1. Introduction

The primary purpose of this Interaction Profile for chlorinated dibenzo-*p*-dioxins (CDDs), hexachlorobenzene, *p,p'*-DDE, methylmercury, and polychlorinated biphenyls (PCBs) 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, 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's Division of Toxicology (DT) 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.

Breast-feeding is widely recognized as offering the developing infant the benefits of balanced nutrition and passive immunization against microbial infections (Pohl and Tylenda 2000), but the detection of persistent environmental pollutants in breast milk samples from general populations in the United States, the Netherlands, Sweden, and elsewhere has led to concerns that these chemicals may have detrimental effects on the health and/or development of breast-fed children. Environmental chemicals that have been detected in samples of human breast milk include mercury and methyl mercury, lead, cadmium, PCBs, CDDs, chlorinated dibenzofurans (CDFs), brominated diphenylethers (BDEs), and persistent forms of organochlorine pesticides such as *p,p'*-dichlorodiphenylether (*p,p'*-DDE), hexachlorobenzene, mirex, and lindane (Abadin et al. 1997; Hooper and McDonald 2000; Pohl and Hibbs 1996; Pohl and Tylenda 2000). Chemicals that are lipophilic and resistant to metabolic degradation have a tendency to increasingly accumulate with increasing levels of the food chain and to distribute to fatty tissue and breast milk within the human body.

Five persistent chemicals or chemical classes detected in human milk (CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs) were selected for the purposes of reviewing available data on their joint actions in producing toxic effects following oral exposure. PCBs, *p,p'*-DDE, and hexachlorobenzene were selected because they have been detected with very high frequency in breast milk in a recent study of women residing in the U.S. Great Lakes region (Kostyniak et al. 1999), in a study of the general population in North Carolina (Rogan et al. 1986a), and in a recent Canadian study of the general population that included the Great Lakes basin (Newsome et al. 1995). It is also expected that breast milk of fish-eating populations in the U.S. Great Lakes region may contain CDDs and methylmercury, because these chemicals have been detected in Great Lakes fish (ATSDR 2001c). Whereas recent U.S. monitoring studies have not focused on the presence of CDDs and methylmercury in breast milk, CDDs have been detected in earlier U.S. studies, as well as in studies in the Netherlands, Canada, Germany, New Zealand, Japan, and Russia (Pohl and Hibbs 1996), and methylmercury has been detected in breast milk samples from Japan, Germany, and Sweden (Abadin et al. 1997). In addition, elevated levels of PCBs and mercury were detected in samples of breast milk from mothers residing in the North Atlantic Faroe Islands where the seafood diet includes pilot whale meat and blubber (Grandjean et al. 1995a).

Another reason for selecting these five chemicals is that there is a fair amount of overlap in the wide range of endpoints or organs that these chemicals affect in humans and/or animals (see Appendices A–E, Table 1). This overlap leads to concern that, following exposure to mixtures of the five chemicals in breast milk or other food sources, all five may jointly act to produce altered neurological development, suppression of immune competence, or cancer, and three (CDDs, *p,p'*-DDE, and PCBs) may jointly act to alter development of reproductive organs (Table 1).

This profile begins with a brief review of recent studies designed to examine whether or not detrimental effects on the health and/or development of breast-fed children may be associated with persistent chemicals detected in breast milk. Available data on the joint toxic actions of the five chemicals of concern are next reviewed. The weight of evidence is assessed concerning whether binary mixtures of these chemicals may be expected to jointly act in additive, less-than-additive, or greater-than-additive manners. Following the review of these data, their relevance to public health concerns associated with exposures to mixtures of these chemicals is discussed, and recommendations are made for exposure-based assessments of joint toxic actions of this mixture of chemicals.

Table 1. Health Effects Observed in Humans or Animals after Oral Exposure to Chemicals of Concern. (See Appendices A, B, C, D, and E for More Details.)

