INTERACTION PROFILE FOR:

CHLORINATED DIBENZO-*p*-DIOXINS, POLYBROMINATED DIPHENYL ETHERS, AND PHTHALATES

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

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PREFACE

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

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

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

thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

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All reviewers were selected in conformity with the conditions for peer review specified in CERCLA Section 104(I)(13).

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

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

SUMMARY

The purpose of this profile is to investigate the possible joint actions of chlorinated dibenzo-*p*-dioxins (CDDs), polybrominated diphenyl ethers (PBDEs), and phthalates (also known as phthalate esters) on endocrine, developmental, and neurobehavioral endpoints in humans. Chemicals from all three of these classes are found in human blood, adipose tissue, and breast milk. In assessing the available information on possible interactions between these chemicals, this profile concludes with recommendations for conducting screening-level assessments of public health concerns from join exposures to mixtures of these chemical classes.

CDDs, PBDEs, and phthalates are ubiquitous in the environment. CDDs originate through incomplete combustion processes such as incineration. PBDEs had previous wide use as flame retardants in plastics and textiles. Phthalates are commonly used to make plastics soft and pliable. Oral exposure through food is believed to be the predominant mode of human exposure to these chemicals. CDDs and PBDEs are bio-persistent by virtue of their slow degradation and elimination from the body. Of the PBDEs, the lower brominated forms (primarily tetra- and pentabrominated diphenyl ethers [BDEs]) are the primary forms found in human tissues and fluids. Deca-brominated BDEs (decaBDE) are not readily absorbed into the body. Phthalates are rapidly metabolized and eliminated from the body, but as exposure to phthalates is considered continuous, phthalates and their metabolites are continuously cycling through the body.

Observations in humans and laboratory animals following exposure to each of these chemicals alone raise concern about the nature and magnitude of possible effects associated with concurrent exposure. Exposure to chemicals in each of these classes alone has been associated with disruption of thyroid function in humans and/or animals, and with adverse effects on fetal development, especially fetal endocrine disruption in animals. Animal studies indicate that 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) and lower-brominated PBDEs each disrupt neurobehavioral development, and that 2,3,7,8-TCDD, di-(2-ethylhexyl)phthalate (DEHP), and di-*n*-butyl phthalate (DBP) each disrupt male reproductive structure and function. Animals studies also demonstrate that 2,3,7,8-TCDD and phthalates (DEHP and DBP) disrupt both male and female reproductive development. Both TCDD and lower-brominated PBDEs each disrupt thyroid function in gestationally exposed animals.

Of the CDDs, 2,3,7,8-TCDD is widely believed to be the most toxic, and is considered representative of the class. There is a large body of evidence that supports a pivotal role for the aryl hydrocarbon receptor (AhR) in the mechanism of TCDD-induced toxicity. Due to structural similarities to 2,3,7,8-TCDD, PBDEs have been investigated for dioxin-like activity; however, a group of expert scientists assembled under the aegis of the World Health Organization (WHO) in 2005 concluded that PBDEs do not meet commonly accepted criteria to be considered "dioxin-like" with regard to their toxicity. Recent *in vitro* investigations from a variety of mammalian cell lines have demonstrated that PBDEs have negligible ability to bind to the AhR and are incapable of activating it to induce the cascade of events (*the AhR signal transduction pathway*) leading to induction of enzymes that are the hallmark of dioxin-like activity. However, companion *in vitro* studies designed to investigate the joint action of PBDEs and 2,3,7,8-TCDD on various stages of the AhR signal transduction pathway indicate that the lower PBDE congeners (such as those found in human blood, adipose tissue, and breast milk) antagonize TCDD-induced activation of the AhR signal transduction pathway, but the molecular nature of this antagonism is currently unclear. Given that these observations were made on isolated cells and at concentrations orders of magnitude higher than concentrations of PBDEs found in human body fluids, the environmental relevance of this apparent antagonism is uncertain. There is no evidence to suggest that phthalates interact with the AhR or express dioxin-like toxicity. In fact, the fetal and developmental toxicity of DEHP is likely mediated through the peroxisome proliferator-activated receptor (PPAR).

There are no studies in the literature that address the possible effects of concurrent whole-body exposure of humans or animals to a mixture of CDDs, PBDEs, and phthalates. The available mechanistic understanding of toxicity caused by each class of chemicals alone is not sufficient to reliably predict the direction or magnitude of any interaction between all three chemicals or between any two pairs of chemicals, except for PBDEs and TCDD. Whereas *in vitro* mechanistic evidence indicates that PBDEs antagonize TCDD activation of the AhR signal transduction pathway, there are no studies that address possible joint action of PBDEs and TCDD on any toxicity endpoint. Furthermore, the mechanistic evidence suggesting possible antagonism is offset by thyroid toxicity data for TCDD alone and PBDEs alone that suggest the possibility of joint additivity on the basis of a common non-AhR-mediated mode of action (i.e., inhibition of thyroxine [T_4] binding by hydroxylated intermediates). There are no physiologically based toxicokinetic (PBTK) models that can be used to predict interactions between any pairs or sets of three chemicals from these three chemical classes.

Given the co-occurrence of CDDs, PBDEs, and phthalates in humans and the commonality of certain classes of effects, ATSDR recommends that the default assumption of joint additivity be employed to assess mixtures of these chemicals using a modified hazard index (HI) approach. To facilitate the use of this approach, target toxicity doses (TTDs) have been derived for thyroid disruption in adults, developmental endocrine disruption (either thyroid or reproductive hormone disruption), and neurodevelopmental toxicity for 2,3,7,8-TCDD, lower-brominated PBDEs, decaBDE, DEHP, di-*n*-octyl phthalate (DNOP), and DBP, where toxicity data were indicative of the effect of concern and were suitable for quantification of effect levels. No TTDs were derived for diethyl phthalate (DEP) or decaBDE due to the lack of effects of concern.

Exposure to CDDs should be determined as the sum of all congeners converted by toxic equivalence to TCDD. Exposure to PBDEs should be evaluated separately for the sum of the lower-brominated congeners and decaBDE mixtures. Exposure to DEHP, DNOP, and DBP should each be determined. The HI for each relevant endpoint (endocrine, neurobehavioral, and developmental) can be derived by summing the ratio of exposure to TTD for each chemical in the mixture that is associated with the effect of concern. HIs in excess of 1 indicate the potential for the mixture to be of greater concern than any individual component, usually resulting in the need for further study or limiting exposure through remedial action or education of the community regarding related issues.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AGD	anogenital distance
Ah	aryl hydrocarbon
AhR	aryl hydrocarbon receptor
ATSDR	Agency for Toxic Substances and Disease Registry
BDE	Brominated diphenyl ether
BINWOE	binary weight-of-evidence
BTEX	benzene, toluene, ethylbenzene, and xylene
CDD	chlorinated dibenzo-*p*-dioxin
CDF	chlorinated dibenzofuran
DBP	di-*n*-butyl phthalate
DEP	Diethyl phthalate
DEHP	di-(2-ethylhexyl) phthalate
DNA	deoxyribonucleic acid
DNOP	Di-*n*-octyl phthalate
DRE	dioxin-responsive element
EGF	epidermal growth factor
EPA	Environmental Protection Agency
EROD	ethoxyresorufin O-deethylase
GD	gestation day
HI	Hazard Index
HQ	Hazard Quotient
IARC	International Agency Research on Cancer
IRIS	Integrated Risk Information System
kg	kilogram
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
MBP	monobutylphthalate
MEHP	monoethylhexylphthalate
mg	milligram
MRL	Minimal Risk Level
mRNA	messenger ribonucleic acid
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
PBDD	polybrominated dibenzo-*p*-dioxin
PBDE	polybrominated diphenyl ether
PBDF	polybrominated dibenzofuran
PBPD	physiologically based pharmacodynamic
PBTK	physiologically based toxicokinetic
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofurans
PCR	polymerase chain reaction
PND	postnatal day
POD	point of departure
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion
ppm	parts per million

ppt	parts per trillion
PVC	polyvinyl chloride
RfC	reference concentration
RfD	reference dose
T_3	triiodothyronine
T_4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	Toxic Equivalence Factor
TEQ	toxic equivalents
TGF	transforming growth factor
THR	thyroid receptor
TSH	thyroid stimulating hormone
TTD	target-organ toxicity dose
TTR	transthyretin
UDP	uridine-5'-diphosphate
U.S.	United States
WHO	World Health Organization
XRE	xenobiotic-responsive element
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to

1. Introduction

The primary purpose of this Interaction Profile for chlorinated dibenzo-*p*-dioxins (CDDs), polybrominated diphenyl ethers (PBDEs), and phthalates is to evaluate data on the toxicology of the "whole" mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern (i.e., endocrine disruption, neurobehavioral effects, and developmental toxicity), adequacy of the data as the basis for a mixture minimal risk level (MRL), and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Division of Toxicology and Human Health Sciences (DTHHS) recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.

Interactions between CDDs, PBDEs, and phthalates are of interest to ATSDR because these chemicals are ubiquitous in the environment, are detected in human biological samples from the general population, and cause similar types of certain adverse health effects in humans or animals. The national data suggest that PBDE and phthalate exposures continue to increase while dioxin toxic equivalents (TEQ) exposures have decreased. Nevertheless, there are site-specific opportunities for high dioxin TEQ exposures. These elevated exposure cases now occur while the nationwide baseline exposures to PBDE and phthalates are higher than in the past. Such situations underscore the need to consider the interaction of these chemicals. This profile focuses on neurobehavioral effects, developmental toxicity, and endocrine disruption, as these are important toxic effects observed in common among these chemical classes.

CDDs are widely present in air, water, and soil primarily due to combustion processes, especially waste incineration (ATSDR 1998). PBDEs previously had widespread use as flame retardants (ATSDR 2017).

Phthalates are most commonly used to make plastics flexible, and as such, are present in food storage containers, automobiles, household goods, and medical tubing (ATSDR 1995, 1997, 2001, 2002). Although all of these chemicals have been detected in air samples, the main source of human exposure to these chemicals is likely to be dietary. CDDs and PBDEs (especially the lower brominated diphenyl ethers [BDEs]) are persistent in fatty animal tissues. Phthalate esters are rapidly metabolized and eliminated, but due to their ubiquitous presence in the environment, they are continuously present in body fluids and tissues.

CDDs, PBDEs, and phthalates are lipophilic and have been detected in human biological samples. ATSDR (1998, 2012) reported that the average concentration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the adipose tissue of the U.S. population is 5.8 pg/g lipid (Orban et al. 1994). For all CDD congeners, excluding dioxin-like polychlorinated biphenyls (PCBs), the national average was approximately 28 pg TEQ/g lipid (see Section 2.2.1.1 for a brief discussion of TEQ). A background exposure level of approximately 0.7 pg 2,3,7,8-TCDD/kg/day (assuming a 70-kg reference body weight) has been estimated for the general population in the United States (Travis and Hattemer-Frey 1987 as cited in ATSDR 1998). If other CDD and chlorinated dibenzofuran (CDF) congeners are included, the background exposure level increases to approximately 18–192.3 pg TEQ/day (0.26–2.75 pg/kg/day using a 70-kg reference body weight) (Schecter et al. 1994b as cited in ATSDR 1998). Schecter et al. (2005) reported that CDD levels in blood serum have decreased since 1973. The concentration of CDD reported for a pooled blood serum sample drawn from U.S. citizens in 2003 was 449 ppt lipid (pg TEQ/g). This value is lower than previously detected in the pooled serum sample from 1973 (3,979 ppt lipid). A large number of studies in the general population in the United States, Canada, Germany, and France during 1972–1999 show a trend of substantial (almost 10-fold) decreases in human TCDD-only body burden over that time period (Aylward and Hays 2002). Considering the long half-life of TCDD, a one-compartment pharmacokinetic model estimated that the decrease in intake must have been more than 95%.

