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

## 2.1 Mixture of Concern

No studies were located that examined health effects in humans or animals exposed to mixtures exclusively containing CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs. No physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models were found for mixtures of these five chemicals. There are, however, several 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. Review of these studies follows.

Concerns that biopersistent and lipophilic chemicals accumulating in breast milk may present health problems offsetting benefits of breast feeding have led to studies examining possible relationships between adverse effects in breast-fed children and chemicals detected in breast milk, and to studies examining several health endpoints in animals following exposure to mixtures of biopersistent chemicals during gestation and/or lactation. Biopersistent, potentially toxic chemicals that have been detected in breast milk include PCBs, CDDs, CDFs, pesticides or their persistent metabolites such as *p,p'*-DDE and hexachlorobenzene, and metals including cadmium, lead, and mercury (Abadin et al. 1997; DeKoning and Karmaus 2000; Kostyniak et al. 1999; Newsome et al. 1995; Pohl and Hibbs 1996; Pohl and Tylenda 2000; Rogan 1996).

Results from studies examining concentrations of CDDs, *p,p'*-DDE, hexachlorobenzene, mercury, and PCBs in breast milk indicate that mean or median concentrations show a 10- to 100-fold range among studies for each of these chemicals (Table 2). This variation has been ascribed to numerous factors including spatially and temporally related differences in exposure of individuals and groups of individuals, differences in sampling, and differences in analytical techniques across studies (Abadin et al. 1997; DeKoning and Karmaus 2000; Pohl and Hibbs 1996; Pohl and Tylenda 2000; Rogan 1996). Breast milk monitoring studies conducted in Sweden for the past 20–30 years indicate that exposure to certain persistent chemicals may be decreasing during this period, but exposure to others may be increasing. For example, average concentrations of CDDs, CDFs, and PCBs (Hooper and McDonald 2000) and *p,p'*-DDE (Table 2; Pohl and Tylenda 2000) in Swedish breast milk samples have been decreasing, while levels of polybrominated diphenyl ethers have been increasing (Hooper and McDonald 2000).

**Table 2. Levels of Chemicals of Concern in Human Breast Milk Samples from General Populations**

| Chemical | Range of mean or median concentrations (ng/g lipid) | Newborn intake via breast milk[a] (µg/kg/day) | Region | Reference |
|---|---|---|---|---|
| CDDs and CDFs | 0.013–0.028[b] | 0.00009–0.00057[c] | United States, Canada, Germany, New Zealand, Japan, Russia | Pohl and Hibbs 1996 |
| CDDs and CDFs | 0.162–0.485[b] | 0.00115–0.00344[a] | South Vietnam (1970–1973) | Pohl and Hibbs 1996 |
| Mercury (total) | 130–793[c] | 0.922–5.625 | Japan, Germany, Sweden | Abadin et al. 1997 |
| Hexachloro-benzene | 5–63 | 0.035–0.447 | New Zealand, Brazil, Arkansas, Australia, Canada, Mexico, Quebec Caucasians | Pohl and Tylenda 2000 |
| Hexachloro-benzene | 100–>1,000 | 0.709–>7.094 | France, Spain, Quebec Inuits, Slovak Republic, Czech Republic | Pohl and Tylenda 2000 |
| *p,p'*-DDE | 500<br>1,200<br>2,000 | 3.547<br>8.513<br>14.188 | Sweden 1989<br>Sweden 1979<br>Sweden 1967 | Pohl and Tylenda 2000 |
| *p,p'*-DDE | 300–>3,000 | 2.128–21.281 | New Zealand, Brazil, France, Australia, Quebec Caucasians and Inuits, Arkansas, Canada, Slovak Republic, Czech Republic, Germany, North Carolina | Pohl and Tylenda 2000; Rogan et al.1986a |
| PCBs | 167–1,770 | 1.185–12.556 | Japan, Quebec Caucasians and Inuits, New York, Michigan, Netherlands, Poland, Finland, Croatia, North Carolina | DeKoning and Karmaus 2000 |

[a]Converted from 0.6–3.6 µg Hg/dL, using a conversion factor of 45.4 g lipid/10 dL milk (DeKoning and Karmaus 2000). Organic forms accounted for about 7–50% of total mercury (Abadin et al. 1997).
[b]Measured in 2,3,7,8-TCDD toxic equivalents (TEQs).
[c]Calculated, based on assumptions of 3.2 kg body weight, 45.4 g fat/L milk and 0.5 milk/day (DeKoning and Karmaus 2000), as follows: 5 ng/g fat x 45.4 g fat/L x 0.5 L/day x 1/3.2 kg x 1 µg/1,000 ng=0.035 µg/kg/day.

Results from a North Carolina study (Gladen and Rogan 1991; Gladen et al. 1988; Rogan et al. 1986a, 1986b, 1987) and a Dutch study (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994a, 1994b, 1996; Patandin et al. 1998, 1999a, 1999b) of breast-fed children provide some evidence that exposure to mixtures of biopersistent chemicals in human breast milk at exposure levels in the upper range of background levels or exposure during gestation via placental transfer may be associated with mild neuro-developmental delays in some children. Results from these studies are suggestive that the observed delays may have been associated with gestational exposure rather than lactational exposure. Public health agencies concur that the benefits of breast-feeding appear to outweigh the risks for most people (Abadin et al. 1997; Pohl and Hibbs 1996; Pohl and Tylenda 2000; Rogan 1996).

The North Carolina study, started in 1978, measured PCBs and *p,p'*-DDE in breast milk, maternal serum, and cord blood, and followed children to assess whether variability in growth, development, and duration of lactation were associated with levels of these chemicals in breast milk at birth (Gladen and Rogan 1991; Gladen et al. 1988; Rogan et al. 1986a, 1986b, 1987). The maternal participants (n = 880) were volunteers who were planning to deliver at one of three participating hospitals. They were not a random sample of the North Carolina population, but were a cohort expected to have normal exposure (i.e., not elevated relative to the general population) to biopersistent chemicals. Fifty-three percent had a college education, 41% were professionals, 18% smoked, 40% drank alcohol at least once a week, 90% were white with ages ranging from 16 to 41 years, 21% reported eating sport fish at least once during pregnancy, and 88% breast-fed their child, at least to some extent (Rogan et al. 1986a). PCBs and *p,p'*-DDE were detected in about 90 and 99% of breast milk samples, respectively. PCB levels were below detection limits in most samples of cord serum (88%) and placenta (97%), whereas *p,p'*-DDE levels were detected in more than 90% of cord serum and placenta samples. Amounts of PCBs and *p,p'*-DDE in breast milk at birth were used as an index of maternal body burden to examine possible relationships between prenatal exposure to these chemicals and variability in growth, development, and duration of lactation. Median PCB and DDE levels in breast milk at birth were 1.8 and 2.4 ppm (1,770 and 2,400 ng/g fat), respectively. Levels of both chemicals in milk declined by about 20 and 40% over 6 and 18 months of lactation.

Birth weight, head circumference, and neonatal jaundice showed no association with PCB and DDE levels in milk at birth, in a multiple regression analysis that included potential cofounding variables such as previous pregnancies, race, sex, age, and sport-fish consumption (Rogan et al. 1986b). Multivariate regression analyses indicated that increasing levels of PCBs and DDE in milk at birth were significantly associated with decreasing scores on two of seven cluster scores on the Brazelton Neonatal Behavioral

Assessment Scale administered to the newborn infants within 3 weeks of delivery (Rogan et al. 1986b). The affected scores were for tonicity involving measures of motor maturity (i.e., general muscle tone, pull-to-sit, activity, and defensive movements) and for reflexes (20 physical reflexes were assessed). Mothers with the highest levels of PCBs or DDE in milk had shorter median durations of lactation (13 or 10 weeks, respectively) than mothers with the lowest levels of these chemicals (26 weeks), but, in multivariate regression analyses, the association between PCB or DDE levels and duration of lactation was not significantly significant (Gladen et al. 1988). No statistically significant associations were found between levels of PCBs or DDE in milk and weight gain or frequency of illness-related physician visits for the children in the first year after birth (Rogan et al. 1987). Children were also assessed with the Bayley Scales of Infant Development at 6, 12, 18, and 24 months (Gladen et al. 1988; Gladen and Rogan 1991). Multivariate regression analyses indicated that decreasing psychomotor development index scores at 6 and 12 months of age, but not mental development index scores, were significantly associated with increasing PCB levels in milk at birth (Gladen et al. 1988). Psychomotor scores at 6 and 12 months were not significantly associated with *p,p'*-DDE levels in milk at birth, or with measures of postnatal PCB or *p,p'*-DDE exposures (Gladen et al. 1988). At 18 and 24 months of age, scores for psychomotor or mental development were not statistically significantly associated with measures of prenatal or postnatal exposure to PCBs or *p,p'*-DDE (Gladen and Rogan 1991).

The Netherlands study, started in 1990, measured PCBs in cord and maternal plasma sampled in the last month of gestation and PCBs and CDDs in breast milk sampled in the second week after delivery and followed children to assess whether variability in birth size, growth, neurological development, and thyroid hormone status could be associated with levels of these chemicals in the biological fluids (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994a, 1994b, 1996; Lanting et al. 1998a, 1998b; Patandin et al. 1998, 1999a, 1999b). The study population included 418 mother/child pairs: 207 pairs from Rotterdam (105 breast-fed and 102 formula-fed) and 211 pairs from Groningen (104 breast-fed and 107 formula-fed). Plasma samples were analyzed for four PCB congeners assessed as non-planar (2,3',4,4',5-pentachlorobiphenyl, 2,2', 3,4,4',5'- and 2,2',4,4',5,5'-hexachlorobiphenyl, and 2,2',3,4,4',5,5'-heptachlorobiphenyl). Milk samples were analyzed for 17 2,3,7,8-substituted CDDs and CDFs, 3 PCBs assessed as planar and 23 PCBs assessed as non-planar (Huisman et al. 1995a).

Within 4 weeks of birth, newborns from the Rotterdam and Groningen groups were examined for neurological deficits and assigned a reflex score (based on 10 reflex measures ), a postural tone score (based on 11 muscle tone measures), and a neurological optimality score (based on 60 neurological measures) (Huisman et al. 1995a). Logistic regression analyses that adjusted for potential confounding

variables such as maternal age, smoking, and alcohol consumption indicated that scores for reflex, postural tone, and neurological optimality were not significantly associated with levels of the four non-planar PCBs in maternal or cord plasma (Huisman et al. 1995a). In contrast, significant associations were found for increased incidence of non-optimal neurological scores and increased breast milk levels of 5/7 CDDs, 2/10 CDFs, 1/3 planar PCBs, and 10/23 non-planar PCBs (including 7/17 diortho-substituted PCBs) (Huisman et al. 1995a). Increasing incidence of postural tone scores assessed as hypotonia was significantly associated with increasing breast milk levels of planar PCBs expressed as TCDD toxicity equivalents (i.e., TEQs), but no significant associations were found between reflex scores and breast milk levels of PCBs or other analyzed chemicals (Huisman et al. 1995a). Multivariate regression analysis of the results of a 57-item examination focusing on motor functions of the children at 18 months of age indicated that decreasing neurological optimality scores were significantly associated with increasing levels of PCBs in cord plasma, but no significant associations were found between neurological optimality scores and breast milk levels of PCBs and/or CDDs (Huisman et al. 1995b).

Children from the Rotterdam group were also assessed for mental and psychomotor development using the Dutch version of the Bayley Scales of Infant Development at 3, 7, and 18 months of age (Koopman-Esseboom et al. 1996). Multivariate regression analyses that included potential confounding variables such as maternal education found no significant association at 3, 7, or 18 months between mental development scores and levels of four PCBs in maternal or cord plasma, levels of PCBs in breast milk, or levels of total PCB and dioxin TEQs in breast milk. At 3 months of age, decreasing psychomotor development scores were significantly associated with increasing PCB levels in maternal plasma, but this relationship was not statistically significant at 7 or 18 months. Multivariate regression analyses revealed no significant associations between psychomotor scores at 3, 7, or 18 months and breast milk levels of PCBs or total PCB and dioxin TEQs. At 7 months of age, increasing duration of breast feeding was significantly associated with increasing psychomotor development scores and mental development scores (Koopman-Esseboom et al. 1996).

Neurological status was also assessed in children from the Rotterdam and Groningen groups at 42 months of age (Lanting et al. 1998a, 1998b; Patandin et al. 1999a, 1999b). A neurological optimality score for each child was determined based on a 56-item examination focusing on motor functions (Lanting et al. 1998a, 1998b). Multivariate regression analyses showed no significant associations between neurological optimality score based on motor functions and PCB levels in maternal or cord plasma or levels of dioxins, PCBs, or PCB and dioxin TEQs in breast milk (Lanting et al. 1998a, 1998b). A beneficial effect of breast feeding was found on tests of fluency of movements (Lanting et al. 1998b). Cognitive abilities were also

assessed at 42 months using the Kaufman Assessment Battery for Children (Patandin et al. 1999a). Multivariate regression analyses showed a statistically significant association between decreasing scores on all three scales of the examination and increasing concentrations of PCBs in maternal plasma sampled during gestation, but no statistically significant associations were found between cognitive scores and breast milk levels of non-dioxin-like PCBs or total PCB and dioxin TEQs (Patandin et al. 1999a). The breast-fed group of children at 42 months of age showed a higher median plasma concentration of four sentinel PCBs (0.75 μg/L; range=0.23–5.90) than the median concentration (0.21 μg/L; range=0.08–0.46) of the formula-fed children (Patandin et al. 1999b), but no significant associations were found between cognitive scores and children's plasma PCB levels at 42 month of age (Patandin et al. 1999a). The authors concluded that (1) *in utero* exposure to PCBs was associated with poorer cognitive functioning at 42 months; (2) maternal PCB body burden should be reduced; and (3) breast-feeding should not be discouraged (Patandin et al. 1999a).

Other findings from the Dutch study include:

- statistically significant associations between increasing cord and serum levels and decreasing birth weight or growth rate from birth to 3 months, but no significant association between breast milk levels of PCBs or dioxins (at 2 weeks after birth) and birth weight or 3-month postnatal growth rates (Patandin et al. 1998);

- significant associations between increasing PCB and dioxin TEQ levels in breast milk and decreasing maternal plasma levels of triiodothyronine ($T_3$) or thyroxine ($T_4$) or increasing plasma levels of thyroid stimulating hormone (TSH) in infants at 2 weeks and 3 months after birth (Koopman-Esseboom et al. 1994a); and

- no statistically significant associations between PCB levels in maternal or cord plasma or PCB levels in milk and symptoms of respiratory tract infection of the infants in the first 18 months of life or levels of antibodies to mumps, measles, or rubella, but significant associations between changes in T cell subpopulations in the infants at 18 months of age and levels of PCB and dioxin TEQs in maternal or cord plasma (Weisglas-Kuperus et al. 1995).

The neurological effects observed in the North Carolina and Dutch studies were somewhat similar. Neurological deficits associated with motor function in the Bayley Scales of Infant Development were associated with increasing levels of persistent chemicals in cord serum or breast milk samples only at birth or ages <18 months. Assessments at later ages up to 42 months found no significant associations

between decreasing motor function scores and increasing concentrations of persistent chemicals in maternal milk at birth. The Dutch study also assessed cognitive function in the Kaufman Assessment Battery for Children at 42 months; significant associations were found for decreasing scores with increasing indices of prenatal exposures to PCBs, CDDs, and CDFs, but not with increasing indices of post-natal exposure. The Dutch study was also designed to compare breast feeding with formula feeding and found an advantageous effect of breast feeding on fluency of movement at 18 and 42 months. Members of both groups of investigators recommended that breast feeding should not be discouraged, but that maternal exposures to toxic persistent environmental chemicals should be reduced (Patandin et al. 1999b; Rogan 1996).

Prospective studies of children whose mothers frequently consumed Lake Michigan (Jacobson and Jacobson 1996; Jacobson et al. 1984, 1985, 1990a) or Lake Ontario (Lonky et al. 1996; Stewart et al. 1999, 2000b) sport fish contaminated with complex mixtures of persistent chemicals found statistically significant associations between prenatal exposure to PCBs (the only chemicals investigated) from maternal consumption of fish and deficits in neonatal behavioral development (Jacobson et al. 1984; Lonky et al. 1996; Stewart et al. 1999, 2000b), in short-term memory during infancy (Jacobson et al. 1985), in short-term memory during early childhood (Jacobson et al. 1990a), and in general intellectual ability during early school years (Jacobson and Jacobson 1996). Due to several study design limitations, the weight of the evidence from these studies is insufficient to establish causal relationships between fish consumption and adverse health effects in humans, but the hypothesis of a possible association between PCB exposure from maternal Great Lakes fish consumption and altered childhood neurological development is plausible based on the findings. Other hypotheses, however, have been proposed, including the possible involvement of other persistent chemicals in contaminated fish or synergistic interactions between PCBs and other neurotoxicants in fish. (See the ATSDR [2001c] Interaction Profile on Persistent Chemicals Found in Fish for more detailed discussion of these and other studies on possible associations between health effects and consumption of contaminated fish.)

A cohort study of neurobehavioral development in children of mothers residing in the North Atlantic Faroe Islands where pilot whale meat and blubber are components of the diet reported that mild deficits in neuropsychological development of the children at 7 years of age were associated with increasing mercury concentrations in maternal cord blood (Grandjean et al. 1997). In a companion nested matched case-control study, average neuropsychological performance of children whose mothers had a hair-mercury concentration of 10–20 μg/g was compared with that of “control” children whose mothers had hair concentrations below 3 μg/g (Grandjean et al. 1998). The case group showed mild cognitive deficits,

compared with controls, in the domains of motor function, language, and memory (Grandjean et al. 1998). The design of these studies precluded determining the possible contribution of postnatal exposure to neurotoxicants in breast milk or other components of the postnatal diet. More detailed discussion of these studies can be found elsewhere (ATSDR 1999b). In contrast, the early attainment of the ability to sit, creep, and stand in Faroe Island infants through 12 months of age was associated with breast feeding, which was associated with increased hair-mercury concentrations (Grandjean et al. 1995b). In addition, infants who reached these developmental milestones early had significantly higher hair-mercury concentrations than children who attained them later. These results suggest that although breast feeding may have led to higher mercury exposure, the benefits of breast feeding may have offset or masked possible neurodevelopmental effects from mercury.

Although the epidemiological studies point to gestational exposure to persistent toxic chemicals being more important than lactational exposure in affecting neurological development, a study of monkeys found long-term, exposure-related behavioral deficits in formula-fed monkeys exposed to PCBs from birth to 20 weeks (Rice 1997, 1998, 1999a, 1999b; Rice and Haywood 1999). Male monkeys were given oral doses of 0 or 7.5 μg/kg/day of a PCB mixture in a liquid diet formula. The mixture contained 15 congeners representing about 80% of the PCB content of Canadian samples of human breast milk; relative concentrations in the mixture were similar to those in human milk (Arnold et al. 1999; Rice 1997). Monkeys were hand-reared from birth and received only the liquid diet during the first 2 weeks. The liquid diet was supplemented with solid food during the remaining 18 weeks of exposure. Monkeys were assessed between 2.5 and 5 years of age for performance on a series of behavioral tasks. Exposed monkeys showed blood concentrations of PCBs at the end of exposure (1–3 ppb) that were within the range (2–10 ppb) of values for general human populations (Rice 1999a). Daily PCB intakes of the exposed monkeys were within the range of estimates of human newborn PCB intakes from breast milk cited in Table 2. Exposed monkeys showed deficits in a spatial delay alternation task with no indication of a deficit in spatial memory *per se* and performance deficits on a fixed interval schedule of reinforcement. Rice (2000) discussed the parallels between the features of attention deficit hyperactivity disorder (ADHD) in human children and the deficits noted in PCB- and lead-exposed monkeys (impaired discrimination reversal and spatial delayed alternation performance and impaired performance on a fixed interval schedule of reinforcement). Rice (1999a) concluded that “these results have implications for the potential contributions of exposure to PCBs through breast milk to later impairment in cognitive function”.

## 2.2 Component Mixtures

No studies were located that examined health effects in humans or animals exposed to four- or three-membered mixtures of the five components of concern. No PBPK/PD models were found for four-, three-, or two-membered mixtures of these chemicals. The following subsections present evaluations of health effects data and discussions of mechanistic information pertinent to the joint toxic action of each pair of components.

### 2.2.1 2,3,7,8-TCDD and Hexachlorobenzene

No studies were located that compared effects on health endpoints following oral exposure to binary mixtures of 2,3,7,8-TCDD (or other CDDs) and hexachlorobenzene with effects following exposure to the compounds alone.

Hexachlorobenzene competitively inhibited the binding of 2,3,7,8-TCDD to Ah-receptor sites in *in vitro* rat hepatic cytosol preparations (Hahn et al. 1989). The affinity of hexachlorobenzene for the Ah receptor was about 10,000-fold lower than that of 2,3,7,8-TCDD. Rats fed a diet containing 3,000 ppm hexachlorobenzene for 4–7 days showed a decrease in TCDD-binding specific activity in hepatic cytosol preparations compared with activity in preparations from control rats (Hahn et al. 1989). The decrease was principally due to a decrease in the number of Ah-receptor-binding sites in the cytosol (Hahn et al. 1989). Such a decrease in cytosolic Ah-receptor levels has been observed following administration of other Ah receptor ligands (such as TCDD and 3-methylcholanthrene) and has been proposed to be due to movement of ligand-receptor complexes into the nucleus (see Appendix A).

