

## 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 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., octaCDDs) are poorly absorbed compared with less chlorinated CDDs such as tetraCDDs (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 the 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 T<sub>4</sub> 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 associated with endocrine disruption include alterations in sex ratios primarily resulting 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), earlier 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 ( $\geq 0.7$  µg/kg) or decreased survival ( $\geq 0.00064$  µg/kg) (ATSDR 1998), decreased fetal and newborn body weight ( $\geq 0.7$  µg/kg) (ATSDR 1998), increased incidence of cleft palate and skeletal anomalies ( $\geq 0.1$  µg/kg) (ATSDR 1998), hydronephrosis ( $\geq 0.5$  µg/kg) (ATSDR 1998), immunotoxicity (thymic atrophy and immunosuppression) ( $\geq 1.5$  µg/kg) (ATSDR 1998), altered learning and memory ( $\geq 0.1$  µg/kg) (Markowski et al. 2002; Seo et al. 1999, 2000), altered brain development ( $\geq 0.1$  µg/kg) (Hojo et al. 2006; Hood et al. 2006; Nishijo et al. 2007; Zareba et al. 2002), and altered social behavior ( $\geq 0.00012$  µg/kg) (ATSDR 1998).

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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 thyroid stimulating hormone (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 WHO as an indicator of potential thyroid problems in neonates. The authors noted that higher TCDD exposures across all three zones of different contamination showed increased TSH concentrations. The group mean of 39 ppt TCDD was associated with TSH levels above the standard. Plasma TCDD was 5.2 ppt (95% CI 4.1–6.7) in newborns with b-TSH  $\leq 5 \mu\text{U}/\text{ml}$  and 39.0 ppt (95% CI 8.9–173) in those with b-TSH  $> 5 \mu\text{U}/\text{ml}$  ( $p = 0.005$ ).”

## 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]- $\alpha$ , and TGF- $\beta$ 1) and increased expression of EGF receptor, and changes in thyroid hormone metabolism leading to lowered thyroid hormone levels (ATSDR 1998; Devito and Birnbaum 1994; Van den Berg et al. 1994). Many of the toxic and biological responses to CDDs are thought to be initially mediated through the binding of the parent compounds to a

soluble intracellular protein, the AhR (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-AhR 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 AhR 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 AhR and demonstrations that genetic differences in AhR 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 AhR. In this mechanistic scheme, the TCDD-AhR's transcriptional regulation of gene expression is thought to indirectly lead to reduced levels of several growth factors (EGF, TGF- $\alpha$ , and TGF- $\beta$ 1) and increased expression of the EGF receptor, which subsequently lead to altered medial cell proliferation in the developing palatal shelves (Abbott et al. 1994). TCDD-induced disruptions in the development of the kidney and male and female reproductive organs, observed in animal experiments, are also thought to be associated with TCDD-induced changes in levels of growth factors and receptors (ATSDR 1998). The molecular events between TCDD-induced changes in gene expression and reduced levels of growth factors remain to be elucidated (ATSDR 1998).

Changes in serum T<sub>4</sub> levels from acute exposure to 2,3,7,8-TCDD has been postulated to involve TCDD induction of UDP-glucuronyltransferase, through the AhR, and subsequently increased metabolism and clearance of T<sub>4</sub> (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 AhR 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 AhR mediation, such as interference, by TCDD metabolites, of T<sub>4</sub> 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 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 AhR 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 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 in ATSDR (2001, Section 2.3.2). The derivations are based on data provided in ATSDR (1998), and in particular, the oral Levels of Significant Exposure (LSE) table. 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.

##### **Thyroid Effects**

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 ( $0.7 \times 10^{-9}$  mg/kg/day) can be used as a  $TTD_{\text{THYROID-DEVELOPMENTAL}}$  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  $TTD_{\text{NEURODEVELOPMENTAL}}$  for 2,3,7,8-TCDD is the chronic oral MRL of 0.000001  $\mu\text{g/kg/day}$  ( $1 \times 10^{-9}$  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)

$TTD_{\text{THYROID}} = 0.00009 \mu\text{g/kg/day}$  ( $9 \times 10^{-8} \text{ mg/kg/day}$ )

Chronic oral MRL (based on neurodevelopmental effects) =  $0.000001 \mu\text{g/kg/day}$  ( $1 \times 10^{-9} \text{ mg/kg/day}$ )

$TTD_{\text{DEVELOPMENTAL}} = 0.00002 \mu\text{g/kg/day}$  ( $2 \times 10^{-8} \text{ mg/kg/day}$ )

## A.5 References

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## Appendix B: Background Information for PBDEs

PBDEs are brominated organic compounds that were previously 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 materials into the environment when conditions are ideal. The primary source for Appendix B information is the ATSDR (2017) Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs). The chemical structures of PBDEs are described in Appendix D.