Schecter et al. (2005) reported that PBDE levels in blood samples taken from U.S. citizens have risen significantly since 1973, when they were essentially non-detectable (detection limits=0.03–1 ppb lipid), to a level in 2003 that was the highest detected anywhere in the world (61.84 ppb lipid or ng/g, total PBDE in pooled whole blood sample). Schecter et al. (2005) reported the highest concentrations in pooled whole-blood samples for BDE-47 (44.2 ng/g lipid), BDE-99 (12.8 ng/g lipid), and BDE-153 (11.2 ng/g lipid); other BDE congeners detected in humans include BDE-209, BDE-183, BDE-154, BDE-138, BDE-100, BDE-85, BDE-77, BDE-28, and BDE-17. BDE-209 is decaBDE, and is the predominant

congener in formerly manufactured and used commercial decaBDE mixtures of flame retardants. Lipid-adjusted serum levels collected in NHANES 2003–2004 also reported the highest geometric means for BDE-47 (20.5 ng/g lipid), with a second highest geometric mean for BDE-153 (5.7 ng/g lipid); BDE-28, BDE-99, BDE-47, BDE-100, and BDE-153 were in ≥60% of participants (Sjödin 2008). PBDEs are also detected in human milk samples at similarly high concentrations, with reported concentrations for total PBDEs ranging from 19.948 to 67.8 ng/g lipid in U.S. and Canadian samples collected between 2002 and 2012 (Guo et al. 2015; Ryan and Rawn 2014; Schecter et al. 2005). In earlier studies, the tetra- and pentabrominated PBDEs have been the predominant congeners detected in breast milk samples, but more recent studies that assayed for a wider range of PBDE congeners found evidence for distribution of hepta, octa, or decaBDEs into cord serum and breast milk (ATSDR 2017). As reported by ATSDR (2017), the composition of BDE detected in human biological samples is determined by environmental and metabolic factors, and does not reflect the composition of any commercial PBDE-containing flame retardant mixture.

Ambient human exposure to the predominant phthalate ester used in the manufacture of plastics, di-2-(ethylhexyl)-phthalate (DEHP), has been estimated to be on the order of 0.21–21 mg/day (3–30 μg/kg body weight/day for a 70-kg adult) (Appendix C: David 2000; Doull et al. 1999; Huber et al. 1996; Kohn et al. 2000; Tickner et al. 2001). DEHP was present in human adipose tissues sampled from accident victims at a concentration of 0.3–1.0 ppm (Appendix C: Mes et al. 1974) and in 48% of the adipose tissue specimens from cadavers autopsied in 1982 as part of the Human Adipose Tissue Survey from the National Human Monitoring Program (Appendix C: EPA 1989b). A significantly higher intake of DEHP was calculated for children (n=254) than for adults (n=85) in the general population (Koch et al. 2006). Exposures at the 95th percentile (25 and 21 μg/kg/day) exceeded the reference dose (RfD) of 20 μg/kg/day.

CDDs, PBDEs, and phthalates, have been associated with adverse effects on endocrine systems, particularly the thyroid and reproductive organs. There is also evidence that PBDEs and CDDs adversely affect neurobehavioral development. Consequently, this profile focuses specifically on possible joint actions related to endocrine disruption, neurobehavioral effects, and developmental toxicity. With regard to developmental toxicity, there is a degree of overlap between the chemicals of concern and disruption of endocrine systems (thyroid and reproductive) following gestational exposures. Appendices to this profile provide background information on health effects and toxicokinetics of CDDs (Appendix A), PBDEs (Appendix B), and phthalates (Appendix C).

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

PBDEs have 209 different molecular configurations (also known as congeners). Certain PBDEs are considered environmentally relevant due to their use in flame retardant mixtures (since the 1970s) and appearance in environmental media and biological samples. Three commercial PBDE mixtures have been produced: decabromodiphenyl ether (decaBDE), octabromodiphenyl ether (octaBDE), and pentabromodiphenyl ether (pentaBDE). DecaBDE has accounted for more than 80% of PBDE usage. The composition of commercial decaBDE is ≥97% of the pure congener (BDE-209) with the remainder mainly nonaBDE. Commercial octaBDE is a mixture of congeners ranging from nona- to hexaBDE, and mixtures of pentaBDE are comprised of tetra-, penta-, and hexaBDE congeners (ATSDR 2004a). People are environmentally exposed to lower PBDEs (e.g., tetra- and pentabrominated congeners) due to differential partitioning and transformation of the individual congeners in the environment, including transformation in animals that are consumed. PBDEs are likely to be retained in the body for long periods of time (years) because they are lipophilic and some congeners are not readily metabolized. Individual environmentally relevant PBDEs that have been studied include BDE-47, BDE-77, BDE-99, BDE-100, BDE-119, BDE-126, BDE-153, BDE-154, and BDE-183 (see ATSDR 2017 for details). Some studies have focused on commercially available mixtures of PBDEs, including octaBDE, pentaBDE, and decaBDE. The European Union banned use of pentaBDE and octaBDE as of August 2004. PentaBDE and octaBDE mixtures were voluntarily withdrawn from the U.S. marketplace by their manufacturers at the end of 2004 and decaBDE was not to be manufactured or imported into the United States after

December 31, 2013 (EPA 2013). Consistent with ATSDR's toxicological profile for PBDEs, this interaction profile considers the effects associated with exposure to the lower PBDEs (predominantly tetra- and pentaBDEs) separately from effects associated with decaBDE. The distinction between decaBDE and "lower" PBDEs is made for two primary reasons. First, lower PBDEs and decaBDE are handled differently in the body, resulting in lower bioavailability of decaBDE. Lower PBDEs preferentially distribute to body fat, while decaBDE tends to distribute to more highly perfused tissue (and to a lesser extent, body fat). However, both lower PBDEs and decaBDE have been detected in human breast milk samples, and have been shown to transfer from dams to fetuses and neonates in animal studies following exposure during gestational and nursing periods. Second, studies in laboratory animals generally indicate that toxicity associated with decaBDE exposure is less pronounced than for lower PBDEs (see Appendix B for more details).

This profile considers the phthalate esters previously assessed in toxicological profiles published by ATSDR, including DEHP, diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), and di-*n*-octyl phthalate (DNOP). These phthalates have been considered separately due to some important differences in the types and severity of adverse effects each has been demonstrated to cause in mammalian systems. Of the phthalates considered by ATSDR, DEHP and DBP have been associated with endocrine (thyroid and reproductive), fetotoxic, and developmental endocrine effects (reproductive) in animals or humans, and are thus the most relevant phthalates considered in this interaction profile. DNOP has been associated with thyroid changes, but not with adverse developmental effects. DEP has not been associated with the neurodevelopmental, developmental endocrine, or thyroid effects of concern in this profile and is thus given less weight of consideration.

The above restrictions with regard to the representative chemicals in each class considered for this profile did not apply to the searches for interaction data in the available literature database. The search strategy included all possible chemicals in each of the three classes (CDDs, PBDEs, and phthalates) in order to ensure that the available studies addressing possible interactions between members of each class would be identified. To further enhance the possibility of locating available literature relevant to interactions between the chemical classes of interest, searches were not restricted with regard to toxic endpoint, even though this profile is focused on endocrine disruption, developmental toxicity, and neurobehavioral effects.

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

2.1 Mixture of Concern

No data were located regarding health or pharmacokinetic endpoints in humans or animals exposed to mixtures containing at least one of the chemicals from each of the three classes: CDDs, PBDEs, and phthalates.

No physiologically based toxicokinetic/pharmacodynamic (PBTK/PD) models were found for tertiary mixtures of at least one chemical from each of the three classes.

2.2 Component Mixtures

No PBTK/PD models were found for binary mixtures of these chemicals. While there are models for some of the individual chemicals under consideration in this profile, there are no data regarding potential pharmacokinetic interactions between any of the pairs of chemicals. Thus, pharmacokinetic models for pairs of chemicals within the chemical classes of concern were not located, and no pharmacokinetic data were located that might be useful for developing "interaction" PBTK models.

The following subsections present relevant information on the joint toxic action of combinations of the components. This profile is focused on interactions pertaining to endocrine disruption, neurobehavioral effects, and developmental toxicity. The endocrine, neurobehavioral and developmental effects associated with each class of chemicals separately are discussed in Appendix A (CDDs), Appendix B (PBDEs), and Appendix C (DEHP, DBP, DEP, and DNOP).

2.2.1 CDDs and PBDEs

No studies designed to investigate interactions between PBDEs and CDDs on specific endocrine disruption or developmental or neurotoxic/neurobehavioral endpoints were identified in the available literature. However, the vast body of literature suggesting that dioxins adversely impact these and other endpoints subsequently has led to investigations of mechanistic-based interactions between dioxins and chemicals with structural similarities to the dioxins, including several investigations of the impact of specific PBDEs and PBDE mixtures on TCDD's effects on various stages in the AhR signal transduction pathway. An overview of the relevance of PBDEs to dioxin-like toxicity is presented in Section 2.2.1.1.

An overview and evaluation of studies of interactions between 2,3,7,8-TCDD and PBDEs on various steps in the AhR signal transduction pathway are presented in Section 2.2.1.2.

2.2.1.1 Toxicity Equivalence for Dioxin-like Mixtures: The Relevance of PBDEs

Based on structural and toxicological similarities, mixtures of dioxin-like compounds typically are evaluated in reference to the toxicity of 2,3,7,8-TCDD by a TEQ methodology that has undergone development since the mid-1980s. The TEQ methodology assumes that the concentrations of dioxin-like chemicals within a mixture are additive with respect to their ability to cause toxicity. A full discussion of the scientific justification for additivity and the TEQ methodology is beyond the scope of this profile, but has been widely published in the available literature (see Van den Berg et al. 2006 as a gateway review) and is discussed in the ATSDR (1998) toxicological profile for CDDs. Essential points are discussed throughout this section by way of assessing whether or not PBDEs should be considered dioxin-like in character, and as such, should be included in assessment of toxic equivalence for a mixture of dioxin-like compounds.

In 2005, the World Health Organization (WHO) International Programme on Chemical Safety convened a panel of experts to review the toxicity equivalence factors (TEFs) for dioxin-like compounds (Van den Berg et al. 2006). A TEF is a specific value (<1) assigned to a chemical based on the relative effective potency for a given toxicological endpoint relative to a reference compound, usually 2,3,7,8-TCDD (TEF=1). TEFs are used to derive a TEQ for a mixture of dioxin-like chemicals by adding together the sum of the TEF times the concentration for each chemical in the mixture. Thus, the TEQ for a mixture is an estimate of the total 2,3,7,8-TCDD-like activity of the mixture.

To be considered as a dioxin-like compound and included in the TEQ scheme, a compound must meet the following criteria (Van den Berg et al. 2006):

- It must share a structural similarity with polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs);
- It must be persistent in the environment and bioaccumulate in the food chain;
- It must bind to the AhR; and
- It must induce AhR-mediated biochemical and toxic responses.

In short, the toxic endpoints produced by dioxin-like chemicals are believed to be mediated by the AhR, but binding to AhR alone is not sufficient to cause toxicity. The sequence believed to occur generally involves the binding of a chemical (also known as a ligand) to AhR in the cytoplasm of a cell. The ligand-bound AhR in turn, associates with other proteins to form a complex that is translocated across the nuclear membrane. Once inside the nucleus, AhR separates from the ligand-protein complex and binds to a nuclear translocator protein (Arnt) and specific DNA sequences known as dioxin-responsive elements (DRE) or xenobiotic-responsive elements (XRE). Formation of the AhR:Arnt:DRE complex leads to the transcription of gene sequences leading to the expression of proteins such as cytochrome P4501A1 (CYP1A1)[1]. This biochemical process, also known as AhR signal transduction, is the common denominator of dioxin-induced toxicity.

