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

This chapter provides a review and evaluation of the literature pertinent to joint toxic action of the mixture and its components.

## 2.1 Mixture of Concern

Toxicological data or PBPK models were not available for the complete mixture of concern.

## 2.2 Component Mixtures

Toxicological and mechanistic data, but no PBPK models, were available for all of the binary mixtures, but were limited. For some binary mixtures, few or no pertinent mammalian studies were available. For others, the data were conflicting, and durations and routes of chemical administration (e.g., intravenous) were of questionable relevance.

Because of the relative paucity of information for chlorpyrifos and lead or mercury/methylmercury, the literature searches were broadened to include other organophosphorus insecticides. Data on joint action with lead or mercury/methylmercury were identified for methyl chlorpyrifos, parathion, methyl parathion, diazinon, fenitrothion, and fenthion, which, like chlorpyrifos, are phosphorothioates (organophosphorus compounds that contain the P=S functional group, which requires metabolism to P=O for anticholinesterase activity). Additional similarities to chlorpyrifos are that these triester compounds have one aryl and two alkyl substituents. See Appendix D for the structure of chlorpyrifos and its active metabolite, chlorpyrifos oxon, as well as the structure of methylmercury. Data on joint action with lead or mercury/methylmercury also were identified for two phosphorodithioate insecticides: malathion and dimethoate. These compounds are less similar to chlorpyrifos; in addition to the double bonded sulfur atom, they contains a sulfur rather than an oxygen in one of the ester linkages, and do not have an aryl substituent. In addition, unlike chlorpyrifos, malathion contains two carboxylic acid ester groups, which are susceptible to metabolism by carboxylesterases, and dimethoate contains an amide group.

In the following sections on the binary mixtures, the studies that focus on more relevant toxic endpoints are discussed first, with priority given to those conducted by simultaneous longer-term oral exposure in mammals, followed by studies of less relevant endpoints (e.g., acute lethal effects), and then studies of chemical interactions and of effects on tissue distribution or metabolism. At the end of each binary mixture section, the experimental results that may be used to support conclusions regarding joint toxic action are summarized in tables. For each listed endpoint and study, the tables present a conclusion regarding the direction of interaction for the influence of each chemical on the toxicity of the other. These conclusions include: additive (dose addition, response addition, or no effect), greater than additive (synergism or potentiation), less than additive (antagonism, inhibition, or masking), or indeterminate (ambiguous, conflicting, or no data).

### 2.2.1 Chlorpyrifos and Lead

No studies of the joint action of lead and chlorpyrifos were located. A few studies of the joint action of lead with other phosphorothioate or phosphorodithioate insecticides and their oxons were available and are reviewed in this section. The more relevant studies are summarized in tables at the end of this section.

The only pertinent human study was a limited cross-sectional epidemiological study of patients at an andrology clinic (Swart et al. 1991). This study investigated the potential association of lead and/or organophosphorus pesticide exposure with abnormal sperm morphology. The study included 22 men with an abnormally low percentage of morphologically normal spermatozoa as compared with 18 men with a normal percentage of morphologically normal spermatozoa. There were no differences in blood lead concentrations or serum cholinesterase (a biomarker for organophosphorus pesticide exposure) between these two groups, indicating no differences in exposure to lead or organophosphorus pesticides. Therefore, this study is not suitable for inclusion in the summary table.

A developmental neurotoxicological study of lead and dimethoate, a phosphorodithioate insecticide, provides limited evidence that the joint toxic action of these chemicals on electrophysiological endpoints may be additive or less than additive (Nagymajtenyi et al. 1998). Rats were given lead alone at 80 or 320 mg/kg/day (from lead acetate), dimethoate alone at 7 or 28 mg/kg/day, or combination treatments of the high dose of one chemical with the low dose of the other (80 mg/kg/day of lead plus 28 mg/kg/day of dimethoate, or 320 mg/kg/day of lead plus 7 mg/kg/day of dimethoate) on days 5–15 of gestation and days 2–28 of lactation. Litter size on the 4$^{th}$ day was adjusted to eight, with up to five males per litter. The weaned male offspring were continued on the same treatment as their dams for an additional 8 weeks,