In immature rats given single intraperitoneal doses of hexachlorobenzene (400 μmole/kg in corn oil = 113.9 mg/kg), two days before injection of 10 or 30 μg/kg doses of 2,3,7,8-TCDD in corn oil, decreases in body weight gains and relative thymus weights, compared with controls, were much larger than in rats given 10 or 30 μg/kg 2,3,7,8-TCDD (0.031 or 0.093 μmole/kg) alone (Li et al. 1989). For example, 13 days after injection of 30 μg/kg TCDD, rats with hexachlorobenzene pretreatment had lost an average of about 10 grams of body weight and had an average relative thymus weight <0.01% of body weight (some of the rats in this group were athymic) compared with a body weight gain of about 40 grams and a relative thymus weight of 0.15% in rats given 30 μg/kg 2,3,7,8-TCDD alone. During this 13-day period, control rats showed a body weight gain of 80 grams and relative thymus weight of 0.25%. Single doses of hexachlorobenzene alone, as high as 3,000 μmole/kg, had no effect on body weight gain or relative

thymus weight.

Because hexachlorobenzene exposure alone showed no effects on body weight gain or thymus weight, the data appear to indicate that hexachlorobenzene pretreatment potentiates these effects of 2,3,7,8-TCDD, which have been proposed to be mediated via the Ah receptor. Three days after injection of 400 μmole/kg hexachlorobenzene, cytosolic levels of the Ah receptor in the thymus, lung, and kidney (but not in the liver) were decreased by about 50% compared with vehicle controls. After 14 days, thymic Ah receptor levels returned to levels similar to control levels, but remained depressed in the lung and kidney. Li et al. (1989) also observed that sole administration of hexachlorobenzene or TCDD induced hepatic cytochrome P450 (CYP) enzyme activity levels (arylhydrocarbon hydroxylase [AHH] and ethoxyresorufin *O*-deethylase [EROD]) that persisted 15 days after dose administration, but enzyme levels induced by hexachlorobenzene at the maximum level tested (3,000 μmole/kg) were 50% lower than maximum levels induced by 30 μg/kg 2,3,7,8-TCDD. Li et al. (1989) noted that the mechanistic significance of hexachlorobenzene-induced changes in Ah receptor levels, CYP enzyme induction patterns, and potentiation of 2,3,7,8-TCDD toxicity was unknown.

One possible expectation of the effect of a hexachlorobenzene pretreatment, or simultaneous exposure to large doses of hexachlorobenzene and small doses of 2,3,7,8-TCDD, is that, by depleting cytosolic levels of the Ah receptor, hexachlorobenzene may inhibit formation of TCDD-Ah receptor complexes and subsequent development of TCDD toxic effects. This is the opposite, however, of what was observed in the Li et al. (1989) rat study. Subsequent studies providing confirming evidence for hexachlorobenzene potentiation of acute TCDD toxicity and a plausible mechanistic explanation were not located. One possible partial explanation of the observation is that the potentiation may involve hexachlorobenzene interacting with some unidentified component, other than the Ah receptor, of the mechanism by which acute exposure to TCDD produces body weight wasting and thymic atrophy.

It is unknown if the apparent potentiation of TCDD toxicity by hexachlorobenzene is dependent on the absolute dose levels of the two agents. TCDD alone at the tested dose levels produced impaired body weight gain and decreased thymus relative weights, whereas hexachlorobenzene alone, at dose levels up to 7- to 8-fold higher than the dose which was potentiating, was without effect on these endpoints. Other animal studies have reported that oral exposure to hexachlorobenzene can produce thymic atrophy and decreased body weight gain that may be similar to the wasting syndrome produced by 2,3,7,8-TCDD (Barnett et al. 1987; Courtney 1979; Smith et al. 1987; Vos 1986), but intraperitoneal doses of hexachlorobenzene alone that were examined in the Li et al. (1989) study were not high enough to cause these

effects in the rat strain that was studied. Other issues of uncertainty regarding the observed apparent potentiation include whether it is dependent on:

- the sequence of exposure (will it also occur with simultaneous exposure to TCDD and hexachlorobenzene?);

- the relative dose levels of the two compounds (the potentiating dose of hexachlorobenzene was approximately 4,000- or 13,000-fold greater than the dose levels of TCDD on a μmole/kg basis—is this a requirement for the apparent potentiation?);

- the duration and route of exposure (will long-term co-exposure to oral hexachlorobenzene and TCDD produces the same potentiation as acute, intraperitoneal exposure?); and

- the type of TCDD-induced effect. (For example, although hexachlorobenzene potentiated TCDD-induced thymic atrophy, it is uncertain that hexachlorobenzene will potentiate TCDD immunosuppression. In humans, childhood and adult thymectomy produces no adverse effects on immune function. In addition, thymectomy of adult animals did not modify TCDD-induced suppression of antibody response to sheep red blood cells, and suppression of immune responses occurred at dose levels significantly lower than those required to produce thymic atrophy in adult animals [see Kerkvliet 1994 for review]).

In summary, an acute intraperitoneal administration study of rats found that pretreatment with hexachlorobenzene potentiated 2,3,7,8-TCDD-induced effects on body weight and thymus weight (Li et al. 1989), but the study design has several limitations (e.g., incomplete characterizations of the dose-response relationships for the individual compounds and dependence of the apparent potentiation on relative dose levels) that do not allow a full characterization of the possible potentiation of hexachlorobenzene on these TCDD effects. No additional studies were located that further examined or replicated this effect, and a plausible mechanistic explanation of the reported potentiation is not readily apparent.

Studies designed to examine the possible influence of 2,3,7,8-TCDD on hexachlorobenzene toxicity were not located, and mechanistic understanding is inadequate to support a reliable projection of the mode of joint action of CDDs and hexachlorobenzene on any toxicity target. Oxidative metabolism of hexachlorobenzene is important to the expression of hexachlorobenzene induction of hepatic porphyria and perhaps other hexachlorobenzene-induced effects, but the involvement of the parent material in some effects has

also been proposed (e.g., thyroid disruption; see van Raaji et al. 1993 and Appendix B). 2,3,7,8 TCDD is well known as a potent inducer of CYP1A isozymes (Kohn et al. 1996), but studies of the effects of tri-acetyloleandomycin (TAO), a selective inhibitor of CYPIIIA, in rats indicate that CYPIIIA enzyme activities are important for the expression of hexachlorobenzene hepatic porphyria (den Besten et al. 1993). den Besten et al. (1993) postulated that uroporphyrinogen decarboxylase is inhibited by an as yet unidentified reactive intermediate that is formed in the liver during the CYPIIIA-catalyzed transformation of hexachlorobenzene to pentachlorophenol, based on observations that repeated exposure of rats to high doses of pentachlorobenzene (which is also metabolized to pentachlorophenol and tetrachlorohydro-quinone) did not induce porphyria. Even if TCDD can induce enzymes involved in the metabolism of hexachlorobenzene, capabilities of downstream enzymes (e.g., Phase II enzymes) might be sufficient, or may also be induced, so that increased concentrations of toxic metabolites may not occur with co-exposure to TCDD relative to hexachlorobenzene alone.

Mechanistic understanding of other hexachlorobenzene-induced health effects (such as neurological effects, decreased circulating levels of thyroid hormones, and disruption of female reproductive organs) is insufficient to clearly indicate whether TCDD induction of CYP or Phase II enzymes involved in hexa-chlorobenzene metabolism will influence hexachlorobenzene toxicity. Although it is known that hexa-chlorobenzene can bind to the Ah receptor *in vitro* with an affinity that is 10,000-fold less than 2,3,7,8-TCDD (Hahn et al. 1989), the degree to which the Ah receptor is involved in the expression of hexachlorobenzene toxicity is unknown.

A brief summary of the toxicological interaction data for 2,3,7,8-TCDD and hexachlorobenzene is provided in Table 3.

**Table 3. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of Hexachlorobenzene and the Influence of Hexachlorobenzene on Toxicity/Carcinogenicity of 2,3,7,8-TCDD by Sequential Exposure**

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Intraperitoneal Exposure (mg/kg/day) | | | | | | |
| 2,3,7,8-TCDD Influence on Toxicity/Carcinogenicity of Hexachlorobenzene | | | | | | |
| Acute | thymus and body weight | | 0.010 or 0.030+113.9[a] (r)[b] | | No apparent interaction, but hexachlorobenzene treatment alone at doses tested was without effect on these endpoints. | Li et al. 1989 |
| Hexachlorobenzene Influence on Toxicity/Carcinogenicity of 2,3,7,8-TCDD | | | | | | |
| Acute | thymus and body weight | 113.9+0.010 or 0.030[a] (r)[b] | | | Greater than additive | Li et al. 1989 |

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat

### 2.2.2 2,3,7,8-TCDD and *p,p'*-DDE

2,3,7,8-TCDD and *p,p'*-DDE have both been demonstrated to disrupt the development of the male rat reproductive system (i.e., cause antiandrogenic effects) and are thought to disrupt development by different mechanisms (see Table 1 and Appendices A and C). Administration of single doses of 2,3,7,8-TCDD as low as 0.16–1 μg/kg to pregnant rats on gestation day 15 (a treatment that is thought to produce *in utero* and lactational exposure of offspring) produced a host of effects on the reproductive system of male offspring without affecting plasma androgen levels (Bjerke et al. 1994; Gray et al. 1997; Roman et al. 1995, 1998a, 1998b). Observed antiandrogenic effects include decreased accessory sex organ weights including prostate, decreased daily sperm production, and decreased cauda epididymal sperm number; decreased responsiveness of the adult prostate to androgenic stimulation; shortened anogenital distance, and decreased messenger ribonucleic acid (mRNA) levels of prostatic androgen-regulated genes. It has been hypothesized that 2,3,7,8-TCDD, via an initial interaction with the Ah receptor, may indirectly affect androgen signaling by altering growth factor pathways, but specific molecular events have not been determined (Gray et al. 1995; Roman et al. 1998b). Antiandrogenic effects observed in male offspring rats following *in utero* and lactational exposure to *p,p'*-DDE include shortened anogenital distance, increased nipple retention, decreased prostate weight and cauda epididymal sperm number, and delayed attainment of puberty (see Loeffler and Peterson 1999 for review). These effects, like those from 2,3,7,8-TCDD, are observed without changes in circulating androgen levels, but *p,p'*-DDE is a direct competitive inhibitor of ligand binding to the androgen receptor (Kelce et al. 1995, 1997), whereas 2,3,7,8-TCDD is not expected to interfere with androgen receptor-ligand binding (Roman et al. 1998b).

Co-exposure of pregnant rats to oral doses of 2,3,7,8-TCDD and *p,p'*-DDE in a corn oil (95%)/ acetone (5%) vehicle produced a statistically significant greater percentage reduction in the average relative weights of the ventral or dorsolateral prostates of male offspring at weaning (65 and 70% of controls at postnatal day 21, respectively), compared with TCDD exposure alone (81 and 86% of controls) or *p,p'*-DDE exposure alone (83 and 83% of controls) (Loeffler and Peterson 1999). The effects of these compounds alone or in combinations on prostate weight were transient (observed at 21 postnatal days) and were not observed at postnatal days 49 or 63. Pregnant rats were exposed to 0.25 μg 2,3,7,8-TCDD alone/kg on gestation day 15, 100 mg *p,p'*-DDE alone/kg on gestation days 14–18, or a combination of these two protocols. As expected, serum levels of androgens (3-alpha diol, testosterone) in male offspring were not affected (compared with controls) by *in utero* and lactational exposure to either chemical alone or to the mixture. Cauda epididymal sperm numbers at postnatal day 63 in male offspring were

significantly decreased by exposure to 2,3,7,8-TCDD or *p,p'*-DDE alone (decreased by 16.7 or 17.6% compared with controls, respectively), but mixed exposure did not significantly decrease the number further (22%). Patterns of immunostaining with anti-androgen receptor antibody in prostate tissue of male offspring exposed to the mixture showed qualitative characteristics of the effects of both compounds individually. Several other measures of antiandrogenic activity were examined, but no significant exposure-related effects were observed in any of the exposed groups. These endpoints have been demonstrated to be affected in male rat offspring at dosage levels of 2,3,7,8-TCCD or *p,p'*-DDE higher than those used in this study and included anogenital distance, age of puberty, weights of other accessory sex organs (seminal vesicles, epididymides, and testes), daily sperm production at postnatal days 49 or 63, and prostate levels of mRNA for several androgen-regulated genes.

The authors variously referred to the response on prostrate weight to the mixture of TCDD and *p,p'*-DDE as augmented, potentiated, and additive, but acknowledged that the design of the study is inadequate to make definitive conclusions regarding the mode of joint action (additive, greater-than-additive, or less-than-additive) (Loeffler and Peterson 1999). To conduct a more rigorous examination, the authors noted that "several doses of each compound would have to be tested in various combinations (i.e., isobolographic design)". A simple non-statistical analysis of the data provides a rough indication that the organ weight response to the mixture may be explained by additivity, but the reliance on an assumption of a linear dose-response relationship in this analysis and the lack of a statistical test precludes discarding the possibility of greater-than-additive or less-than-additive joint action on this endpoint. In this analysis, the differences in the average 21 day postnatal relative prostate weights for the TCDD alone group and the control group mean (about 0.11 mg/g body weight calculated from data in Figure 3 of Loeffler and Peterson 1999) and the *p,p'*-DDE group mean and the control mean (about 0.12 mg/g body weight) are summed (0.23 mg/g body weight) and compared with the change in relative prostate weight produced by the mixture (about 0.20 mg/g body weight). The predicted "additive" response of the individual components (0.23 mg/g body weight) is similar to the observed response to the mixture (0.20 mg/g body weight), but application of statistical tests (that account for experimental and biological variability) to compare these values is not possible.

The sum of changes in relative prostate weights induced by 2,3,7,8-TCDD and *p,p'*-DDE alone compared with the response to the binary mixture suggests that these compounds may additively affect male rat prostate weight development, but more definitive tests of this hypothesis would require an experimental design with several doses of each compound and a statistical test of additivity. The available results provide no evidence for marked synergistic or antagonistic interactions between concurrent oral exposure

to doses of 2,3,7,8-TCDD and *p,p'*-DDE. Mixed exposure did not affect several other measures of antiandrogenic activity that have been demonstrated to be adversely affected by doses of the individual compounds higher than those used in this study.

A brief summary of the toxicological interaction data for 2,3,7,8-TCDD and *p,p*'-DDE is provided in Table 4.

**Table 4. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of *p,p'*-DDE and the Influence of *p,p'*-DDE on Toxicity/Carcinogenicity of 2,3,7,8-TCDD by Simultaneous Exposure**

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) | | | | | | |
| 2,3,7,8-TCDD Influence on Toxicity/Carcinogenicity of *p,p'*-DDE | | | | | | |
| Acute | prostate weights in offspring | | 0.00025+100[a] (r)[b] | | Additive action is suggested, but the study design precludes discarding the possibility of greater-than or less-than-additive action. | Loeffler and Peterson 1999 |
| *p,p'*-DDE Influence on Toxicity/Carcinogenicity of 2,3,7,8-TCDD | | | | | | |
| Acute | prostate weights in offspring | | 100+0.00025 (r) | | Additive action is suggested, but the study design precludes discarding the possibility of greater-than or less-than-additive action. | Loeffler and Peterson 1999 |

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: r = rat

### 2.2.3 Hexachlorobenzene and *p,p'*-DDE

No *in vitro* or *in vivo* studies were located regarding possible interactions between hexachlorobenzene and *p,p'*-DDE in affecting health-related endpoints in humans or animals.

Sensitive shared targets of hexachlorobenzene and *p,p'*-DDE oral toxicity include the liver (hepatomegaly and degenerative histological effects), immune system (suppression of humoral and cell-mediated immunological responses), and pre- and post-natal neurological development (altered neurobehavior) (see Table 1 and Appendices B and C). Both chemicals produce cancer in orally exposed animals.

Hepatic porphyria from repeated exposure to hexachlorobenzene has been postulated to be dependent on CYPIIIA- or CYP1A-mediated metabolism and to involve an unidentified reactive intermediate formed during transformation to pentachlorophenol (see Appendix B). DDT, DDE, and DDD have been demonstrated in rats to induce hepatic CYPIIB, and to a lesser degree CYPIIIA, but not CYP1A isozymes (see Appendix C). If simultaneous exposure to DDE and hexachlorobenzene cause an increased induction of CYPIIIA enzymes (compared with hexachlorobenzene alone) so that capabilities of Phase II enzymes to control liver concentrations of the reactive hexachlorobenzene metabolite are exceeded, then a potentiation of hexachlorobenzene-induced liver toxicity may occur. No studies were located that investigated hepatic metabolic interactions between hexachlorobenzene and *p,p'*-DDE (or DDT), but this projection is not reliable given that hexachlorobenzene can induce its own metabolism and downstream Phase II enzymes would need to be saturated for any potentiation to occur.

In the absence of pertinent data on possible mode of joint actions or sufficient mechanistic understanding, an unambiguous projection of interactions between *p,p'*-DDE and hexachlorobenzene cannot be made. Future studies designed to examine possible interactions of hexachlorobenzene and *p,p'*-DDE in affecting neurological, developmental, hepatic, immunological, or cancer endpoints following oral exposure may help to determine if interactions occur.

### 2.2.4 2,3,7,8-TCDD and Methylmercury

2,3,7,8-TCDD, other CDDs, CDFs, and PCBs have been demonstrated to produce immunotoxic effects in animals such as lymphoid tissue depletion and increased susceptibility to infectious agents (Kerkvliet 1994; see Appendix A). Mechanistic studies indicate that immunotoxic effects induced by acute or subacute exposures to 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons are likely to involve

initial mediation by the Ah receptor and multiple sites within the immune system (Kerkvliet 1994). Animal studies with mercury indicate that mercuric salts and methylmercury can cause both an autoimmune stimulation and a suppression of the immune system depending on dose and genetic characteristics (ATSDR 1999b; see Appendix D). For example, 2-week oral exposure to 14.8 mg Hg/kg/day as mercuric chloride decreased thymus weight in mice, 0.7 mg Hg/kg/day as mercuric chloride increased lymphoproliferative responsiveness to T-cell mitogens in another mouse strain known to be sensitive to mercury-induced autoimmunity, and 0.5 mg Hg/kg/day as methylmercury in the diet suppressed natural killer cell activity in another strain of mouse that is resistant to mercury-induced autoimmunity (ATSDR 1999b; Ilback 1991).

Two *in vitro* studies of immunological endpoints in rat cultured lymphocytes found no evidence for interactions between methylmercury and a synthetic mixture of CDDs, CDFs, and PCBs at low concentrations that were reflective of concentrations in flesh of St. Lawrence River fish (Omara et al. 1997, 1998).

In the first experiment (Omara et al. 1997), cell viability, cell proliferation in response to T- and B-cell mitogens, and intracellular calcium concentrations were measured in cultured rat lymphocytes exposed for 72 hours to methylmercury, CDDs+CDFs, PCBs, or a methylmercury/CDD/CDF/PCB mixture. Culture-medium concentrations were reflective of the extremes of the ranges of concentrations found in flesh of fish from the St. Lawrence River: methylmercury (0.1 or 2 μg/mL), a CDD/CDF mixture of 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin, 2,3,7,8-TCDF, and 1,2,3,7,8-pentachlorodibenzofuran in a 20:5:2.5:80:15 weight proportion (0.01 or 0.5 μg/mL), and a PCB mixture of Aroclor 1242, 1254, and 1260 in a 3:4:3 weight proportion (0.01 or 0.5 μg/mL). Mixed exposures included all possible combinations of these concentrations of methyl-mercury, the CDD/CDF mixture, and the PCB mixture (e.g., low methylmercury+high CDD/CDF+high PCB; low methylmercury+high CDD/CDF+low PCB; etc.; n=8).

No significant effects on lymphocyte cell viability or calcium contents were found following exposure to either concentrations of the CDD/CDF or PCB mixtures alone, or to methylmercury alone at the lower concentration or in combination with the CDD/CDF/PCB mixtures (Omara et al. 1997). Exposure to the CDD/CDF or PCB mixtures alone, at the tested concentrations, did not suppress the lymphocytic response to any of the test mitogens, but stimulated the response of splenic and peripheral blood lymphocytes to one of the tested mitogens, concanavalin A. In contrast, methylmercury, alone at 2 μg/mL, significantly decreased viability of the lymphocytes after 4 or 24 hours of exposure, and, at

0.1 μg/mL, significantly suppressed the responses of the different types of lymphocytes to several T- and B-cell mitogens (Omara et al. 1997). The effects of methylmercury on lymphocyte viability and lymphocyte mitogenic ability were not significantly different in the presence of the CDD/CDF/PCB mixtures.

In a similar design, the second experiment measured rat splenocyte mixed leukocyte reaction, splenic natural killer cell activity, and phagocytic activities of splenic, peritoneal, and peripheral blood lymphocytes after 24- or 72-hour exposure (Omara et al. 1998). The high concentration of methyl-mercury (2 μg/mL), alone or in combination with the CDD/CDF/PCB mixtures, was cytolethal to rat splenocytes, peritoneal lymphocytes, and peripheral blood lymphocytes. Exposure to the lower methyl-mercury concentration (0.1 μg/mL), alone or in combination with the CDD/CDF/PCB mixtures, caused no significant suppression of splenocyte mixed leukocyte reaction, splenic natural killer cell-mediated lysis of Yac-1 cells, or phagocytosis of fluorescent beads by splenic, peritoneal, or peripheral blood lymphocytes.

No other *in vitro* or *in vivo* studies were located regarding possible interactions between 2,3,7,8-TCDD (or other CDDs) and methylmercury (or other forms of mercury) in affecting other health-related endpoints in humans or animals.