While PBDEs have structural similarities to dioxins, are persistent in the environment, and may bind weakly to AhR, they do not induce the AhR-mediated enzymes typical of dioxin-like compounds. Studies conducted with PBDE mixtures in different mammalian cell lines suggested that while PBDEs may bind weakly to AhR, the resulting complex fails to catalyze the other steps necessary to up-regulate DNA and induce the signature enzymes (e.g., EROD, CYP1A1), which are the hallmark of dioxin-like activity (Peters et al. 2004, 2006a, 2006b). Potential polybrominated dibenzo-*p*-dioxin (PBDD) and polybrominated dibenzofuran (PBDF) contamination of PBDE mixtures is of concern. Studies conducted with various PBDE-containing flame-retardant mixtures and PBDE congeners with varying amounts of PBDD and PBDF contamination demonstrated that up-regulation of CYP1A1 activity is proportional to PBDD/PBDF contamination (Brown et al. 2004; Sanders et al. 2005). Details of these studies as they relate to the interaction between PBDEs and TCDD are discussed in more detail in Section 2.2.1.2.

The WHO expert panel that evaluated TEFs for dioxin-like chemicals reviewed the available studies for PBDEs. They concluded that PBDEs are not AhR agonists (i.e., do not induce the biochemical process associated with binding to the AhR) and should not be included in the TEQ for dioxin-like chemicals (Van den Berg et al. 2006). However, the panel expressed concern that commercial mixtures of PBDEs contain PBDD and PBDF impurities that produce AhR-mediated effects such as induction of CYP1A, and raised concern that photochemical and combustion processes involving PBDEs could result in the production of additional PBDD and PBDF contamination.

[1]Induction of EROD is often used as a marker for CYP1A1 activity. EROD induction is commonly assessed to determine whether a chemical has dioxin-like activity (i.e., is an AhR agonist).

It should be noted that another class of chemicals (i.e., PCBs) consists of congeners that are "dioxin-like" (i.e., the effects they induced are AhR mediated) and congeners that are not dioxin-like. However, both groups share some toxicity endpoints (i.e., not all the thyroid and neurodevelopmental disrupting activity is attributable to the classic Ah receptor pathway). That is why a new (alternative) TEF system was proposed recently based on the thyroxine hormone levels as biomarker of effects that should be useful for non-dioxin-like PCBs (Yang et al. 2010). Such a system may be useful for PBDEs, as well.

2.2.1.2. Toxicological Interactions Between PBDEs and TCDD

The potential effects of PBDEs alone on the AhR signal transduction pathway, and the impact of PBDEs on TCDD's effects on various stages of the AhR signal transduction pathway have been investigated in four *in vitro* studies.

1. Chen and Bunce (2003) used isolated rat hepatocytes to study whether PBDEs could act as either agonists or antagonists at several stages of AhR signal transduction (i.e., the process of AhR binding and activation of deoxyribonucleic acid (DNA) transcription and translation leading to production of CYP1A1 protein). As such, they looked at the formation of the AhR-ARNT-DRE complex, induction of CYP1A1 messenger ribonucleic acid (mRNA) (detected by Northern blot analysis of isolated RNA with a human CYP1A1 cDNA probe), and induction of CYP1A1 protein (detected by Western blot analysis of SDS-PAGE separated proteins with a goat antirat CYP1A1 polyclonal antibody) in freshly isolated cultured rat hepatocyte cells exposed for 24 hours to PBDE alone (0.1–100 μM), TCDD alone (10 nM), or combinations of PBDE (at selected concentrations depending on the endpoint) plus TCDD (at selected concentrations depending on the endpoint). Commercial PBDE mixtures (penta-, octa-, and decaBDE) as well as individual congeners (BDE-3, BDE-15, BDE-17, BDE-47, BDE-71, BDE-75, BDE-77, BDE-99, BDE-85, BDE-100, BDE-119, BDE-126, BDE-153, BDE-154, BDE-156, and BDE-183) were tested in this study.

2. Peters et al. (2004) studied the AhR-mediated induction of CYP1A1 mRNA levels and EROD activity (as an enzymatic activity marker of CYP1A1 induction) in human breast carcinoma (MCF-7), human hepatocellular carcinoma (HepG2), and rat hepatoma (H4IIE) cells exposed for 72 hours to various PBDE congeners alone (0.01–10 μM), to TCDD alone (0.001–2.5 nM), or combinations of PBDE and TCDD (same range of concentrations as for each alone). This study tested the following highly purified PBDE congeners: BDE-47, BDE-77, BDE-99, BDE-100,

BDE-153, BDE-154, BDE-183, and BDE-209. mRNA levels were measured with real-time polymerase chain reaction (PCR) amplification methods and fluorescent CYP1A1 cDNA probes.

3. Peters et al. (2006a) investigated induction of EROD activity by TCDD, PBDEs, and combinations of TCDD and PBDEs in isolated hepatocytes from male or female cynomolgus monkeys exposed to test concentrations for 48 hours. The highly purified PBDE congeners and PBDE and TCDD concentrations tested in this study were the same as those tested in Peters et al. (2004).

4. To further investigate the mechanism of inhibition by PBDEs of TCDD induction of CYP1A1 protein, Peters et al. (2006b) created genetically modified cell lines to directly assess the impact of PBDEs on TCDD effects on the expression of specific DNA sequences involved in the AhR signal transduction pathway. Mouse, rat, and human hepatoma cell lines were modified by transient transfection with various gene sequences for XREs or promoter regions. The cells were modified to respond via fluorescence or other quantifiable means when a ligand (TCDD or TCDD agonists) activated the appropriate receptor or sequence. This allowed the investigators to directly assess binding and activation at specific points in the AhR signal transduction pathway alongside traditional indicators of AhR activity such as EROD induction. PBDEs (0.1–10 μM) alone, TCDD alone (0.001–1nM), and combinations of PBDE and TCDD were tested in the modified rodent and human cell lines exposed for 24 hours. The PBDE congeners tested were the same as those tested by Peters et al. (2004).

The results from these studies are summarized as follows.

- ***TCDD induced various stages of the AhR signal transduction pathway at low (picomolar to nanomolar) concentrations.*** TCDD was maximally effective in activating investigated stages of the AhR signal transduction pathway in mammalian cell lines at concentrations ranging from 0.1 to 10 nM depending on the endpoint. Within this range of concentrations, TCDD induced formation of the AhR-ARNT-DRE complex (Chen and Bunce 2003), CYP1A1 mRNA (Chen and Bunce 2003; Peters et al. 2004), CYP1A1 protein (Chen and Bunce 2003), and EROD enzymatic activities (Chen and Bunce 2003, Peters et al. 2004, 2006a, 2006b). TCDD was also maximally effective in inducing the expression of various reporter genes associated with various phases of AhR signal transduction within this concentration range in both human and rodent cell lines (Peters et al. 2006b).

- ***PBDE congeners and PBDE mixtures did not effectively induce stages of the AhR signal transduction pathway.*** Early studies with isolated rat hepatocytes reported that several PBDE congeners (BDE-77, BDE-119, and BDE-126) induced AhR-ARNT-DRE complex formation, CYP1A1 mRNA, and CYP1A1 protein to levels equivalent to levels induced by the maximal TCDD concentration (10 nM), but this occurred at PBDE concentrations that were 1,000–100,000-fold higher than maximal concentrations of TCDD (Chen and Bunce 2003). Other tested PBDE congeners, including the environmentally relevant BDE-47 and BDE-99 congeners and the pentaBDE commercial mixture, did not activate these stages of the AhR signal transduction pathway (Chen and Bunce 2003). BDE-47 and BDE-99 are principal congeners detected in human blood, breast-milk, and fat tissue samples and principal constituents of the commercial pentaBDE mixture (Chen and Bunce 2003; Schecter et al. 2005). Later studies, using more highly purified PBDE congeners, found no PBDE induction of CYP1A1 mRNA levels or EROD activity in cultured human or rat cancer cells (Peters et al. 2004) and no EROD activity in isolated hepatocytes from cynomolgus monkeys (Peters et al. 2006a). These results obtained by Peters et al. (2004, 2006a) suggest that possible contaminants (e.g., PBDDs and PBDFs) in the test materials used by Chen and Bunce (2003) may have been responsible for the weak induction activity (compared with TCDD) of some of the PBDE congeners (Brown et al. 2004; Sanders et al. 2005). These results are consistent with the conclusions of the WHO expert panel that PBDEs are not AhR agonists and should not be included in the TEQ for dioxin-like chemicals (Van den Berg et al. 2006).

- ***Lower-brominated PBDEs strongly inhibited TCDD-induced formation of the AhR-ARNT-DRE complex.*** PentaBDE mixture, BDE-47, and BDE-99 (at 10 µM) inhibited the formation of the complex by 10 nM TCDD, by about 50, 100, and 100%, respectively, in freshly isolated rat hepatocytes (Chen and Bruce 2003). BDE-119 at concentrations up to 10 µM did not inhibit TCDD induction of complex formation, and BDE-77, BDE-126, BDE-100, BDE-153, and BDE-156 “mildly” inhibited TCDD induction of complex formation (Chen and Bunce 2003). In a later study using mouse (H1G1.1c3) and rat (H4G1.1c2) hepatoma cells lines that are genetically modified to produce a fluorescent protein (EFGP) following AhR activation by ligands, the presence of most of the tested PBDE congeners (BDE-47, BDE-77, BDE-99, BDE-100, BDE-153, and BDE-154, but not BDE-183) inhibited (maximally at concentrations of 10 µM) induction of AhR-EGFP expression by 0.1 or 1 nM TCDD (Peters et al. 2006b). The degree of inhibition increased with increasing bromination of the PBDE congeners; BDE-47 and BDE-

77 were the strongest inhibitors of TCDD induction of AhR-EGFP expression. BDE-183 did not inhibit TCDD-induced AhR-EGFP expression in replicate experiments (Peters et al. 2006b). Similar evidence for PBDE inhibition of TCDD induction of the AhR signal transduction pathway was found in studies with a human hepatoma cell line (HepG2) transfected with a AhR-responsive luciferase reporter gene DNA construct. The results from the study by Peters et al. (2006b) are taken as indirect evidence of an antagonistic interaction of lower-brominated PBDEs on TCDD induction of the formation of the active AhR-ARNT-DRE complex, because AhR-EGFP expression and luciferase expression in the modified cell lines require the formation of the active AhR-ARNT-DRE complex.

- ***No PBDE congeners or PBDE mixtures have shown any impact on TCDD induction of CYP1A1 mRNA levels.*** At a concentration of 10 µM, individual PBDEs (BDE-77, BDE-119, BDE-47, or pentaBDE) did not inhibit the induction of CYP1A1 mRNA by 0.1 nM TCDD in rat hepatocytes, but the impact of PBDE congeners at higher concentrations of TCDD (i.e., 1 or 10 nM) was not studied (Chen and Bunce 2003). Similarly, in studies using human breast carcinoma cells (MC-7) or human hepatocellular carcinoma cells (HepG2), PBDE congeners (BDE-47, BDE-77, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, or BDE-209), at concentrations ranging from 0.1 to 10 µM, did not inhibit the induction of CYP1A1 mRNA by 1 nM TCDD (Peters et al. 2004). Both studies reported that CYP1A1 mRNA levels in co-exposed cells (i.e., PBDE+TCDD) and TCDD-only exposed cells were not statistically significantly different.

- ***Lower-brominated PBDEs inhibited TCDD induction of CYP1A1 protein in rat hepatocytes.*** The presence of BDE-47 or the pentaBDE mixture (at 10 µM) inhibited the induction of CYP1A1 protein by 1 nM TCDD by about 25 and 60%, respectively, whereas BDE-77 and BDE-119 did not significantly impact the protein induction by 1 nM TCDD (Chen and Bunce 2003). This study did not examine the impact of PBDE congeners on TCDD induction of CYP1A1 protein at higher TCDD concentrations.