### B.1 Toxicokinetics

No studies are available regarding the extent or rate of absorption of PBDEs in humans (ATSDR 2017). Information regarding oral absorption in animals is available from studies of commercial PBDE mixtures and individual <sup>14</sup>C-labeled tetra-, penta-, hexa-, and decaBDE congeners. As reviewed by ATSDR (2017), the most recent and best available estimates of oral absorption efficiencies following gavage administration in lipophilic vehicles indicate a range of 70–85% for tetra- (BDE-47), penta- (BDE-99, BDE-100), and hexa- (BDE-153, BDE-154) congeners, and 10–26% for decaBDE (BDE-209).

No studies were located that examined tissue distribution of PBDEs in humans following controlled oral exposure (ATSDR 2017). Evidence from studies evaluating maternal blood, cord blood, and breast milk from pregnant and nursing mothers exposed to environmental PBDEs (most likely via dust and food ingestion) indicated that PBDEs can transfer from the mother to the developing fetus or nursing infant (ATSDR 2017). In general, the tetra- and pentabrominated PBDEs have been the predominant congeners detected in maternal and cord serum samples and breast milk samples, but more recent studies assaying for a wider range of PBDE congeners found evidence for distribution of hepta-, octa-, or decaBDEs into cord serum and breast milk (ATSDR 2017).

Tissue distribution studies in animals orally exposed to <sup>14</sup>C-labeled BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-209 indicated that decaBDE is distributed among tissues somewhat differently than tetra-, penta-, and hexaBDEs (ATSDR 2017). While lower-brominated BDE congeners are preferentially accumulated in adipose tissues following absorption and an initial wide distribution, absorbed decaBDE is less readily distributed to adipose tissues and appears to preferentially distribute to highly perfused tissues. Although less likely to partition to adipose tissues, decaBDE was still found in

low quantities in adipose tissues in these studies, and has been shown to transfer from dams to fetuses and neonates from exposure during gestational and nursing periods (ATSDR 2017).

Oxidative hydroxylation of PBDEs is a principal metabolic transformation that occurs in humans and laboratory animals. Hydroxylated PBDEs have been identified in samples of human biological fluids, including blood and breast milk (ATSDR 2017). Hydroxylated PBDEs also have been identified in feces, carcasses, or bile of laboratory rodents exposed to <sup>14</sup>C-labeled tetra-, penta-, hexa-, or decaBDEs (ATSDR 2017).

Information from *in vivo* toxicokinetic studies with rodents is inadequate to describe detailed metabolic pathways, but is adequate to propose that cytochrome P450s are involved in the formation of hydroxylated metabolites and hydroxylated debrominated metabolites of BDE-47, BDE-99, BDE-100, BDE-154, and BDE-209 (ATSDR 2017). Recent *in vitro* studies with human or rat liver microsomes or hepatocytes, and human or rat recombinant CYPs, provide more detailed information adequate for proposing metabolic pathways for BDE-47, BDE-99, and BDE-100 in humans showing CYP2B6 mediation for hydroxylation, debromination, and ether bond cleavage for BDE-47, hydroxylation and ether bond cleavage for BDE-99, and hydroxylation for BDE-100 (ATSDR 2017). However, no clear metabolic pathways were identified for BDE-153 or BDE-209 using these methods (ATSDR 2017).

Apparent half-lives of PBDE congeners in blood of PBDE-exposed workers during non-exposed vacation periods ranged from 15 days for BDE-209, 18–39 days for nonabrominated congeners, and 37–94 days for octabrominated congeners (ATSDR 2017). The detection of PBDEs in human breast milk samples indicates that elimination via milk is an elimination route for nursing women, but several studies examining PBDE concentrations during lactation do not provide a clear account of the degree to which PBDEs are cleared from the body during lactation (ATSDR 2017). Results from animal studies given single oral doses of <sup>14</sup>C-labeled PBDE congeners or PBDE mixtures indicate that biliary excretion into the feces is the principal route of elimination in rats, and that the urine and feces are principal routes of elimination of orally absorbed PBDEs in mice (ATSDR 2017).