Results of studies of Omara et al. (1997, 1998) provide no evidence of interactions or additivity between methylmercury and Ah-receptor-interacting halogenated aromatic hydrocarbons in affecting a number of immunological endpoints in rat lymphocytes under low-level, *in vitro* conditions. The levels of CDDs, CDFs, and PCBs examined in these experiments were below thresholds for immunosuppressive effects. This feature of the study design restricts conclusions that can be made from the data about the modes (additive, greater-than-additive, or less-than-additive) of possible joint action of the components on the endpoints examined. In summary, the data show that at the doses tested, CDDs, CDFs, and PCBs did not significantly change (enhance or antagonize) the *in vitro* immunosuppressive effects (suppression of lymphocyte viability and mitogenic ability) caused by methylmercury alone and did not jointly act with methylmercury to produce effects that were not caused by methylmercury alone. CDDs, CDFs, PCBs, and methylmercury have all been demonstrated to produce various adverse effects on the immune system, but the available results provide no information on how methylmercury may influence the effects of CDDs, CDFs, or PCBs on the evaluated immune system endpoints and thus, only limited information on joint action CDDs and methylmercury on the immune system.

### 2.2.5 Hexachlorobenzene and Methylmercury

No *in vitro* or *in vivo* studies were located regarding possible interactions specifically between hexachlorobenzene and methylmercury, but two published studies have looked for possible acute-exposure interactions between hexachlorobenzene, HCB, and mercuric chloride, $HgCl_2$ (Lecavalier et al. 1994; Renner 1980). Given that the health effects from methylmercury are thought to be mediated by the divalent mercuric ion, these studies are evaluated herein with the recognition that they can provide no information of potential interactions with hexachlorobenzene at steps involved in the absorption, distribution, and metabolism of methylmercury, processes that are responsible for the greater potency of methylmercury compared with mercuric salts (ATSDR 1999b; see Appendix D).

In the most extensive study (with respect to endpoints examined), groups of 10 female Sprague-Dawley rats were exposed to single gavage doses of 0, 400, or 600 mg HCB/kg; 0, 10, or 12.5 $HgCl_2$/kg; or (400 mg HCB+10 mg $HgCl_2$)/kg, (400 mg HCB+12.5 mg HgCl2)/kg, (600 mg HCB+10 mg $HgCl_2$)/kg, or (600 mg HCB+12.5 mg $HgCl_2$)/kg (Lecavalier et al. 1994). Endpoints included mortality by the end of a 14-day observation period, necropsy examination, histopatholgical examination of about 30 tissues and organs from surviving animals, hematological variables, serum clinical chemistry variables, several hepatic microsomal CYP enzyme activities (e.g., ethoxyresorufin deethylase), and residues of hexachlorobenzene and mercury in samples of brain, liver, kidney, spleen, serum, and fat.

Deaths occurred in the 600-mg/kg hexachlorobenzene groups, but incidences were not markedly influenced by co-exposure to mercury (1/10, 1/10, and 2/10 deaths occurred in mixed 600-mg HCB/kg groups with 0, 10, or 12.5 mg $HgCl_2$/kg). Liver weights were significantly increased in hexachlorobenzene-exposed groups without significant elevations in hepatic CYP enzymes or serum enzyme activities indicative of liver damage. Histopathological examinations showed mild to moderate hepatic cytoplasmic vacuolation, anisokaryosis, and pyknotic hepatocytes in hexachlorobenzene-exposed groups. Mercury exposure alone did not significantly change liver endpoints compared with controls. Lecavalier et al. (1994) reported that co-administration of mercury did not markedly change the liver endpoints compared with hexachlorobenzene alone. Hexachlorobenzene-exposed groups also showed histopathological changes in the thyroid (reduced follicle size and colloid density and increased epithelial height) and thymus (reduced cortical and medullary volume), whereas mercury-exposed groups showed renal histopathological changes (protein casts, cellular casts, and interstitial sclerosis). The “severity of these changes” was reported to be “additive in nature” for the mixed exposure groups, but the data were not presented in a way that this contention could be quantitatively assessed. Levels of hexachlorobenzene or

mercury in serum, brain, kidney, liver, or fat were not significantly different in the mixed exposure groups compared with the groups exposed to hexachlorobenzene or mercury alone. Although the design and reporting of this study are inadequate to fully characterize the modes of possible joint actions of hexachlorobenzene and mercury on indices of liver damage and histopathological changes in tissues, the results indicate that mercury co-exposure (at doses of 10 or 12.5 mg/kg) did not change the low incidence of lethality in the high-dose (600 mg/kg) hexachlorobenzene groups and that mercury co-exposure did not change liver responses to hexachlorobenzene.

In an earlier study, groups of 10 female Sprague-Dawley rats were given single doses (in a corn oil vehicle) of 0, 400, 600, or 800 mg HCB/kg; and 0, 3.6, 6, 8, 10, 12.5, or 18 mg $HgCl_2$/kg (Renner et al. 1980). Other groups were given mixtures of 3.6, 6, 8, 10, 12.5, or 18 mg $HgCl_2$/kg with 400 mg HCB/kg or 6, 12.5, or 18 mg $HgCl_2$/kg with 600 mg HCB/kg. No mortality occurred in groups exposed to hexachlorobenzene alone. Mortalities occurred in groups exposed to mercuric chloride alone at 12.5 mg/kg (1/10) and 18 mg/kg (5/10). Mortalities occurred in the presence of 400 mg hexachlorobenzene and mercuric chloride at doses of 10 mg/kg (10/10), 12.5 mg/kg (1/10), and 18 mg/kg (10/10). In the presence of 600 mg hexachlorobenzene and mercuric chloride, no rats died with co-exposure to 6 mg $HgCl_2$/kg, but all rats died with co-exposure to 12.5 or 18 mg $HgCl_2$/kg. The study design is inadequate to evaluate the mode of possible joint action of mercury and hexachlorobenzene in producing lethality in rats (e.g., a dose-response relationship for lethality from hexachlorobenzene was not characterized to any degree), but some evidence is presented that co-exposure to 400 or 600 mg/kg hexachlorobenzene increased mercury-induced lethality at 4 or 5 mercury dose levels of 10 mg/kg or greater.

In summary, Renner (1980) reported that 400 or 600 mg/kg doses of hexachlorobenzene potentiated mercuric chloride acute lethality in rats (given 10, 12.5, or 18 mg$HgCl_2$ /kg doses), but Lecavalier et al. (1994) did not find that 10 or 12.5 mg $HgCl_2$/kg produced lethality in rats in the presence or absence of 400 or 600 mg/kg hexachlorobenzene. Examining other endpoints after acute oral exposure scenarios involving several dose levels of hexachlorobenzene and mercuric chloride (alone or in combination), Lecavalier et al. (1994) reported that no evidence was found in rats that mercuric chloride affected hexachlorobenzene-induced liver, thyroid, or thymus effects or that hexachlorobenzene affected mercury-induced kidney effects, but the results were inadequately reported to allow quantitative assessment. No studies were located regarding potential interactions between these chemicals under repeated exposure scenarios, and no information was found regarding interactions between hexachlorobenzene and methylmercury under any exposure scenario.

Because no data regarding the toxicological interactions of hexachlorobenzene with *methylmercury* were available, Table 5 provides a brief summary of the data for hexachlorobenzene and *mercuric chloride*.

**Table 5. Summary of Available Data on the Influence of Hexachlorobenzene on Toxicity/Carcinogenicity of Mercuric Chloride and the Influence of Mercuric Chloride on Toxicity/Carcinogenicity of Hexachlorobenzene by Simultaneous Exposure[a]**

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) | | | | | | |
| Hexachlorobenzene Influence on Toxicity/Carcinogenicity of Mercuric Chloride | | | | | | |
| Acute | lethality | 400[b]+10 (r)[c]<br>600+10 (r) | <br>400+10 (r)<br>600+10 (r) | | Indeterminate due to conflicting results from similar experiments. | Renner 1980<br>Renner 1980<br>Lecavalier 1994<br>Lecavalier 1994 |
| Mercuric Chloride Influence on Toxicity/Carcinogenicity of Hexachlorobenzene | | | | | | |
| Acute | hepatocyte vacuolation | | 10+400 (r)<br>12.5+400 (r)<br>10+600 (r)<br>12.5+600 (r) | | Additivity was reported; data were not presented, so conclusion could not be evaluated. | Lecavalier 1994<br>Lecavalier 1994<br>Lecavalier 1994<br>Lecavalier 1994 |

[a]Data for the pair, *methylmercury* and hexachlorobenzene, were not located, so available data for mercuric chloride and hexachlorobenzene were evaluated as explained in text.
[b]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[c]Species code: r = rat

### 2.2.6 *p,p'*-DDE and Methylmercury

No *in vitro* or *in vivo* studies were located regarding possible interactions between *p,p'*-DDE and methylmercury (or other forms of mercury) in affecting health-related endpoints in humans or animals.

Oral exposure to either *p,p'*-DDE or methylmercury has been shown to adversely influence pre- and postnatal neurological development, and both compounds are known to produce adverse effects on the fully developed neurological system (see Table 1 and Appendices C and D). Joint actions between these two compounds in producing deficits in neurological developmental or function are plausible, but modes of possible joint action are unknown and unstudied. Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects on mitosis through impairment of microtubule assembly, inhibition of various enzymes such as protein kinase C, inhibition of transport mechanisms in developing brain cells, and alteration of synaptosomal release of neurotransmitters (e.g., dopamine) that may involve non-specific changes in membrane integrity or disruption of calcium homeostasis and subsequent activation of second messenger system or cell death (Appendix D). *p,p'*-DDE's actions on the developing and mature neurological system are poorly understood (see Appendix C), but, as with *p,p'*-DDT, interference with sodium channels and potassium gates in neuronal membranes and inhibition of neuronal ATPases may be involved. Obvious cellular or molecular sites of possible interactions between *p,p'*-DDE and methylmercury are not apparent.

### 2.2.7 PCBs and 2,3,7,8-TCDD

Mixtures of PCBs display a wide array of effects that have considerable similarities with the wide array of effects produced by 2,3,7,8-TCDD and other CDDs (see Appendices A and E). Some PCB congeners are thought to produce toxic effects through initial mechanisms of action (e.g., Ah-receptor mediation and induction of hepatic CYP1A1, 1A2, and 1B1, and associated Phase II enzymes) that are shared with 2,3,7,8-TCDD, although 2,3,7,8-TCDD is generally much more potent than PCB mixtures or individual PCB congeners. Thus, the Ah receptor represents a likely molecular site for interactions between PCBs and 2,3,7,8-TCDD that can have toxicological significance. Based on assumptions of a shared initial mechanism involving the Ah receptor and additive joint action, a component-based Toxic Equivalency Factor (TEF) approach to evaluating health hazards from complex mixtures containing CDDs, CDFs, and PCBs has been used to some extent (see Appendices A and E). However, PCB congeners with Ah-receptor affinity are minor components in commercial and environmental PCB mixtures, and there is

increasing evidence that PCB congeners can produce toxic effects through both Ah receptor dependent and independent mechanisms (see Appendix E). In addition, there is some evidence for a few endpoints, reviewed herein, that there may be greater-than-additive and less-than-additive interactions between 2,3,7,8-TCDD and PCB mixtures or congeners. Consequently, it has been recommended that the TEF approach should be used cautiously, especially for complex mixtures in which PCBs may predominate (Hansen 1998; Safe 1998a, 1998b). To conduct exposure-based health assessments involving mixtures of CDDs, CDFs, and PCBs, ATSDR (1998) has recommended using the TEF, but also has derived MRLs for PCB mixtures for the same purpose (ATSDR 2000). The PCB MRLs are based on health effects from commercial PCB mixtures (e.g., Aroclor 1254) or synthetic mixtures of PCBs designed to mimic composition of environmental PCB mixtures. They were derived because PCB congeners with Ah-receptor affinity are minor components in environmental mixtures, multiple mechanisms may be involved in the development of PCB-induced health effects, and non-additive joint actions on toxicity targets may exist between specific PCB congeners and between PCB congeners and CDDs (ATSDR 2000).

***Complex PCB mixtures and 2,3,7,8-TCDD***

Studies designed to examine possible binary interactions between complex PCB mixtures and 2,3,7,8-TCDD are restricted to two intraperitoneal-exposure studies examining impaired immune responses to sheep red blood cells in C57BL/6J mice (Bannister et al. 1987; Davis and Safe 1989) and a gavage-exposure study examining cleft palate incidences in offspring of C57BL/6J mice (Haake et al. 1987). Results from this limited database suggest that PCB mixtures antagonize the acute immuno-toxicity and the acute developmental toxicity (producing cleft palate) of 2,3,7,8-TCDD, when PCB:TCDD dose ratios were >1,000:1 (see Table 6). Similarly designed studies examining repeated exposure scenarios or other endpoints potentially shared by complex PCB mixtures and 2,3,7,8-TCDD (e.g., cancer or neurodevelopment) do not appear to be available. Whereas the designs of these studies are inadequate to fully characterize the mode of possible joint action of PCB mixtures and 2,3,7,8-TCDD on impairing the immune response to sheep red blood cells and producing cleft palate, they adequately describe conditions under which CDD-effects were antagonized by PCB mixtures and provide some information that the antagonism is dependent on the relative proportions of the PCB mixture dose and the TCDD dose (Davis and Safe 1989).

In response to injection with sheep red blood cells, mice given single intraperitoneal doses of 3.72 or 11.1 nmol 2,3,7,8-TCDD/kg (0.0011 or 0.0036 mg/kg) produced only 156 or 62 plaque-forming cells, respectively, per million viable spleen cells (PFC/$10^6$ spleen cells), compared with the response of

562 PFC/$10^6$ spleen cells in non-exposed controls (Bannister et al. 1987). Aroclor 1254 alone displayed minimal potency in affecting the immune response. Immune responses were not significantly different from control values in mice injected with doses of 5, 15, 75, or 150 μmol Aroclor 1254/kg alone (1.6, 4.9, 24.6, or 49.3 mg/kg), but the high-dose response was marginally impaired (561, 465, 594, or 394 PFC/$10^6$ spleen cells). Co-injection of 3.72 nmol 2,3,7,8-TCDD/kg with 5, 15, or 75 μmol Aroclor 1254/kg completely restored the immune response to control values (491, 480, or 558 PFC/$10^6$ spleen cells), whereas co-exposure with 150 μmol Aroclor 1254/kg partially restored the response (372 PFC/$10^6$ spleen cells). Co-exposure to 11.2 nmol 2,3,7,8-TCDD/kg and 75 μmol Aroclor 1254/kg antagonized the TCDD-induced effect to a limited degree (170±10 versus 62±12 PFC/$10^6$ spleen cells for TCDD alone).

In the other intraperitoneal study, mice given 3.7 nmol 2,3,7,8-TCDD/kg (1.2 μg/kg) showed a response of 180 PFC/$10^6$ spleen cells compared with 912 PFC/$10^6$ spleen cells in non-exposed control mice, and co-exposure with 25 mg/kg of Aroclor 1242, 1248, 1254, or 1260 partially antagonized the TCDD-induced impairment (Davis and Safe 1989). Average respective immune responses in the co-exposed mice were 440, 427, 459, and 459 PFC/$10^6$ spleen cells; these were significantly higher than the response in mice exposed to TCDD alone. Co-exposure with 25 mg/kg Aroclor 1232 did not significantly change the immune response compared with TCDD exposure alone (244 PFC/$10^6$ spleen cells). A synthetic mixture of PCB congeners detected in human milk samples also significantly antagonized the immunotoxicity of 3.7 nmol 2,3,7,8-TCDD/kg, at a dose of 50 mg/kg, but not at dose levels of 5 or 25 mg/kg.

Co-treatment of pregnant mice with gavage doses of 20 μg 2,3,7,8-TCDD/kg (on gestation day 10) plus 244 mg Aroclor 1254/kg (on gestation day 9) produced 8.2% fetuses with cleft palate per litter compared with 62% per litter in mice treated with TCDD alone (Haake et al. 1987). Exposure to Aroclor 1254 alone or to the corn-oil vehicle produced no fetuses with cleft palate. The same dose level of Aroclor 1254 did not antagonize cleft palate formation induced by 90 mg/kg dexamethasone, a synthetic glucocorticoid hormone analog that is thought to produce developmental effects through an initial binding with glucocorticoid receptors. A lower dose of Aroclor 1254 (250 μmol/kg or 82 mg/kg) was less effective as a TCDD antagonist, but specific data were not reported (Biegel et al. 1989a).

***Less complex PCB mixtures and 2,3,7,8-TCDD***

To examine possible interactions between mixtures of PCBs and 2,3,7,8-TCDD associated with repeated exposure, liver tumor promotion activity in partially hepatectomized rats exposed to a mixture containing 68 ppm 2,3,7,8-TCDD, 223-ppm 1,2,3,7,8-pentachloro-*p*-dioxin, 1,151-ppm 2,3,4,7,8-pentachloro-dibenzofuran, 4,130 ppm 3,3',4,4',5-pentachlorobiphenyl, 866,604 ppm 2,3',4,4',5-pentachlorobiphenyl, and 127,824 ppm 2,3,3',4,4',5-hexachlorobiphenyl was compared with predicted tumor promotion activity using TEFs based on tumor promotion activity of the individual components compared to TCDD activity (van der Plas et al. 1999). The mixture composition was reflective of relative concentrations, and accounted for approximately 90% of TCDD toxic equivalents (TEQs), found in Baltic Sea fish samples. Tumor promotion activity was measured in groups of female Sprague-Dawley rats (number and volume of glutathione S-transferase-positive foci in liver) following 20 weekly subcutaneous injections of 0.1 µg 2,3,7,8-TCDD/kg, or 1 µg TEQ/kg of the five-component mixture noted above. Promotion was preceded by initiation with single intraperitoneal injections of 30 mg/kg diethylnitrosamine. Mean foci volume and volume fraction of hepatic foci in the 1-TEQ/kg mixture-exposed groups were about one-half of values for the group promoted with TCDD alone. A six-component mixture was also examined; it contained in addition to the components noted above, 20,000 g 2,2',4,4',5,5'-hexachlorobiphenyl per g of 2,3,7,8-TCDD. Tumor promotion activity for this mixture was greater than that of the five-component mixture, but was still less than that predicted by the TEF method. One possible explanation of the difference between the observed and TEF predicted values is that the components may have interacted in a less-than-additive manner (e.g., less potent PCBs may antagonize tumor promotion by the more potent 2,3,7,8-TCDD), but equally as plausible is the possibility that the TEFs are inaccurate and overestimate tumor promotion potencies.

***PCB congeners and 2,3,7,8-TCDD—Acute exposures***

A study of possible binary *in vitro* interactions between 2,3,7,8-TCDD and two PCB congeners in promoting malignant transformation of carcinogen-initiated cultured mouse fibroblasts found that one congener (2,2',4,4',5,5'-hexachlorobiphenyl—a congener without Ah-receptor-agonist activity) antagonized TCDD promotion of transformation, whereas 3,3',4,4',5-pentachlorobiphenyl (which displays Ah-receptor-agonist activity) added to promotion of transformation in the presence of 2,3,7,8-TCDD (Wolfle 1998). These *in vitro* observations of apparent additive joint action of 3,3',4,4',5-pentachloro-biphenyl and 2,3,7,8-TCDD in promoting malignant transformation of mouse fibroblasts concur with observations from subcutaneous-exposure studies observing apparent additive joint action of these

specific congeners in promoting liver tumors in rats (Hemming et al. 1995).

Blockage of ovulation, reduction of ovarian weight gain, and changes in preovulatory hormone levels have been observed in gonadotropin-primed immature female rats given single doses of 2,3,7,8-TCDD and other CDDs alone and in combination (Gao et al. 1999). The slopes of the dose-response relationships for the CDD components were similar to the slope for an equipotent mixture (expressed in total toxic equivalents [TEQ] relative to 2,3,7,8-TCDD), indicating additive joint action by a common mechanism. Comparable similarities in slopes were observed for dose-response relationships for 3,3',4,4',5-pentachlorobiphenyl and an equipotent mixture containing this PCB plus 2,3,4,7,8-pentachlorodibenzofuran, 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (Gao et al. 2000). Another PCB congener (2,2',4,4'-tetrachlorobiphenyl) was found to be inactive in this reproductive toxicity assay, but the effect of its presence in a mixture with the effective components was not examined (Gao et al. 2000).

There are several studies examining possible interactions within binary mixtures of a few PCB congeners and 2,3,7,8-TCDD in suppressing immune responses to sheep red blood cells and producing cleft palate during *in utero* development, using similar acute exposure protocols and the same mouse strain as studies observing antagonism by complex PCB mixtures of TCDD effects on these endpoints (see Table 6). Examined PCB congeners either did not produce these effects or were so much less potent than 2,3,7,8-TCDD that, with co-exposure treatments, TCDD effects predominated. The limited information from these studies indicates that non-additive interactions, both potentiation and antagonism, can occur between PCB congeners and 2,3,7,8-TCDD, depending on the endpoint, the PCB congener (some are antagonistic, one has been identified as synergistic, and some show no effect on TCDD toxicity), the PCB dose, and/or the PCB:TCDD dose ratio. Mechanistic understanding of these non-additive interactions is poor, and extensive studies examining how PCB molecular structure parameters may be associated with these interactions have not been carried out. Nevertheless, the limited number of congeners and endpoints examined indicate the possibility of wide variance in how individual PCB congeners may interact with 2,3,7,8-TCDD in producing these acute effects.