- ***Several PBDE congeners inhibited TCDD induction of EROD activity.*** In studies with human (MCF-7, HepG2) or rat (H411E) cultured cancer cells, the presence of any tested PBDE congener (BDE-47, BDE-77, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, or BDE-209) inhibited the induction of EROD activity by 1nM TCDD (Peters et al. 2004). Data for BDE-153 were shown in the original report. At a concentration of 10 µM, the presence of BDE-153 inhibited the

induction of EROD activity by 1 nM TCDD by about 50, 50, and 30% in MCF-2, HepG2, and H411E cells, respectively (Peters et al. 2004). Data for the other PBDE congeners were not shown by Peters et al. (2004), but were reported to show "similar inhibitory effects on EROD activity after co-exposure, though quantitative differences were observed." Similar results were reported for studies with freshly isolated monkey hepatocytes (Peters et al. 2006a) and with H1G1.1c3 mouse or H4G1.1c2 rat hepatoma cell lines (Peters et al. 2006b). The inhibition of EROD activity by PBDEs does not appear to be a direct effect on the catalytic capability of CYP1A1 activity (with the exception of BDE-183). The evidence for the latter conclusion is based on the observation that exposure of MCF-7, HepG2, or H411E cells to PBDEs after exposure to TCDD had no effect on the induction of EROD activity following exposure to TCDD alone. In these studies, cells were first exposed to 1nM TCDD for 72 hours, followed by exposure to PBDEs for 5 minutes prior to measurement of EROD activity (Peters et al. 2004). However, there is some evidence that BDE-183 may inhibit EROD activity via catalytic inhibition. In support of this hypothesis are the observations that BDE-183 inhibits TCDD-induced EROD activity, but does not inhibit the TCDD-induced AhR-EGFP gene expression that would be consistent with Ah-mediated expression of EROD activity in the same cell lines (Peters et al. 2006b). The lower-brominated congeners tested both inhibited TCDD-induced AhR-EGFP expression and TCDD-induced EROD activity.

In summary, the results from these studies provide evidence that PBDEs do not activate the AhR signal transduction pathway, but may antagonize TCDD-induced biochemical activity mediated by the AhR when exposure to these chemicals is simultaneous. The mechanism by which this antagonism occurs is unknown, and is complicated by the observation that PBDEs inhibited TCDD activation of DNA sequences and related TCDD-induced gene products (e.g., CYP1A1 protein levels, AhR-responsive EGFP or luciferase, EROD activities), but did not inhibit TCDD-induced mRNA formation. The relevance of these molecular observations with respect to the joint action of PBDEs and TCDD in producing potential neurobehavioral toxicity, endocrine disruption, or developmental toxicity in the human population is unstudied and unknown.

Adding to the uncertainty surrounding the meaning of the aforementioned *in vitro* studies with regard to human health risk assessment are the high concentrations of PBDEs and TCDD tested relative to concentrations found in biological fluids. Peters et al. (2004) estimated that the ratio of PBDE to TCDD concentrations tested in their studies are 10–1000 times higher than PBDE or TCDD concentrations found in human blood. This observation applies to the other studies as well, because all of these investigators

used similar test concentrations. And finally, based on the observation that TCDDs and PBDEs are already present in the human body, the impact of further exposure to a mixture of PBDEs and TCDD is uncertain. The evidence from the above *in vitro* studies indicates that antagonism of TCDD-induced AhR-mediated activity occurs only when exposure to PBDEs and TCDD is simultaneous.

2.2.2 CDDs and Phthalates

A study pertaining to potential interactions between CDDs and phthalates with regard to endocrine disruption and developmental toxicity was published recently. Sprague-Dawley rats were used to study disruption of the androgen and AhR signaling pathways in .male reproductive tract by chemicals with different mechanisms of toxicity (Rider et al. 2010). Groups of dams were treated with either TCDD (2 μg/kg/day) or vehicle on gestation day (GD) 14 and with DBP (500 mg/kg/day) or vehicle on GDs 14–18. Other groups were treated with the binary mixture of either 2 μg TCDD/kg/day and 500 mg DBP/kg/day or 1.3 μg TCDD /kg/day and 320 mg DBP/kg/day. The incidence of malformed organs for both mixtures exceeded response addition for the epididymal, testicular, vas deferens, hypospadias, and liver malformations. However, only one result was statistically significant: the reduction in epididymal weights ($p<0.05$). The reported liver malformations associated with exposure to the mixtures were not observed following treatments with the individual chemicals.

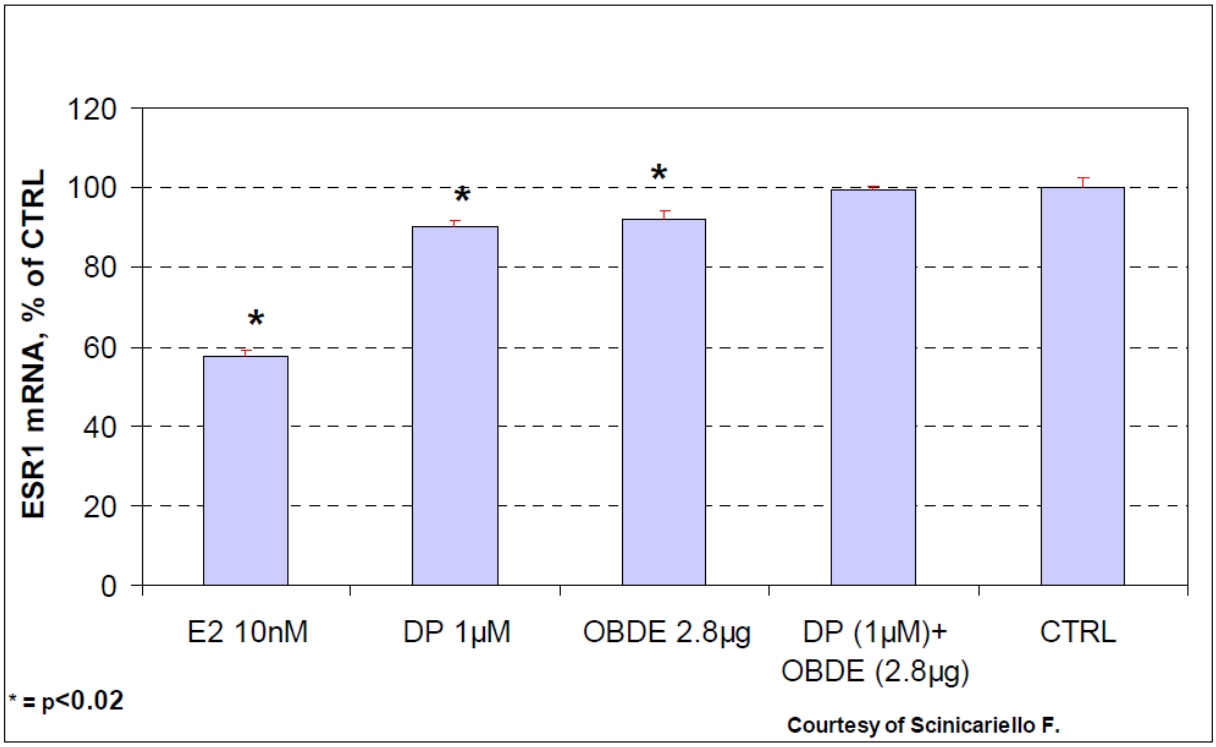
In contrast, in an older study, there was some evidence that DEHP may antagonize TCDD-induced fatty liver, hyperlipidemia, and mortality in rats (Tomaszewski et al. 1988). Treatment of F344 rats with TCDD alone (160 μg/kg) resulted in an increase in serum triglycerides and cholesterol levels, while treatment with DEHP alone (2 g/kg/day) caused a decrease in triglycerides and cholesterol levels as compared to the controls. Pre- or post-treatment with DEHP resulted in a decrease in the TCDD-induced hyperlipidemia. The authors suggested that the mechanism was an increase in hepatic peroxisomal beta-oxidation and decreased hepatic lipid synthesis due to DEHP administration. Another suggestion of possible inhibitory effects comes from a study that involved “a similar mixture” to the mixture assessed in this document (see ATSDR 2004a). The effects of fetal and neonatal exposures on neurodevelopmental endpoints were studied in ICR mouse dams and their pups (Tanida et al. 2009). Specifically, the authors analyzed the tyrosine hydroxylase (TH) and Fos-immunoreactive neurons and the intensity of TH-immunoreactivity in midbrain dopaminergic nuclei following oral exposure to 5 mg/kg/day of bisphenol A (GDs 8–18 and postnatal days [PNDs] 1–7), 1 mg/kg/day of DEHP (GDs 8–18 and PNDs 1–7), and a single dose of 8 ng/kg/day TCDD (GD 8) either individually, or in a trinary mixture. Administration of individual chemicals caused significant changes as compared to the controls. However, these effects were

not detected following exposure to the mixture, suggesting inhibitory interactions. The mechanism of the interactions was not established. Since bisphenol A and PBDEs are different chemicals, the outcome of the respective trinary interactions (i.e., bisphenol A, DEHP, and TCDD versus PBDEs, DEHP, and TCDD) may be different. Nevertheless, this study is important as an example of interactions between three endocrine disruptors with different mechanisms of action that are often found in the environment.

2.2.3 PBDEs and Phthalates

No extensive studies were located in the available literature pertaining to potential interactions between PBDEs and phthalates with regard to endocrine disruption, developmental toxicity, or neurotoxicity (or any other endpoints related to toxicity of CDDs or phthalates in mammals).

Preliminary results of an *in vitro* study were reported (Pohl 2009). MCF-7 cells were grown in phenol red-free IMDM medium and 5% charcoal treated calf serum for 24 hours with either 10 nM of estradiol, or 1 μM DNOP, or 2.8 μg octaBDE, or a solution containing 1 μM DNOP and 2.8 μg octaBDE. ESR1 mRNA was determined by real time reverse-transcriptase PCR. The mRNA was quantified using the "delta-delta Ct" method. Results are presented as percent of control cells and represent the mean of nine experiments ±standard error (t-test used for statistical evaluation) (see Figure 1). The individual chemicals downregulate the ESR1 mRNA. When present together in the medium, there was no difference in ESR1 mRNA compared to the control. Less-than-additivity was suggested. However, lower doses need to be tested to show the potential for additivity and/or interaction.



CTRL = percent of control cells; DP = di-*n*-octyl phthalate; E2 = estradiol; ESR1 = estrogen receptor-alpha; mRNA = messenger ribonucleic acid; OBDE = octabromodiphenyl ether

Figure 1. Effect of Di-*n*-octyl Phthalate and OctaBDE on the Expression of ESR1 mRNA

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

No studies were located that examined health effects in humans or animals exposed to three-component mixtures containing CDDs, PBDEs, and phthalates. While there are PBTK models for some of the individual chemicals under consideration in this profile, there are no data examining or identifying potential pharmacokinetic interactions between any chemicals from the three chemical classes under consideration. Thus, pharmacokinetic models for pairs of chemicals (or sets of three chemicals) from the chemical classes of concern were not located, and no pharmacokinetic data were located that might be useful for developing "interaction" PBTK models.

The health effects relevant to endocrine disruption, neurotoxicity, and developmental toxicity associated with each of the chemical classes under investigation in this profile are summarized in Table 1.

Table 1. Health Effects Observed in Humans or Animals after Oral Exposure to Chemicals of Concern

	Chemical of concern[b]		
Effect of concern[a]	2,3,7,8-TCDD	PBDEs	Phthalates (relevant form)
Thyroid disruption (pre- and/or postnatal)	A	**H**[b,c]	**H**[d] (DEHP, DBP, DNOP)
Male reproductive organ disruption	A	A	A (DEHP, DBP)
Altered neurological development (pre- and/or postnatal)	A[e]	A	
Altered female reproductive organ development, sexual maturity	A	**H**	**H** (DEHP)
Altered male reproductive organ development (testicular degeneration, feminization)	A	A	**H** (DEHP, DBP)
Other developmental effects (malformations or fetotoxicity)	A[f]	A[g]	A[h] (DEHP, DBP)

[a]Restricted to endpoints relevant to endocrine disruption, neurotoxicity, and developmental toxicity that occur for at least two chemical classes. See Appendices A, B, and C for more details.
[b]Upper case and bolded **H** indicates that effects have been observed clearly in humans (evidence unsupported by statistical verification of an effect outside the normal control range is not considered demonstrative of an effect in humans). Upper case and non-bolded A indicates that effects have been observed only in animals.
[c]Human evidence comes from *in vitro* binding studies with human transthyretin (TTR) and thyroid receptor (THR) proteins; animal studies demonstrate treatment-related thyroid disruption in developing fetuses as well as in adults.
[d]Meeker et al. (2007) demonstrated a correlation between urinary MEHP levels and decreased serum T_3 and T_4 in a cohort of men in Boston, Massachusetts. Huang et al. (2007) demonstrated a correlation between urinary MBP and decreased serum T_3/T_4 in pregnant women.
[e]Indicates that these are the most sensitive noncancer health effects from oral exposure (i.e., they occur at lower dose levels than other noncancer effects).
[f]Cleft palate, hydronephrosis, immunotoxicity, and death were most common.
[g]Variations in skeletal ossification.
[h]Reduced fetal body weight, increased rates of abortion and fetal resorptions, and skeletal malformations.