## **B.2 Health Effects**

Most health effects data on PBDEs are from studies of orally exposed laboratory animals and human studies in which the main exposure route was unknown, but expected to be oral (ATSDR 2017). Based

on a comprehensive evaluation of available human and animal data, ATSDR (2017) concluded that the following are targets of concern from oral exposure to PBDEs:

- the developing nervous system expressed as changes in neurobehavior (decaBDE and lower-brominated PBDEs);
- the developing and mature thyroid (decaBDE and especially lower-brominated PBDEs);
- the pancreas and its importance in insulin regulation (decaBDE and lower-brominated PBDEs);
- the developing and mature liver (decaBDE and lower-brominated PBDEs); and
- the developing male and female reproductive systems (decaBDE and especially lower-brominated PBDEs).

Information on carcinogenic effects of PBDEs in animals is limited to results from three chronic bioassays of decaBDE mixtures in rats and mice. Significantly increased incidences of neoplastic liver nodules in rats and combined hepatocellular adenomas and carcinomas in mice were reported in one of the three chronic bioassays. No carcinogenicity studies of lower-brominated PBDEs were located in the available literature. An EPA (2008) IRIS Toxicological Review concluded that the available data for decaPBDE provided *suggestive evidence of carcinogenic potential*, principally based on no studies of cancer in humans exposed to decaBDE and evidence for increased incidences of neoplastic nodules in rats and hepatocellular adenomas and carcinomas (combined) in mice exposed to decaBDE. The NTP (2016) 14<sup>th</sup> Report on Carcinogens does not contain a cancer classification for any PBDE.

## **B.2 Mechanisms of Action**

As reviewed by ATSDR (2017), the main targets of concern following PBDE exposure in humans are the developing nervous and reproductive systems, the developing and mature endocrine system, the liver, and the male reproductive system. Other potential targets are the female reproductive system, the adult nervous system, and the developing and adult immune system; however, ATSDR (2017) concluded that evidence for these endpoints is limited. ATSDR (2017) reviewed available mechanistic data related to general mechanisms (e.g., hepatic enzyme induction, AhR-mediated effects), endocrine disruption, and neurological effects and concluded that definitive mechanisms underlying these effects have not been elucidated. For other effects, including reproductive toxicity, immunotoxicity, and hepatotoxicity, only limited mechanistic data were available (ATSDR 2017).

**General Mechanisms of Toxicity.** The non-coplanar molecular characteristic of PBDEs, relative to the coplanar molecular characteristics of dioxins and dioxin-like compounds, accounts, at least in part, for marked differences in toxicological properties between dioxin-like halogenated compounds and PBDEs (ATSDR 2017). For example, 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 (ATSDR 2017). A WHO panel of experts concluded that PBDEs had negligible ability to bind and activate the AhR and induce CYP1A1 activity, and as such, should not be included in the TEQ scheme for evaluating dioxin-like toxicity (Van den Berg et al. 2006). Further information on the mechanisms by which PBDEs induce neurotoxic or hepatic effects were not available (ATSDR 2017).

**Endocrine Disruption Effects.** As reviewed in detail by ATSDR (2017), mechanistic studies show that PBDEs and/or their metabolites are capable of acting as thyroid hormone transporters or receptors and are weakly estrogenic, anti-androgenic, anti-prostagentic, and anti-glucocorticogenic. However, these findings were not always consistent between different congeners, metabolites, and studies. Therefore, ATSDR (2017) concluded that mechanisms of endocrine disruption by PBDEs have not been fully elucidated.

**Neurodevelopmental Effects.** Developmental exposure to PBDEs has been associated with altered neurodevelopment and behavior later in life in both humans and animals (ATSDR 2017). As reviewed in detail by ATSDR (2017), mechanisms for these behavioral and cognitive effects have not been elucidated; however, proposed mechanisms include neuroendocrine disruption (including altered thyroid hormone homeostasis), alterations in neurotransmitter systems (cholinergic, dopaminergic, glutamatergic, and/or GABAergic), altered calcium homeostatic mechanisms, altered intracellular communication, oxidative stress, and cell death. Additionally, monohydroxylated metabolites are more potent than the parent PBDEs in several of the mechanistic assays, suggesting that bioactivation by oxidative metabolism contributes to the neurotoxic potential of PBDEs (see ATSDR 2017 for more in-depth discussion).

