions.

In the absence of appropriate neurobehavioral data, the $TTD_{\text{DEVELOPMENTAL}}$ of 0.03 mg/kg/day can be used as $TTD_{\text{NEUROBEHAVIORAL}}$ for the following reasons. The $TTD_{\text{DEVELOPMENTAL}}$ is based on thyroid effects in rat offspring exposed *in utero* to technical pentaBDE mixture (Zhou et al. 2002). It is well known that thyroid hormones play a crucial role in the proper development of the nervous system (Zoeller and Rovet 2004). Uncorrected maternal hypothyroidism resulting in fetal hypothyroidism may result in impaired neurodevelopment of the fetus with severe long-lasting implications. For example, Pop et al. (1999) studied a cohort of 220 healthy children and found that children of women with FT_4 levels below the 5th and 10th percentiles at 12 weeks of gestation showed impaired psychomotor development at 10 months of age. In women with the lowest 10th percentile FT_4 concentrations at 12 weeks of gestation, maternal FT_4 concentrations was positively correlated with the children's psychomotor development. Haddow et al. (1999) measured TSH levels in serum collected from 25,216 women and found that the 7–9-year-old children of the 62 women with high TSH levels performed less well in 15 tests relating to intelligence, attention, language, reading ability, school performance, and visual-motor performance than children of women with normal TSH values. Their full-scale IQ scores on the Wechsler Intelligence Scale for Children averaged 4 points lower than those of the children of matched control women. Given this information, it seems appropriate to use the $TTD_{\text{DEVELOPMENTAL}}$ also as $TTD_{\text{NEUROBEHAVIORAL}}$. It should also be noted that in one of the abstracts that presented neurobehavioral data mentioned above (Taylor et al. 2002), no changes in motor and sensory behaviors were reported in offspring from rats exposed to a technical pentaBDE mixture during pregnancy at levels that did affect T_4 in the offspring, suggesting that a $TTD_{\text{DEVELOPMENTAL}}$ (specifically based on thyroid parameters) can be protective of neurobehavioral effects.

Summary (TTDs for DecaBDE)

TTD_{THYROID} = Not derived: thyroid hyperplasia was observed but thyroid function was not evaluated.

$TTD_{\text{DEVELOPMENTAL}}$ = Not derived: no evidence that deca-BDE causes developmental toxicity

$TTD_{NEUROBEHAVIORAL}$ = Not derived: no evidence that deca-BDE causes neurobehavioral effects under normal conditions of exposure where it is not appreciably absorbed

Summary (TTDs for lower PBDEs)

$TTD_{THYROID}$ = 0.02 mg/kg/day

$TTD_{DEVELOPMENTAL}$ = 0.03 mg/kg/day (based on thyroid development) = acute oral MRL

$TTD_{NEUROBEHAVIORAL}$ = 0.03 mg/kg/day (based on thyroid development)

B.6 References

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Appendix C: Background Information for Phthalates

Appendix C-1: Background Information for DEHP

DEHP is a synthetic chemical used as a plasticizer. DEHP is present in plastic products such as wall coverings, tablecloths, floor tiles, furniture upholstery, shower curtains, garden hoses, swimming pool liners, rainwear, baby pants, dolls, some toys, shoes, automobile upholstery and tops, packaging film and sheets, sheathing for wire and cable, medical tubing, and blood storage bags. In plastics, DEHP is physically mixed into the product, rather than chemically bound. Therefore, it has the potential to migrate from the plastic matrix into the surrounding media when conditions are appropriate (ATSDR 2002).

C-1.1 Toxicokinetics

Human data indicate that gastrointestinal absorption of DEHP and its metabolites might amount to approximately 20–25% of an orally-administered dose (ATSDR 2002). Trace amounts of DEHP might be absorbed through the skin. No human data were available regarding the toxicokinetics of inhaled DEHP, although some degree of absorption from respiratory tissues would be expected. Animal data generally support the human findings. DEHP is hydrolyzed in the small intestines and absorbed as MEHP and 2-ethylhexanol. At high concentrations, a limited amount of unchanged DEHP might be absorbed. The degree of gastrointestinal absorption varies among animal species and is apparently greater in rodents than in monkeys. Animal studies indicate that DEHP might be absorbed through the skin in minute quantities. Absorption via the respiratory tract has also been indicated, although quantitative absorption studies have not been published.

Limited human data from autopsies have indicated the presence of DEHP in adipose tissues and kidneys. Studies in animals have shown the liver, kidneys, and testes to be major distribution sites for DEHP metabolism or utilization. Metabolic pathways for DEHP involve a number of reactions. Hydrolytic cleavage of DEHP results in the formation of MEHP and 2-ethylhexanol. The esterases responsible for these hydrolytic steps are found in numerous body tissues, but highest levels occur in the pancreas (hydrolytic reactions occur more readily following oral exposure because of the high content of esteratic activity within the gastrointestinal tract). MEHP is further metabolized via numerous oxidative reactions, resulting in the formation of 30 or more metabolites, some of which can be conjugated with glucuronic acid for excretion. Oxidation of 2-ethylhexanol primarily yields 2-ethylhexanoic acid and several keto acid derivatives, which are excreted in the urine.