DBP = di-*n*-butyl phthalate; DEHP = di-(2-ethylhexyl) phthalate; DNOP = di-*n*-octyl phthalate; MEHP = mono-(2-ethylhexyl) phthalate; PBDE = polybrominated diphenyl; T_3 = triiodothyronine; T_4 =thyroxine; 2,3,7,8-TCDD = tetrachlorodibenzo-*p*-dioxin

As shown in Table 1, CDDs, PBDEs, and phthalates have been shown to disrupt thyroid function, raising concern that these chemicals may act jointly to disrupt thyroid functioning following simultaneous oral exposure. Recent case studies indicating a strong association between levels of urinary monoesters of DEHP and DBP (primary metabolites of phthalates: monoethylhexyl phthalate [MEHP] and monobutyl phthalate [MBP], respectively) and decreased serum triiodothyronine (T_3) and thyroxine (T_4) levels in a cohort of men in Boston (MEHP; Meeker et al. 2007) and in a cohort of pregnant women (MBP; Huang et al. 2007) add strength to the notion that phthalates adversely affect thyroid functioning in humans. Based on the commonality of observed toxic endpoints, the following joint toxic actions may also be possible: (1) 2,3,7,8-TCDD and certain phthalates (DEHP or DBP) may disrupt male organ structure and function; (2) 2,3,7,8-TCDD and lower PBDEs may disrupt neurological development; (3) phthalates

(DEHP, DBP) and TCDD may disrupt the development of male and female reproduction tissues or organs; and (4) 2,3,7,8-TCDD and lower PBDEs may disrupt thyroid development.

In addition, 2,3,7,8-TCDD, lower PBDEs, and certain phthalates (DEHP and DBP) all cause fetotoxicity, but the types of effects observed are somewhat different for each chemical, and the modes of toxic action are likely to be different.

On the basis of these observations, intermediate-duration target toxicity doses (TTDs) are developed in this profile for thyroid disruption in adults (PBDEs, TCDD, and phthalates), disruption of neurobehavioral development (PBDEs and TCDD), and developmental endocrine disruption (based on thyroid disruption for PBDEs, and disruption of reproductive hormones for phthalates and TCDD). The use of TTDs is discussed in Section 3, and the derivation of TTDs for each of the chemicals is discussed in the Appendices.

The basis for existing MRLs for representative chemicals from each of the chemical classes is shown in Table 2. Table 2 reflects the differences between CDDs, PBDEs, and phthalates with regard to the most sensitive toxic endpoints relevant to a given duration of exposure for each chemical class.

Table 2. Health Effects Forming the Basis of ATSDR Oral MRLs for Chemicals of Concern

Duration of exposure	2,3,7,8-TCDD	Lower PBDEs	DecaBDE	DEHP	DBP	DNOP
Acute	Immuno-suppression (susceptibility to influenza A) in rats	Maternal thyroid effects (decreased serum T_4), developmental reproductive effects, developmental neurobehavioral effects in rat dams and their offspring	Developmental neurobehavioral effects in mice exposed during early postnatal development	Not derived due to insufficient dose-response data on development of the male reproductive system	Testicular atrophy and feminization of gestationally exposed male fetal rats	Liver effects
Intermediate	Immune effects (decreased thymus weight) in rats	Reduced serum testosterone in adult male rats	Increased serum glucose in adult rats (associated with insulin dysregulation)	Reduced male fertility, testicular atrophy, abnormal sperm	None derived due to observation of fetal death at lower doses	Liver effects
Chronic	Neuro-behavioral changes in monkey offspring	None derived due to the lack of a sufficient chronic study	None derived due to the lack of a sufficient chronic study	Testicular pathology in male rats	None derived due to sensitivity of gestational endpoints	None derived

Limited data exist regarding interactions between CDDs, PBDEs, and phthalates; however, the studies do not properly elucidate the mechanisms of interactions and their magnitude.

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

With component-based approaches to assessing health hazards from mixtures of chemicals, it is important to assess the joint additive action assumption and consider the possibility that less-than-additive or greater-than-additive joint actions may occur among the components of the mixture. With this purpose in mind, the available data on the possible joint actions of pairs of the chemicals of concern were reviewed

in Section 2.2. Available data on possible binary interactions among these three chemicals are limited or absent for most of the pairs and "interaction" PBTK models for pairs of the chemicals (or sets of three chemicals from the three classes) are not available. Using the classification scheme summarized in Table 3 and ATSDR (2004a), Tables 4, 5, 6, 7, 8, and 9 describe binary weight-of-evidence determinations (BINWOEs) for the pairs of the three chemicals of concern. The conclusions presented in these tables were based on the evaluations of results from the available interaction literature presented in Section 2.2. A summary of the BINWOEs is presented in Table 10. The BINWOEs focus on simultaneous oral exposure as this is the exposure scenario of most interest for public health concerns for the subject chemicals and their mixture.

As noted in Table 4, there is limited evidence that the effect of TCDD on PBDE exposure could be additive with respect to thyroid disruption and neurobehavioral development. As discussed in Table 5, there is limited evidence that the effect of PBDE on TCDD toxicity is antagonistic with regard to toxicity mediated through AhR. However, due to conflicting evidence from *in vitro* mechanistic studies (suggesting antagonism) and studies of each chemical alone on thyroid functioning (suggesting additivity due to possible common modes of inhibition of T_4 binding by hydroxylated intermediates), the direction or nature of the effect of PBDEs on TCDD thyroid disruption is too uncertain to predict with any reliability. Given that thyroid disruption is associated with adverse impacts on neurobehavioral development, it is similarly too uncertain to predict the direction or nature of the effect of PBDEs on the effects of TCDD on neurobehavioral development.

As discussed in detail in tables that follow, there is no mechanistic evidence that can reliably be used to predict the direction of possible interaction (i.e., greater than additive or less than additive) between PBDEs and phthalates (Tables 8 and 9) or between TCDD and phthalates (Tables 6 and 7). However, some literature data suggest that interactions do occur.

On the basis of the existing data as summarized in the BINWOE tables, ATSDR recommends that the default assumption of joint additive action at shared targets of toxicity be employed to assess potential adverse health outcomes associated with concurrent exposures to CDDs, PBDEs, and phthalates. There is limited evidence that PBDEs antagonize AhR signal transduction, but no evidence to support how this observation might relate to joint action in causing toxicity. Data for each chemical alone relevant to thyroid disruption suggest additivity, rather than antagonism, on the basis of a common mode of action (inhibition of T_4 binding by hydroxylated metabolites) that does not involve the AhR signal transduction pathway.

Table 3. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

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

Source: ATSDR 2004a

Table 4. Effect of 2,3,7,8-TCDD on PBDEs
BINWOE: =IIIC for thyroid effects
BINWOE: =IIIC for neurodevelopmental effects

Direction of Interaction – There are no studies that investigate toxicity following joint exposure to TCDDs and PBDEs. However, joint additive action on thyroid function (mediated by hydroxylated metabolites) is plausible based on limited mechanistic understanding of thyroid toxicity not mediated by AhR. Based on the hypothetical adverse effects of thyroid disruption on neurological development, it follows that PBDEs and TCDD could have joint additive action on neurodevelopmental toxicity.

Mechanistic Understanding – Results from *in vitro* studies with various types of rat and primate cells indicate that PBDE congeners are not effective agonists for TCDD in activating the AhR signal transduction pathway (Chen and Bunce 2003; Peters et al. 2004, 2006a, 2006b). Thus, health effects from exposure to PBDEs are not expected to be mediated through the AhR signal transduction pathway (Van den Berg et al. 2006), and there is no evidence that the impact of TCDD on this pathway will influence the toxicity of PBDE congeners.

Exposure to TCDD alone and to PBDEs alone causes thyroid toxicity through inhibition of circulating T_4. For TCDD, the mechanism by which this occurs is postulated to involve: (1) AhR-mediated induction of uridine 5'-diphosphate (UDP)-glucuronyl transferase and subsequent increased metabolism and elimination of T_4 and (2) inhibition of T_4 binding to plasma transport proteins by hydroxylated metabolites (Appendix A.3). PBDEs are known to inhibit the binding of T_4 to plasma proteins, but do not induce AhR-mediated signal transduction (Appendix B.3). Joint additive action is consistent with the observation that both PBDEs and TCDD may disrupt T_4 homeostasis through their respective hydroxylated intermediates. However, there are no studies involving co-exposure to TCDD and PBDEs to validate the notion of joint additivity on thyroid endpoints. Therefore, a rating of III is assigned for limited mechanistic understanding of possible thyroid toxicity through additive joint action.

TCDD-induced developmental toxicity in animal studies (e.g., cleft palate formation) is thought to involve AhR-mediated regulation of gene expression leading to reduced levels of several growth factors (Appendix A.3). In contrast, PBDEs do not cause cleft palate and only cause fetotoxicity at high doses that also cause maternal toxicity (Appendix B.3). Neurodevelopmental effects have been observed in studies with TCDD alone and with several types of PBDEs alone. Although the mechanism of neurodevelopmental toxicity is uncertain for either chemical (Appendices A.3 and B.3), it is plausible that TCDD and PBDEs may additively disrupt thyroid hormone function, which in turn may additively affect neurological development. This hypothesis cannot be confirmed due to the lack of interaction studies of endocrine or neurodevelopmental endpoints following co-exposure to PBDEs and TCDD. Therefore, a rating of III is assigned for limited mechanistic understanding of possible neurodevelopmental toxicity through additive joint action.

Toxicologic Significance – No studies were located that were designed to compare responses of relevant toxicity targets (i.e., endocrine organs, nervous system, developing fetus) to mixtures of TCDD and PBDE with responses to either compound alone. No studies were located in which pretreatment with TCDD before PBDE exposure was examined for possible effects on PBDE toxicity. Joint actions on the developing nervous system, developing fetus and thyroid are plausible (see Appendices A and B), but whether the actions would be additive, greater-than-additive, or less-than-additive is unstudied. Therefore, a rating of C is assigned for toxicological significance.

Additional Uncertainties – The available modifying factors do not apply (no studies that address potential toxicity following co-exposure to TCDD and PBDEs are available). The uncertainty surrounding the limited information for the potential joint toxic action of these chemicals is reflected in the ratings for mechanistic understanding and toxicological significance.

Table 5. Effect of PBDEs on 2,3,7,8-TCDD
BINWOE: <IIIC2b for AhR-mediated TCDD effects
BINWOE: Indeterminate (?) for thyroid effects
BINWOE: Indeterminate (?) for neurodevelopmental effects

Direction of Interaction – *In vitro* mechanistic data indicate that PBDEs may antagonize TCDD induction of the AhR signal transduction pathway. This pathway is linked to several toxic effects associated with TCDD effects including developmental effects (e.g., cleft palate) and decreased T_4 due to AhR-mediated induction of UDP-glucuronyl transferase. Therefore, the direction of interaction is assigned to be "<" for the effects of PBDEs on AhR-mediated toxicity.

However, as discussed below, due to conflicting mechanistic evidence (i.e., *in vitro* studies of AhR mediated signal transduction indicating antagonism, versus common modes of toxic action indicating additivity), the direction of the interaction for both thyroid effects and neurodevelopmental effects is indeterminate.