#### **B.4 Health Guidelines**

ATSDR (2017) derived an intermediate-duration inhalation MRL of 0.006 mg/m<sup>3</sup> for lower PBDEs based on a NOAEL of 1.1 mg/m<sup>3</sup> for changes in thyroid hormones in rats intermittently exposed to a commercial octaBDE product for 13 weeks. The MRL of 0.006 mg/m<sup>3</sup> was derived by dividing the NOAEL<sub>HEC</sub> of 0.53 mg/m<sup>3</sup> 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 (2017) did not derive acute- or chronic-duration inhalation MRLs for lower PBDEs due to the lack of appropriate data. Likewise, ATSDR (2017) did not derive inhalation MRLs for decaBDE for acute, intermediate, or chronic durations of exposure.

ATSDR (2017) derived an acute-duration oral MRL of 0.00006 mg/kg/day for lower PBDEs based on a LOAEL of 0.06 mg/kg/day for multiple effects observed in rat dams and offspring following a single exposure to 2,2',4,4',5-pentaBDE (BDE-99) on GD 6 via gavage, including thyroid effects (decreased serum T<sub>4</sub> levels) in rat dams and developmental reproductive effects (male and female) and developmental neurobehavioral effects in adult F1 offspring (Kuriyama et al. 2005, 2007; Talsness et al. 2005). The MRL was estimated by dividing the 0.06 mg/kg LOAEL by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for animal to human extrapolation, and 10 for human variability).

ATSDR (2017) derived an intermediate-duration oral MRL of 0.000003 mg/kg/day for lower PBDEs based on a minimal LOAEL of 0.001 mg/kg/day for a 34% reduction in serum testosterone in male rats exposed to 2,2',4,4'-tetraBDE (BDE-47) for 8 weeks via gavage (Zhang et al. 2013b). The MRL was estimated by dividing the 0.001 mg/kg/day minimal 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 (2017) did not derive a chronic-duration oral MRL for lower-brominated PBDEs due to insufficient data.

ATSDR (2017) derived an acute-duration oral MRL of 0.01 mg/kg/day for decaBDE based on a NOAEL of 1.34 mg/kg for neurobehavioral effects in 2–4-month-old mice following a single exposure to 2,2',3,3',4,4',5,5',6,6'-decaBDE (BDE-209) on PND 3 (Buratovic et al. 2014; Johansson et al. 2008). The MRL was estimated by dividing the 1.34 mg/kg NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

ATSDR (2017) derived an intermediate-duration oral MRL of 0.0002 mg/kg/day for decaBDE based on a minimal LOAEL of 0.05 mg/kg/day for a 12% increase in serum glucose in adult rats exposed to 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE-209) for 8 weeks via gavage (Zhang et al. 2013a). The change in serum glucose level was taken as a minimally adverse sign of insulin dysregulation observed at higher dose levels. The MRL was estimated by dividing the 0.05 mg/kg/day 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 (2017) did not derive a chronic-duration oral MRL for decaBDE due to the lack of appropriate data. Data from the NTP (1986) 2-year dietary rat and mouse bioassays were not used as the basis for an MRL because the lowest tested dose was a LOAEL for precancerous liver lesions in male rats.

The EPA IRIS program derived oral RfDs for several individual PBDE congeners based on altered neurobehavior in 2–8-month-old mice following single exposure to individual congeners via gavage on PND 3 or 10 (IRIS 2008a, 2008b, 2008c, 2008d). An RfD of 0.0001 mg/kg/day was derived for 2,2',4,4',5-pentaBDE based on a  $BMDL_{1SD}$  of 0.29 mg/kg (IRIS 2008a); an RfD of 0.0001 mg/kg/day was derived for 2,2',4,4'-tetraBDE based on a  $BMDL_{1SD}$  of 0.35 mg/kg (IRIS 2008b); an RfD of 0.0002 mg/kg/day was derived for 2,2',4,4',5,5'-hexaBDE based on a NOAEL of 0.45 mg/kg (IRIS 2008c); and an RfD of 0.007 mg/kg/day was derived for 2,2',3,3',4,4',5,5',6,6'-decaBDE based on a NOAEL of 2.22 mg/kg (IRIS 2008d). For each congener, an uncertainty factor of 3,000 (10 for intraspecies variability, 10 for interspecies variability, 10 for extrapolating from animals to humans and 3 for extrapolating from single-dose to lifetime exposure) was used.