In orally-exposed humans, approximately 65% of DEHP metabolites are excreted in the urine as glucuronide conjugates. The aglycone moiety of these conjugates as well as the nonconjugated DEHP metabolites excreted by humans are similar to those found in urine and feces of laboratory animals, although relative proportions might differ with species, dose, and time. No studies were located regarding fecal excretion of DEHP metabolites in humans. However, significant amounts of DEHP were noted in the feces of animals given DEHP by the oral route; it presumably represents unmetabolized DEHP. MEHP and other metabolites were frequently found in feces of DEHP-exposed animals, in some cases associated with biliary excretion products.

Because of their lipophilic nature, both DEHP and MEHP can accumulate in breast milk and subsequently be transferred to suckling offspring. This has been directly demonstrated in animals. DEHP has been detected in human breast milk.

C-1.2 Health Effects

Limited information was located regarding effects of DEHP on the relevant endpoints evaluated in this mixture in humans. Rais-Bahrami et al. (2004) assessed the onset of puberty and sexual maturity in male and female adolescents who had been exposed to DEHP as neonates through extracorporeal membrane oxygenation. Other endpoints assessed included thyroid function as well as measurements of sex hormones. The results showed no significant adverse effects on physical growth and pubertal maturity; thyroid, liver, renal, and male and female gonadal functions were within normal range for age and sex distribution; exposure data were not available. A more recent study found that serum levels of DEHP were significantly higher in precocious girls compared with normal children and that DEHP in serum of precocious girls was positively correlated with the volume of the uterus and ovaries (Qiao et al. 2007). Information on thyroid function is also available in a recent small study of adult males in the United States that reported an inverse association between MEHP urinary concentration and free T₄ and T₃ levels (Meeker et al. (2007). Reduced follicle size and colloid density in the thyroid were reported in male and female rats dosed with 375 and 419 mg/kg/day DEHP, respectively, via the diet for 13 weeks (Poon et al. 1997), but no histopathological changes were observed in the thyroid from rats (at 939 mg/kg/day) and mice (at 1458 mg/kg/day) treated with DEHP in their feed for 2 years (David et al. 2000a, 2000b). Studies in animals, mostly rodents, have shown that DEHP induces abnormal development of the male reproductive tract following perinatal exposure (ATSDR 2002). A variety of effects have been observed in androgen-sensitive tissues of young male rats, including reduced (female-like) anogenital

distance and permanent nipples, vaginal pouch, penile morphological abnormalities, hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands. In general, these effects were reported after perinatal exposure to ≥ 300 mg/kg/day (ATSDR 2002). These morphological effects, as well as reduced fetal and neonatal testosterone levels and adult sexual behavioral changes in male rats following gestational and lactational exposure, are consistent with an antiandrogenic action of DEHP. A more recent study (Borch et al. 2006) reported that administration of 300 mg/kg/day DEHP to pregnant rats resulted in reduced steroidogenesis in fetal testes leading to low fetal testosterone levels. This was accompanied by alteration in the expression of a number of testicular mRNA steroidogenesis factors. Both function and development of the reproductive system were adversely affected in male offspring of rats that were orally exposed to DEHP in a two-generation study. The changes in the development, structure, and function of the male reproductive tract observed in various studies indicate that effects of DEHP on reproduction and development are interrelated. Perinatal exposure (GD 7 to PD 16) of Wistar rats to 10 mg/kg/day of DEHP by gavage caused decreased anogenital distance, increased incidence of nipple retention, reduced prostate weight, and mild dysgenesis of external genitalia in pups (Christiansen et al. 2010). Effects on female reproductive development have also been reported in recent studies. For example, Grande et al. (2006) reported that exposure of rats throughout gestation and lactation induced a significant delay in the age of pubertal onset in female offspring; these effects occurred at doses ≥ 15 mg/kg/day, but not at 5 mg/kg/day. In another recent study, exposure of prepubertal female rats to DEHP by inhalation advanced the age of vaginal opening and first estrus cycle (Ma et al. 2006). Developmental toxicity studies also have shown that gestational exposure to DEHP can be embryotoxic and teratogenic in rats and mice. A range of effects have been observed including intrauterine deaths, skeletal and cardiovascular malformations, neural tube closure defects, increased perinatal mortality, and developmental delays. No information was located regarding neurobehavioral effects of DEHP.

C-1.3 Mechanisms of Action

Male reproductive system development. Considerable research has been conducted to elucidate the mechanism(s) by which exposure to DEHP during gestation and lactation alters the development of the reproductive system in male rat offspring. The reported effects observed in androgen-sensitive tissues of male neonates and infants, including female-like anogenital distance and permanent nipples, vaginal pouch, penile morphological abnormalities, hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands (Gray et al. 1999, 2000; Moore et al. 2001; Parks et al. 2000) are consistent with an antiandrogenic action of DEHP. Consistent with this