Seven congeners (six hexachlorobiphenyls and one pentachlorobiphenyl) were examined for binary joint action with 2,3,7,8-TCDD in producing immune suppression (Biegel et al. 1989b; Davis and Safe 1990; Smialowicz et al. 1997). Three (2,3,3',4,5,5'- and 2,2',4,4',5,5'-hexachlorobiphenyl, and 2,3,3',4,5'-pentachlorobiphenyl) were partially antagonistic (i.e., they did not completely prevent the immune suppression). Relationships between PCB:TCDD dose ratio and antagonism varied among the

antagonistic congeners. For example, antagonism by 2,2',4,4',5,5'-hexachlorobiphenyl was observed at weight ratios above about 330,000:1, but not at ratios below about 40,000:1 (Biegel et al. 1989b; Smialowicz et al. 1997). In contrast, antagonism by 2,3,3',4,5,5'-hexachlorobiphenyl occurred at ratios above about 15,000:1, but not at 6,000:1 (Davis and Safe 1990). Among the non-antagonistic congeners, some were immunotoxic, showing variable potencies (e.g., the potency of 2,3,3',4,4',5-hexachlorobiphenyl was about 1,000-fold greater than that of 2,2',4,4',5,5'-hexachlorobiphenyl), and others were not immunotoxic (2,2',4,4',6,6'-hexachlorobiphenyl).

The mechanism involved in PCB antagonism of TCDD acute immunosuppression is unknown. Davis and Safe (1990) suggested that it may occur by mechanisms other than through the Ah receptor, as some antagonistic PCB congeners (e.g., 2,2',4,4',5,5'-hexachlorobiphenyl) do not displace TCDD from the Ah receptor. Smialowicz et al. (1997) proposed that the antagonism was a functional antagonism (two chemicals producing opposite effects on the same physiological function) based on observations that oral exposure to 2,2',4,4',5,5'-hexachlorobiphenyl alone enhanced the immune response compared with control values. However, intraperitoneal exposure to 2,2',4,4',5,5'-hexachlorobiphenyl alone did not enhance the immune response to sheep red blood cells in another study (Biegel et al. 1989b).

Three congeners, none of which caused cleft palate by themselves, have been examined for interaction with TCDD-induction of cleft palate and show distinctly different interactions (see Table 6). 2,3,3',4,4',5-Hexachlorobiphenyl markedly potentiated TCDD-induced cleft palate (Birnbaum et al. 1985). For example, gavage doses of 0.003 mg 2,3,7,8-TCDD/kg alone on gestation days 10–13 produced 4.2% of fetuses with cleft palate per litter, but combined exposure with 40 or 80 mg 2,3,3',4,4',5-hexachlorobiphenyl/kg produced 19.9 and 43.1%, respectively. In contrast, 2,2',4,4',5,5'-hexachlorobiphenyl and 2,2',4,4'-tetrachlorobiphenyl were antagonistic (Biegel et al. 1989a, 1989b; Birnbaum et al. 1985; Morrissey et al. 1992). The relationship of antagonism with dose of the 2,2',4,4',5,5'-congener was shown to have an inverted U shape. Percentage of fetuses with cleft palate in groups exposed to 0.015 mg 2,3,7,8-TCDD/kg and 0, 62.5, 125, 250, 500, or 1,000 mg 2,2',4,4',5,5'-hexachlorobiphenyl/kg were 35, 23, 5, 1, 0, and 38% (Morrissey et al. 1992). Results from the earlier studies (Biegel et al. 1989b; Birnbaum et al. 1985) indicated that the examined PCB congeners or TCDD alone induced increased incidence of hydronephrosis in mouse offspring and provided no evidence of non-additive interactions. However, after examining a wider range of 2,2',4,4',5,5'-hexachlorobiphenyl doses in combination with 0.015 mg 2,3,7,8-TCDD/kg, partial antagonism of TCDD-induced hydronephrosis was observed to have a sharp inverted U shaped relationship with PCB dose. Percentages of fetuses per litter with hydronephrosis were 99, 98.1, 97.6, 63.5, and 100% for groups exposed to mixtures of

0.015 mg 2,3,7,8-TCDD plus 0, 62.5, 250, 500, or 1,000 mg 2,2',4,4',5,5'-hexachlorobiphenyl/kg (Morrissey et al. 1992).

The mechanisms of PCB congener antagonism or synergism of TCDD developmental toxicity are unknown. No other data are available regarding how other congeners or other complex PCB mixtures may influence TCDD-induced cleft palate and kidney malformations in mouse offspring.

***PCB congeners and 2,3,7,8-TCDD—Intermediate exposures***

A series of studies of female Sprague-Dawley rats exposed to binary mixtures in the diet for 13 weeks examined possible interactions between 2,3,7,8-TCDD and three PCB congeners (one with no ortho chlorines—3,3',4,4',5-pentachlorobiphenyl; one with a single ortho chlorine—2,3,3',4,4',5-hexachlorobiphenyl; and one with two ortho chlorines—2,2',4,4',5,5'-hexachlorobiphenyl) in affecting several endpoints including serum levels of thyroid hormones, body and organ weights, and hepatic levels of porphyrins and retinoids (van Birgelen et al. 1992, 1994a, 1994b, 1996a). The PCB:TCDD concentration ratios administered in these studies were chosen to reflect relative concentrations in human milk and fat samples (1.5–450:1, 240–2,400:1, and 2,000–200,000:1 for the 3,3',4,4',5-, 2,3,3',4,4',5-, and 2,2',4,4',5,5'-congeners, respectively).

Results from these studies provide no evidence for synergistic interactions between 3,3',4,4',5-pentachlorobiphenyl and 2,3,7,8-TCDD or 2,3,3',4,4',5-hexachlorobiphenyl and 2,3,7,8-TCDD in decreasing thyroid hormone levels, body weights, thymus weights, or hepatic retinoid levels or in increasing relative liver weights or hepatic levels of porphyrin (see Tables 8 and 9). Some evidence for less-than-additive joint action was found for these endpoints, but these apparent interactions may have been due to near-maximal effects occurring at the dose levels used. For example, hepatic retinol levels after 13 weeks were 14.9, 6.2, 1.5, and 0.6 mg/g liver in rats provided 3,3',4,4',5-pentachlorobiphenyl alone at dietary concentrations of 0, 7, 50, or 180 ppb; whereas in groups whose diet additionally included 0.4 or 5 ppb 2,3,7,8-TCDD, hepatic retinol concentrations were 8.2, 10.7, 4.0, and 4.8 mg/g liver and 2.2, 0.9, 1.8, and 1.4 mg/g liver, respectively (van Birgelen et al. 1994b). Similar results were found for the combined effects of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-TCDD on the same endpoints, with the notable exception of evidence for synergistic action in decreasing thyroid hormone levels (van Birgelen et al. 1992) and increasing hepatic porphyrin levels (van Birgelen et al. 1996a). The apparent synergism was especially marked for joint action on hepatic porphyrin levels. Average hepatic levels of porphyrins after 13 weeks on diets containing 0, 10, 30, or 100 ppm 2,2',4,4',5,5'-hexachlorobiphenyl were 1.9, 2.8, 1.4,

and 3.0 μg/g liver (Van Birgelen et al. 1996a). In contrast, porphyrin levels were 1.9, 300, 969, and 1,223 μg/g liver and 2.5, 22, 1,527, and 1,094 μg/g liver in rats fed the same respective 2,2',4,4',5,5'-hexachlorobiphenyl concentrations plus 0.5 or 5 ppb 2,3,7,8-TCDD in the diet (van Birgelen et al. 1996a).

Tables 6 and 7 summarize the available interactions data for PCBs and 2,3,7,8-TCDD.

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD**

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Parenteral Exposure (mg/kg/day) – PCB Mixtures | | | | | | |
| Acute | impaired immune response to sheep red blood cells (intra-peritoneal) | | | 1.6[a]+0.0012 (m)[b]<br>4.9+0.0012 (m)<br>24.6+0.0012 (m)<br>49.3+0.0012 (m)<br>24.6+0.0036 (m) | PCB mixtures, with the exception of Aroclor 1232, antagonized the acute immunotoxicity of 2,3,7,8-TCDD in mice, at dose ratios >1,000:1. | Bannister et al. 1987 (Aroclor 1254) |
| | | | | 25+0.0012 (m) | | Davis and Safe 1989 (Aroclors 1242, 1248, 1254, 1260) |
| | | | 25+0.0012 (m) | | | Davis and Safe 1989 (Aroclor 1232) |
| | | | 5+0.0012 (m)<br>25+0.0012 (m) | 50+0.0012 (m) | | Davis and Safe 1989 (PCB mix reflective of human milk) |

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Parenteral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl) | | | | | | |
| Acute | impaired immune response to sheep red blood cells (intra-peritoneal) | | 0.36–36+0.0012 (m)<br>7.2+0.0012 (m)<br>16–32+0.0012 (m)<br>25–100+0.0012 (m)<br>36–361+0.0012 (m)<br>180–361+0.0012(m)<br><br>36.1+0.0012 (m) | 18–72+0.0012 (m)<br>65+0.0012 (m)<br><br>400–1,000+0.0012 (m) | 3/7 Congeners examined partially antagonized the acute immunotoxicity of TCDD; relationships between dose (or dose ratio) and antagonism were different for the antagonistic congeners. | Davis and Safe 1990<br>(2,3,3',4,4',5-HCB)<br>(2,3,3',4,5,5'-HCB)<br>(2,3,3',4,5'-PeCB)<br>(2,3',4,4',5',6-HCB)<br>(2,2',4,4',5,6'-HCB)<br>(2,2',4,4',6,6'-HCB)<br><br>Biegel et al. 1989b<br>(2,2',4,4',5,5'-HCB) |
| Inter-mediate | liver tumor promotion activity (sub-cutaneous) | | µg/kg/week<br>1.00+0.100 (r)<br>3.16+0.316 (r)<br>10.0+1.00 (r) | | Combined exposure with TCDD did not show obvious deviation from additive effect on development of γ-glutamyl transpeptidase altered foci. | Hemming et al. 1995<br>(3,3',4,4',5-PeCB) |

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Mixtures | | | | | | |
| Acute | increased offspring with cleft palate | | | 244+0.020 (m) | A complex mixture of PCBs completely antagonized TCDD-induced cleft palate formation at examined dose ratio (12,200:1). | Haake et al. 1987 (Aroclor 1254) |
| Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl) | | | | | | |
| Acute | increased offspring with cleft palate | 40–80+0.012 (m)<br>10–20+0.003 (m) | 25–50+0.003 (m) | | 1/3 Congeners (2,3,3',4,4',5-) potentiated TCDD-induced cleft palate formation; 2/3 congeners were partially antagonistic (2,2',4,4',5,5'- and 2,2',4,4'-). | Birnbaum et al. 1985 (2,3,3',4,4',5-HCB) (2,2',4,4',5,5'-HCB)<br>Biegel et al. 1989a, 1989b (2,2',4,4',5,5'-HCB) (2,2',4,4'-TCB) |
| | | | 90.2+0.020 (m) | 270.7+0.020 (m)<br>217.5+0.020 (m) | Relationship between dose and antagonism for 2,2',4,4',5,5'-HCB increased to a maximum then declined to no effect (inverted U shape). | |
| | | | 62.5+0.015 (m)<br>1,000+0.015 (m) | 125–500+ 0.015 (m) | | Morrissey et al. 1992 (2,2',4,4',5,5'-HCB) |

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; TCB = tetrachlorobiphenyl) | | | | | | |
| Acute | increased offspring with hydronephrosis | | 62.5–250+0.015 (m)<br>1,000+0.015 (m)<br><br>90.2+0.020<br>270.7+0.020 | 500+0.015 (m) | Relationship between dose and partial antagonism appeared to be a steep inverted U. | Morrissey et al. 1992 (2,2',4,4',5,5'-HCB)<br><br>Biegel et al. 1989b (2,2',4,4',5,5'-HCB) |
| Acute | impaired immune response to sheep red blood cells | | 3.58+0.001 (m)<br>35.8+0.001 (m)<br>3.58–358+0.010 (m) | 358+0.001 (m) | Antagonism of low dose of TCDD observed at dose ratio of 358,000:1. No antagonism of high TCDD dose. | Smialowicz et al. 1997 (2,2',4,4',5,5'-HCB) |

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Congeners<br>(HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl) | | | | | | |
| Inter-mediate | decreased thyroid hormone levels (total and free T4) | 13 weeks<br>0.98–9.8+0.00005 (r)<br>0.98–9.8+0.0005 (r) | 4 weeks<br>0.98–9.8+0.00005 (r)<br>0.98–9.8+0.0005 (r) | | Joint action of 2,2',4,4',5,5'-HCB and TCDD to decrease T4 levels was synergistic at 13 weeks. | van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB) |
| | | | | 13 weeks<br>0.08–0.7+0.0003 (r) | Other two congeners showed less-than-additive joint action with TCDD on thyroid hormone levels. | van Birgelen et al. 1994a (2,3,3',4,4',5-HCB) |
| | | | | 13 weeks (µg/kg/day)<br>0.5–10.1+0.03 (r)<br>0.4–9.7+0.3 (r) | | van Birgelen et al. 1994b (3,3',4,4',5-PeCB) |

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl) | | | | | | |
| Inter-mediate | decreased body weight and thymus weight; increased relative liver weight | | 13 weeks<br>0.98–9.8+0.00005 (r)<br>0.98–9.8+0.0005 (r) | 13 weeks<br>0.08–0.7+0.0003 (r)<br><br>13 weeks (µg/kg/day)<br>0.5–10.1+0.03(r)<br>0.4–9.7+0.3 (r) | 2,2',4,4',5-HCB did not influence TCDD effects on body weight and thymus weight, and additively affected relative liver weight.<br><br>Other 2 congeners showed less than additive joint action with TCDD on these endpoints. | van der Kolk et al. 1992 (2,2',4,4',5,5'-HCB)<br><br>van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)<br><br>van Birgelen et al. 1994b (3,3',4,4',5-PeCB) |

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Congeners<br>(HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl) | | | | | | |
| Inter-mediate | increased hepatic porphyrin levels | 13 weeks<br>0.7–6.4+0.00003 (r)<br>0.6–5.9+0.0003 (r) | 13 weeks<br>0.08–0.7+0.0003 (r)<br><br>13 weeks (µg/kg/day)<br>0.5–10.4+0.03 (r)<br>0.4–9.7+0.3 (r) | | 2,2',4,4',5,5'-HCB + TCDD, at individual non-effective doses, jointly increased liver porphyrin levels by about 11–800 times control levels. Other examined congeners showed no such synergism with TCDD. | van Birgelen et al. 1996a<br>(2,2',4,4',5,5'-HCB)<br><br>(2,3,3',4,4',5-HCB)<br><br>(3,3',4,4',5-PeCB) |

**Table 6. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of 2,3,7,8-TCDD** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl; TCB = tetrachlorobiphenyl) | | | | | | |
| Inter-mediate | decreased hepatic retinoid levels | | | 13 weeks<br>0.98–9.8+0.00005 (r)<br>0.98–9.8+0.0005 (r)<br><br>13 weeks<br>0.08–0.7+0.0003 (r)<br><br>13 weeks (μg/kg/day)<br>0.5–10.1+0.03 (r)<br>0.4–9.7+0.3 (r) | All 3 congeners alone decreased hepatic levels of retinol and retinylpalmitate. In combination with TCDD, less than additive action was indicated, but near maximal decreases occurred at TCDD doses alone. | van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB)<br><br>van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)<br><br>van Birgelen et al. 1994b (3,3',4,4',5-PeCB) |

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: m = mouse; r = rat

**Table 7. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs**

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Parenteral Exposure (mg/kg/day) – PCB Mixtures (no data) | | | | | | |
| Parenteral Exposure (mg/kg/day) – PCB Congeners (PeCB = pentachlorobiphenyl) | | | | | | |
| Inter-mediate | liver tumor promotion activity (subcutaneous) | | μg/kg/week<br>0.100[a]+1.00 (r)[b]<br>0.316+3.16 (r)<br>1.00+10.0 (r) | | Combined exposure with TCDD did not show obvious deviation from additive effect on development of γ-glutamyl transpeptidase altered foci. | Hemming et al. 1995 (3,3',4,4',5-PeCB) |

**Table 7. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Mixtures (no data) | | | | | | |
| Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl) | | | | | | |
| Inter-mediate | decreased thyroid hormone levels (total and free T4) | 13 weeks<br>0.00005+0.98–9.8 (r)<br>0.0005+0.98–9.8 (r) | 4 weeks<br>0.00005+0.98–9.8 (r)<br>0.0005+0.98–9.8 (r) | 13 weeks<br>0.0003+0.08–0.7 (r)<br><br>13 weeks (µg/kg/day)<br>0.03+0.5–10.1 (r)<br>0.3+0.4–9.7 (r) | Joint action of 2,2',4,4',5,5'-HCB and TCDD to decrease T4 levels was synergistic at 13 weeks.<br><br>TCDD with other 2 PCB congeners showed less-than-additive joint action on thyroid hormone levels. | van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB)<br><br>van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)<br><br>van Birgelen et al. 1994b (3,3',4,4',5-PeCB) |
| Inter-mediate | decreased body weight and thymus weight; increased relative liver weight | | 13 weeks<br>0.00005+0.98–9.8 (r)<br>0.0005+0.98–9.8 (r) | 13 weeks<br>0.0003+0.08–0.7 (r)<br><br>13 weeks (µg/kg/day)<br>0.03+0.5–10.1 (r)<br>0.3+0.4–9.7 (r) | TCDD with 2,2',4,4',5-HCB additively affected relative liver weight.<br><br>TCDD with other 2 congeners showed less than additive joint action on these endpoints. | van der Kolk et al. 1992 (2,2',4,4',5,5'-HCB)<br><br>van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)<br><br>van Birgelen et al. 1994b (3,3',4,4',5-PeCB) |

**Table 7. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Mixtures (no data) | | | | | | |
| Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl) | | | | | | |
| Inter-mediate | increased hepatic porphyrin levels | 13 weeks<br>0.00003+0.7–6.4 (r)<br>0.0003+0.6–5.9 (r) | <br><br><br><br>13 weeks<br>0.0003+0.08–0.7 (r)<br><br>13 weeks (μg/kg/day)<br>0.03+0.5–10.4 (r)<br>0.3+0.4–9.7 (r) | | 2,2',4,4',5,5'-HCB + TCDD, at individual non-effective doses, jointly increased liver porphyrin levels by about 11–800 times control levels. TCDD with other 2 congeners showed no such synergism. | van Birgelen et al. 1996a<br>(2,2',4,4',5,5'-HCB)<br><br>(2,3,3',4,4',5-HCB)<br><br>(3,3',4,4',5-PeCB) |

**Table 7. Summary of Available Data on the Influence of 2,3,7,8-TCDD on Toxicity/Carcinogenicity of PCBs** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) – PCB Mixtures (no data) | | | | | | |
| Oral Exposure (mg/kg/day) – PCB Congeners (HCB = hexachlorobiphenyl; PeCB = pentachlorobiphenyl) | | | | | | |
| Inter-mediate | decreased hepatic retinoid levels | | | 13 weeks<br>0.00005+ 0.98–9.8 (r)<br>0.0005 + 0.98–9.8 (r)<br><br>13 weeks<br>0.0003+0.08–0.7 (r)<br><br>13 weeks (µg/kg/day)<br>0.03+0.5–10.1 (r)<br>0.3+0.4–9.7 (r) | All 3 congeners or TCDD alone decreased hepatic levels of retinol and retinylpalmitate. In combination, less than additive action was indicated, but near maximal decreases occurred at TCDD doses alone. | van Birgelen et al. 1992 (2,2',4,4',5,5'-HCB)<br><br>van Birgelen et al. 1994a (2,3,3',4,4',5-HCB)<br><br>van Birgelen et al. 1994b (3,3',4,4',5-PeCB) |

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: m = mouse; r = rat

### 2.2.8 PCBs and Hexachlorobenzene

As discussed in Appendices B and E, health effects associated with exposure to PCBs or hexachlorobenzene that are common to both include hepatic porphyria, porphyria cutanea tarda, liver hypertrophy, disruption of thyroid hormone homeostasis, immunosuppression, impaired neurological development, and liver cancer. Physiological and molecular processes leading to the development of these effects involve potential sites at which PCBs and hexachlorobenzene may interact in affecting these health endpoints, but no *in vitro* or *in vivo* studies were located that were designed to examine possible interactions between PCBs and hexachlorobenzene. Processes in which potential interactions may occur include: induction of Phase I and II enzymes that may influence porphyria, liver hypertrophy, tissue damage from reactive oxygen species, and liver cancer development; disruption of thyroid hormone homeostasis via binding to thyroid hormone transport proteins, altering thyroid hormone metabolism, or damaging thyroid tissue; and disruption of sex hormone homeostasis via estrogenic, anti-estrogenic, androgenic, or anti-androgenic actions. In general, mechanistic understanding of the processes involved in the development of the common toxicity targets is too incomplete to reliably predict whether mixtures of PCBs and hexachlorobenzene may jointly act in additive, less-than-additive, or greater-than-additive fashions.

### 2.2.9 PCBs and *p,p'*-DDE

Results from animal (and some human) studies identify several sensitive shared targets of PCBs and *p,p'*-DDE oral toxicity including the liver (hepatomegaly, degenerative histological effects, and liver cancer), immune system (suppression of cell-mediated immunological responses), neurological development (altered neurobehavior in offspring exposed *in utero* or during nursing periods), and altered reproductive function or development (see Table 1 and Appendices C and E). A limited amount of *in vitro* and *in vivo* data regarding possible interactions between PCBs and *p,p'*-DDE is available as reviewed below, but the data do not provide information relevant to how PCBs and *p,p'*-DDE may jointly act in affecting shared sensitive targets of public health concern.