Mechanistic Understanding – Many effects of TCDD are thought to be mediated via the AhR signal transduction pathway (Appendix A.3). Although PBDEs are not effective agonists for the AhR signal transduction pathway, *in vitro* studies indicate that PBDEs antagonize TCDD-induced biochemical activities (CYP1A1 protein, AhR responsive expression of reporter genes, EROD enzymatic activity) mediated by the AhR when exposure to these chemicals is simultaneous (Chen and Bunce 2003; Peters et al. 2006a, 2006b, 2004; Van den Berg et al. 2006; Section 2.2.1.2.). The mechanism by which this antagonism occurs is uncertain, and is complicated by the observation that PBDEs inhibited TCDD activation of DNA sequences and related TCDD-induced gene products (e.g., CYP1A1 protein levels, AhR-responsive EGFP or luciferase, EROD activities) but did not inhibit TCDD-induced CYP1A1 mRNA formation. Antagonist activity decreased with increasing bromination and was maximal at PBDE concentrations (10 µM) that were 1,000–100,000-fold greater than maximal TCDD inducing concentrations (0.1–10 nM) (Peters et al. 2006a, 2006b, 2004; Chen and Bunce 2003). The relevance of the *in vitro* findings with regard to resulting toxic endpoints that could be manifest in animals and humans following joint exposure to TCDD and PBDEs is unstudied and unknown. However, because PBDEs have been demonstrated to antagonize AhR-mediated signal transduction *in vitro*, a value of III is assigned for limited mechanistic understanding of the effect of PBDEs on TCDD-induced toxicity mediated by AhR.

Exposure to TCDD alone and to PBDEs alone causes thyroid toxicity through inhibition of circulating T_4. For TCDD, the mechanism by which this occurs is postulated to involve two mechanisms: (1) AhR-mediated induction of UDP-glucuronyl transferase and subsequent increased metabolism and elimination of T_4 and (2) inhibition of T_4 binding to plasma transport proteins by hydroxylated metabolites (Appendix A.3). PBDEs are known to inhibit the binding of T_4 to plasma proteins, but do not induce AhR-mediated signal transduction (Appendix B.3). These observations result in conflicting predictions about the nature of an interaction between PBDEs and TCDD as follows. Joint additive action is consistent with the observation that both PBDEs and TCDD may disrupt T_4 homeostasis through their respective hydroxylated intermediates. However, antagonistic action is consistent with the *in vitro* studies indicating that PBDEs antagonize TCDD-induced activation of AhR-mediated signal transduction: There are no *in vivo* studies that address thyroid toxicity (or any other toxicity) associated with co-exposure to PBDEs and TCDD. Therefore, the direction of interaction is not known and subsequent classifications for mechanistic understanding and toxicological significance cannot be assigned.

TCDD-induced developmental toxicity in animal studies (e.g., cleft palate formation) is thought to involve AhR-mediated regulation of gene expression leading to reduced levels of several growth factors (Appendix A.3). In contrast, PBDEs do not cause cleft palate and only causes fetotoxicity at high doses that also cause maternal toxicity (Appendix B.3). Neurodevelopmental effects have been observed in studies with TCDD alone and with several types of PBDEs alone. No studies on the effect of co-exposure to TCDD and PBDEs have been conducted. Although the mechanism of neurodevelopmental toxicity is uncertain for either chemical (Appendices A.3 and B.3), both TCDDs alone and PBDEs alone

disrupt thyroid hormone function, which in turn may additively affect neurological development. As discussed in the previous paragraph, the lines of evidence for the effects of PBDEs on TCDD-induced thyroid toxicity are conflicting (i.e., effects on AhR-mediated toxicity indicate antagonism, while effects on T_4 indicate additivity). Therefore, as for thyroid effects, the potential effects of PBDEs on TCDD-induced neurodevelopmental toxicity are indeterminate in direction, and unknown with regard to mechanistic understanding (i.e., no category is assigned).

Toxicologic Significance – No studies were located that were designed to compare responses of relevant toxicity targets (i.e., endocrine organs, nervous system, developing fetus) to mixtures of TCDD and PBDE with responses to either compound alone. No studies were located in which pretreatment with PBDE before TCDD exposure was examined for possible effects on TCDD toxicity. Joint actions on the developing nervous system, developing fetus and thyroid are plausible (see Appendices A and B), but the nature of these actions is unknown and unstudied. Based on limited evidence of PBDE antagonism of TCDD-induced actions on the AhR and the lack of confirming data examining toxicity endpoints, a factor of C is assigned for toxicological significance.

Additional Uncertainties (AhR-mediated toxicity only) – A modifying factor of 2 is assigned for different duration of exposure. A modifying factor of b is assigned for *in vitro* studies.

Table 6. Effect of 2,3,7,8-TCDD on Phthalates
BINWOE: >IIIB for developmental effects
BINWOE: <IIIB for hepatic effects

Direction of Interaction – The predominant direction of possible interactions cannot be predicted. Two studies were located that examined interactions of TCDD and phthalates in rats; the results were conflicting for the different effects in each study, two separate BINWOEs were derived.

Mechanistic Understanding – Impaired reproductive function and development have been associated with oral exposure to TCDD and oral exposure to DEHP or DBP (see Appendices A and C). Thyroid disruption is also associated with oral exposure to TCDD and oral exposure to DEHP, DBP, or DNOP. There is no evidence for a common mechanism of action for phthalate- and TCDD-induced toxicity for any of these endpoints. The mechanisms responsible for TCDD-induced impairment of reproductive development are thought to be mediated through the AhR and subsequent changes in levels of growth factors and receptor interactions. Thyroid disruption by TCDD is postulated to occur through two mechanisms: (1) AhR-mediated upregulation of UDP-glucuronyltransferase and subsequently increased metabolism and elimination of T_4 and (2) interference of hydroxylated metabolites with binding of T_4 to transport proteins. There is no evidence that phthalates bind to the AhR. There is evidence that DEHP-induced fetotoxicity and teratogenicity is mediated through the peroxisome proliferator activated receptor (PPAR), and evidence that DEHP does not bind to, or directly interfere with, androgen receptors (unlike TCDD, which is an androgen receptor antagonist) (ATSDR 2002). There is no clear mechanistic understanding of potential thyroid disruption associated with phthalate exposure. Animal studies with DEHP reported histopathological changes in thyroid tissue (reduced colloid density and follicle size after 90 days but not after 2 years of exposure) that could have been transient, and did not measure serum thyroid hormone levels. A 90-day study with DNOP reported the same histopathological changes noted in the 90-day study with DEHP, and similarly did not measure serum thyroid hormones. A 90-day study with DBP failed to note any significant histopathological changes in the thyroid, but reported a significant reduction in T_3, but no treatment-related effect on T_4. Recent human studies reported an inverse correlation between serum T_3 and T_4 and urinary MBP (Huang et al. 2007), and between serum T_3 and T_4 and urinary MEHP (Meeker et al. 2007).

Toxicologic Significance – Two studies were located that examined interactions between TCDD and phthalates in rats. Greater-than-additive interaction was reported in inducing male developmental effects (decreased epididymal weights) in reproductive systems of pups prenatally exposed to TCDD and DBP (Rider et al. 2010). The study also reported liver malformations following exposure to the mixture. This effect was not observed following administration of individual chemicals. In contrast, pretreatment or post-treatment with DEHP resulted in a decrease in the TCDD-induced hyperlipidemia (i.e., potential liver effect) (Tomaszewski et al. 1988). The former study used much lower TCDD dose (2 or 1.3 µg/kg) than the latter one (160 µg/kg).

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

Table 7. Effect of Phthalates on 2,3,7,8-TCDD
BINWOE: >IIIB for developmental effects
BINWOE: <IIIB for hepatic effects

Direction of Interaction – The direction of possible interactions cannot be predicted. Two studies were located that examined interactions of TCDD and phthalates in rats. The results were conflicting for two different effects; two separate BINWOEs were derived.

Mechanistic Understanding – Impaired reproductive function and development have been associated with oral exposure to TCDD and oral exposure to DEHP or DBP (see Appendices A and C). Thyroid disruption is also associated with oral exposure to TCDD and oral exposure to DEHP, DBP, or DNOP. There is no evidence for a common mechanism of action for phthalate- and TCDD-induced toxicity for any of these endpoints. The mechanisms responsible for TCDD-induced impairment of reproductive development are thought to be mediated through the AhR and subsequent changes in levels of growth factors and receptor interactions. Thyroid disruption by TCDD is postulated to occur through two mechanisms: (1) AhR-mediated upregulation of UDP-glucuronyltransferase and subsequently increased metabolism and elimination of T_4 and (2) interference of hydroxylated metabolites with binding of T_4 to transport proteins. There is no evidence that phthalates bind to the AhR. There is evidence that DEHP-induced fetotoxicity and teratogenicity is mediated through the PPAR, and evidence that DEHP does not bind to, or directly interfere with, androgen receptors (unlike TCDD, which is an androgen receptor antagonist). There is no clear mechanistic understanding of potential thyroid disruption associated with phthalate exposure. Animal studies with DEHP reported histopathological changes in thyroid tissue (reduced colloid density and follicle size after 90-days but not after 2 years of exposure) that could have been transient, and did not measure serum thyroid hormone levels. A 90-day study with DNOP reported the same histopathological changes noted in the 90-day study with DEHP, and similarly did not measure serum thyroid hormones. A 90-day study with DBP failed to note any significant histopathological changes in thyroid, but reported a significant reduction in T_3, but no treatment-related effect on T_4. Recent human studies reported an inverse correlation between serum T_3 and T_4 and urinary MBP (Huang et al. 2007), and between serum T_3 and T_4 and urinary MEHP (Meeker et al. 2007).

Toxicologic Significance – Two studies were located that examined interactions between TCDD and phthalates in rats. Greater-than-additive interaction was reported in inducing male developmental effects (decreased epididymal weights) in reproductive systems of pups prenatally exposed to TCDD and DBP (Rider et al. 2010). The study also reported liver malformations following exposure to the mixture. This effect was not observed following administration of individual chemicals. In contrast, pretreatment or post-treatment with DEHP resulted in a decrease in the TCDD-induced hyperlipidemia (i.e., potential liver effect) (Tomaszewski et al. 1988). The former study used much lower TCDD dose (2 or 1.3 µg/kg) than the latter one (160 µg/kg).

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

Table 8. Effect of Phthalates on PBDEs
BINWOE: Indeterminate (?)

Direction of Interaction – The direction of possible interactions cannot be predicted because there are no relevant *in vivo* or *in vitro* data examining modes of joint action of phthalates and PBDEs on several shared toxicity targets, and the available mechanistic understanding for phthalates and for PBDEs does not support reliable projections of possible interactions.

Mechanistic Understanding – Separate studies have shown that oral exposure to PBDEs and oral exposure to DEHP or DBP adversely affects the developing fetal skeleton (see Appendices B and C). Thyroid disruption has been associated with oral exposure to lower PBDEs and oral exposure to DEHP, DBP, or DNOP. There is no evidence for a common mechanism of action for either thyroid disruption or effects on the developing fetal skeleton. There is evidence that DEHP-induced fetotoxicity and teratogenicity are mediated through the PPAR. The mechanism of PBDE-induced fetotoxicity is not likely to be mediated by the AhR and is otherwise unknown. There is no clear mechanistic understanding of potential thyroid disruption associated with phthalate exposure (ATSDR 2002). Animal studies with DEHP reported histopathological changes in thyroid tissue (reduced colloid density and follicle size after 90 days but not after 2 years of exposure) that could have been transient, and did not measure serum thyroid hormone levels. A 90-day study with DNOP reported the same histopathological changes noted in the 90-day study with DEHP, and similarly did not measure serum thyroid hormones. A 90-day study with DBP failed to note any significant histopathological changes in thyroid, but reported a significant reduction in T_3, but no treatment-related effect on T_4. Recent human studies reported an inverse correlation between serum T_3 and T_4 and urinary MBP (Huang et al. 2007), and between serum T_3 and T_4 and urinary MEHP (Meeker et al. 2007). PBDEs disrupt thyroid function by decreasing circulating levels of T_4. There is some evidence that this may occur through hydroxylated intermediates that interfere with binding of T_4 at the receptor site or transport proteins. Taken together, this information is too tentative to be useful in predicting the direction or nature of joint actions of phthalates and PBDEs on either developing fetuses or thyroid function.

