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.