Incubation of an estrogen receptor preparation from alligator oviducts with a mixture of *p,p'*-DDE, *p,p'*-DDD, Aroclor 1242, *trans* nonachlor, and *cis* nonachlor or a mixture of these agents plus dieldrin, toxaphene, and chlordane inhibited the binding of tritium-labeled 17β-estradiol to estrogen receptors (Vonier et al. 1996). The individual agents, at the concentrations used in these mixtures, did not inhibit the *in vitro* binding of 17β-estradiol to the estrogen receptors. In the absence of other information, these observations do not provide sufficient evidence of interactions between *p,p'*-DDE and Aroclor 1242 that

may influence reproductive functions or development.

Combined dietary exposure of mallards to 40 ppm *p,p'*-DDE and Aroclor 1254 did not alter DDE-induced egg shell thinning, but appeared to decrease the number of intact eggs that were produced compared with values for control groups or groups exposed to either agent alone (Risebrough and Anderson 1975). Dietary exposure of groups of mallards (4 drakes and 10 hens) to 40 ppm *p,p'*-DDE or 40 ppm *p,p'*-DDE +40 ppm Aroclor 1254 for 5 months caused 17 and 19% reduction in mean egg shell thickness compared with control groups (Risebrough and Anderson 1975). Exposure to 40 ppm Aroclor 1254 alone did not affect egg shell thickness. Combined exposure reduced total egg production over the study period by about 35% compared with controls. Egg production in the first 7 weeks was similar in all groups, but markedly dropped thereafter in the DDE+Aroclor 1254 group. About 25% of the decline in egg production in the combined exposure group was attributed to egg eating. Further information or studies regarding this apparent synergism between *p,p*'-DDE and Aroclor 1254 were not located. This apparent synergism is unlikely to be relevant to possible alterations of reproductive performance in mammals exposed to mixtures of PCBs and *p,p'*-DDE.

### 2.2.10 PCBs and Methylmercury

PCBs and methylmercury are both neurotoxicants, each affecting pre- and post-natal neurological development, as well as function of the developed neurological system. Effects on neurodevelopment are among the most sensitive effects produced by both PCBs and methylmercury (see Table 1 and Appendices D and E), and their joint toxic action as neurotoxicants is of public health interest. Each chemical's mechanisms of action are thought to involve disruption of calcium homeostatic mechanisms in neural cells leading to activation of various second messenger systems and subsequent changes in neuro-transmitter release (e.g., dopamine), cell damage, or cell death (Bemis and Seegal 1999; Kodavanti and Tilson 1997; Kodavanti et al. 1993, 1996a). For example, *in vitro* exposure of rat cerebellar cells to methylmercury (Marty and Atchison 1997) or 2,2'-dichlorobiphenyl or 3,3',4,4',5-pentachlorobiphenyl (Kodavanti et al. 1993) caused elevations in intracellular $Ca^{+2}$ concentrations. A recent *in vitro* study with rat striatal tissue provides evidence of a synergistic joint action to decrease tissue levels of dopamine (Bemis and Seegal 1999).

Four-hour exposures of punches from freshly excised rat striatal slices to either methylmercury or a 1:1 mixture of Aroclors 1254 and 1260 (Aroclor1254/1260) caused decreased dopamine tissue concentrations, and increased dopamine media concentrations, consistent with proposed actions of these

agents on calcium homeostasis and subsequent effects on neurotransmitter release (Bemis and Seegal 1999). Exposure levels were 1, 4, 10, 14, 20, or 40 μM methylmercury and 10, 20, 40, 100, or 200 ppm Aroclor 1254/1260 (4 or 6 punches per exposure level). These concentrations are higher than PCB (0.84–1.9 ppm) or mercury (0.34 ppm) concentrations reported to occur in Great Lakes fish (Bemis and Seegal 1999). Dopamine tissue concentrations showed statistically significant ($p<0.05$) decreases, compared with controls, with each incremental increase in Aroclor 1254/1260 level ranging from about 90% of control values at 10 ppm to 60% at 200 ppm. In contrast, statistically significant decreases from methylmercury were only observed at the two highest concentrations. Dopamine levels in tissues exposed to 14, 20, and 40 μM methylmercury were 84 (not significantly different from controls), 20, and 1% of control values, indicating a steep dose-response relationship between 14 and 20 μM. Conversely, media levels of dopamine increased with increasing levels of Aroclor 1254/1260 in the media. Media levels of lactate dehydrogenase, measured as indices of the integrity of plasma membranes and viability of the tissue, were not significantly different from control levels at all Aroclor concentrations, but were significantly elevated by about 20% at 20 μM methylmercury.

Striatal tissue samples were also simultaneously exposed to methylmercury at 4, 10, or 14 μM plus Aroclor 1254/1260 at 10, 20, 40, 100, or 200 ppm (Bemis and Seegal 1999). Data for tissue dopamine levels or media dopamine levels were analyzed by a two-factor analysis of variance at each of the three levels of methylmercury. The analysis assumed a linear response-addition model of two factors and an interaction term (personal communication with R. Seegal, 02/23/01). The analysis of tissue concentrations indicated that the interaction term was statistically significant at 4 ($p<0.05$), 10 ($p<0.001$), and 14 ($p<0.01$) μM methylmercury; similar results were obtained for the media concentration interaction term. This study design and statistical analysis provides qualitative information that interactions occurred. Observed combined-exposure mean tissue dopamine concentrations (expressed as a percentage of control values in Figure 1 of Bemis and Seegal 1999) were compared with predicted values that were a sum of the mean dopamine concentrations of tissues exposed to respective concentrations of the individual agents. The observed values were lower than the predicted values at Aroclor 1254/1260 concentrations $\geq$40 ppm in the presence of 10 or 14 μM methylmercury and the predicted values at Aroclor 1254/1260 concentrations $\geq$100 ppm in the presence of 4 μM methylmercury. This comparison is suggestive of a synergistic effect. For example, the observed mean dopamine concentration at 14 μM methylmercury plus 200 ppm Aroclor 1254/1260 was about 20% of control values, whereas the predicted value was 40% of control (based on approximate observed responses of 60% to 200 ppm Aroclor 1254/1260 alone and about 80% to 14 μM methylmercury alone). A statistical test of the comparison of the observed and predicted concentrations, however, cannot be constructed because of limitations in the study

design and the reporting of the data in the Bemis and Seegal (1999) report.

In an *in vivo* study of possible interactions between PCBs and methylmercury, groups of pregnant female JCL-ICR mice were fed a normal diet or one containing 500 ppm Kanechlor 500 from gestation day 0 to day 21 after delivery and received gavage doses of methylmercury in corn oil (0, 0.4, or 4 mg Hg/kg/day) from gestation day 15 to day 21 after delivery (Tanimura et al. 1980). An approximate dose of 940 mg/kg/day Kanechlor 500 is estimated based on a calculated food consumption rate of 0.06 kg/day (EPA 1988) and an approximate average body weight of 0.032 kg for the dams. These dose levels are expected to be considerably higher than those experienced by people consuming contaminated fish from the Great Lakes or Baltic Sea. The dose levels were selected based on preliminary studies indicating that dietary exposure of mice to 500 ppm Kanechlor 500 throughout gestation produced no teratogenicity, but some embryolethality and decreased learning ability, and that gavage methylmercury doses of 4 mg Hg/kg/day would cause body weight changes, but no overt pathological changes, in offspring (8 mg Hg/kg/day produced paralysis, gait problems, and lethality in all pregnant mice). At sacrifice on day 21 after delivery, maternal mice exposed to 500 ppm Kanechlor alone or in combination with methylmercury showed similar increased body weights and increased liver weights compared with controls, but no gross pathological changes were detected in autopsies. These effects were not seen in maternal mice exposed to methylmercury alone.

Offspring survival through day 21 after delivery in all treated groups was not significantly different from control group survival, except for the Kanechlor 4 mg Hg/kg group, which showed 82.5% survival at day 21 compared with 98.9% in the control group. Survival of male offspring in all Kanechlor groups showed a marked decline, compared with controls, at about 5 weeks after birth; at 10 weeks after birth, male offspring survival percentages were about 60, 60, and 40% for the groups with Kanechlor plus 0, 0.4, and 4 mg Hg/kg, respectively, compared with >90% in the control and methylmercury alone groups. Autopsies of expired offspring revealed no obvious or specific cause of death. Survival data for female offspring were reported to have been similar. At birth, there were no significant exposure-related differences in offspring body weights, but the rate of offspring body weight gain through weaning was decreased in all Kanechlor groups compared with controls. The depression in early offspring body weight gain was most pronounced in the Kanechlor plus 4 mg Hg/kg group.

A battery of developmental tests including negative geotaxis, righting on the surface, cliff avoidance, swimming, auditory startle, and hindlimb support were administered on several preweaning days to all offspring (Tanimura et al. 1980). Statistically significant exposure-related effects were restricted to

decreased hindlimb support test scores in all Kanechlor groups at day 14 and in only the Kanechlor plus 4 mg Hg/kg group at days 17 and 21, and decreased proportion of correct responses in a visual placing test in only the Kanechlor plus 4 mg Hg/kg group at day 21. Tests of general activity (open field), and learning ability (water filled multiple T-maze and conditioned avoidance response) were administered to two male offspring from each litter at several post-weaning intervals. No consistent, statistically significant differences in test performances were found between control and exposed groups, although learning ability in the conditioned avoidance test was reported to have been "slightly inhibited" in methylmercury groups and "tended to be lower, compared to the untreated controls" in the PCB-treated groups.

Reproductive performance and a measure of developmental toxicity were also evaluated in the male and female F1 offspring with a first mating at 10 weeks of age (Tanimura et al. 1980). No statistically significant exposure-related changes were observed in first mating success, live birth index, number of live F2 newborns, F2 survival through day 21 after birth, and F2 offspring body weight through day 21 after birth. Reproductive performance was continuously surveyed in one randomly selected F1 female per litter until 48 weeks of age. No significant exposure-related effects were found on reproductive efficiency, litter sizes, survival rate, growth rate, or prevalence of anomalies in offspring sacrificed on day 21 after birth.

In summary, the mouse study by Tanimura et al. (1980) found no evidence for obvious synergism or additive joint action between Kanechlor 500 and methylmercury in affecting several endpoints evaluated in offspring of female mice exposed during lactation and gestation. Post-natal survival was affected to a greater degree by combined exposure (at a dose level of 4, but not 0.4 mg Hg/kg/day) than exposure to Kanechlor 500 alone; methylmercury alone did not affect post-natal survival. The combined-exposure effect on post-natal survival could be explained by a possible potentiation of Kanechlor 500 lethality by methylmercury, but the possibility of some other mode of joint action cannot be precluded due to design limitations of this study. Other examined endpoints included righting and swimming ability, hindlimb support, general open-field activity, and learning ability in offspring at several postnatal periods, reproductive performance in F0 and F1 generations, and prevalence of developmental anomalies. The study did not include doses of the individual agents that influenced most of the examined variables, did not provide any information on dose-response relationships for the individual agents, and provided no information on dose-response relationships for combined exposure and most of the variables. Thus, no meaningful comparisons (statistically based or otherwise) could be made between observed combined-exposure responses and predicted responses based on some concept of joint action. Thus, very limited

information is provided concerning possible modes of joint action of PCBs and methylmercury on post-natal survival, neurobehavior, reproductive performance, and prevalence of developmental anomalies.

Reproductive endpoints, serum thyroid hormone levels (T3 and T4), and histology of brain, kidney, adrenals, pituitary, and thyroid were evaluated in groups of adult ranch-bred mink fed a commercial mink food supplemented with 0 or 1 ppm Aroclor 1254, 1 ppm Hg as methylmercury, 1 ppm Aroclor 1254 +1 ppm methylmercury, or 0.5 ppm Aroclor 1254 +0.5 ppm methylmercury for 8 months that spanned one breeding period (December 1984 through June 1985) (Wren et al. 1987a, 1987b). Exposed groups contained 12 females and 4 males; the control groups had 15 females and 5 males. Food intake and body weight data were not reported, but gross estimates of 0.2 mg/kg/day Aroclor 1254 and 0.2 mg Hg/kg/day are derived for the 1-ppm treatment based on a food intake of 150 g/day and body weight of 0.9 kg for minks (Aulerich et al. 1987). During the third month of exposure, eight females and one male in the 1-ppm methylmercury group, and three females in the 1-ppm Aroclor + methylmercury group died, displaying obvious signs of mercury intoxication (e.g., convulsions, tremors, and lethargy). The mortality was attributed to a combination of cold stress and methylmercury poisoning, and surviving minks were fed diets containing 1 ppm methylmercury every other day for the remainder of the study. No exposure-related effects were found on the thyroid, pituitary, adrenal glands, or serum T4 or T3 levels in adult minks that survived the 8-month exposure period. Fertility of adult male mink, percentage of females whelped, or number of offspring born per female were not significantly affected by any of the treatments. The average number of offspring per female at weaning (5 weeks after birth) was significantly ($p<0.05$) lower in the 1 ppm Aroclor + methylmercury group (2.1 offspring/female) than in the control (4.5), 1 ppm Aroclor (5.0), 1 ppm methylmercury (4.0), or 0.5 ppm Aroclor + 0.5 ppm methylmercury groups (3.6), indicating that post-natal offspring mortalities were increased by combined exposure to the high levels of methylmercury and Aroclor 1254.

Wren et al. (1987b) concluded that these observations showed a synergistic effect of Aroclor 1254 and methylmercury on post-natal survival of mink offspring, but without more information about dose-response relationships on this endpoint, the data do not allow a rigorous conclusion regarding joint action. The data indicate that the two agents, at respective non-effective exposure levels of 1 ppm, added together to induce post-natal mortality, but it is not possible to discern if they added together in a less-than-additive, additive, or greater-than-additive manner. Given that the individual agents were administered at concentrations that did not affect post-natal mortality, demonstration of synergism assuming response addition requires that the response to the 0.5 ppm Aroclor + 0.5 ppm methylmercury treatment would have been significantly greater than the control value; however, post-natal mortality was not changed,

compared with control, by this treatment.

There is evidence to suggest that induction of CYP1A and CYP2B enzymes by PCB mixtures is counteracted by simultaneous exposure to methylmercury in rats and quails (Leonzio et al. 1996a; Takabatake et al. 1980).

In Wistar rats fed a diet containing 50 ppm of a 1:1 mixture of Kanechlors 400:500 for 14 days and subcutaneously exposed to 10 mg Hg/kg/day as methylmercuric chloride on the last 2 days, induction of hepatic levels of CYP and several associated oxygenases (aminopyrine N-demethylase, aniline hydroxylase, p-nitroanisole O-demethylase) was curtailed compared with levels in rats treated with 50 ppm Kanechlor alone (Takabatake et al. 1980). Estimated daily doses of 4.6 mg Kanechlor/kg/day were calculated assuming a food consumption rate of 0.02 kg/day and body weight of 0.217 kg for Wistar rats. Rats fed a normal diet and treated similarly with methylmercuric chloride showed a decrease in hepatic levels of CYP and associated oxygenases compared with rats fed a normal diet (Takabatake et al. 1980).

In quail fed a diet containing 25 ppm methylmercury and 100 ppm Aroclor 1260 for 21 days, induction of hepatic levels of benzoxylresorufin-O-deethylase (BROD, an indicator of CYP2B) and ethoxyresorufin-O-deethylase (EROD, an indicator of CYP1A) was curtailed compared with levels in quail fed a diet containing 100 ppm Aroclor 1260 (Leonzio et al. 1996a). (Information provided in the report was insufficient to calculate estimated doses.) Exposure to 10 ppm Aroclor 1260 induced hepatic levels of these enzymes to a lesser degree than 100 ppm, but this level of induction was not markedly influenced by simultaneous exposure to 2.5 ppm methylmercury in the diet. Levels of BROD and EROD were increased to a small degree, compared with control values, in quail fed a diet containing 2.5 ppm methyl-mercury alone, but activities were non-significantly decreased in quail fed a diet containing 25 ppm methylmercury alone. Exposure to 25 ppm methylmercury and 100 ppm Aroclor 1260 produced a decrease in serum cholesterol that was associated with a decrease in hepatic levels of Aroclor 1260 compared with exposure to Aroclor 1260 alone (Leonzio et al. 1996a).

A companion study found that a 3-week exposure of quail to 2.5 or 25 ppm methylmercury in the diet or 10 or 100 ppm Aroclor 1260 in the diet increased mean porphyrin levels in liver or excreta and that combined exposure to 2.5 ppm methylmercury +10 ppm Aroclor 1260 or 25 ppm +100 ppm Aroclor 1260 appeared to additively increase porphyrin levels (Leonzio et al. 1996b). For example, differences in total porphyrins in excreta between groups treated with 25 ppm methylmercury alone or 100 ppm

Aroclor 1260 and control values were 3,801 and 2,831 pmol/g of excreta. For the combined exposure group, the difference was 6,070 pmol/g of excreta, which is similar to a predicted value of 6,632 pmol/g based on an additivity assumption.

The results of the studies by Leonzio et al. (1996a) and Takabatake et al. (1980) suggest that simultaneous exposure to methylmercury may counteract the induction of hepatic CYP enzymes by PCBs, but it is unclear if this is due to direct mercury inhibition of enzyme activity, methylmercury inhibition of PCB uptake or distribution, or some other mechanism. Both compounds appear to cause porphyria in quail, and limited data suggest that they may jointly act in an additive manner (Leonzio et al. 1996b).

A summary of available *in vivo* interactions data for PCBs and methylmercury is presented in Tables 8 and 9.

**Table 8. Summary of Available Data on the Influence of PCBs on Toxicity/Carcinogenicity of Methylmercury**

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) | | | | | | |
| Inter-mediate | impaired learning capability in offspring | | 940[a]+4 (m)[b] | | Data were inadequate for definitive conclusions on joint action. | Tanimura et al. 1980 (Kanechlor 500) |
| Inter-mediate | decreased neonatal survival in offspring | | 0.2+0.2 (i) | | Data indeterminate to determine joint action; decreased survival not observed with (0.1+0.1) mg/kg/day. | Wren et al. 1987b (Aroclor 1254) |
| Inter-mediate | increased porphyrins in liver and excreta | | 2.5 ppm+10 ppm (q)<br>25 ppm+100 ppm (q) | | Results consistent with additivity in producing porphyria. | Leonzio et al. 1996b (Aroclor 1260) |

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: i = mink; m = mouse; q = quail; r = rat

**Table 9. Summary of Available Data on the Influence of Methylmercury on Toxicity/Carcinogenicity of PCBs**

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) | | | | | | |
| Acute | induction of hepatic CYP | | | 10[a] subcutaneous+4.6 (r)[b] | Inhibition of PCB induction of hepatic CYP. | Takabatake et al. 1980 (Kanechlors 400/500) |
| Inter-mediate | induction of hepatic CYP | | 2.5 ppm+10 ppm (q) | 25 ppm+100 ppm (q) | Inhibition of PCB induction of hepatic CYP at high, but not low, dietary concentrations. | Leonzio et al. 1996a (Aroclor 1260) |
| Inter-mediate | impaired learning capability in offspring | | 4+940 (m) | | Study design inadequate for definitive conclusions on joint action. | Tanimura et al. 1980 (Kanechlor 500) |

**Table 9. Summary of Available Data on the Influence of Methylmercury on Toxicity/Carcinogenicity of PCBs** (*continued*)

| Duration | Endpoint | Results | | | Conclusions | References |
|---|---|---|---|---|---|---|
| | | Greater than additive | Additive/no effect | Less than additive | | |
| Oral Exposure (mg/kg/day) | | | | | | |
| Inter-mediate | decreased neonatal survival in offspring | 4+940 (m) | 0.4+940 (m)<br><br>0.2+0.2 (i) | | Methylmercury showed no effect at low dose; possible potentiation of PCB effect at 4 mg/kg, but study designs are inadequate for definitive conclusions on joint action. | Tanimura et al. 1980 (Kanechlor 500)<br><br>Wren 1987b (Aroclor 1254) |
| Oral Exposure (mg/kg/day) | | | | | | |
| Inter-mediate | decreased body weight gain in neonates | 4+940 (m) | 0.4+940 (m) | | Methylmercury showed no effect at low dose; possible potentiation at 4 mg/kg, but study design is inadequate for definitive conclusions on joint action. | Tanimura et al. 1980 (Kanechlor 500) |
| Inter-mediate | porphyria | | 2.5 ppm+10 ppm (q)<br>25 ppm+100 ppm (q) | | Results consistent with additivity. | Leonzio et al. 1996b (Aroclor 1260) |

[a]First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b]Species code: i = mink; m = mouse; q = quail; r = rat

## 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 five-component mixtures containing CDDs, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs. Furthermore, PBPK/PD models have not been developed to predict dispositional and toxicological outcomes of joint action of mixtures of these five components.

As discussed in the appendices and illustrated in Table 1, oral exposure to each component of the mixture of concern (2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, methylmercury, and PCBs) can produce a wide range of health effects that is dependent on dose level, duration of exposure, and genetic, gender, and developmental status of the exposed individual. There is a fair amount of overlap in the endpoints or organs that these chemicals affect (Table 1). This observation is reflected in the endpoints that form the basis of the oral MRLs for these chemicals (Table 10).

The observations reflected in Tables 1 and 10 lead to concerns that, following oral exposure to mixtures of these five chemicals: (1) all five chemicals may jointly act to produce altered neurological development, suppression of immune competence, or cancer; (2) four (2,3,7,8-TCDD, hexachlorobenzene, *p,p'*-DDE, and PCBs) may jointly act to produce liver damage; (3) four (2,3,7,8-TCDD, hexachlorobenzene, methylmercury, and PCBs) may jointly act to disrupt female reproductive organ function; (4) four (2,3,7,8-TCDD, *p,p'*-DDE, methylmercury, and PCBs) may jointly act to disrupt male reproductive organ function; and (5) three (2,3,7,8-TCDD, *p,p'*-DDE, and PCBs) may jointly act to alter development of male reproductive organs.