view are the results of a study in which exposure to DEHP from gestation day 14 to postnatal day 3 caused significantly reduced testicular testosterone production and reduced testicular and whole-body testosterone levels in fetal and neonatal male rats (Parks et al. 2000). Histological examinations of the testes in these rats showed that DEHP induced increased numbers of multifocal areas of Leydig cell hyperplasia, as well as multinucleated gonocytes, at gestation day 20 and postnatal day 3. However, *in vitro* assays have shown that neither DEHP nor its metabolite MEHP displayed significant affinity for the human androgen receptor (Paganetto et al. 2000; Parks et al. 2000). The available evidence indicates that DEHP is not an androgen receptor antagonist, but itself acts as an antiandrogen during a critical stage of reproductive tract differentiation by reducing testosterone to female levels in the fetal male rat. Parks et al. (2000) hypothesized that DEHP, or a metabolite, reduces testosterone production either by directly acting on the Leydig cells to reduce testosterone synthesis, or by interfering with Sertoli cell paracrine factors that regulate Leydig cell differentiation and function. Regardless of the mechanism, if the Leydig cells in exposed males continue to divide rather than differentiate for only a brief period of sexual differentiation, this could delay the onset of Leydig cell testosterone production and lead to malformations of the reproductive tract, external genitalia, and other androgen-dependent tissues (e.g., nipples) (Parks et al. 2000). Recent studies have suggested that alterations in testicular testosterone production are, in turn, due to alterations in the expression of a number of mRNA steroidogenesis-related factors (Borch et al. 2006). In general, results from *in vivo* and *in vitro* studies indicate that DEHP has negligible estrogenic potency relative to the endogenous hormone, 17 β -estradiol.

Fetotoxicity/teratogenicity. The mechanism(s) of fetotoxicity/teratogenicity of DEHP has not been elucidated, but there are studies that sought to determine whether these effects are mediated by the Peroxisome Proliferator Activated Receptor (PPAR α). Peters et al. (1997) assessed pregnancy outcome in female F₄C57BL/6N x Sv/129, wild type (+/+), and PPAR α -null (-/-) mice on gestation days 10 and 18 after administration of DEHP by gavage on gestation days 8 and 9. PPAR α -null mice lack expression of PPAR α protein and are refractive to peroxisomal proliferators (Lee et al. 1995). Relative to controls, DEHP significantly decreased the percentage of live fetuses, increased the percentage of resorptions, decreased fetal weight, and increased the percentage of fetuses with external malformations in both mice strains. On gestation day 10, maternal liver CYP4A1 mRNA was significantly elevated in DEHP-treated (+/+) mice but not in (-/-) mice, consistent with their respective phenotype. Mean maternal liver metallothionein and zinc levels were significantly higher in DEHP-treated mice (both strains) compared to controls. Maternal plasma zinc was not significantly altered as a result of treatment with DEHP. Embryonic zinc was significantly reduced in conceptus from both mice strains. These findings indicated that DEHP-induced fetotoxicity and teratogenicity and altered zinc metabolism are not mediated through

PPAR α -dependent mechanisms, and that alterations in zinc metabolism might contribute to the mechanism underlying DEHP-induced fetotoxicity and teratogenicity.

C-1.4 Health Guidelines

ATSDR (2002) derived an MRL of 0.1 mg/kg/day for intermediate-duration oral exposure to DEHP based on a NOAEL of 14 mg/kg/day for decreased fertility in mice. This derivation used an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR (2002) derived an MRL of 0.06 mg/kg/day was derived for chronic-duration oral exposure to DEHP based on a NOAEL of 5.8 mg/kg/day for testicular pathology in male rats that were exposed to DEHP in the diet for up to 104 weeks in a chronic toxicity study. The chronic MRL was derived by dividing the 5.8 mg/kg/day NOAEL by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

EPA (IRIS 2007) derived a chronic oral RfD of 0.02 mg/kg/day for DEHP based on a LOAEL of 19 mg/kg/day for hepatic effects in guinea pigs fed a diet containing DEHP for 1 year. The RfD was derived by applying an uncertainty factor of 1,000 (10 each for inter and intraspecies extrapolation and 10 for using a LOAEL) to the LOAEL.

NTP (2004) determined that DEHP may reasonably be anticipated to be a human carcinogen. IARC (2006) has classified DEHP in Group 3, *cannot be classified as to its carcinogenicity to humans*. The EPA (IRIS 2007) classified DEHP in Group B2, *probable human carcinogen* based on inadequate data in humans and sufficient evidence in animal studies. Based on updated guidelines for carcinogen risk assessment (EPA 2005), this compound is classified as a chemical that is *likely to be carcinogenic to humans*.

C-1.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

The endpoints of concern for DEHP in this mixture are endocrine, neurobehavioral, and developmental. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2004a). The derivations are based primarily on data provided in ATSDR (2002), and in particular the LSE tables.

Thyroid Effects (Adults)

Limited information is available for humans. Rais-Bahrami et al. (2004) reported no alterations in thyroid function in a group of male and female adolescents who had been exposed to DEHP as neonates through extracorporeal membrane oxygenation. A study of adult males from the U.S. population reported an inverse association between the concentration of MEHP in the urine and free T₄ and T₃ levels, although the relationship did not appear to be linear when MEHP concentrations were categorized by quintiles (Meeker et al. 2007). The lowest LOAEL for thyroid effects in animals is 375 mg/kg/day for reduced follicle size and mild reduction in colloid density in male Sprague-Dawley rats in a 90-day study (Poon et al. 1997). A 2-year dietary study reported no gross or microscopic alterations in the thyroid from female Fisher 344 rats and female B6C3F1 mice that received doses of 939 and 1,458 mg/kg/day, respectively (David et al. 2000a, 2000b). Neither of these studies measured serum levels of thyroid hormones or TSH. Since no histological alterations were seen in the 2-year study, it would appear that the alterations seen in the 90-day study may have been transient and without long-lasting consequences for the animal. The NOAEL in the 90-day study was 38 mg/kg/day and can be used to derive a TTD_{THYROID}. Applying an uncertainty factor of 100 (10 animal to human extrapolation and 10 for human variability) to the NOAEL of 38 mg/kg/day results in a TTD_{THYROID} of 0.4 mg/kg/day for DEHP. An additional uncertainty factor to account for extrapolation from intermediate-duration to chronic-duration exposure is not necessary on the grounds that the effects observed after 90 days of exposure were not present after 2 years of exposure to significantly higher doses.