The EPA IRIS program also derived oral RfDs for two commercial PBDE mixtures based on hepatic enzyme inductions in rats in 90-day gavage studies (IRIS 2002, 2003b). An RfD of 0.002 mg/kg/day was derived for a commercial pentaBDE mixture based on a NOAEL of 1.77 mg/kg/day (IRIS 2002) and an RfD of 0.003 mg/kg/day was derived for a commercial octaBDE mixture based on a NOAEL of 3.13 mg/kg/day (IRIS 2003b). For each commercial mixture, an uncertainty factor of 1,000 (10 for intraspecies variability, 10 for interspecies variability, and 10 for subchronic to chronic extrapolation) was used.

NTP (2016) and IARC (2016) do not include PBDEs in their listings. The EPA IRIS program has evaluated several individual congeners for carcinogenic potential, and determined that there is *suggestive evidence of carcinogenic potential* for 2,2',3,3',4,4',5,5',6,6'-decaBDE based on no human data and limited evidence of carcinogenicity in animals (IRIS 2008d) and *inadequate information to assess carcinogenic potential* for 2,2',4,4'-tetraBDE, 2,2',4,4',5-pentaBDE, and 2,2',4,4',5,5'-hexaBDE (IRIS 2008 a, 2008b, 2008c). An oral slope factor of  $7 \times 10^{-4}$  per mg/kg-day and a drinking water unit risk of  $2 \times 10^{-8}$  per  $\mu\text{g/L}$  were derived based on the incidence of hepatic tumors in rats (IRIS 2008d); quantitative estimates of carcinogenic risk are not available for tetra-, penta-, or hexaBDE congeners. The EPA IRIS

program determined that commercial mixtures of nonaBDE, octaBDE, hexaBDE, pentaBDE, tetraBDE, triBDE, *p,p'*-diBDE, and *p*-BDE are *not classifiable as to human carcinogenicity*, based on no human data or animal data (2002a, 2002b, 2002c, 2003a, 2003b, 2003c, 2005, 2006). 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 Interaction Profile mixture of chemicals 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 (2017), in particular the LSE tables.

### Thyroid Effects (Adults)

**DecaBDE.** Dose-related increases in thyroid hyperplasia were reported for male Sprague-Dawley rats exposed to 80 and 800 mg/kg/day for 30 days (Norris et al. 1973, 1975b), but not in rats exposed to  $\leq 90$  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  $\geq 80$  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 B6C3F<sub>1</sub> 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 after repeated oral exposure to decaBDE is difficult to ascertain.

Decreased serum T<sub>4</sub> and T<sub>3</sub> levels were observed in male mice after 35-day exposures to 950 mg/kg/day decaBDE, but not to 750 mg/kg/day (Sarkar et al. 2015). Similarly, decreased serum T<sub>4</sub> levels were observed in pregnant mice given 1,500 mg/kg/day, but not 750 mg/kg/day, on GDs 7–9 (Chi et al., 2011). Serum T<sub>4</sub> levels were not changed in adult rats exposed to gavage doses up to 600 mg/kg/day decaBDE for up to 90 days (Lee et al. 2010; Van der ven et al. 2008; Wang et al. 2010, 2011; Zhou et al. 2001), and serum T<sub>3</sub> levels were not changed in adult male rats exposed to decaBDE at doses up to 100 mg/kg/day



via gavage for 90 days (Wang et al. 2010, 2011). Van der ven et al. (2008) reported significantly *increased* serum T<sub>3</sub> levels in adult female, but not male, rats exposed to decaBDE at 60 mg/kg/day (but not at 30 mg/kg/day) via gavage for 28 days; however, this finding is inconsistent with evidence of decreased serum T<sub>3</sub> levels in other studies of decaBDE and lower PBDEs. Additionally, Van der ven et al. (2008) indicate that there are “no observations to explain” elevated T<sub>3</sub> levels. Therefore, this study was not considered for derivation of the oral TTD<sub>THYROID</sub>.

The lowest LOAEL for significant decreases in serum levels of thyroid hormones in adult animals among these oral studies is 950 mg/kg/day for decreased serum T<sub>3</sub> and T<sub>4</sub> levels in adult male mice (Sarkar et al. 2015). The associated NOAEL of 750 mg/kg/day serves as the point of departure (POD) for the oral TTD<sub>THYROID</sub> for decaPBDE. Dividing the POD by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) arrives at a value of 7.5 mg/kg/day decaBDE.