As discussed in Section 2.1 of this profile, the detection of all five of these, and other, chemicals in breast milk, combined with the knowledge that each is able to alter neurological development, has led to epidemiological studies in Michigan, North Carolina, New York, and the Netherlands examining if there are associations between increasing concentrations of several of these chemicals in maternal cord serum and breast milk (e.g., PCBs, *p,p'*-DDE, and CDDs) and deficits in measures of motor and cognitive function in children. All four studies demonstrated statistically significant associations between concentrations of these persistent chemicals in maternal fluid samples and deficits in neurological development of the children; however, the Netherlands study also was able to demonstrate beneficial effects of breast feeding on neurological development. The results from the human studies are thought to implicate gestational exposure to the persistent chemicals to a greater degree than lactational exposure.

**Table 10. Health Effects Forming the Basis of ATSDR Oral MRLs for Chemicals of Concern. (See Appendices A, B, C, D, and E for More Details.)**

| Duration of Exposure | 2,3,7,8-TCDD | Hexachlorobenzene | *p,p'*-DDE[a] | Methylmercury | PCBs |
|---|---|---|---|---|---|
| Acute | immuno-suppression in rats | neurobehavioral changes in rat offspring | neurobehavioral changes in mouse offspring | none derived, inadequate data | none derived, inadequate data |
| Inter-mediate | immuno-suppression in rats | reproductive organ changes in female monkeys | hepatomegaly in rats | None derived, inadequate data | neurobehavioral changes in monkey offspring |
| Chronic | neurobehavioral changes in monkey offspring | liver degeneration and porphyria in rats | none derived, inadequate data | neurobehavioral changes in human offspring | immuno-suppression in monkeys |

[a]No MRLs were derived specifically for *p,p'*-DDE, but MRLs for DDT (listed in the DDE column of this table) were based on effects due to *p,p'*-DDT and are expected to be relevant to *p,p'*-DDE.

However, a study of formula-fed monkeys found long-term neurobehavioral deficits in monkeys exposed from birth to 20 weeks to a PCB mixture representative of patterns of congeners found in human breast milk. It is plausible that the monkey study was able to discern PCB-induced effects on postnatal neurological development because they were not masked by potential beneficial effects of breast feeding.

In the absence of studies that examine relevant endpoints and describe dose-response relationships following oral exposures to mixtures that contain these five chemicals (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 approach or the target-organ toxicity dose modification of the hazard index approach). Likewise, a component-based approach assuming response additivity appears reasonable for assessment of cancer risks from oral exposure to mixtures of these five chemicals. 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.

It is recommended that these approaches treat mixtures of PCB congeners (i.e., total PCBs) as a single component of concern. As discussed in the Introduction of this profile, this approach is consistent with ATSDR's approaches to deriving oral MRLs for PCBs, which are based on data linking health effects with exposure to PCB mixtures (Appendix E; ATSDR 2000). 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.

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 five chemicals are limited for most of the pairs and PBPK models for pairs of the chemicals are not available. Tables 12–31 describe binary weight-of-evidence determinations (BINWOEs) for the pairs of the five chemicals of concern using the classification scheme summarized in Table 11 and ATSDR 2001a. The numerical scale of this scheme ranges from -1 for high confidence that a less-than-additive joint action will occur, through 0 for evidence that additive joint action will occur or for indeteministic evidence for the mode of joint action, up to +1 for high confidence that a greater-than-additive joint action will occur. The conclusions presented in these tables were based on the evaluations of the pertinent literature presented in Section 2.2. An overview of the BINWOEs is presented in Table 32. 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. A summary discussion of the BINWOES follows this paragraph and precedes the descriptive tables.

There are no pertinent interaction data and understanding of mechanisms of action is too incomplete to make projections of interactions between the following pairs of chemicals:

- *p,p'*-DDE and hexachlorobenzene (Tables 16 and 17);
- *p,p'*-DDE and methylmercury (Tables 22 and 23);

- PCBs and hexachlorobenzene (Tables 26 and 27); and
- PCBs and *p,p'*-DDE (Tables 28 and 29).

Lack of interaction data, conflicting interaction data, and/or incomplete understanding of mechanisms of action also preclude projecting interactions for the following:

- the effect of 2,3,78-TCDD on hexachlorobenzene toxicity (Table 12);
- the effect of hexachlorobenzene on methylmercury toxicity (Table 20);
- the effect of PCBs on the following TCDD-induced effects: thyroid hormone disruption, porphyria, impaired reproductive organ function and development, impaired neurological function and development, tumor initiation and promotion (Table 24);
- the effect of 2,3,7,8-TCDD on the following PCB-induced effects: immunosuppression, thyroid hormone disruption, porphyria, developmental toxicity (cleft palate formation and hydronephrosis), impaired reproductive organ function and development, impaired neurological function and development, tumor initiation and promotion (Table 25); and
- the effect of methylmercury on the following PCB-induced effects: immunosuppression, thyroid hormone disruption, developmental toxicity not related to neurological deficits or decreased neonatal survival, impaired reproductive performance, tumor initiation and promotion (Table 31).

Evidence of varying quantity and quality is available supporting projections of additive joint action (or no interactive effect) for the following:

- 2,3,7,8-TCDD and *p,p'*-DDE for noncancer effects and cancer (Tables 14 and 15);
- methylmercury and 2,3,7,8-TCDD for noncancer effects and cancer (Tables 18 and 19);
- methylmercury on hexachlorobenzene toxicity (Table 21);
- PCBs and 2,3,7,8-TCDD for inducing body and thymus weight changes, hepatomegaly, and decreased liver levels of retinoids (Tables 24 and 25); and
- methylmercury and PCBs for inducing hepatic porphyria (Tables 30 and 31).

Evidence is also available supporting the following possible interactions:

- hexachlorobenzene potentiation (greater-than-additive interaction) of TCDD reduction in body weight and thymus weight (Table 13);
- antagonism (less-than-additive interaction) by PCB mixtures of TCDD induction of immune system suppression and developmental effects (cleft palate and hydronephrosis) (Table 24); and
- synergism (greater-than-additive interaction) between PCBs and methylmercury in disrupting regulation of brain levels of dopamine, a process influencing neurological function and development (Tables 30 and 31).

In summary, there is only a limited amount of evidence that non-additive interactions may exist between a few of the chemical pairs: hexachlorobenzene potentiation of TCDD reduction of body and thymus weights; PCB antagonism of TCDD immunotoxicity and developmental toxicity; and synergism between PCBs and methylmercury in disrupting neurological function and development. For the remaining pairs, additive joint action at shared targets of toxicity is either supported by data (for a few pairs) or is recommended as a public health protective assumption due to lack of interaction data, conflicting interaction data, and/or lack of mechanistic understanding to reliably project potential non-additive interactions.

**Table 11. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions**

| Classification | | Factor |
|---|---|---|
| **Direction of Interaction** | | **Direction** |
| = | Additive | 0 |
| > | Greater than additive | +1 |
| < | Less than additive | –1 |
| ? | Indeterminate | 0 |
| **Quality of the Data** | | **Weighting** |
| **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. | 1.0 |
| II. | Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur is 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. | 0.71 |
| III. | Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have. | 0.32 |
| **Toxicological Significance** | | |
| A. | The toxicological significance of the interaction has been directly demonstrated. | 1.0 |
| B. | The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals. | 0.71 |
| C. | The toxicological significance of the interaction is unclear. | 0.32 |
| **Modifiers** | | |
| 1. | Anticipated exposure duration and sequence. | 1.0 |
| 2. | Different exposure duration or sequence. | 0.79 |
| a. | *In vivo* data | 1.0 |
| b. | *In vitro* data | 0.79 |
| i. | Anticipated route of exposure | 1.0 |
| ii. | Different route of exposure | 0.79 |

*Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05*
*BINWOE = Direction Factor x Weighting Factor: Ranges from −1 through 0 to +1*

Source: ATSDR 2001a

Table 12. Effect of **2,3,7,8-TCDD** on **Hexachlorobenzene**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of the interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information clearly indicating that possible pharmacokinetic interactions with 2,3,7,8-TCDD will influence hexachlorobenzene toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of the mode of joint action of 2,3,7,8-TCDD and hexachlorobenzene on any toxicity target.

*Mechanistic Understanding* - Hepatic porphyria from repeated exposure to hexachlorobenzene has been proposed to be dependent on CYPIIIA-mediated metabolism and to involve an unidentified reactive intermediate (that inhibits the heme biosynthetic pathway from uroporphyrinogen) during biotransformation to pentachlorophenol (den Besten et al. 1993). Alternatively, it has been proposed that induction of CYP1A isozymes (via the Ah receptor) leads to a stimulation of the oxidation of uroporphyrinogen to uroporphyrin and an inhibition of heme synthesis from uroporphyrinogen (Sinclair et al. 1997). 2,3,7,8-TCDD is a potent inducer of CYP1A, but does not appear to induce CYPIIIA isozymes. Both compounds can produce hepatic porphyria, but the mode of their joint action has not been studied, and it is unknown whether they would jointly produce hepatic porphyria in an additive, greater-than-additive, or less-than-additive manner.

Mechanistic understanding of other hexachlorobenzene-induced health effects (such as altered neurological development, decreased circulating levels of thyroid hormones, and disruption of female reproductive organs) is insufficient to clearly indicate whether TCDD induction of CYP or Phase II enzymes will potentiate hexachlorobenzene toxicity (see Appendix B). Although hexachlorobenzene binds to the Ah receptor (with much less affinity than 2,3,7,8-TCDD), the degree to which hexachlorobenzene-induced toxic effects are mediated by the Ah receptor is unknown.

Joint action at several shared target organs (e.g., liver, developing neurological system, reproductive organs, thyroid) is plausible, but whether the action would be additive, greater-than-additive, or less-than-additive is unknown and unstudied.

*Toxicological Significance* - No studies were located that were designed to compare responses of pertinent toxicity targets to mixtures of TCDD and hexachlorobenzene with responses to either compound alone. No studies were located in which pretreatment with TCDD before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity.

*Additional Uncertainties* - If 2,3,7,8-TCDD induces CYP enzymes involved in hexachlorobenzene metabolism, an alternative possibility is that TCDD co-exposure may potentiate hexachlorobenzene-induced porphyria and perhaps other TCDD effects that may be caused by a reactive metabolite. This speculation would lead to a greater-than-additive judgement with very high uncertainty. This alternative judgement does not appear warranted because 2,3,7,8-TCDD appears to induce CYP1A enzymes, rather than CYPIIIA enzymes, and possible increased rates of formation of the toxic metabolite may not exceed capacity of downstream enzymes to control concentrations in the liver.

Table 13. Effect of **Hexachlorobenzene** on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: > IIIC1aii** (0.32 x 0.32 x 1.0 x 1.0 x 0.79 = +0.08)
for body and thymus weight effects from acute exposure
**BINWOE: > IIIC2aii** (0.32 x 0.32 x 0.79 x 1.0 x 0.79 = +0.06)
for body and thymus weight effects from non-acute exposure
**BINWOE: ?** (0)
for other effects

*Direction of Interaction* - Greater-than-additive (>) action of hexachlorobenzene on TCDD-induced body and thymus weight effects is based on the observation that pretreatment of rats with single intraperitoneal doses of hexachlorobenzene (400 μmole/kg) potentiated effects on body and thymus weights produced by intraperitoneal doses (0.031 or 0.093 μmole/kg) of 2,3,7,8-TCDD (Li et al. 1989). The mode of possible joint toxic action with hexachlorobenzene on other TCDD toxicity targets is unknown and unstudied. Mechanistic understanding is inadequate to support reliable projections of the mode of joint action of 2,3,7,8-TCDD and hexachlorobenzene on other toxicity targets.

*Mechanistic Understanding* - The apparent potentiation is opposite to an expectation that pretreatment with hexachlorobenzene, at doses up to 10,000-fold higher (on a mole/kg basis) than 2,3,7,8-TCDD doses, would have inhibited TCDD-induced effects mediated by the Ah receptor, based on the observation that this pretreatment decreased cytosolic levels of the Ah receptor in several tissues. This illustrates that the mechanistic understanding of TCDD toxic effects and the observed interaction are inadequate to provide a reliable explanation or support reliable inferences based on possible biochemical interactions between hexachlorobenzene and 2,3,7,8-TCDD. Thus, the highest uncertainty category (III) was selected for mechanistic understanding. The results suggest that the interaction may occur at some site other than the Ah receptor.

Joint action on several shared target organs is plausible, but mechanistic understanding of toxic actions on other potential shared toxicity targets (e.g., liver, reproductive organs, immune system) is inadequate to support a reliable projection of mode of joint toxic action (additive, greater-than-additive, or less-than-additive).

*Toxicological Significance* - The apparent potentiation by hexachlorobenzene was demonstrated for TCDD effects on body and thymus weight from acute exposure in one study using one species (rat) and a mixed exposure scenario in which doses of hexachlorobenzene were far greater than doses of TCDD. The response appears to be a potentiation since hexachlorobenzene, even at doses 7.5 times higher than the potentiating dose, did not affect body or thymus weights (Li et al. 1989). The highest uncertainty category (C) was selected due to the lack of corroborative results from replicate studies or other studies using other dose levels, other mixture proportions of hexachlorobenzene and TCDD, or other species.

The mode by which hexachlorobenzene may jointly act with TCDD on other more sensitive effects from acute or repeated exposure (immunosuppression, female reproductive organ disruption, or altered neurological development) is unknown and unstudied; thus, the indeterminate (?) direction of interaction category is appropriate.

Table 13. Effect of **Hexachlorobenzene** on **2,3,7,8-TCDD** *(continued)*

*Modifying Factors* - Because of the biopersistence of these chemicals, sequential administration may produce similar interactions as simultaneous exposure. For acute exposure, a “1" is assigned for duration and sequence. For intermediate or chronic exposure, a “2" is assigned, because the interaction was demonstrated for acute exposure. A “ii” is assigned to the oral BINWOEs, because the observed interaction is from intraperitoneal exposure.

*Additional Uncertainties* - The apparent potentiation does not appear to involve an interaction at the Ah receptor, a mechanistic component that is thought also to be involved in the development of several other effects from TCDD. The modes of possible joint toxic actions between TCDD and hexachlorobenzene on these other toxicity targets are unknown. The modifying factors reflect additional uncertainties regarding the applicability of the single, sequential, intraperitoneal dosing protocol to simultaneous oral exposure and durations longer than acute.

Table 14. Effect of **2,3,7,8-TCDD** on ***p,p'*-DDE**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: =IIIC** (0)
for anti-androgenic effects
**BINWOE: ?** (0)
for other effects

*Direction of Interaction* - Support for additive joint toxic action of TCDD and *p,p'*-DDE on male reproductive organ development and function is restricted to an observation that combined exposure to TCDD and *p,p'*-DDE decreased prostate weight in male rat offspring to a greater degree than either compound alone (Loeffler and Peterson 1999). Mechanistic information suggests that they may act on a molecular scale by independent anti-androgenic mechanisms. Modes of possible joint toxic action on several other toxicity targets are unknown and unstudied. Mechanistic understanding is inadequate to support reliable projections of modes of joint actions on other toxicity targets.

*Mechanistic Understanding* - Anti-androgenic effects from *p,p'*-DDE are postulated to involve inhibition of androgen-binding to androgen receptors (Kelce et al. 1995, 1997), whereas TCDD is not expected to interfere with androgen receptor-ligand binding and may indirectly affect androgen signaling by altering growth factor pathways (Roman et al. 1998b). An additive mode of joint toxic action on male reproductive organ function, and development, is plausible on a whole organ level of organization, but is not supported by these hypotheses of *independent* molecular-scale mechanisms of action. Thus, the highest uncertainty category (III) for mechanistic understanding was selected.

Joint actions on several other shared target organs are plausible (e.g., liver, immune system), but mechanistic understanding is inadequate to support a reliable projection of mode of joint toxic action (additive, greater-than-additive, or less-than-additive).

*Toxicological Significance* - Simultaneous administration of oral doses of 0.25 μg/kg 2,3,7,8-TCDD and 100 mg/kg *p,p'*-DDE during gestation transiently decreased prostrate weight in male rat offspring at postnatal day 21 to a greater degree than administration of either compound alone (Loeffler and Peterson 1999). Whereas additive joint action on this endpoint is a plausible explanation of the results (see Section 2.2.2), the study design is inadequate to rule out possible greater-than-additive or less-than-additive joint actions. Due to study design limitations and a lack of corroborative data from other studies, the highest uncertainty category (C) was selected for toxicological significance.

Joint actions at several shared target organs (e.g., liver, immune system) are plausible, but studies designed to characterize modes of possible joint action (additive, greater-than-additive, or less-than-additive) were not located.

*Additional Uncertainties* - Confidence in the additivity projection for anti-androgenic effects would be strengthened with better designed studies that included several dose levels of each compound, alone and in mixture, to more conclusively determine the mode of joint toxic on male reproductive development.

Table 15. Effect of ***p,p'*-DDE** on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: =IIIC** (0)
for anti-androgenic effects
**BINWOE: ?** (0)
for other effects

*Direction of Interaction* - Support for additive joint toxic action of *p,p'*-DDE and TCDD on male reproductive organ development and function is restricted to an observation that combined exposure to TCDD and DDE decreased prostate weight in male rat offspring to a greater degree than either compound alone (Loeffler and Peterson 1999). Modes of possible joint toxic action on several other toxicity targets are unknown and unstudied. Mechanistic understanding is inadequate to support reliable projections of modes of joint actions on other toxicity targets.

*Mechanistic Understanding* - Anti-androgenic effects from *p,p'*-DDE are postulated to involve inhibition of androgen-binding to androgen receptors (Kelce et al. 1995, 1997), whereas TCDD is not expected to interfere with androgen receptor-ligand binding and may indirectly affect androgen signaling by altering growth factor pathways (Roman et al. 1998b). An additive mode of joint toxic action on male reproductive organ function, and development, is plausible on a whole organ level of organization, but is not supported by these hypotheses of *independent* molecular-scale mechanisms of action. Thus, the highest uncertainty category (III) for mechanistic understanding was selected.

Joint actions on several other shared target organs are plausible (e.g., liver, immune system), but mechanistic understanding is inadequate to support a reliable projection of mode of joint toxic action (additive, greater-than-additive, or less-than-additive).

*Toxicological Significance* - Simultaneous administration of oral doses of 0.25 μg/kg 2,3,7,8-TCDD and 100 mg/kg *p,p'*-DDE during gestation transiently decreased prostrate weight in male rat offspring at postnatal day 21 to a greater degree than administration of either compound alone (Loeffler and Peterson 1999). Whereas additive joint action on this endpoint is a plausible explanation of the results (see Section 2.2.2), the study design is inadequate to rule out the possibility of greater-than-additive or less-than-additive joint actions. Due to study design limitations and a lack of corroborative data from other studies, the highest uncertainty category (C) was selected for toxicological significance.

Joint actions at several shared target organs (e.g., liver, immune system) are plausible, but studies designed to characterize modes of possible joint action (additive, greater-than-additive, or less-than-additive) were not located.

*Additional Uncertainties* - Confidence in the additivity projection for anti-androgenic effects would be strengthened with better designed studies that included several dose levels of each compound, alone and in mixture, to more conclusively determine the mode of joint toxic on male reproductive development.

Table 16. Effect of ***p,p'*-DDE** on **Hexachlorobenzene**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that pharmacokinetic interactions with *p,p'*-DDE will influence hexachlorobenzene toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of the mode of joint toxic action of *p,p'*-DDE and hexachlorobenzene on any toxicity target.

*Mechanistic Understanding* - Joint actions of *p,p'*-DDE and hexachlorobenzene in producing several similar effects (liver damage, immunosuppression, male reproductive organ disruption, altered neurological development, and cancer) are plausible, but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action. Hexachlorobenzene-induced hepatic porphyria is thought to involve an unidentified reactive intermediate produced either by CYPIIIA-mediated or CYP1A-mediated metabolism (den Besten et al. 1993; Sinclair et al. 1997; see Appendix B). DDE induces hepatic CYPIIB and, to a lesser degree, CYPIIIA in rats. If simultaneous exposure to DDE and hexachlorobenzene cause an increased induction of CYPIIIA enzymes (compared with hexachlorobenzene alone) so that capabilities of downstream Phase II enzymes to control liver concentrations of the reactive hexachlorobenzene metabolite are exceeded, then a potentiation of hexachlorobenzene-induced liver toxicity may occur. No studies were located that investigated hepatic metabolic interactions between hexachlorobenzene and *p,p'*-DDE (or DDT), but this projection is not reliable given that hexachlorobenzene can induce its own metabolism and downstream Phase II enzymes would need to be saturated for the potentiation to occur.

*Toxicological Significance* - No studies were located that were designed to compare responses of pertinent toxicity targets to mixtures of DDE and hexachlorobenzene with responses to either compound alone. No studies were located in which pretreatment with DDE before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., liver, nervous system, immune system, thyroid—see Appendices B and C), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied.

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

Table 17. Effect of **Hexachlorobenzene** on ***p,p'*-DDE**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that possible pharmacokinetic interactions with *p,p'*-DDE will influence hexachlorobenzene toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of the mode of joint toxic action of *p,p'*-DDE and hexachlorobenzene on any toxicity target.