Effects	Chemicals of concern[a]				
	2,3,7,8-TCDD	Hexachloro-benzene	*p,p'*-DDE	Methyl-mercury	PCBs
Wasting syndrome	x	x			x
Kidney damage				x	
Liver damage	**X**	**X**[b]	x[b]		x
Immunosuppression	x[b]	x	x	x	x[b]
Thyroid hormone disruption	**X**	**X**			x
Female reproductive organ disruption	x	**X**[b]		x	x
Male reproductive organ disruption	**X**		x	x	x
Neurological impairment	**X**	**X**	**X**	**X**	x
Altered neurological development (pre- and/or post-natal)	x[b]	**X**[b]	x[c]	**X**[b]	**X**[b]
Altered female reproductive organ development	x				x
Altered male reproductive organ development	x		x[b]		x
Other developmental effects (malformations or fetotoxicity)	x	**X**	x	x	x
Cancer[d]	x	x	x	x	x

[a]Upper case and bolded **X** indicates that effects have been observed in humans. Lower case and non-bolded x indicates that effects have been observed only in animals.
[b]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).
[c]No data are available for *p,p'*-DDE effects on this endpoint, but altered neurobehavior was observed in adult rats following exposure to single oral doses of 0.5 mg *p,p'*-DDT/kg on postnatal day 10 (Eriksson et al. 1990, 1992).
[d]Carcinogenic responses have been demonstrated in animals exposed to each of the chemicals. EPA has derived oral slope factors for humans exposed to 2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, and PCBs based on tumor responses in animals (see Appendices A, B, C, and E). EPA did not derive a slope factor for humans exposed to methylmercury based on evidence that effects on the nervous system and its development would occur at exposure levels much lower than those necessary to produce cancer (see Appendix D).

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 Ah receptor (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 (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 (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 (Viluksela et al. 1998a, 1998b).

Like CDDs, oral exposure of animals to PCB mixtures elicits a broad array of effects, including a body weight wasting syndrome involving thymic atrophy, induction of hepatic Phase I (CYP oxygenases) and Phase II (e.g., UDP-glucuronyltransferases) enzymes, liver damage and enlargement, porphyria, kidney damage, immunosuppression, thyroid hormone disruption, disruption of female and male reproductive organs, altered development of female and male reproductive organs, neurological impairment, altered neurological development (associated with pre- or post-natal exposure), and cancer (Appendix E; ATSDR 2000). In contrast to CDDs, Ah-receptor mediation may account for only a subset of the wide array of PCB-induced effects. There is increasing evidence from animal studies that several PCB-induced effects may involve multiple mechanisms (ATSDR 2000; Fischer et al. 1998; Hansen 1998; Li and Hansen 1997; Safe 1994b). PCB-induced effects that appear to predominately involve Ah-receptor dependent mechanisms include: induction of hepatic activities of CYP1A1, 1A2, and 1B1 (Connor et al. 1995; Hansen 1998; Safe 1994b); body weight wasting and thymic atrophy from acute exposure (Hori et al. 1997; Safe 1994b); and porphyria and porphyria cutanea tardea (Smith et al. 1990b). PCB-induced effects involving Ah-receptor independent mechanisms include: induction of hepatic activities of CYP2B1, 2B2, 2A1, and 3A (Connor et al. 1995; Hansen 1998); neurological and neurodevelopmental effects involving changes in brain dopamine levels (Seegal 1996b, 1998) and/or changes in brain cell intracellular calcium homeostasis and related signal transduction processes (Tilson and Kodavanti 1997;

Tilson et al. 1998; Wong and Pessah 1996, 1997; Wong et al. 1997); and tissue injury related to activation of neutrophils (Brown and Ganey 1995; Ganey et al. 1993; Tithof et al. 1995).

The profile does not focus on a representative PCB congener (or congeners) or subclasses of PCBs to discuss interactions with the other components of the subject mixture, because it is likely that: (1) multiple mechanisms are involved in PCB-induced health effects; (2) different PCB congeners may produce effects by different and multiple mechanisms; and (3) humans are exposed to complex mixtures of PCB congeners with differing biological activities. PCB mixtures are discussed as the entity of concern in parallel with ATSDR's PCB MRLs, which are derived for exposure to PCB mixtures (see Appendix E).