**Lower PBDEs.** The acute MRL of 0.00006 mg/kg/day is adopted for the oral TTD<sub>THYROID</sub> for lower PBDEs. The acute-duration oral MRL is based on a LOAEL for decreased serum T<sub>4</sub> levels in rat dams exposed to 0.06 mg/kg pentaBDE on GD 6 via gavage (as well as for developmental reproductive and neurobehavioral effects in adult F1 offspring) (ATSDR 2017). While this is a single-exposure study, the LOAEL is markedly lower than the lowest LOAELs for thyroid effects in available repeated-dose studies in adult animals exposed to lower PBDEs, which were  $\geq 3$  mg/kg/day (ATSDR 2017). Based on available data, the acute-duration study is the most sensitive study on which to base the oral TTD<sub>THYROID</sub>.

## **Developmental Endocrine Effects**

**DecaBDE.** Testicular lesions (increased incidence of slight/moderate vacuolization in interstitial cells) were observed in adult male offspring of mouse dams exposed to decaBDE at >10 mg/kg/day (lowest dose) GDs 0–17 (Tseng et al. 2013). Additional effects observed at 1,500 mg/kg/day included slight-severe vacuolization in seminiferous tubules, almost complete loss of spermatozoa and spermatids in seminiferous tubules, and increased abnormal sperm heads (Tseng et al. 2013). In other studies, no exposure-related changes were observed in male or female reproductive development in rats following gestational and lactation exposure to doses up to 1,000 mg/kg/day (anogenital distance [AGD], onset of puberty [preputial separation or vaginal opening], estrous parameters, and/or reproductive organ weight and histology) (Biesecker et al. 2011; Fujimoto et al. 2011) or in mice following early postnatal exposure up to 30 mg/kg/day (AGD or onset of puberty) (Reverte et al. 2014; Rice et al. 2007).

Disruption in thyroid homeostasis occurred in developing animals following exposure to decaBDE, but the lowest LOAELs were higher than those observed for developmental reproductive effects. In young male rats exposed to decaBDE on PNDs 10–42, serum levels of T<sub>3</sub> and T<sub>4</sub> were significantly decreased at doses as low as 100 mg/kg/day, the lowest dose tested (Lee et al., 2010). In addition, decreased serum T<sub>3</sub> levels were decreased in offspring exposed during gestation and lactation to doses as low as 146 mg/kg/day decaBDE in rats and 1,500 mg/kg/day in mice; no changes were observed in serum T<sub>4</sub> or TSH (Fujimoto et al. 2011; Tseng et al. 2008).

The lowest LOAEL for altered endocrine development among these oral studies is 10 mg/kg/day for testicular lesions in adult male offspring of pregnant mice exposed on GDs 0–17 (Tseng et al. 2013). This LOAEL serves as the POD for the oral TTD<sub>DEVELOPMENTAL</sub> for decaPBDE. Dividing the POD by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) arrives at a value of 0.01 mg/kg/day decaBDE.

**Lower PBDEs.** The acute MRL of 0.00006 mg/kg/day is adopted for the oral TTD<sub>DEVELOPMENTAL</sub> for lower PBDEs. The acute-duration oral MRL is based on a LOAEL for developmental reproductive effects in adult F1 offspring of rat dams exposed to 0.06 mg/kg pentaBDE on GD 6 via gavage (as well as for decreased serum T<sub>4</sub> levels in rat dams and neurobehavioral effects in adult F1 offspring) (ATSDR 2017). While this is a single-exposure study, the LOAEL is markedly lower than the lowest LOAELs for developmental reproductive effects reported in repeated-dose studies in developing animals ( $\geq 10.2$  mg/kg/day) and lower than developmental thyroid effects in available pre- and peri-natal exposure studies ( $\geq 0.3$  mg/kg/day) (ATSDR 2017). Based on available data, the acute-duration study is the most sensitive study on which to base the oral TTD<sub>DEVELOPMENTAL</sub>.

## Neurobehavioral Effects

**DecaBDE.** The acute MRL of 0.01 mg/kg/day is adopted for the oral TTD<sub>NEUROBEHAVIORAL</sub> for decaBDE. The acute-duration oral MRL for decaBDE is based on a NOAEL of 1.34 mg/kg for neurobehavioral effects in 2–4-month-old mice following a single exposure to decaBDE on PND 3 (ATSDR 2017). While this is a single-exposure study, the lowest LOAEL for neurobehavioral changes following repeated exposures during early postnatal development was 20 mg/kg/day (delayed ontogeny of reflexes, increased locomotion), with an associated NOAEL of 6 mg/kg/day (Rice et al. 2007). Based on available data, the acute-duration study is the most sensitive study on which to base the oral TTD<sub>NEUROBEHAVIORAL</sub>.