*Mechanistic Understanding* - Joint actions of *p,p'*-DDE and hexachlorobenzene in producing several similar effects (liver damage, immunosuppression, altered neurological development, and cancer) are plausible (see Appendices B and C), but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action. Toxic actions of *p,p'*-DDE are thought to involve the parent compound disrupting functions of membranes in various target organs (e.g., disruption of transport mechanisms in neuronal membranes, disruption of ultrastructure of hepatic mitochondrial membranes; see Appendix C). The possible influence of hexachlorobenzene on DDE molecular mechanisms of toxic action is unstudied.

*Toxicological Significance* - No studies were located that were designed to compare responses of pertinent toxicity targets to mixtures of DDE and hexachlorobenzene with responses to either compound alone. No studies were located in which pretreatment with DDE before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., liver, nervous system, immune system, thyroid; see Appendices B and C), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied.

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

Table 18. Effect of **2,3,7,8-TCDD** on **Methylmercury**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: =IIIBbii** (0)
for immune system suppression
**BINWOE: ?** (0)
for other effects

*Direction of Interaction* - There is *in vitro* evidence that a synthetic mixture of CDDs, CDFs, and PCBs at concentrations that were reflective of concentrations in fish from the St. Lawrence River did not change the effects of methylmercury on rat lymphocyte viability and mitogenic ability (Omara et al. 1997). The additive direction of interaction is selected to reflect a projected lack of effect of CDDs on methylmercury immunotoxicity. For other methylmercury effects, a direction of interaction cannot be reliably projected due to the absence of pertinent joint toxic action data, absence of information that possible pharmacokinetic interactions with TCDD may influence methylmercury toxicity, and inadequate mechanistic understanding supporting a reliable projection of the mode of possible joint toxic action of 2,3,7,8-TCDD and methylmercury on other toxicity targets.

*Mechanistic Understanding* - CDDs are postulated to produce immunotoxic effects such as lymphoid tissue depletion and increased susceptibility to infectious agents via an initial mediation by the Ah receptor and unknown subsequent molecular events within the immune system (Kerkvliet 1994; Appendix A). Mercuric salts and methylmercury have been demonstrated to cause both autoimmune stimulation and a suppression of the immune system, but the mechanisms that may be involved are unknown (Appendix D). Pertinent molecular sites of possible interactions between TCDD and methylmercury are thus unidentified, and the limited mechanistic understanding suggests that CDDs may produce immune effects by different mechanisms than methylmercury (i.e., methylmercury immunotoxicity is not expected to involve Ah receptor mediation). The highest uncertainty category (III) was therefore selected for mechanistic understanding.

Mechanistic understanding of methylmercury-induced critical effects (altered neurological development, immunosuppression, and cancer) is insufficient in itself to project possible interactions with TCDD, although interactions at the Ah receptor do not seem likely.

*Toxicological Significance* - *In vitro* studies of immunological endpoints in rat cultured lymphocytes found no evidence for interactions between methylmercury and a synthetic mixture of CDDs, CDFs, and PCBs at low concentrations reflective of concentrations in St. Lawrence River fish (Omara et al. 1997, 1998), but study design limitations preclude definitive conclusions regarding the mode of possible joint toxic actions on the immune system. No other studies (*in vitro* or *in vivo*) to support or refute the results of this single study were located. A moderate confidence rating for toxicological significance (B) is selected to reflect the lack of supporting data, design limitations of the single available study, and the plausibility that the observed lack of effect of CDDs, CDFS, and PCBs on methylmercury immunotoxicity is relevant to pertinent environmental exposure levels such as fish consumption.

No studies were located that were designed to compare responses of other pertinent toxicity targets to mixtures of TCDD and methylmercury with responses to either compound alone. No studies were located in which pretreatment with TCDD before methylmercury exposure was examined for possible effects on methylmercury toxicity at any target organ. Joint actions at several shared target organs are

Table 18. Effect of **2,3,7,8-TCDD** on **Methylmercury** *(continued)*

*Toxicological Significance (continued)* - plausible (e.g., nervous system, thyroid; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) for other effects reflects this lack of data.

*Modifying Factors* - The modifying data quality factor of b was selected to reflect that the only available data on joint action is from an *in vitro* study.

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

Table 19. Effect of **Methylmercury** on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: = ?**

*Direction of Interaction* - A direction of interaction cannot be reliably projected due to the absence of pertinent joint toxic action data, absence of information that possible pharmacokinetic interactions with methylmercury may influence TCDD toxicity, and inadequate mechanistic understanding supporting a reliable projection of the mode of possible joint toxic action of 2,3,7,8-TCDD and methylmercury on other toxicity targets.

*Mechanistic Understanding* - CDDs are postulated to produce several types of effects via an initial mediation by the Ah receptor and subsequent molecular events within target organs (Kerkvliet 1994; Appendix A). Mercuric salts and methylmercury have been associated with effects that occur in some target organs that overlap with CDD toxicity targets, but molecular mechanisms that may be involved are unknown or poorly understood (Appendix D). Pertinent molecular sites of possible interactions between TCDD and methylmercury are thus unidentified, and the limited mechanistic understanding suggests that CDDs may produce effects by different mechanisms than methylmercury (e.g., methylmercury immunotoxicity is not expected to involve Ah receptor mediation).

Interactions at the Ah receptor do not seem likely, but whether methylmercury may interact with TCDD at other cellular or molecular sites involved in the development of TCDD health effects is unknown.

*Toxicological Significance* - *In vitro* studies of immunological endpoints in rat cultured lymphocytes found no evidence for interactions or additivity between methylmercury and a synthetic mixture of CDDs, CDFs, and PCBs at low concentrations reflective of concentrations in St. Lawrence River fish (Omara et al. 1997, 1998). However, the doses of the mixture used in the study were without effect on the examined endpoints in the absence of methylmercury and the study provides no information on possible effects of methylmercury on CDD-induced effects (see Section 2.2.4).

No studies were located that compared responses of other pertinent toxicity targets to mixtures of methylmercury and TCDD with responses to either compound alone. No studies were located in which pretreatment with methylmercury before TCDD exposure was examined for possible effects on TCDD toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., nervous system, thyroid; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

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

Table 20. Effect of **Hexachlorobenzene** on **Methylmercury**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of the interaction cannot be predicted for any toxicity target, because (1) data regarding possible interactions between hexachlorobenzene and methylmercury are restricted to one study (Renner 1980) reporting that hexachlorobenzene potentiated the acute lethality of mercuric chloride in rats, and another study with a similar exposure protocol that did not find similar results (Lecavalier et al. 1994), (2) there is no information indicating that pharmacokinetic interactions with hexachlorobenzene will influence methylmercury toxicity or carcinogenicity, and (3) mechanistic understanding is inadequate to support a reliable projection of interaction.

*Mechanistic Understanding* - Joint actions of methylmercury and hexachlorobenzene in producing effects on common target organs (immune suppression, nervous system impairment including altered neurological development, and cancer; see Appendix B and D) are plausible, but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action.

*Toxicological Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and methylmercury with responses to either compound alone. No studies were located in which pretreatment with hexachlorobenzene before methylmercury exposure was examined for possible effects on methylmercury toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

An acute oral exposure lethality study in rats (Renner 1980) reported that 400 or 600 mg/kg doses of hexachlorobenzene increased incidence of lethality in rats given 10, 12.5, or 18 mg$HgCl_2$ /kg doses (compared with mercuric chloride alone), but Lecavalier et al. (1994) reported that 10 or 12.5 mg $HgCl_2$/kg did not produce lethality in rats in the presence or absence of 400 or 600 mg/kg hexachlorobenzene. The lethality endpoint in these studies is unlikely to be relevant to the development of altered neurological development, the methylmercury-induced health effect of most concern to public health.

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

Table 21. Effect of **Methylmercury** on **Hexachlorobenzene**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: =IIIC** (0)
for liver effects
**BINWOE: ?** (0)
for other effects

*Direction of Interaction* - The additive direction of interaction category is selected to reflect a projected lack of effect of methylmercury on hexachlorobenzene hepatotoxicity. Support of this projection is restricted to the observation that co-exposure to acute 10- or 12-mg/kg doses of mercuric chloride did not change liver effects from acute 400- or 600-mg/kg doses of hexachlorobenzene in rats (Lecavalier et al. 1994).

For other hexachlorobenzene effects, the direction of the interaction cannot be predicted in the absence of pertinent joint toxic action data, information clearly indicating that possible pharmacokinetic interactions with methylmercury will influence hexachlorobenzene toxicity or carcinogenicity, and adequate mechanistic understanding supporting a reliable projection of modes of joint action of methylmercury and hexachlorobenzene.

*Mechanistic Understanding* - Joint actions of methylmercury and hexachlorobenzene in producing effects on common target organs (immune suppression, nervous system impairment including altered neurological development, and cancer; see Appendix and D) are plausible, but mechanistic understanding is inadequate to support reliable projections of modes of joint toxic action. Hexachlorobenzene-induced hepatic porphyria is thought to involve an unidentified reactive intermediate produced either by CYPIIIA-mediated or CYP1A-mediated metabolism (den Besten et al. 1993; Sinclair et al. 1997; see Appendix B), but no information was located indicating that methylmercury or mercury may alter metabolism of hexachlorobenzene. Reliable mechanistic inferences could not be made due to inadequate understanding of the mechanism(s) involved in other hexachlorobenzene-induced critical effects (immunosuppression, female reproductive organ disruption, altered neurological development, and cancer).

*Toxicological Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and methylmercury with responses to either compound alone. No studies were located in which pretreatment with methylmercury before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices A and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

A high-dose acute oral study in rats reported that co-exposure to 10 or 12.5 mg/kg doses of mercuric chloride did not change liver effects (increased liver weight and hepatocyte vacuolization) from exposure to 400 or 600 mg/kg hexachlorobenzene (Lecavalier et al. 1994), but the results were inadequately reported to allow quantitative assessment. The limited evidence supports a projection that methylmercury will not affect the hepatotoxicity of hexachlorobenzene and selection of the “additive” direction of interaction category for the effect of methylmercury on hexachlorobenzene. The lowest confidence rating for toxicological significance (“C”) was selected to reflect the absence of data on methylmercury and hexachlorobenzene, the poor reporting of the results of the Lecavalier et al. study, and the lack of corroborative results from other studies or species.

Table 22. Effect of ***p,p'*-DDE** on **Methylmercury**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that pharmacokinetic interactions with *p,p'*-DDE will influence methylmercury toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of interactions between *p,p'*-DDE and methylmercury.

*Mechanistic Understanding* - Oral exposure to either *p,p'*-DDE or methylmercury has been shown to adversely influence pre- and post-natal neurological development, and both compounds are known to produce adverse effects on the fully developed neurological system (see Table 1 and Appendices C and D). Joint actions between these two compounds in producing deficits in neurological developmental or function are plausible, but modes of possible joint action are unknown and unstudied. Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects on mitosis through impairment of microtubule assembly, inhibition of various enzymes such as protein kinase C, inhibition of transport mechanisms in developing brain cells, and alteration of synaptosomal release of neurotransmitters (e.g., dopamine) that may involve non-specific changes in membrane integrity or disruption of calcium homeostasis and subsequent activation of second messenger system or cell death (Appendix D). *p,p'*-DDE's actions on the developing and mature neurological system are poorly understood (see Appendix C), but, as with *p,p'*-DDT, may involve interference with sodium channels and potassium gates in neuronal membranes and inhibition of neuronal ATPases. Obvious cellular or molecular sites of possible interactions between *p,p'*-DDE and methylmercury are not apparent. Reliable mechanistic inferences could not be made due to inadequate understanding of the mechanism(s) involved in methylmercury-induced critical effects (altered neurological development, immunosuppression, and cancer).

*Toxicological Significance* - No studies were located that compared responses to mixtures of *p,p'*-DDE and methylmercury with responses to either compound alone. No studies were located in which pretreatment with *p,p'*-DDE before methylmercury exposure was examined for possible effects on methylmercury toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices C and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

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

Table 23. Effect of **Methylmercury** on ***p,p'*-DDE**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of interaction cannot be predicted in the absence of (1) pertinent joint toxic action data, (2) information indicating that pharmacokinetic interactions with methylmercury will influence *p,p'*-DDE toxicity or carcinogenicity, or (3) mechanistic understanding supporting a reliable projection of possible modes of joint toxic actions between *p,p'*-DDE and methylmercury.

*Mechanistic Understanding* - Oral exposure to either *p,p'*-DDE or methylmercury has been shown to adversely influence pre- and post-natal neurological development, and both compounds are known to produce adverse effects on the fully developed neurological system (see Table 1 and Appendices C and D). Joint actions between these two compounds in producing deficits in neurological developmental or function are plausible, but modes of possible joint action are unknown and unstudied. Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects on mitosis through impairment of microtubule assembly, inhibition of various enzymes such as protein kinase C, inhibition of transport mechanisms in developing brain cells, and alteration of synaptosomal release of neurotransmitters (e.g., dopamine) that may involve non-specific changes in membrane integrity or disruption of calcium homeostasis and subsequent activation of second messenger system or cell death (Appendix D). *p,p'*-DDE's actions on the developing and mature neurological system are poorly understood (see Appendix C), but, as with *p,p'*-DDT, may involve interference with sodium channels and potassium gates in neuronal membranes and inhibition of neuronal ATPases. Obvious cellular or molecular sites of possible interactions between *p,p'*-DDE and methylmercury are not apparent. Reliable mechanistic inferences could not be made due to inadequate understanding of the mechanism(s) involved in *p,p'*-DDE-induced critical effects (altered neurological development, liver degeneration, immunosuppression, and cancer).

*Toxicological Significance* - No studies were located that compared responses to mixtures of *p,p'*-DDE and methylmercury with responses to either compound alone. No studies were located in which pretreatment with methylmercury before *p,p'*-DDE exposure was examined for possible effects on *p,p'*-DDE toxicity at any target organ. Joint actions at several shared target organs are plausible (e.g., immune system, nervous system; see Appendices C and D), but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

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

Table 24. Effect of **PCBs** on **2,3,7,8-TCDD**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: <IIIB1aii** (0.32 x 0.79 x 1 x 1 x 0.79 = -0.20)
immune effects (suppression of cell-mediated immune response), acute
**BINWOE: <IIIB2aii** (0.32 x 0.79 x 0.79 x 1 x 0.79 = -0.16)
immune effects (suppression of cell-mediated immune response), non-acute
**BINWOE: <IIIC1ai** (0.32 x 0.32 x 1 x 1 x 1 = -0.10)
developmental toxicity (cleft palate, hydronephrosis in offspring), acute
**BINWOE: <IIIC2ai** (0.32 x 0.32 x 0.79 x 1 x 1 = -0.08)
developmental toxicity (cleft palate, hydronephrosis in offspring), non-acute
**BINWOE: =IIIC** (0)
body and thymus weight changes, hepatomegaly, decreased hepatic retinoids
**BINWOE: ?** (0)
thyroid hormone disruption, porphyria
**BINWOE: ?** (0)
female reproductive organ development
**BINWOE: ?** (0)
tumor promotion
**BINWOE: ?** (0)
other effects

*Direction of Interaction* - PCB mixtures antagonized TCDD-induced immunosuppression and developmental toxicity in mice. Intermediate-duration dietary exposure of rats to binary mixtures of TCDD plus each of three PCB congeners produced no synergism on changes in body and organ weights and levels of retinoids in liver indicating that PCB mixtures may additively act with 2,3,7,8-TCDD on these endpoints, but one congener (and not the other two) synergistically acted with 2,3,7,8-TCDD to increase hepatic porphyrin levels and deplete serum T4 levels. Available data are inconclusive regarding joint action of PCB mixtures and 2,3,7,8-TCDD in adversely affecting female reproductive organ development and promoting tumors.

*Mechanistic Understanding* - Oral exposures to PCBs or CDDs such as 2,3,7,8-TCDD are associated with wide arrays of health effects that show considerable overlap. Although some PCB congeners have been demonstrated to produce some effects via a common initial mechanistic step with 2,3,7,8-TCDD and other CDDs (binding to the Ah receptor), mechanistic understanding of ensuing processes is too incomplete to provide reliable projections of net physiological responses to joint exposure of PCB mixtures and 2,3,7,8-TCDD. In addition, there is evidence that other PCB congeners produce adverse effects via mechanisms that are independent of Ah receptor mediation, and some PCB congeners counteract effects of other PCB congeners and TCDD. Thus, mechanistic understanding for all directional BINWOEs was assigned a low data quality factor (III) to reflect the inability of available mechanistic understanding to support reliable projections of modes of joint actions between PCB mixtures and TCDD.

*Toxicologic Significance* - PCB mixtures antagonized TCDD-induced immunosuppression (intra-peritoneal exposure) and cleft palate formation (oral exposure) in mice (Bannister et al. 1987; Davis and Safe 1989; Haake et al.1987). There is evidence that individual PCB congeners vary in how they interact with TCDD in affecting these endpoints; some antagonize, some do not, and one was shown to potentiate TCDD-induced cleft palate formation (Biegel et al. 1989a, 1989b; Birnbaum et al. 1985; Morrissey et al. 1992). To reflect uncertainty that the observed antagonisms may occur with environmental PCB mixtures of varying composition and that antagonism will occur on other immune endpoints, a moderate data quality factor (B) was assigned to the BINWOE for immune effects

Table 24. Effect of **PCBs** on **2,3,7,8-TCDD** (*continued*)

*Toxicologic Significance (continued)* - (several PCB mixtures were demonstrated to antagonize TCDD inhibition of cell-mediated immune response, but one [Aroclor 1232] did not), whereas a low data quality factor (C) was assigned for developmental toxicity (the only PCB mixture examined for joint action with TCDD was Aroclor 1254).

A 13-week dietary exposure rat studies of binary joint action of TCDD with each of three PCB congeners (expected to have various mechanisms of action) found no evidence of synergism on body or organ weight changes or Vitamin A depletion in the liver (van Birgelen et al. 1992, 1994a, 1994b, 1996a). Evidence for less-than-additive joint action on these endpoints (with each of the three PCB:TCDD binary mixtures examined) was found, but this may have been due to near-maximal effects occurring at the dose levels used. However, evidence was found for synergistic effects between one of the congeners (2,2',4,4',5,5'-hexachlorobiphenyl, but not the others) and TCDD on depletion of serum T4 levels and increased accumulation of porphyrins in liver (van Birgelen et al. 1992, 1996a). The mechanistic basis for the apparent synergism between TCDD and this PCB congener without Ah-receptor affinity is unknown. Because of the variability between PCB congeners and the lack of data examining joint action of PCB mixtures and TCDD on T4 depletion and porphyria, the direction of interaction for the BINWOE was judged to be indeterminate.

3,3',4,4',5-Pentachlorobiphenyl appears to affect ovulation, ovarian weight, and circulating hormone levels in immature rats in an additive manner in combination with 2,3,7,8-TCDD and other dioxins (Gao et al. 2000), but the effect of the presence of ineffective PCB congeners, such as 2,2',4,4'-tetra-chlorobiphenyl, in influencing these endpoints is unexamined.

The joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors is unexamined with the exception of one study that found evidence for less-than-additive joint action in a mixture of several PCBs with 2,3,7,8-TCDD, 1,2,3,7,8-pentachloro-*p*-dioxin, and 2,3,4,7,8-pentachlorodibenzofuran (van der Plas et al. 1999). There are data suggesting that one PCB congener (3,3',4,4',5-pentachlorobi-phenyl) additively promotes tumors with TCDD and another (2,2,4,4',5,5'-hexachlorobiphenyl) antagonizes TCDD-promotion of tumors (Hemming et al. 1995; Wolfle 1998). The available data are inconclusive regarding the joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors.

*Modifying Factors* - The “ii” of the immune effects BINWOEs reflects the intraperitoneal exposure data that is its basis and the expected use for oral exposures; the “2" in the non-acute BINWOE reflects the acute data basis. No modifying factors were used in the BINWOE for body and thymus weight changes, hepatomegaly, and decreased hepatic retinoids because the 13-week dietary exposure data are expected to be directly relevant to oral repeated exposure scenarios.

*Additional Uncertainties* - The BINWOEs were derived to assess how environmental PCB mixtures may influence TCDD toxicity. PCB mixtures are the entity of concern, because humans are exposed to complex PCB mixtures and ATSDR PCB MRLs are based on data for PCB mixtures. There is a large degree of uncertainty in the BINWOEs, given evidence that the composition of environmental PCB mixtures can vary substantially, evidence that PCB congeners can vary in potency, mechanisms of action, and how they interact with TCDD, evidence that interactions between PCBs and TCDD can display complex relationships with dose and dose proportions, and the limited number of studies that have examined how mixtures of PCBs jointly act with TCDD in influencing the wide array of shared toxicity targets.

Table 25. Effect of **2,3,7,8-TCDD** on **PCBs**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: =IIIC** (0)
body and thymus weight changes, hepatomegaly, decreased hepatic retinoids
**BINWOE: ?** (0)
thyroid hormone disruption, porphyria
**BINWOE: ?** (0)
immune suppression, developmental toxicity
**BINWOE: ?** (0)
female reproductive organ development
**BINWOE: ?** (0)
tumor promotion
**BINWOE: ?** (0)
other effects

*Direction of Interaction* - Intermediate-duration dietary exposure of rats to binary mixtures of TCDD plus each of three PCB congeners produced no synergism on changes in body and organ weights and levels of retinoids in liver indicating that PCB mixtures may additively act with 2,3,7,8-TCDD on these endpoints, but one congener (and not the other two) synergistically acted with 2,3,7,8-TCDD to increase hepatic porphyrin levels and deplete serum T4 levels. Available studies of joint action of PCB mixtures and 2,3,7,8-TCDD on immune suppression and developmental toxicity do not discern how TCDD may influence PCB effects on these endpoints. Available data are inconclusive regarding joint action of PCB mixtures and 2,3,7,8-TCDD in adversely affecting female reproductive organ development and promoting tumors.