Toxicologic Significance – Less-than-additivity was reported in an *in vitro* study when DNOP and octaBDE were tested together for their action as endocrine disruptors on human breast cancer cells (Pohl 2009). However, the results were preliminary and lower doses have to be tested to obtain the full understanding of the interaction. Joint actions on the thyroid and developing fetus are plausible, but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects the lack of data.

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

Table 9. Effect of PBDEs on Phthalates
BINWOE: Indeterminate (?)

Direction of Interaction – The direction of possible interactions cannot be predicted because there are no relevant *in vivo* or *in vitro* data examining modes of joint action of phthalates and PBDEs on several shared toxicity targets, and the available mechanistic understanding for phthalates and for PBDEs does not support reliable projections of possible interactions.

Mechanistic – Separate studies have shown that oral exposure to PBDEs and oral exposure to DEHP or DBP adversely affects the developing fetal skeleton (see Appendices B and C). Thyroid disruption has been associated with oral exposure to lower PBDEs and oral exposure to DEHP, DBP, or DNOP. There is no evidence for a common mechanism of action for either thyroid disruption or effects on the developing fetal skeleton. There is evidence that DEHP-induced fetotoxicity and teratogenicity are mediated through the PPAR. The mechanism of PBDE-induced fetotoxicity is not likely to be mediated by the AhR and is unknown. There is no clear mechanistic understanding of potential thyroid disruption associated with phthalate exposure. Animal studies with DEHP reported histopathological changes in thyroid tissue (reduced colloid density and follicle size after 90 days but not after 2 years of exposure) that could have been transient, and did not measure serum thyroid hormone levels. A 90-day study with DNOP reported the same histopathological changes noted in the 90-day study with DEHP, and similarly did not measure serum thyroid hormones. A 90-day study with DBP failed to note any significant histopathological changes in thyroid, but reported a significant reduction in T_3, but no treatment-related effect on T_4. Recent human studies reported an inverse correlation between serum T_3 and T_4 and urinary MBP (Huang et al. 2007), and between serum T_3 and T_4 and urinary MEHP (Meeker et al. 2007). PBDEs disrupt thyroid function by decreasing circulating levels of T_4. There is some evidence that this may occur through hydroxylated intermediates that interfere with binding of T_4 at the receptor site or to transport proteins. Taken together, this information is too tentative to be useful in reliably predicting the direction or nature of joint actions of phthalates and PBDEs on either developing fetuses or thyroid function.

Toxicologic Significance – Less-than-additivity was reported in an *in vitro* study when DNOP and octaBDE were tested together for their action as endocrine disruptors on human breast cancer cells (Pohl 2009). However, the results were preliminary and lower doses have to be tested to obtain the full understanding of the interaction. Joint actions on the thyroid and developing fetus are plausible, but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects the lack of data.

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

Table 10. Matrix of BINWOE Determinations for Repeated Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF		
		2,3,7,8-TCDD	PBDEs	Phthalates
EFFECT OF	2,3,7,8-TCDD		**=IIIC2 (thyroid toxicity) =IIIC2 (neurodevelopmental toxicity)**	**>IIIB (developmental toxicity) <IIIB (hepatic toxicity)**
	PBDEs	**<IIIC2b (AhR-mediated toxicity) ? (thyroid toxicity) ? (neurodevelopmental toxicity)**		**?**
	Phthalates	**>IIIB (developmental toxicity) <IIIB (hepatic toxicity)**	**?**	

LEGEND FOR TABLE 10

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

DIRECTION: = additive; > greater than additive; < less than additive; ? indeterminate

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

TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint;
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals;
C: toxicologic significance of interaction is unclear.

MODIFYING FACTORS:
1: anticipated exposure duration and sequence;
2: different exposure duration or sequence;
a: *in vivo* data;
b: *in vitro* data;
i: anticipated route of exposure;
ii: different route of exposure.

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

To conduct exposure-based assessments of possible endocrine, neurotoxic, or developmental health hazards from oral exposures to mixtures of CDDs, PBDEs, and phthalates, ATSDR recommends the use of a component-based approach (i.e., HI approach), because there are no direct data available to characterize health hazards (and dose-response relationships) from exposure to any mixtures of CDDs, PBDEs, and phthalates. In addition, "interaction" PBTK/PD models have not yet been developed that would predict appropriate target doses of the components.

Recommendations focus on oral exposure because it is the most relevant route with respect to health concerns from bio-persistent chemicals. CDDs and PBDEs are bio-persistent due to their resistance to metabolism and elimination from bodily tissues. Phthalates are metabolized and eliminated much more rapidly than CDDs and PBDEs, but are continuously present in body tissues due their ubiquitous presence in the environment.

As discussed by ATSDR (1992, 2004d), the exposure-based assessment of a potential health hazard is a screening approach, to be used in conjunction with evaluation of community-specific health outcome data, consideration of community health concerns, and biomedical judgment, to assess the degree of public health hazard presented by mixtures of substances released into the environment. In a component-based approach for noncancer health effects: (1) joint additive actions of the components on shared targets of toxicity are assumed; (2) oral intakes are calculated based on measured concentrations of the components in media of concern; (3) intakes are divided by MRLs or TTDs; and (4) resulting hazard quotients (HQs) are summed to arrive at a HI.

TTDs are developed for an endpoint of concern when the critical effect levels for those effects are higher than those associated with the most sensitive endpoint. When the most sensitive endpoint is the effect of concern, the MRL is used as the reference toxicity benchmark for estimating the effect-specific hazard index (ATSDR 2004a). The derivation of TTDs is analogous to the derivation of MRLs and follows applicable ATSDR guidance. Based on the commonality of specific effects and targets within the general categories of endocrine disruption, neurobehavioral effects, and developmental toxicity, separate chemical-specific TTDs have been derived for the most sensitive endpoints encompassing developmental neurobehavioral effects (PBDEs and TCDD), developmental endocrine effects (TCDD, PBDEs, and phthalates), and thyroid disruption in adults (TCDD, PBDEs, and phthalates). In some cases, the

endpoint-specific TTDs are equivalent to MRLs (for TCDD, the $TTD_{NEUROBEHAVIORAL}$ = chronic MRL, for lower PBDEs, the $TTD_{THYROID}$ and $TTD_{DEVELOPMENTAL}$ = acute MRL, and for decaBDE, the $TTD_{NEUROBEHAVIORAL}$ = acute MRL). The relevant TTDs are summarized in Table 11; details on derivation can be found in Appendices A–C.

Table 11. Target Organ Toxicity Doses (TTDs) for Repeated Oral Exposure to Chemicals of Concern (Concentrations are mg/kg Body Weight/Day)

	2,3,7,8-TCDD	Lower-brominated PBDEs	DecaBDE	DNOP	DEHP	DBP	DEP
Neurobehavioral development	$1x10^{-9}$	$3x10^{-5}$	0.01	NA, ND	NA, ND	NA, ND	NA, ND
Reproductive	$2x10^{-8}$ (male repro-duction)	$6x10^{-5}$ (male and female repro-duction)	0.01 (male repro-duction)	NA, ND	0.05 (female repro-duction)	0.008 (male and female repro-duction)	NA, ND
Thyroid disruption	$9x10^{-8}$	$6x10^{-5}$	7.5	0.4	0.4	1.5	NA, ND

See Appendices A, B, and C, for details of derivations.

DBP = di-*n*-butyl phthalate; DecaBDE = decabromodiphenyl ether; DEHP = di-(2-ethylhexyl) phthalate; DEP = diethyl phthalate; DNOP = di-*n*-octyl phthalate; NA = not applicable; ND = not derived; PBDE = polybrominated diphenyl ether; 2,3,7,8-TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

For the assessment of the CDDs, concentrations in the media of concern should be converted to TEQs and summed to arrive at exposure levels that can be converted to oral intakes and compared with oral MRLs (or TTDs) for the reference dioxin, 2,3,7,8-TCDD (ATSDR 1998). For the assessment of PBDEs, lower-brominated congeners should be summed and assessed together, with a separate assessment of decaBDE (consistent with ATSDR 2017 profile). For phthalates, only DEHP, DBP, and DNOP should be considered because these are the only phthalate esters that have been associated with the common effects of concern. Exposure and HQs should be determined for each of these esters as follows. For thyroid effects in adults, exposure concentrations should be estimated for DEHP, DBP, and DNOP, and hazard quotients should be derived using the specific $TTD_{THYROID}$ for each phthalate ester. For developmental endocrine effects, exposures should be estimated for DEHP and DBP, and HQs should be derived for each on the basis of the $TTD_{DEVELOPMENTAL}$ for each ester.

The calculation of a screening-level HI for assessing a mixture of chemicals under the assumption of joint additivity involves a modification of the HI approach as follows. Exposure estimates are made for each

chemical of concern in the mixture. Chemical-specific TTDs or MRLs are similarly defined, where possible, for each endpoint of concern. Finally, HIs are calculated for each endpoint of concern for joint exposure to the mixture by summing the ratio of exposure to endpoint-specific TTD or MRL for each chemical in the mixture to generate the HQ[2]. This procedure is described in ATSDR (2004a, Section 2.3.2). For example, the HI for thyroid effects of a mixture of TCDD, PBDEs, DEHP, and DBP would be calculated as follows:

$$HI_{THY} = \frac{E_{TCDD}}{TTD_{TCDD\ THY}} + \frac{E_{PBDE(lower)}}{TTD_{PBDE\ (lower)\ THY}} + \frac{E_{decaBDE}}{TTD_{decaBDE\ THY}} + \frac{E_{DEHP}}{TTD_{DEHP\ THY}} + \frac{E_{DBP}}{TTD_{DBP\ THY}},$$

where HI_{THY} is the HI for thyroid toxicity, E_{TCDD} is the exposure to 2,3,7,8-TCDD (expressed in the same units as the corresponding TTD), $TTD_{TCDD\ THY}$ is the TTD for 2,3,7,8-TCDD, which is based on thyroid toxicity ($1x10^{-9}$ mg/kg/day), $E_{PBDE(lower)}$ is the exposure to PBDE (expressed in the same units as the corresponding TDD), $TTD_{PBDE(lower)\ THY}$ is the TTD for the thyroid toxicity of PBDEs, and so forth. A similar approach is recommended to generate HI values for neurodevelopmental toxicity (PBDEs and TCDD) and developmental endocrine toxicity (TCDD, PBDEs, DEHP, and DBP).

The proposed approach could overestimate actual risks to human health with regard to joint TCDD and PBDE exposure. While the toxicity data for exposure to TCDD alone and to PBDEs alone indicate possible joint additivity with respect to thyroid disruption and neurobehavioral developmental toxicity, there is *in vitro* evidence that PBDEs could antagonize TCDD-induced toxicity mediated through the AhR signal transduction pathway. However, due to the lack of any studies that investigate thyroid or neurobehavioral endpoints following joint exposure to TCDD and PBDEs (compared with TCDD alone and PBDEs alone) and the lack of information to quantitatively assess the conflicting weights of evidence for additivity and PBDE antagonism of TCDD thyroid and neurodevelopmental toxicity, it is highly uncertain whether the resultant joint action of TCDD and PBDEs on these endpoints would be either additive or less-than-additive. Furthermore, there are no data to predict what effects, if any, the presence of phthalate esters would have on PBDE inhibition of TCDD-induced AhR signal transduction, or on potential toxic outcomes.

[2]The ratio of exposure to TTD is known as the HQ.

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

The addition of HQs for a particular exposure scenario assumes that less-than-additive (e.g., antagonistic or inhibitory) or greater-than-additive (e.g., synergistic or potentiating) interactions do not occur among the components of the mixture. As discussed in Section 2.3, there is very limited evidence to reliably predict the nature of interactions between CDDs, PBDEs, and phthalates on endocrine disruption, developmental toxicity, or neurobehavioral effects following joint exposure. *In vitro* mechanistic evidence indicates that PBDEs may antagonize TCDD-related toxic effects that are mediated through the AhR signal transduction pathway, but there are no studies that address possible joint action of PBDEs and TCDD on any toxicity endpoint. Furthermore, the mechanistic evidence suggesting possible antagonism is offset by thyroid toxicity data for TCDD alone and PBDEs alone that suggest the possibility of joint additivity on the basis of a common non-AhR-mediated mode of action (i.e., inhibition of T_4 binding by hydroxylated intermediates). Therefore (as discussed previously), the HI for thyroid toxicity could possibly overestimate, but would not likely underestimate, actual risks to human health.