Developmental Endocrine Effects

The lowest LOAEL for developmental effects was identified in a recent study that evaluated reproductive development of female offspring from rats treated daily with doses of up to 405 mg/kg/day DEHP by gavage from gestation day 6 to lactation day 22 (Grande et al. 2006). A significant delay in the age at vaginal opening (approximately 2 days) was observed at ≥ 15 mg DEHP/kg/day, as well as a trend for a delay in the age at first estrus at ≥ 135 mg/kg/day (approximately 2 days); the NOAEL was 5 mg/kg/day. Applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to the NOAEL of 5 mg/kg/day results in a TTD_{DEVELOPMENTAL} of 0.05 mg/kg/day for DEHP.

Neurobehavioral Effects

A TTD_{NEUROBEHAVIORAL} cannot be derived due to the lack of information on the potential neurobehavioral effects of DEHP.

Summary (TTDs for DEHP)

TTD_{THYROID} = 0.4 mg/kg/day

TTD_{DEVELOPMENTAL} = 0.05 mg/kg/day (based on reproductive endocrine effects)

TTD_{NEUROBEHAVIORAL} = not derived

C-1.6 References

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Appendix C-2: Background Information for DBP

DBP is a synthetic chemical used as a plasticizer. The plastics that DBP is used most in are PVC plastics and nitrocellulose lacquers. These plastics are used to make products such as carpets, paints, glue, insect repellents, hair spray, nail polish, and rocket fuel. In plastics, DBP is physically mixed into the product, rather than chemically bound. Therefore, it has the potential to migrate from the plastic matrix into surrounding media under appropriate conditions (ATSDR 2001).

C-2.1 Toxicokinetics

The only information regarding toxicokinetics of DBP in humans is that from a study in which volunteers given an oral dose of 0.255 to 0.510 mg DBP excreted approximately 70% as mono butyl phthalate (MBP) in the urine after 24 hours (NTP 2000). This suggests that DBP is absorbed and metabolized (or metabolized and absorbed) by humans. Data from animal studies suggest that airborne DBP may be rapidly absorbed through the lungs and that oral doses are rapidly and extensively absorbed (ATSDR 2001). A study in rats showed that approximately 60% of a single dermal dose was absorbed during a 7-day period. Animal data suggest that following inhalation, oral, or dermal exposure, DBP is widely distributed throughout the body and does not accumulate in the body. There are no data on transplacental transfer or transfer via maternal milk. There is some evidence to suggest that DBP and its metabolites are rapidly cleared from the body. Thus, it is unlikely that DBP will be stored in maternal tissues and released during pregnancy or lactation. In animals, the metabolism of DBP proceeds mainly by hydrolysis of one butyl ester bond to yield MBP. The product that appears in the urine is mainly MBP conjugated with glucuronic acid, with lower levels of unconjugated MBP, various oxidation products of MBP, and a small amount of the free phthalic acid. Studies in rats, hamsters, and guinea pigs indicate that excretion of a single oral dose is essentially complete within 48 hours of dosing, mostly (63–97%) via the urine.

C-2.2 Health Effects

Limited information is available for humans regarding effects of DBP on the relevant endpoints evaluated in this mixture. A study reported that serum levels of DBP and DEHP were significantly higher in precocious girls compared with normal children and that DBP and DEHP in serum of precocious girls was positively correlated with the volume of the uterus and ovaries (Qiao et al. 2007). An additional

study found negative associations between urinary levels of MBP, the main metabolite of DBP, and serum levels of thyroid hormones in pregnant women during the second trimester, after adjusting for age, BMI, and gestation (Huang et al. 2007). In rats and mice, administration of up to 2,964 and 4,278 mg/kg/day DBP, respectively, in the diet for 13 weeks did not cause any significant gross or microscopic changes in the thyroid (NTP 1995). In another 90-day dietary study in rats, doses of 752 mg/kg/day DBP induced a significant decrease in total T₃, but did not affect total T₄ or the microscopic morphology of the thyroid gland; the NOAEL was 152 mg/kg/day (Schilling et al. 1992). Animal studies have also shown that acute- and intermediate-duration oral exposure to DBP causes a number of developmental effects, including increases in postimplantation losses, decreases in the number of live fetuses per litter, decreases in fetal/pup body weights, and increases in incidences of external, skeletal, and internal malformations (ATSDR 2001). The lowest levels at which these effects were seen varied widely. Decreases in the number of live pups/litter were seen following doses of 80 mg/kg/day in rats and 1,950 mg/kg/day in mice. Perinatal administration of DBP causes alterations in the development of the reproductive system of rodents (ATSDR 2001). Recent studies indicate that these effects can occur at relatively low levels of exposure. For example, administration of ≥ 2.5 mg/kg/day DBP during gestation and lactation reduced testicular spermatocyte development and mammary gland changes in male and female offspring on postnatal day 21 (Lee et al. 2004). In another study, perinatal administration of 12 mg/kg/day DBP to rats induced a significant delay in vaginal opening in female pups (Salazar et al. 2004). In yet another study, doses of 50 mg/kg/day DBP, but not 10 mg/kg/day, administered to rats on gestation days 12–19 significantly reduced fetal testicular testosterone (Lehmann et al. 2004). Higher doses, in the range 100–500 mg/kg/day, administered perinatally, induced a variety of effects in male offspring including decreased anogenital distance, retention of areolas or nipples, small sex accessory glands, and reduced testes weight, and also produced malformations of the reproductive tract (ATSDR 2001). Similarly, when the impact of DBP exposure during the masculinization programming window was tested in rats, DBP significantly decreased the penis size, the ventral prostate and seminal vesicles sizes, and reduced the anogenital distance (Macleod et al. 2010). No information was located regarding neurobehavioral effects of DBP.

