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.

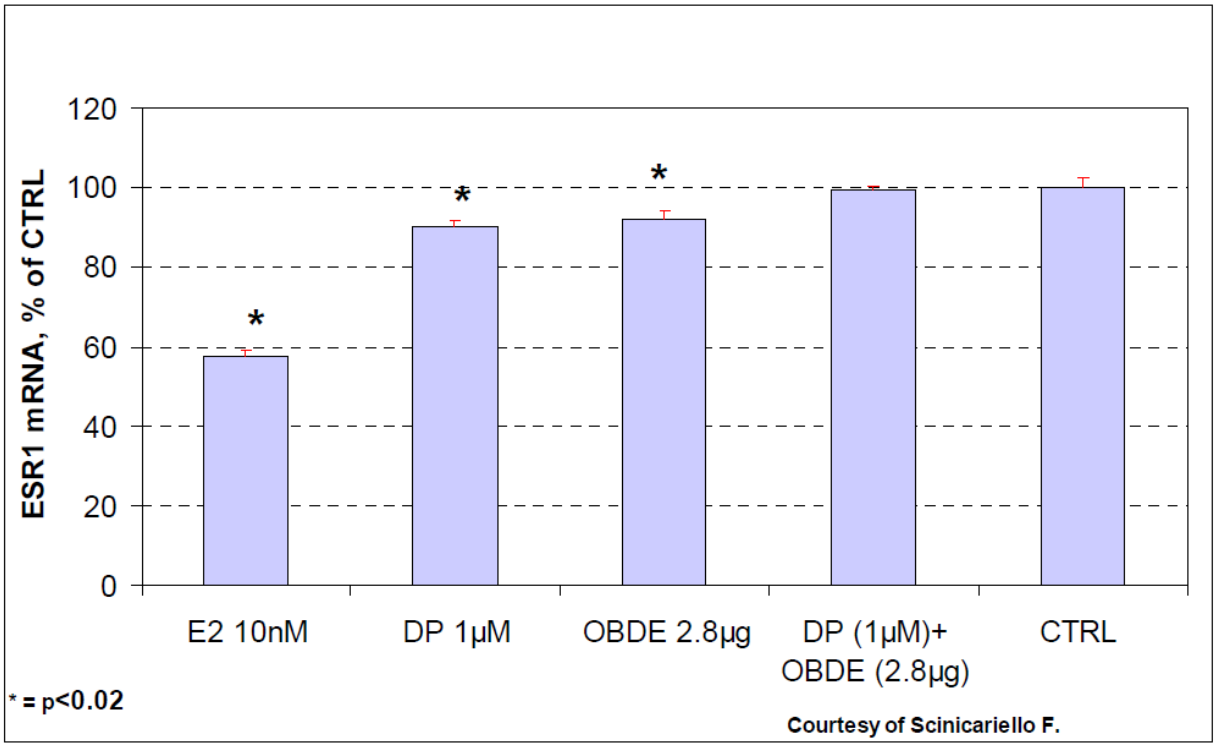
In contrast, in an older study, there was some evidence that DEHP may antagonize TCDD-induced fatty liver, hyperlipidemia, and mortality in rats (Tomaszewski et al. 1988). Treatment of F344 rats with TCDD alone (160 μg/kg) resulted in an increase in serum triglycerides and cholesterol levels, while treatment 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.