**Lower PBDEs.** Animal studies reported neurodevelopmental effects following pre- and peri-natal exposures to lower-brominated PBDEs (tetra or penta BDEs) at doses ranging from 0.03 to 18 mg/kg/day, including neurobehavioral alterations, delayed ontogeny of reflexes, impaired learning, biochemical changes in the hippocampus, and decreased sociability (Blanco et al. 2013; Branchi et al. 2005; Cheng et al. 2009; Koenig et al. 2012; Ta et al. 2011; Woods et al. 2012). The lowest LOAEL value of 0.03 mg/kg/day is selected as the POD for the intermediate-duration  $TTD_{NEUROBEHAVIORAL}$  for lower PBDEs. Three studies identified this value as a LOAEL for neurobehavioral effects: (1) decreased performance in open field tests in PND 60 female offspring of mouse dams exposed to tetraBDE from pre-mating day 28 to PND 2 (Ta et al. 2011); (2) decreased vocalization at PNDs 8–10 and decreased sociability at PND 72 in offspring of mouse dams exposed to tetraBDE from PMD 28 to PND 21 (Woods et al. 2012); and (3) transient changes in open field behavior in PND 34 offspring of mouse dams exposed to pentaBDE between GD 6 and PND 21 (Branchi et al. 2005). The intermediate-duration  $TTD_{NEUROBEHAVIORAL}$  of 0.00003 for lower PBDEs was derived by dividing the POD of 0.03 by an uncertainty factor of 1,000 (10 for using a LOAEL, 10 for animal to human extrapolation, and 10 for human variability).

## TTD Summary

### *DecaBDE*

**$TTD_{THYROID}$ :** 7.5 mg/kg/day

**$TTD_{DEVELOPMENTAL}$ :** 0.01 mg/kg/day

**$TTD_{NEUROBEHAVIORAL}$ :** 0.01 mg/kg/day (acute MRL)

### *Lower PBDEs*

**$TTD_{THYROID}$  = 0.00006 mg/kg/day (acute MRL)**

**$TTD_{DEVELOPMENTAL}$  = 0.00006 mg/kg/day (acute MRL)**

**$TTD_{NEUROBEHAVIORAL}$  = 0.00003 mg/kg/day**

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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 intestine 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; this 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 humans for this Interaction Profile mixture of chemicals. 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 T<sub>4</sub> and T<sub>3</sub> 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 and mice treated with much higher doses 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) AGD 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  $\geq 300$  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 AGD, 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 an earlier start of first estrus in female offspring; these effects occurred at doses  $\geq 15$  mg/kg/day, but not at 5 mg/kg/day. In another recent study, exposure of prepubertal female rats to DEHP by inhalation caused earlier onset 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 AGD 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 GD 14 to PND 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 GD 20 and PND 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 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 $\beta$ -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 PPAR- $\alpha$  (PPAR $\alpha$ ). Peters et al. (1997) assessed pregnancy outcome in female F<sub>4</sub>C57BL/6N x Sv/129, wild type (+/+), and PPAR $\alpha$ -null (-/-) mice on GDs 10 and 18 after administration of DEHP by gavage on GDs 8 and 9. PPAR $\alpha$ -null mice lack expression of PPAR $\alpha$  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 GD 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 $\alpha$ -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 for interspecies and 10 for 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 Interaction Profile mixture of chemicals 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 in 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 T<sub>4</sub> and T<sub>3</sub> 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 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 rats and 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<sub>THYROID</sub>. Applying an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability) to the NOAEL of 38 mg/kg/day results in a TTD<sub>THYROID</sub> 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 GD 6 to lactation day 22 (Grande et al. 2006). A significant delay in the age at vaginal opening (approximately 2 days) was observed at  $\geq 15$  mg DEHP/kg/day, as well as a trend for a delay in the age at first estrus at  $\geq 135$  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<sub>DEVELOPMENTAL</sub> 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)