C-2.3 Mechanisms of Action

The specific mechanisms by which DBP affects the development of the male reproductive system in animals have not been determined, but since the effects are similar to those induced by DEHP, the mechanisms discussed for DEHP may also be applicable to DBP (see Appendix C-1).

The results from several studies suggest that DBP has anti-androgenic properties (i.e., Ema et al. 1998, 2000; Mylchreest et al. 1999, 2000). This is further supported by the findings of similar, but not identical, effects from DBP exposure as from exposure to linuron, a known androgen receptor ligand (Gray et al. 1999). These effects included delayed preputial separation, reduced fertility, testicular atrophy, and reduced sperm production in treated males, and reduced anogenital distance, increased number of retained nipples, and decreased androgen-dependent tissue weights in male offspring (exposed *in utero* and via lactation only) of treated rats. However, these androgen-related effects do not appear to be mediated by interaction of DBP or its primary metabolite, MBP, with the androgen receptor (Mylchreest et al. 1998, 1999). Parks et al. (2000) hypothesized that the unchanged phthalate ester, or a metabolite, reduces testosterone production either by directly acting on the Leydig cells to reduce testosterone synthesis, or by interfering with Sertoli cell paracrine factors that regulate Leydig cell differentiation and function. Regardless of the mechanism, if the Leydig cells in exposed males continue to divide rather than differentiate for only a brief period of sexual differentiation, this could delay the onset of Leydig cell testosterone production and lead to malformations of the reproductive tract, external genitalia, and other androgen-dependent tissues (e.g., nipples) (Parks et al. 2000).

The results from *in vitro* and *in vivo* assays for estrogenicity have provided evidence of weak estrogenic activity for DBP. In one *in vitro* assay, DBP was approximately 10-million-fold less potent than 17 β -estradiol (Harris et al. 1997). In another *in vitro* assay, DBP was approximately 3,000-fold less potent than 17 β -estradiol (Zacharewski et al. 1998). The negative results obtained *in vivo* may be due, at least in part, to the presence *in vivo* of esterases that metabolize DBP to MBP, which has been reported not to interact with the estrogen receptor (Mylchreest et al. 1998).

C-2.4 Health Guidelines

ATSDR (2001) derived an acute-duration oral MRL of 0.5 mg/kg/day for DBP based on a NOAEL of 50 mg/kg/day for developmental effects in the offspring of rats exposed to DBP on gestational days 12–21. The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

EPA (IRIS 2007) derived an RfD of 0.1 mg/kg/day for DBP based on a NOAEL of 125 mg/kg/day for increased mortality in rats in a 1-year dietary study. An uncertainty factor of 1,000 was applied to the NOAEL (10 for animal to human extrapolation, 10 for human variability, and 10 for less than chronic duration study and study deficiencies).

NTP (2004) and IARC (2006) do not include DBP in their listings. The EPA (IRIS 2007) classified DBP in Group D, *not classifiable as to human carcinogenicity*, based on no human or animal data. Based on updated guidelines for carcinogen risk assessment (EPA 2005), this compound is classified as a chemical for which *there is inadequate information to assess carcinogenic potential*.

C-2.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

The endpoints of concern for DBP in this mixture are thyroid, neurobehavioral, and developmental. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2004a). The derivations are based primarily on data provided in ATSDR (2001), in particular the LSE tables, but more recent information regarding the end points of concern in this mixture that is likely to impact the existing MRLs has also been considered.

Thyroid Effects (adults)

A recent epidemiological study reported negative associations between urinary levels of MBP, the main metabolite of DBP, and serum levels of thyroid hormones in pregnant women during the second trimester, after adjusting for age, BMI, and gestation age (Huang et al. 2007). Exposure doses, however, were not available; thus, this study cannot be used for derivation of a TTD_{THYROID} . The lowest LOAEL from an animal study was 752 mg/kg/day for decreased total serum T_3 in rats in a 90-day dietary study (Schilling et al. 1992). The NOAEL was 152 mg/kg/day and can be used to derive a TTD_{THYROID} of 1.5 mg/kg/day by dividing the NOAEL of 152 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability). An additional uncertainty factor to account for extrapolation from intermediate-duration to chronic-duration exposures does not appear necessary based on the results of 2-year studies in rats and mice exposed to significantly higher doses of the related DEHP (David et al. 2000a, 2000b). These studies, although they did not monitor serum hormone levels, did not find gross or microscopic alterations in the thyroid or clinical signs of hypo- or hyperthyroidism in the animals.