When the screening assessment provides preliminary evidence that the mixture may constitute a health hazard (i.e., one or more endpoint-specific hazard indexes exceed 1, or the mixture cancer risk equals or exceeds $1x10^{-4}$), additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2004a). The additional evaluation includes biomedical judgment, assessment of community-specific health outcome data, and consideration of community health concerns (ATSDR 2004a).

Data Needs for Assessing Joint Toxic Actions of CDDs, PBDEs, and Phthalates. Although there are PBTK models for some individual chemicals within these three classes of chemicals, there are no "interaction" PBTK models like those that exist for benzene, toluene, ethylbenzene, and xylene (BTEX) and certain other volatile organic chemicals (e.g., see ATSDR Interaction Profile for BTEX; ATSDR 2004b). Before such models can be developed, pharmacokinetic points of interactions between members of the subject classes of CDDs, PBDEs, and phthalates must first be identified. However, to date, no common points of pharmacokinetic interaction have been identified for CDDs, PBDEs, and phthalates. If a common point of pharmacokinetic interaction were to be identified, then it would be possible to design the additional studies needed to develop an "interaction" PBTK model for CDDs, PBDEs, and phthalates. Following identification of a common point of pharmacokinetic interaction, *in vivo* studies would have to be conducted to examine the kinetics of internal concentrations of the parent chemicals of concern and

their metabolites following co-exposure, and these studies would have to be compared with similar studies for each chemical alone.

For example, before the "interaction" PBTK model for BTEX was developed, scientists knew that there was a common initial step in the metabolism of each of these chemicals (CYP2E1) in the rat, and that these chemicals were competitive inhibitors of each other's metabolism. As discussed in ATSDR (2004b), the BTEX model (Haddad et al. 1999a) predicts toxicokinetic interactions in the quaternary mixture, as indicated by venous blood levels of chemicals, by using information on binary interactions among the component chemicals. Development of the model initially involved: (1) refining and verifying the validity of existing PBTK models for the four individual chemicals; (2) linking (interconnecting) pairs of the individual chemical PBTK models at the level of hepatic metabolism by introducing binary interaction terms for potential mechanisms of action (competitive, noncompetitive, and uncompetitive metabolic inhibitions); and (3) characterizing the mechanism of interactions in the binary mixtures by optimally fitting model simulations to experimental data on venous blood concentrations of parent chemicals in rats exposed by inhalation to all binary combinations of the four components. Once the PBTK model was developed, it was used to examine at what exposure concentrations the competitive interactions became important.

4. Conclusions

ATSDR recommends a component-based HI approach (modified with TTDs) that assumes additive joint toxic action to assess possible endocrine, neurobehavioral, and developmental health hazards from oral exposure to mixtures of CDDs, PBDEs, and phthalates. No *in vivo* studies were located that examined endocrine, neurobehavioral, or developmental endpoints following exposure to trinary mixtures of CDDs, PBDEs, and phthalates, thereby precluding the derivation of any "whole mixture" MRLs. However, the available toxicity information for chemicals from each of the chemical classes of concern indicates that joint toxic action is plausible with regard to thyroid disruption (2,3,7,8-TCDD, PBDEs, DEHP, DNOP, and DBP), neurodevelopmental effects (2,3,7,8-TCDD and PBDEs), and developmental endocrine effects (i.e., disruption of male or female reproductive function following perinatal exposure [2,3,7,8-TCDD, PBDEs, and DEHP or DBP] or disruption of thyroid functioning, which may influence neurological development [PBDEs and CDDs]). Based on the available toxicity information, separate chemical-specific TTDs have been derived for the most sensitive endpoints encompassing developmental neurobehavioral effects (PBDEs and TCDD), developmental endocrine effects (TCDD, PBDEs, and phthalates), and thyroid disruption in adults (TCDD, PBDEs, and phthalates). For TCDD, the TTD for neurodevelopmental effects is the chronic MRL. ATSDR recommends using these TTDs in screening-level assessments (using the HI approach) for the protection of public health from increased risks for these effects from chronic oral exposure to mixtures of CDDs, PBDEs, and phthalates.

Weight-of-evidence analyses of available data on the joint toxic action of binary mixtures of these components indicate that scientific evidence for greater-than-additive or less-than-additive interactions between TCDD and phthalates and between phthalates and PBDEs is lacking or inadequate to characterize the possible modes of joint action on endocrine disruption, neurobehavioral toxicity, and developmental toxicity. *In vitro* mechanistic evidence indicates that PBDEs may antagonize TCDD-related toxic effects mediated through the AhR signal transduction pathway, but there are no studies that address possible joint action of PBDEs and TCDD on any toxicity endpoint. Furthermore, the mechanistic evidence suggesting possible antagonism is offset by thyroid toxicity data for TCDD alone and PBDEs alone that suggest the possibility of joint additivity on the basis of a common non-AhR-mediated mode of action (i.e., inhibition of T_4 binding by hydroxylated intermediates). Based on these considerations, ATSDR recommends that additivity be assumed in exposure-based screening assessments for the protection of public health from oral exposure to mixtures of these components. When the screening assessment indicates a potential hazard, further evaluation is needed, using biomedical judgment, community-specific health outcome data, and taking into account community health concerns.

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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_4 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 (≥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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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 μU per mL serum. The 5 μU/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 ≤ 5 μU/ml and 39.0 ppt (95% CI 8.9–173) in those with b-TSH > 5 μU/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]-α, and TGF-β1) and increased expression of EGF receptor, and changes in thyroid hormone metabolism leading to lowered thyroid hormone levels (ATSDR 1998; Devito and Birnbaum 1994; Van den Berg et al. 1994). Many of the toxic and biological responses to CDDs 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-α, and TGF-β1) and increased expression of the EGF receptor, which subsequently lead to altered medial cell proliferation in the developing palatal shelves (Abbott et al. 1994). TCDD-induced disruptions in the development of the kidney and male and female reproductive organs, observed in animal experiments, are also thought to be associated with TCDD-induced changes in levels of growth factors and receptors (ATSDR 1998). The molecular events between TCDD-induced changes in gene expression and reduced levels of growth factors remain to be elucidated (ATSDR 1998).

Changes in serum T_4 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_4 (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_4 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 (0.7×10^{-9} mg/kg/day) can be used as a $TTD_{THYROID\text{-}DEVELOPMENTAL}$ 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 $TTD_{NEURODEVELOPMENTAL}$ for 2,3,7,8-TCDD is the chronic oral MRL of 0.000001 µg/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)

$TTD_{THYROID}$ = 0.00009 μg/kg/day ($9x10^{-8}$ mg/kg/day)
Chronic oral MRL (based on neurodevelopmental effects) = 0.000001 μg/kg/day ($1x10^{-9}$ mg/kg/day)
$TTD_{DEVELOPMENTAL}$ = 0.00002 μg/kg/day ($2x10^{-8}$ mg/kg/day)

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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 ^{14}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 ^{14}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 ^{14}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 ^{14}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) 14th 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-prostagenic, 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^3 for lower PBDEs based on a NOAEL of 1.1 mg/m^3 for changes in thyroid hormones in rats intermittently exposed to a commercial octaBDE product for 13 weeks. The MRL of 0.006 mg/m^3 was derived by dividing the $NOAEL_{HEC}$ of 0.53 mg/m^3 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_4 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 $7x10^{-4}$ per mg/kg-day and a drinking water unit risk of $2x10^{-8}$ per µg/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. *D*ose-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 ≤90 mg/kg/day for 90 days, rats exposed to ≤8,000 mg/kg/day for 13 weeks, or mice exposed to ≤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 ≥94% decaBDE composition used in the NTP studies. In chronic-duration studies, thyroid follicular cell hyperplasia was increased at ≥3,200 mg/kg/day in male B6C3F_1 mice that were exposed to ≥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_4 and T_3 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_4 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_4 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_3 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_3 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_3 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_3 levels. Therefore, this study was not considered for derivation of the oral $TTD_{THYROID}$.

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_3 and T_4 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 $TTD_{THYROID}$ 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. *T*he acute MRL of 0.00006 mg/kg/day is adopted for the oral $TTD_{THYROID}$ for lower PBDEs. The acute-duration oral MRL is based on a LOAEL for decreased serum T_4 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 ≥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 $TTD_{THYROID.}$

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) (Biesemeier 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_3 and T_4 were significantly decreased at doses as low as 100 mg/kg/day, the lowest dose tested (Lee et al., 2010). In addition, decreased serum T3 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_4 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 $TTD_{DEVELOPMENTAL}$ 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 $TTD_{DEVELOPMENTAL}$ 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_4 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 (≥10.2 mg/kg/day) and lower than developmental thyroid effects in available pre- and peri-natal exposure studies (≥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 $TTD_{DEVELOPMENTAL}$.

Neurobehavioral Effects

DecaBDE. The acute MRL of 0.01 mg/kg/day is adopted for the oral $TTD_{NEUROBEHAVIORAL}$ 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 $TTD_{NEUROBEHAVIORAL}$.

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 premating 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

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 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_4 and T_3 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 ≥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 ≥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, 17β-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 (PPARα). Peters et al. (1997) assessed pregnancy outcome in female F_4C57BL/6N x Sv/129, wild type (+/+), and PPARα-null (-/-) mice on GDs 10 and 18 after administration of DEHP by gavage on GDs 8 and 9. PPARα-null mice lack expression of PPARα 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 PPARα-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_4 and T_3 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 $TTD_{THYROID}$. 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 $TTD_{THYROID}$ 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 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 ≥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 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_3, but did not affect total T_4 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 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 DBP affect 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 17β-estradiol (Harris et al. 1997). In another *in vitro* assay, DBP was approximately 3,000-fold less potent than 17β-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 in 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 $TTD_{THYROID}$. 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 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 $TTD_{DEVELOPMENTAL}$ 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 by 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_4 (Hinton et al. 1986); serum levels of T_3 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_0 generation mice or up to 8,640 mg/kg/day for 85–105 days to the F_1 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 $TTD_{THYROID}$ 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 $TTD_{THYROID}$ 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 mg/kg/day

$TTD_{DEVELOPMENTAL}$ = not derived

$TTD_{NEUROBEHAVIORAL}$ = 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 ^{14}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 ^{14}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 $TTD_{THYROID}$ 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 $TTD_{DEVELOPMENTAL}$ will not be derived.

Neurobehavioral Effects

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

Summary (TTDs for DEP)

$TTD_{THYROID}$ = not available
$TTD_{DEVELOPMENTAL}$ = not available
$TTD_{NEUROBEHAVIORAL}$ = not available

C-4.6 References

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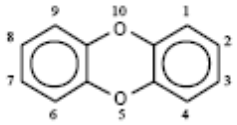
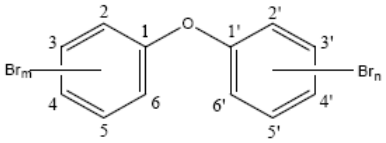
Appendix D: Chemical Structures of Mixture Components

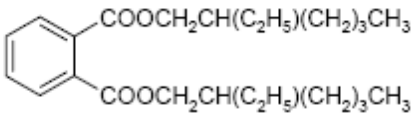
Chlorinated Dibenzo-*p*-Dioxins



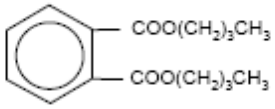
Polybrominated Diphenyl Ethers



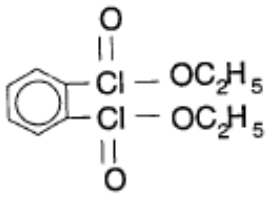
Di-2(ethylhexyl) phthalate



Di-*n*-Butyl phthalate



Diethyl phthalate



Di-*n*-octyl phthalate