5 days/week. The chemicals were administered by gavage. For the combination treatments, the two chemicals were given separately with a 2-hour interval between chemicals; the order was not specified. There were no statistically significant differences in the average number of pups/litter, and birth weight and pup weight gain between the groups. In addition, no clinical signs of toxicity were observed, no macroscopic malformations were seen in the offspring, and brain cholinesterase activity was not significantly decreased in any treated group. Electrophysiological studies, performed at the end of treatment, indicated that both chemicals increased the mean frequency and decreased the ratio of the slow to fast waves [(delta+theta)/($beta_1$+$beta_2$)] of the electrocorticograms, and increased the latency of the evoked potentials in the somatosensory, visual, and auditory centers of the brain in an apparent dose-related manner. Comparisons with controls were consistently statistically significant for the high-dose single-chemical treatments and the combination treatments, and were intermittently significant for the low-dose single-chemical treatments. The combination treatments tended to have a more pronounced effect than the high-dose chemical alone, particularly for the high-lead low-dimethoate combination, but statistical comparisons of the combination treatments with the single-chemical treatments were not presented. Assuming a linear dose response, the electrocorticogram data generally appear to be consistent with less-than-additive joint action, and the evoked potential data generally appear additive, but the experimental design and statistical analyses were not adequate for a definitive determination, and data were not presented for all endpoints. When the treatments were given only during gestation or only during gestation plus lactation, and the offspring tested 8 weeks after weaning, the results showed similar trends, but were not statistically significantly different from controls.

A similar study of electrocorticograms and evoked potentials was conducted on 10-week-old male rats by the same group of investigators (Nagymajtenyi et al. 2000b). The rats were gavaged with lead alone at 80 or 320 mg/kg/day (from lead acetate), dimethoate alone at 5 or 20 mg/kg/day, or combination treatments of 80 mg/kg/day of lead plus 20 mg/kg/day of dimethoate, or 320 mg/kg/day of lead plus 5 mg/kg/day of dimethoate on 5 days/week for 4, 8, or 12 weeks. The high dose of each chemical alone produced statistically significant effects similar to those seen in the 1998 study by the same investigators. The combinations also produced statistically significant effects as compared with controls, and the effects appeared more pronounced than the effects from the high-dose chemicals alone, but were not significantly different from the high-dose chemicals alone. The data, shown only for the somatosensory mean frequency and for latency of the evoked somatosensory response, appeared consistent with less-than-additive joint action.

In a study in which weanling rats of both sexes were fed lead at 0, 2, 20, or 200 ppm lead (approximately 0, 0.2, 2.0, or 20 mg/kg/day of lead; from lead chloride) in the diet through sexual maturity, mating, gestation, and lactation, and the weanling offspring were injected with a single intraperitoneal dose of 0, 0.45, 0.90, 1.80, or 3.60 mg/kg of parathion, the lead treatment alone did not affect serum or brain cholinesterase activity in the offspring (Phillips et al. 1973). Lead treatment also did not affect the depressions in serum and brain cholinesterase activity caused by parathion. In addition, lead did not affect mortality due to the two highest doses of parathion. The dams were continued on the lead diets through 347 days of age and then were injected with a single intraperitoneal dose of 2.5 mg/kg parathion; again, no effect of lead on the parathion-induced serum or brain cholinesterase activity was seen. Statistical analyses were not presented, and the interval (if any) between lead pretreatment and parathion injection was not reported.

A study of immunotoxicological effects of combined exposure to lead and dimethoate (Institoris et al. 2005) reported a possible protective effect of combined exposure, as compared with exposure to each chemical alone. Four-week-old male rats were gavaged with non-immunotoxic and immunotoxic doses of lead acetate alone (at 20 and 80 mg Pb/kg/day), dimethoate alone (at 7.04 and 28.2 mg/kg/day), and combination treatments of the high dose of one component with the low dose of the other (20 mg/kg/day of lead plus 28.2 mg/kg/day of dimethoate, or 80 mg/kg/day of lead plus 7.04 mg/kg/day of dimethoate) on 5 days/week for a 28-day period. For the combined treatments, the animals were treated first with dimethoate, followed by lead 30 minutes later. No clinical signs of toxicity were seen, and no gross pathological changes were seen at necropsy. Body weight in the high-dose dimethoate group was statistically significantly depressed at days 14–28, and lead alone did not affect body weight. Co-administration of the low lead dose with the high dimethoate dose appeared to protect against this effect, in that the body weight depression was less marked and not statistically significantly different from controls. In a series of experiments, the high dose of each chemical alone statistically significantly decreased the humoral response (number of anti-sheep red blood cell plaque-forming cells per $10^6$ cells and per spleen) and decreased the cellular immune response (delayed hypersensitivity assayed as footpad swelling) in at least one experiment. The combination treatments, tested in one of the experiments, either did not significantly decrease these immune responses, or produced a significantly lesser decrease than the high-dose component alone, indicating a possible protective effect. The investigators suggested that the protective effect could be due to effects on the kinetics of the chemicals, but provided no evidence for this hypothesis.