Developmental Endocrine Effects

The lowest developmental LOAEL is from a study by Lee et al. (2004), who administered DBP in the diet to pregnant rats from gestation day 15 to postnatal day 21 and evaluated the development of the reproductive system of male and female pups until postnatal week 20. The dietary concentrations of DBP

were 0, 20, 200, 2,000, and 10,000 ppm. Treatment with DBP (all doses) caused vacuolar degeneration of the areolae of the mammary gland in males evaluated on postnatal week 11. The severity of the lesion was dose-related. Higher doses also disrupted female sexual differentiation as evidenced by histopathological alterations in the prepubertal mammary gland and changes in the pituitary weight and cell populations of pituitary hormones in the adult stage. According to the investigators, the changes in prepubertal and adult stage males suggested an organizational effect of DBP on the male endocrine system, possibly by affecting the hypothalamic-pituitary axis due to testosterone insufficiency. Since the lowest dietary concentration tested, 20 ppm (approximately 2.5 mg/kg/day, estimated by the investigators) caused changes of only minimal severity, it can be considered a minimal LOAEL. Applying an uncertainty factor of 300 (10 for animal to human extrapolation, 10 for human variability, and 3 for a minimal LOAEL) to the LOAEL of 2.5 mg/kg/day results in a $TTD_{DEVELOPMENTAL}$ of 0.008 mg/kg/day.

Neurobehavioral Effects

A $TTD_{NEUROBEHAVIORAL}$ cannot be derived due to the lack of information on the potential neurobehavioral effects of DBP.

Summary (TTDs for DBP)

$TTD_{THYROID} = 1.5$ mg/kg/day

$TTD_{DEVELOPMENTAL} = 0.008$ mg/kg/day (disruption of reproductive endocrine function)

$TTD_{NEUROBEHAVIORAL} =$ not available

C-2.6 References

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Appendix C-3: Background Information for DNOP

DNOP is a synthetic chemical used as a plasticizer. DNOP can be found in carpetback coating, packaging films, medical tubing and blood storage bags, floor tile, wire, cables, adhesives, and also in cosmetics and pesticides. In plastics, DNOP is physically mixed into the product, rather than chemically bound. Therefore, it has the potential to migrate from the plastic matrix into the environment when conditions are ideal (ATSDR 1997).

C-3.1 Toxicokinetics

No information was located regarding the toxicokinetics of DNOP in humans following any route of exposure or in animals following inhalation or dermal exposure. Oral studies in animals provide evidence of gastrointestinal absorption, but quantitative data are lacking on the rate and extent of absorption. A study of the distribution of DNOP in rats reported the identification of mono-octylphthalate in blood and testes within 1–24 hours (peak in plasma at 3 hours and at 6 hours in testes) after dosing, whereas a different study reported the identification of residues of DNOP in liver and adipose tissue. The metabolism of DNOP has been studied *in vivo* and *in vitro*, and the data indicate that, like most phthalate esters, DNOP can be hydrolyzed at one or both ester linkages to produce the monoester as well as phthalic acid (minor metabolite). As with other phthalates, subsequent oxidation of the remaining arylester to short-chain carboxyls, alcohols, and ketones has been demonstrated. Although one study seems to indicate that urine is the major elimination route of DNOP metabolites following oral exposure, no quantitative information on the rate and extent of excretion is available (ATSDR 1997).

C-3.2 Health Effects

No information was located regarding health effects of DNOP in humans. Limited information is available in animal studies regarding the relevant endpoints for DNOP in this mixture. Dietary administration of approximately 2,000 mg/kg/day DNOP (only dose level tested) for 3, 10, or 21 days to rats induced a significant decrease in serum T₄ (Hinton et al. 1986); serum levels of T₃ were not affected. Morphological alterations in the thyroid included an increase in the number and size of lysosomes, enlargement of the Golgi apparatus, and damage to the mitochondria. A 13-week dietary study in rats reported reductions in size of the thyroid follicles and mild decreases in colloid density at 350 mg/kg/day in males and 403 mg/kg/day in females (Poon et al. 1997). The respective NOAELs were 37 and

41 mg/kg/day. No studies were located to determine whether exposure to DNOP might cause neurobehavioral effects in humans or in animals. In a preliminary assessment of the developmental toxicity of DNOP, gavage administration of doses of 9,780 mg/kg/day (only dose level tested) to mice on gestation days 6–13 resulted in a significantly reduced number of pups born alive per litter and reduced pup weight gain (Hardin et al. 1987). However, the investigators noted that the statistical significance may have resulted more from exceptionally high concurrent controls for these two parameters than from chemical toxicity. In studies performed according to the NTP Continuous Breeding Protocol, administration of up to 7,460 mg/kg/day for 105 days DNOP to F₀ generation mice or up to 8,640 mg/kg/day for 85–105 days to the F₁ generation did not result in developmental alterations, as assessed by the number of live pups per litter, the proportion of pups born alive, pup sex ratio, or the live pup mean weight (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985).

C-3.3 Mechanisms of Action

No specific mechanism of toxicity has been identified for DNOP. DNOP does not appear to behave as a peroxisome proliferator, as do other phthalate esters (ATSDR 1997).

C-3.4 Health Guidelines

ATSDR (1997) derived an acute-duration oral MRL of 3 mg/kg/day for DNOP based on a LOAEL of 1,000 mg/kg/day for liver effects in rats administered DNOP by gavage daily for 14 days. The MRL was derived by dividing the LOAEL by an uncertainty factor of 300 (10 for animal to human extrapolation, 10 for human variability, and 3 for using a minimal LOAEL).