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

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

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

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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 polyvinyl chloride (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 from a study in which volunteers given an oral dose of 0.255–0.510 mg DBP subsequently excreted approximately 70% as MBP in the urine after 24 hours (NTP 2000). This suggests that DBP is absorbed and metabolized (or metabolized and absorbed) by humans. Data in animals 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 by 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 for the mixture of chemicals in this IP. 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). A recent systematic review and meta-analysis study reported that exposure of DEHP and DBP might be associated with precocious puberty risk for girls. The authors indicated that the association is of



“moderate strength” (Wen et al. 2015). 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, body mass index, 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 T<sub>3</sub>, but did not affect total T<sub>4</sub> 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  $\geq 2.5$  mg/kg/day DBP during gestation and lactation reduced testicular spermatocyte development and mammary gland changes in male and female offspring on PND 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 GDs 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 AGD, retention of areolas or nipples, small sex accessory glands, and reduced testes weight, and 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 and the ventral prostate and seminal vesicles sizes, and reduced AGD (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 are 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 AGD, 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 $\beta$ -estradiol (Harris et al. 1997). In another *in vitro* assay, DBP was approximately 3,000-fold less potent than 17 $\beta$ -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 GDs 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 the mixture of chemicals in this Interaction Profile 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 endpoints 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, body mass index, and gestation (Huang et al. 2007). Exposure doses, however, were not available; thus, this study cannot be used for derivation of a  $TTD_{\text{THYROID}}$ . 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  $TTD_{\text{THYROID}}$  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 GD 15 to PND 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 caused vacuolar degeneration of the alveolar cells 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 hypothalamus-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  $TTD_{\text{DEVELOPMENTAL}}$  of 0.008 mg/kg/day.

### Neurobehavioral Effects

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

### Summary (TTDs for DBP)

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

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

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

### 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 Interaction Profile mixture of chemicals. 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 T<sub>4</sub> (Hinton et al. 1986); serum levels of T<sub>3</sub> 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 GDs 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 control values 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 F<sub>0</sub> generation mice or up to 8,640 mg/kg/day for 85–105 days to the F<sub>1</sub> 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, although such activity has been shown for 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 Interaction Profile mixture of chemicals 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  $TTD_{\text{THYROID}}$  can be derived by applying an uncertainty factor of 100 (10 for animal to human extrapolation, 10 for human variability) to the NOAEL of 41 mg/kg/day. The  $TTD_{\text{THYROID}}$  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 the 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 animal.

#### 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 GDs 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 post application to an *in vitro* preparation of human abdominal skin. Data in animals are limited. A single application of <sup>14</sup>C-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, 24% was absorbed. In 7 days, total recovery of radioactivity in the urine and feces was about 50% of the applied dose. Dermally absorbed radioactivity derived from <sup>14</sup>C-DEP was minimal (<0.5% of the applied dose) in tissues of rats 1 week after a single application, indicating virtually no absorption under the conditions of the study. No data were located regarding absorption, distribution, or excretion of DEP or 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 Interaction Profile mixture of chemicals. 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 GDs 6–15 resulted in fetuses with a significant increased number of skeletal variations, particularly rudimentary ribs on GD 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 for interspecies extrapolation, 10 for 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  $TTD_{\text{THYROID}}$  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)

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

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

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

## C-4.6 References

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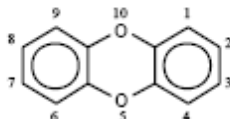
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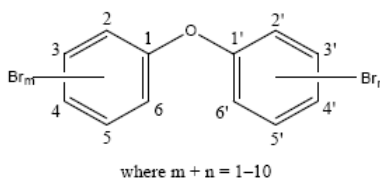
NTP. 2004. Report on carcinogens. 11th ed. Research Triangle Park, NC: National Toxicology Program, U.S. Department of Health and Human Services, Public Health Service. <http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html>. December 14, 2007.

## Appendix D: Chemical Structures of Mixture Components

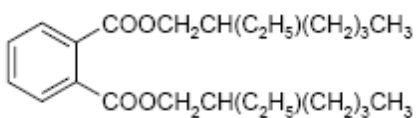
### Chlorinated Dibenzo-*p*-Dioxins



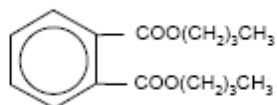
### Polybrominated Diphenyl Ethers



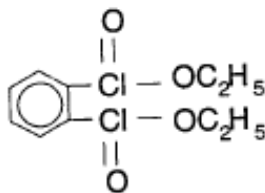
### Di-2(ethylhexyl) phthalate



### Di-*n*-Butyl phthalate



### Diethyl phthalate





Di-*n*-octyl phthalate