*Mechanistic Understanding* - Oral exposures to PCBs or CDDs such as 2,3,7,8-TCDD are associated with wide arrays of health effects that show considerable overlap. Although some PCB congeners have been demonstrated to produce some effects via a common initial mechanistic step with 2,3,7,8-TCDD and other CDDs (binding to the Ah receptor), mechanistic understanding of ensuing processes is too incomplete to provide reliable projections of net physiological responses to joint exposure of PCB mixtures and 2,3,7,8-TCDD. In addition, there is evidence that other PCB congeners produce adverse effects via mechanisms that are independent of Ah receptor mediation, and some PCB congeners counteract effects of other PCB congeners and TCDD. Thus, mechanistic understanding for all directional BINWOEs was assigned a low data quality factor (III) to reflect the inability of available mechanistic understanding to support reliable projections of modes of joint actions between PCB mixtures and TCDD.

*Toxicologic Significance* - A 13-week dietary exposure rat studies of binary joint action of TCDD with each of three PCB congeners (expected to have various mechanisms of action) found no evidence of synergism on body or organ weight changes or Vitamin A depletion in the liver (van Birgelen et al. 1992, 1994a, 1994b, 1996a). Evidence for less-than-additive joint action on these endpoints (with each of the three PCB:TCDD binary mixtures examined) was found, but this may have been due to near-maximal effects occurring at the dose levels used. However, evidence was found for synergistic effects between one of the congeners (2,2',4,4',5,5'-hexachlorobiphenyl, but not the others) and TCDD on depletion of serum T4 levels and increased accumulation of porphyrins in liver (van Birgelen et al. 1992, 1996a). Because of this variability between PCB congeners and the lack of data examining joint action of PCB mixtures and TCDD on T4 depletion and porphyria, the direction of interaction for the BINWOE was judged to be indeterminate (?).

Table 25. Effect of **2,3,7,8-TCDD** on **PCBs** (*continued*)

*Toxicologic Significance (continued)* - 3,3',4,4',5-Pentachlorobiphenyl appears to affect ovulation, ovarian weight, and circulating hormone levels in immature rats in an additive manner in combination with 2,3,7,8-TCDD and other dioxins (Gao et al. 2000), but the effect of the presence of ineffective PCB congeners, such as 2,2',4,4'-tetrachlorobiphenyl, in influencing these endpoints is unexamined. No other data are available regarding PCBs and 2,3,7,8-TCDD joint action on these endpoints.

The joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors is unexamined with the exception of one study that found evidence for less-than-additive joint action in a mixture of several PCBs with 2,3,7,8-TCDD, 1,2,3,7,8-pentachloro-*p*-dioxin, and 2,3,4,7,8-pentachlorodibenzofuran (van der Plas et al. 1999). There are data suggesting that one PCB congener (3,3',4,4',5-pentachlorobiphenyl) additively promotes tumors with TCDD and another (2,2,4,4',5,5'-hexachlorobiphenyl) antagonizes TCDD-promotion of tumors (Hemming et al. 1995; Wolfle 1998). The available data are inconclusive regarding the joint action of PCB mixtures and 2,3,7,8-TCDD in promoting tumors.

*Modifying factors* - No modifying factors were used in the BINWOE for body and thymus weight changes, hepatomegaly, and decreased hepatic retinoids, because the 13-week dietary exposure data are expected to be directly relevant to oral repeated exposure scenarios.

*Additional Uncertainties* - The BINWOEs were derived to assess how 2,3,7,8-TCDD may influence the toxicity of environmental PCB mixtures. PCB mixtures are the entity of concern, because humans are exposed to complex PCB mixtures and ATSDR PCB MRLs are based on data for PCB mixtures. There is a large degree of uncertainty in the BINWOEs, given evidence that the composition of environmental PCB mixtures can vary substantially, evidence that PCB congeners can vary in potency, mechanisms of action, and how they interact with TCDD, evidence that interactions between PCBs and TCDD can display complex relationships with dose and dose proportion, and the limited number of studies that have examined how mixtures of PCBs jointly act with TCDD in influencing the wide array of shared toxicity targets.

Table 26. Effect of **PCBs** on **Hexachlorobenzene**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no *in vivo* or *in vitro* data examining modes of joint action of PCBs and hexachlorobenzene on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatic porphyria, liver hypertrophy, decreased serum T4 levels, impaired immune response to foreign cells, impaired neurological development, reproductive organ dysfunction, and liver cancer have all been associated with oral exposure to either one of these agents (see Appendices B and E). However, there are no *in vitro* or *in vivo* studies designed to examine how these agents may jointly act to produce these effects. Processes in which potential interactions may occur include: induction of Phase I and II enzymes that may influence porphyria, liver hypertrophy, tissue damage from reactive oxygen species, and liver cancer development; disruption of thyroid hormone homeostasis via binding to thyroid hormone transport proteins, altering thyroid hormone metabolism, or damaging thyroid tissue; and disruption of sex hormone homeostasis via estrogenic, anti-estrogenic, androgenic, or anti-androgenic actions. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater than-additive manners.

*Toxicologic Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and PCBs with responses to hexachlorobenzene or PCBs alone. No studies were located in which pretreatment with PCBs before hexachlorobenzene exposure was examined for possible effects on hexachlorobenzene toxicity at any target organ. Joint actions at several shared target organs are plausible, but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

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

Table 27. Effect of **Hexachlorobenzene** on **PCBs**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no *in vivo* or *in vitro* data examining modes of joint action of PCBs and hexachlorobenzene on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatic porphyria, liver hypertrophy, decreased serum T4 levels, impaired immune response to foreign cells, impaired neurological development, reproductive organ dysfunction, and liver cancer have all been associated with oral exposure to either one of these agents (see Appendices B and E). However, there are no *in vitro* or *in vivo* studies designed to examine how these agents may jointly act to produce these effects. Processes in which potential interactions may occur include: induction of Phase I and II enzymes that may influence porphyria, liver hypertrophy, tissue damage from reactive oxygen species, and liver cancer development; disruption of thyroid hormone homeostasis via binding to thyroid hormone transport proteins, altering thyroid hormone metabolism, or damaging thyroid tissue; and disruption of sex hormone homeostasis via estrogenic, anti-estrogenic, androgenic, or anti-androgenic actions. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater-than-additive manners.

*Toxicologic Significance* - No studies were located that compared responses to mixtures of hexachlorobenzene and PCBs with responses to hexachlorobenzene or PCBs alone. No studies were located in which pretreatment with hexachlorobenzene before PCB exposure was examined for possible effects on PCB toxicity at any target organ. Joint actions at several shared target organs are plausible, but whether the actions would be additive, greater-than-additive, or less-than-additive is unknown and unstudied; the indeterminate classification (?) reflects this lack of data.

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

Table 28. Effect of **PCBs** on ***p,p'*-DDE**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?** (0)

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no pertinent *in vivo* or *in vitro* data examining modes of joint action of PCBs and *p,p'*-DDE on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatomegaly, liver cancer, suppression of cell-mediated immune responses, impaired neurological function and development, and impaired reproductive function and development have been associated with oral exposure to PCBs and oral exposure to *p,p'*-DDE (see Appendices C and E). Processes relevant to these shared toxicity targets in which possible interactions may occur include binding to androgen receptors, production of reactive metabolites or metabolic byproducts that damage neurological tissue, and disruption of sex hormone homeostasis. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater-than-additive manners.

*Toxicologic Significance* - Interaction data are limited to reports that *p,p*'-DDE and Aroclor 1242, in combination with several pesticides but not by themselves, inhibit *in vitro* binding of 17β-estradiol to alligator estrogen receptors (Vonier et al. 1996) and that 5-month dietary exposure of mallards to both agents simultaneously did not alter *p,p'*-DDE-induced egg shell thinning, but decreased egg production capabilities compared with dietary exposure to either agent alone (Risebrough and Anderson 1975). In the absence of other information, these data are no expected to be directly relevant to DDE- or PCB-induced reproductive effects in humans or other shared toxicity targets mentioned above.

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

Table 29. Effect of ***p,p'*-DDE** on **PCBs**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: ?**(0)

*Direction of Interaction* - The direction of possible interactions cannot be predicted because there are no pertinent *in vivo* or *in vitro* data examining modes of joint action of PCBs and *p,p'*-DDE on several shared toxicity targets, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Hepatomegaly, liver cancer, suppression of cell-mediated immune responses, impaired neurological function and development, and impaired reproductive function and development have been associated with oral exposure to PCBs and oral exposure to *p,p'*-DDE. Processes relevant to these shared toxicity targets in which possible interactions may occur include binding to androgen receptors, production of reactive metabolites or metabolic byproducts that damage neurological tissue, and disruption of sex hormone homeostasis. Mechanistic understanding of how common effects produced by these agents develop is too incomplete to provide reliable projections of whether these agents may jointly act in additive, less-than-additive, or greater-than-additive manners.

*Toxicologic Significance* - Interaction data are limited to reports that *p,p*'-DDE and Aroclor 1242, in combination with several pesticides but not by themselves, inhibit *in vitro* binding of 17β-estradiol to alligator estrogen receptors (Vonier et al. 1996) and that 5-month dietary exposure of mallards to both agents simultaneously did not alter *p,p'*-DDE-induced egg shell thinning, but decreased egg production capabilities compared with dietary exposure to either agent alone (Risebrough and Anderson 1975). In the absence of other information, these data are no expected to be directly relevant to DDE- or PCB-induced reproductive effects in humans or other shared toxicity targets mentioned above.

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

Table 30. Effect of **PCBs** on **Methylmercury**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: >IICb** (0.79 x 0.32 x 0.79 = +0.20)
for impaired neurological function or development
**BINWOE: ?** (0)
for impaired reproductive performance
**BINWOE: =IIIB**(0)
for hepatic porphyria
**BINWOE: ?** (0)
for decreased postnatal survival
**BINWOE: ?** (0)
for other effects

*Direction of Interaction* - There is *in vitro* evidence from one study that PCBs and methylmercury may synergistically decrease dopamine levels in rat brain cells presumably via disruption of calcium homeostatic mechanisms (Bemis and Seegal 1999), but obvious synergism or additive joint action in affecting neurobehavioral endpoints was not demonstrated in a mouse *in vivo* study (Tanimura et al. 1980). A greater-than-additive joint action on neurological function or development is projected with a moderate degree of uncertainty. The direction of interaction for impaired reproductive performance is indeterminate (?) due to inadequate data on joint action for this toxicity target (Tanimura et al. 1980). Additive joint action to produce hepatic porphyria is supported by evidence from a study of quails exposed to Aroclor 1260 and methylmercury in the diet (Leonzio 1996b). For decreased postnatal survival from combined exposure to PCBs and methylmercury, data from a mouse study (Tanimura et al. 1980) and a mink study (Wren et al. 1987a, 1987b) are inadequate to support a projection of mode of possible joint action. For other effects, the direction of possible interactions cannot be projected because there are no pertinent *in vivo* or *in vitro* data, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Changes in neurological function or development from PCBs and methylmercury have been proposed to at least partly involve disruption of calcium homeostatic mechanisms in neural cells leading to changes in neurotransmitter release (e.g., dopamine) or cell damage. Combined *in vitro* exposure of rat striatal tissue to a methylmercury and a 1:1 mixture of Aroclor 1254/1260 appeared to synergistically deplete tissue levels of dopamine (Bemis and Seegal 1999). These data suggest a possible synergism between PCB mixtures and methylmercury in affecting neurological dysfunction and development. A moderate uncertainty rating (II; i.e., medium confidence rating) was selected to reflect several areas of uncertainty: (1) mechanistic linkages between changes in dopamine release and the development of PCB- or methylmercury-induced changes in neural function and development are poorly understood; (2) obvious synergism was not observed on *in vivo* endpoints of neurological function in mice exposed to mixtures of methylmercury and PCBs (Tanimura et al. 1980); and (3) the Bemis and Seegal (1999) report had some study design and reporting limitations that prevented a formal statistical characterization of the mode of joint action on dopamine release (see Section 2.2.10).

Mechanistic understanding for other potential shared toxicity targets between PCBs and methylmercury (e.g., impaired reproductive performance, hepatic porphyria) is too incomplete to support reliable projections of modes of joint actions.

Table 30. Effect of **PCBs** on **Methylmercury** *(continued)*

*Toxicologic Significance* - Kanechlor 500 and methylmercury, exposure to either agent alone or in combination did not change several measures of F0- and F1-generation reproductive performance, neurobehavior of offspring, or prevalence of developmental anomalies (Tanimura et al. 1980). The study provides no evidence of obvious synergism or additive joint action between Kanechlor 500 and methylmercury in affecting neurobehavior, reproductive performance, or prevalence of developmental abnormalities. Design limitations of this study preclude more definitive conclusions on mode of joint action on these endpoints (see Section 2.2.10). The data quality factor for highest toxicologic significance uncertainty (C) is selected due to the design limitations of the Tanimura et al. (1980) and the lack of other better designed studies examining possible joint actions on neurobehavior, reproductive, or developmental endpoints.

Combined gestational and lactational exposure of mice to Kanechlor 500 plus methylmercury, at 4 (but not 0.4) mg Hg/kg/day, decreased postnatal survival to a greater degree than did exposure to Kanechlor 500 alone; methylmercury alone did not affect postnatal survival (Tanimura et al. 1980). In a mink study, dietary concentrations of 1 ppm Aroclor 1254 alone, 1 ppm methylmercury alone, or 0.5 ppm concentrations of each together in the diet, did not affect postnatal survival, but 1 ppm concentrations of each in the diet decreased postnatal survival compared with controls (Wren et al. 1987a, 1987b). The design of these studies preclude comparisons between observed combined-exposure responses and predicted responses based on a presumed mode of joint action (see Section 2.2.10).

Intermediate-duration exposures of quail to methylmercury or Aroclor 1260 in the diet led to accumulation of porphyrins in liver; hepatic porphyrin levels in quail exposed to both agents simultaneously were similar to levels predicted based on additivity of response (Leonzio 1996b). To reflect uncertainty in extrapolating from quails to mammals and the lack of corroborative data, a moderate data quality factor (B) was selected for toxicological significance of the projection of additive joint action to produce hepatic porphyria.

*Modifying factors* - The modifying factor of “b” for the BINWOE for greater-than-additive joint action in impairing neurological function and development reflects uncertainty associated with the *in vitro* basis of the determination.

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

Table 31. Effect of **Methylmercury** on **PCBs**
(see Table 11 for explanation of BINWOE codes)
**BINWOE: >IICb** (0.79 x 0.32 x 0.79 = +0.20)
for impaired neurological function or development
**BINWOE: ?** (0)
for impaired reproductive performance
**BINWOE: =IIIB** (0)
for hepatic porphyria
**BINWOE: ?** (0)
for decreased postnatal survival
**BINWOE: ?** (0)
for other effects

*Direction of Interaction* - There is *in vitro* evidence from one study that PCBs and methylmercury may synergistically decrease dopamine levels in rat brain cells presumably via disruption of calcium homeostatic mechanisms (Bemis and Seegal 1999), but obvious synergism or additive joint action in affecting neurobehavioral endpoints was not demonstrated in a mouse *in vivo* study (Tanimura et al. 1980). A greater-than-additive joint action on neurological function or development is projected with a moderate degree of uncertainty. The direction of interaction for impaired reproductive performance is indeterminate (?) due to inadequate data on joint action for this toxicity target (Tanimura et al. 1980). Additive joint action to produce hepatic porphyria is supported by evidence from a study of quails exposed to Aroclor 1260 and methylmercury in the diet (Leonzio 1996b). For decreased postnatal survival from combined exposure to PCBs and methylmercury, data from a mouse study (Tanimura et al. 1980) and a mink study (Wren et al. 1987a, 1987b) are inadequate to support a reliable projection of mode of possible joint action. For other effects, the direction of possible interactions cannot be projected because there are no pertinent *in vivo* or *in vitro* data, and mechanistic understanding is too incomplete to support reliable projections of possible interactions.

*Mechanistic Understanding* - Changes in neurological function or development from PCBs and methylmercury have been proposed to at least partly involve disruption of calcium homeostatic mechanisms in neural cells leading to changes in neurotransmitter release (e.g., dopamine) or cell damage. Combined *in vitro* exposure of rat striatal tissue to a methylmercury and a 1:1 mixture of Aroclor 1254/1260 appeared to synergistically deplete tissue levels of dopamine (Bemis and Seegal 1999). These data suggest a possible synergism between PCB mixtures and methylmercury in affecting neurological dysfunction and development. A moderate uncertainty rating (II; i.e., medium confidence rating) was selected to reflect several areas of uncertainty: (1) mechanistic linkages between changes in dopamine release and the development of PCB- or methylmercury-induced changes in neural function and development are poorly understood; (2) obvious synergism was not observed on *in vivo* endpoints of neurological function in mice exposed to mixtures of methylmercury and PCBs (Tanimura et al. 1980); and (3) the Bemis and Seegal (1999) report had some study design and reporting limitations that prevented a formal statistical characterization of the mode of joint action on dopamine release (see Section 2.2.10).

Mechanistic understanding for other potential shared toxicity targets between PCBs and methylmercury (e.g., impaired reproductive performance, hepatic porphyria) is too incomplete to support reliable projections of modes of joint actions.

Table 31. Effect of **Methylmercury** on **PCBs** *(continued)*

*Toxicologic Significance* - In a mouse study involving gestational and lactational exposure to Kanechlor 500 and methylmercury, exposure to either agent alone or in combination did not change several measures of F0- and F1-generation reproductive performance, neurobehavior of offspring, or prevalence of developmental anomalies (Tanimura et al. 1980). The study provides no evidence of obvious synergism or additive joint action between Kanechlor 500 and methylmercury in affecting neurobehavior, reproductive performance, or prevalence of developmental abnormalities. Design limitations of this study preclude more definitive conclusions on mode of joint action on these endpoints (see Section 2.2.10). The data quality factor for highest toxicologic significance uncertainty (C) is selected due to the design limitations of the Tanimura et al. (1980) and the lack of other better designed *in vivo* studies examining possible joint actions on neurobehavior, reproductive, or developmental endpoints.

Combined gestational and lactational exposure of mice to Kanechlor 500 plus methylmercury, at 4 (but not 0.4) mg Hg/kg/day, decreased postnatal survival to a greater degree than did exposure to Kanechlor 500 alone; methylmercury alone did not affect postnatal survival (Tanimura et al. 1980). In a mink study, dietary concentrations of 1 ppm Aroclor 1254 alone, 1 ppm methylmercury alone, or 0.5 ppm concentrations of each together in the diet, did not affect postnatal survival, but 1 ppm concentrations of each in the diet decreased postnatal survival compared with controls (Wren et al. 1987a, 1987b). The design of these studies preclude comparisons between observed combined-exposure responses and predicted responses based on a presumed mode of joint action (see Section 2.2.10).

Intermediate-duration exposures of quail to methylmercury or Aroclor 1260 in the diet led to accumulation of porphyrins in liver; hepatic porphyrin levels in quail exposed to both agents simultaneously were similar to levels predicted based on additivity of response (Leonzio 1996b). To reflect uncertainty in extrapolating from quails to mammals and the lack of corroborative data, a moderate data quality factor (B) was selected for toxicological significance of the projection of additive joint action to produce hepatic porphyria.

Combined exposure of rats or quail to commercial PCB mixtures and methylmercury appears to counteract PCB induction of hepatic CYP enzymes (Leonzio et al. 1996a; Takabatake et al. 1980), but the toxicological significance of this interaction is unclear.

*Modifying factors* - The modifying factor of “b” for the BINWOE for greater-than-additive joint action in impairing neurological function and development reflects uncertainty associated with the in vitro basis of the determination.

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

**Table 32. Matrix of BINWOE Determinations for Repeated Simultaneous Oral Exposure to Chemicals of Concern**

| | | ON TOXICITY OF | | | | |
|---|---|---|---|---|---|---|
| | | 2,3,7,8-TCDD | Hexachloro-benzene | *p,p'*-DDE | Methylmercury | PCBs |
| **EFFECT OF** | 2,3,7,8-TCDD | | **? (0)** | Anti-andro-genic effects **=IIIC(0)** Other effects **? (0)** | Immune suppression **=IIIBbii (0)** Other effects ? (0) | Body and organ weight changes, decreased retinoids in liver **=IIIC(0)** Other effects **? (0)** |
| | Hexachloro-benzene | Body and thymus weight **>IIIA2aii (+0.06)** Other effects: **? (0)** | | **? (0)** | **? (0)** | **? (0)** |
| | *p,p'*-DDE | Anti-androgenic effects **=IIIC(0)** Other effects **? (0)** | **? (0)** | | **? (0)** | **? (0)** |
| | Methylmercury | **? (0)** | Liver effects **=IIIC(0)** Other effects **? (0)** | **? (0)** | | Neurological effects **>IICb (+0.20)** Reproductive performance **? (0)** Porphyria **=IIIB(0)** Other effects **? (0)** |
| | PCBs | Immune suppression **<IIIB2aii (-0.16)** Developmental **<IIIC2ai (-0.08)** Body and organ weight changes, decreased retinoids in liver **=IIIC(0)** Other effects **? (0)** | **? (0)** | **?(0)** | Neurological effects **>IICb (+0.20)** Reproductive performance **? (0)** Porphyria **=IIIB(0)** Other effects **? (0)** | |

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

DIRECTION: = additive (0); > greater than additive (+1): < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data does not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0);
2: different exposure duration or sequence (0.79);
a: *in vivo* data (1.0);
b: *in vitro* data (0.79);
i: anticipated route of exposure (1.0);
ii: different route of exposure (0.79)