ATSDR (1997) derived an intermediate-duration oral MRL of 0.4 mg/kg/day for DNOP based on a NOAEL of 41 mg/kg/day for liver effects in rats exposed to DNOP in the diet for 13 weeks; the LOAEL was 403 mg/kg/day. The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

EPA (IRIS 2007) does not list an RfD of reference concentration (RfC) for DNOP.

NTP (2004) and IARC (2006) do not include DNOP in their listings. The EPA (IRIS 2007) has not classified DNOP as to its carcinogenicity.

C-3.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

The endpoints of concern for DNOP in this mixture are thyroid, neurobehavioral, and developmental. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2004a). The derivations are based primarily on data provided in ATSDR (1997), and in particular the LSE tables.

Thyroid Effects (Adults)

Sprague-Dawley rats exposed to up to 350 mg/kg/day DNOP (females) or up to 403 mg/kg/day (males for 13 weeks in the diet showed mild changes in the thyroid consisting of reduction in the follicle size and decreased colloid density (Poon et al. 1997). These dose levels were also LOAELs for liver effects, and an intermediate-duration oral MRL was based on a NOAEL of 41 mg/kg/day for liver effects in female rats. A TTD_{THYROID} can be derived by applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to the NOAEL of 41 mg/kg/day. The TTD_{THYROID} for DNOP is 0.4 mg/kg/day. An additional uncertainty factor to account for extrapolation from intermediate-duration to chronic-duration exposure is not necessary based on observations made in 2-year studies in rats and mice exposed to the related chemical, DEHP (David et al. 2000a, 2000b). These studies reported no gross or microscopic changes in the thyroid following exposure to dietary levels of DEHP significantly higher than those used by Poon et al. (1997) with DBP. In the 90-day study, Poon et al. (1997) also tested DEHP at levels comparable to DNOP and reported comparable effects (reduction in follicle size and decreased colloid density). Since no histological alterations were seen in the 2-year study with DEHP, it would appear that the thyroid alterations seen in the 90-day studies are transient and without long-lasting consequences for the animals.

Developmental Endocrine Effects

A preliminary assessment of the developmental toxicity of DNOP in mice reported a significantly reduced number of pups born alive per litter and reduced pup weight gain following maternal exposure to 9,780 mg/kg/day DNOP on gestation days 6–13 (Hardin et al. 1987). These results were considered inconclusive due to the unusually high values for these parameters in the control group. Studies that followed a continuous breeding protocol, also in mice, reported no developmental alterations in offspring from mice treated with up to 8,640 mg/kg/day DNOP (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). This dose level constitutes a NOAEL for developmental effects. The lack of studies identifying a

reliable LOAEL for developmental effects for DNOP precludes derivation of a $TTD_{DEVELOPMENTAL}$ for this chemical.

Neurobehavioral Effects

A $TTD_{NEUROBEHAVIORAL}$ cannot be derived due to the lack of information on the potential neurobehavioral effects of DNOP.

Summary (TTDs for DNOP)

$TTD_{THYROID} = 0.4 \text{ mg/kg/day}$

$TTD_{DEVELOPMENTAL} = \text{not derived}$

$TTD_{NEUROBEHAVIORAL} = \text{not derived}$

C-3.6 References

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Appendix C-4: Background Information for DEP

DEP is a synthetic chemical used as a plasticizer. DEP can be found in plastics used to manufacture toothbrushes, automobile parts, tools, toys, and food packaging. It is also used in cosmetics and pesticides. In plastics, DEP is physically mixed into the product, rather than chemically bound. Therefore, it has the potential to migrate from the plastic into the surrounding media when conditions are appropriate (ATSDR 1995).

C-4.1 Toxicokinetics

No information was located regarding the toxicokinetics of DEP in humans, with the exception of a report of 4.8% absorption after 72 hours following application to an *in vitro* preparation of human abdominal skin. Data in animals are limited. A single application of ¹⁴C-DEP to the skin of rats resulted in approximately 24% excretion of the applied radioactivity in the urine in 24 hours, indicating that at least, some absorption occurred. In seven days, total recovery of radioactivity in the urine and feces was about 50% of the applied dose. Dermally absorbed radioactivity derived from ¹⁴C-DEP was minimal (<0.5% of the applied dose) in tissues of rats 1 week after a single application, indicating virtually no accumulation under the conditions of the study. No data were located regarding absorption, distribution, or excretion of DEP or its metabolites in animals following inhalation or oral exposure. No *in vivo* studies were located regarding the metabolism of DEP in humans or animals. Hepatic and intestinal preparations from rats, ferrets, baboons, and humans showed that DEP is mono-deesterified and that the *in vitro* metabolism was qualitatively similar among the various preparations (ATSDR 1995).

C-4.2 Health Effects

No information was located regarding health effects of DEP in humans. Limited information is available in animal studies regarding the relevant endpoints for DEP in this mixture. Exposure of rats to DEP up 3,710 mg/kg/day in the diet for 2 or 16 weeks had no significant effect on the gross or microscopic appearance of the pituitary, adrenals, or thyroid glands (Brown et al. 1978). Serum hormone levels were not measured in this study. No studies were located to determine whether exposure to DEP might cause neurobehavioral effects in humans or in animals. In a preliminary assessment of the developmental toxicity of DEP, gavage administration of doses of 4,500 mg/kg/day (only dose level tested) to mice resulted in no evidence of developmental effects, as assessed by survival, birth weight, and neonatal weight gain (Hardin et al. 1987). Dietary treatment of pregnant rats with 3,210 mg/kg/day DEP during

gestation days 6–15 resulted in fetuses with a significantly increased number of skeletal variations, particularly rudimentary ribs on gestation day 20 (Field et al. 1993). This dose level also caused a significant reduction in maternal food consumption and weight gain during the treatment period; reduced food consumption was, according to the investigators, due to poor palatability of the food. The developmental NOAEL in this study was 1,910 mg/kg/day. In a continuous breeding study in mice, dietary administration of 3,250 mg/kg/day DEP to the parental generation did not alter the number of pups per litter, the proportion of pups alive, or the live pup birth weight (Lamb et al. 1987).

C-4.3 Mechanisms of Action

No specific mechanism of toxicity has been identified for DEP. DEP appears to be a weak peroxisome proliferator compared with other phthalate esters (ATSDR 1995).

C-4.4 Health Guidelines

ATSDR (1995) derived an acute-duration oral MRL of 7 mg/kg/day for DEP based on a minimal LOAEL of 2,000 mg/kg/day for reproductive effects in rats administered DEP by gavage for 2 days. The MRL was derived by dividing the LOAEL by an uncertainty factor of 300 (10 for animal to human extrapolation, 10 for human variability, and 3 for using a minimal LOAEL).

ATSDR (1995) derived an intermediate-duration oral MRL of 6 mg/kg/day for DEP based on a minimal LOAEL of 1,753 mg/kg/day for liver effects in rats exposed to DEP in the diet for 3 weeks. The MRL was derived by dividing the LOAEL by an uncertainty factor of 300 (10 for animal to human extrapolation, 10 for human variability, and 3 for using a minimal LOAEL).

EPA (IRIS 2007) derived an RfD of 0.8 mg/kg/day for DEP based on a NOAEL of 750 mg/kg/day for reduced growth rate, food consumption, and altered organ weight in rats in a subchronic feeding study. An uncertainty factor of 1,000 was used (10 each for inter and intraspecies extrapolation and 10 for using a subchronic study).

NTP (2004) and IARC (2006) do not include DEP in their listings. The EPA (IRIS 2007) has classified DEP in Group D, *not classifiable as to human carcinogenicity*, based on no human data and inadequate data in animals. Based on updated guidelines for carcinogen risk assessment (EPA 2005), DEP is classified as a chemical for which there is *inadequate information to assess carcinogenic potential*.

C-4.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

The endpoints of concern for DEP in this mixture are thyroid, neurobehavioral, and developmental. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2004a). The derivations are based primarily on data provided in ATSDR (1995), and in particular the LSE tables.

Thyroid Effects

The only relevant information located is that from an intermediate-duration dietary study in rats. That study identified a NOAEL of 3,710 mg/kg/day (the highest dose level tested) for gross and microscopic histology of the endocrine glands, including the thyroid (Brown et al. 1978). Because a LOAEL was not identified, a TTD_{THYROID} cannot be derived.

Developmental Endocrine Effects

The highest developmental NOAEL for DEP below a LOAEL is 1,910 mg/kg/day from a gestational exposure study in rats (Field et al 1993). In the group treated with doses of 3,210 mg/kg/day, there was a significant increase in the number of skeletal variations, particularly rudimentary ribs. However, since this dose level also caused a significant reduction in maternal food consumption and weight gain during the treatment period, and there is no evidence that the effect involved an endocrine-related mechanism, a $TTD_{\text{DEVELOPMENTAL}}$ will not be derived.

Neurobehavioral Effects

A $TTD_{\text{NEUROBEHAVIORAL}}$ cannot be derived due to the lack of information on the potential neurobehavioral effects of DEP.

Summary (TTDs for DEP)

TTD_{THYROID} = not available

$TTD_{\text{DEVELOPMENTAL}}$ = not available

$TTD_{\text{NEUROBEHAVIORAL}}$ = not available

C-4.6 References

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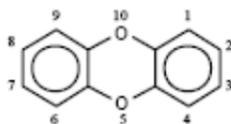
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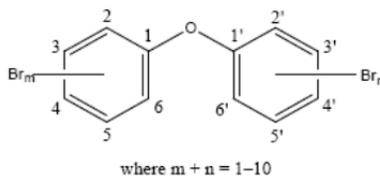
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Appendix D: Chemical Structures of Mixture Components

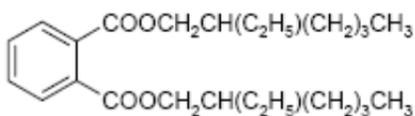
Chlorinated Dibenzo-p-Dioxins



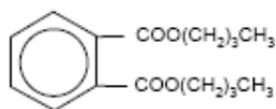
Polybrominated Diphenyl Ethers



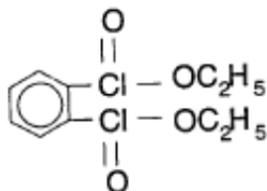
Di-2(ethylhexyl) phthalate



Di-n-Butyl phthalate



Diethyl phthalate



Di-n-octyl phthalate