A study of lead's effect on other phosphorothioate insecticides indicates a potential for a chemical interaction between lead ions and chlorpyrifos. The incubation of methyl chlorpyrifos, methyl parathion, or ronnel with lead (II) (from lead nitrate) in buffered solution resulted in hydrolysis to 3,5,6-trichloro-2-pyridinol, 4-nitrophenol, or 2,4,5-trichlorophenol, respectively, generally at pHs of about 5.5–7.2 (Smolen and Stone 1997). Similar incubation of lead(II) with the methyl chlorpyrifos oxon (the active form of methyl chlorpyrifos) also resulted in hydrolysis to 3,5,6-trichloro-2-pyridinol at pHs of about 4.5–7.3 (Smolen and Stone 1997). For chlorpyrifos and chlorpyrifos oxon, analogous hydrolysis by lead (II) ions would be expected to produce 3,5,6-trichloro-2-pyridinol (and diethyl thiophosphate or diethyl phosphate). These hydrolysis products also are formed during metabolic inactivation, and do not have anticholinesterase activity. The purpose of this study was to investigate the possibility that metals in soil may catalyze the hydrolysis of organophosphorus pesticides. The investigators concluded that although lead may catalyze hydrolysis, its concentration in most agricultural soils would be too low. This conclusion may not be appropriate for other lead exposure scenarios, such as dust and chips from deteriorating lead paint, or soils contaminated by smelters or mining activities, or for hazardous waste sites. Whether co-exposure to inorganic lead from these sources and to phosphorothioate insecticides such as chlorpyrifos would result in hydrolysis of the chlorpyrifos was not tested or discussed.

A study in which rats were exposed to lead at 0, 60, or 120 mg/kg/day (as lead nitrate) in the drinking water for 3 months, followed by a single oral dose of 5 or 10 mg of methyl parathion (phosphorothioate) or 1 mg/kg of methyl paraoxon (activated form), reported that lead ameliorated some of the signs of acute toxicity of these compounds (Hapke et al. 1978). The interval (if any) between the end of lead exposure and the administration of methyl parathion or its oxon was not reported. The lead-treated animals had a longer latency from dosing with methyl parathion or methyl paraoxon to first signs of cholinesterase inhibition (muscle spasms), and a shorter duration of signs of toxicity. Acetylcholinesterase and plasma cholinesterase activities were inhibited by methyl parathion and methyl paraoxon, but not lead, and lead did not significantly influence the inhibition by the organophosphorus compounds. In the same study, increased liver weights and some mortality occurred in groups of rats that were dosed orally with 2.5 or 5 mg/kg/day of methyl parathion for 3 weeks, but the pre-treatment with 0, 60, or 120 mg/kg/day lead did not influence mortality or liver weight increases due to methyl parathion. Because acetylcholinesterase activity, liver weight, mortality data, and statistical comparisons of the data were not presented in the publication, this portion of the study is not included in the summary table.

Further results from this study showed that excretion of 4-nitrophenol in the urine was increased, and the proportion of alkylphosphate present as methyl paraoxon was increased in the liver and decreased in the

skeletal muscle of the lead-treated rats (Hapke et al. 1978). The investigators attributed the effect to an inhibition of GSH-dependent metabolism (although no data regarding this mechanism were provided), and to the protective effect of lead against methyl parathion's inhibition of liver carboxylesterase (observed in this study), leading to greater hydrolysis of methyl parathion to 4-nitrophenol by this enzyme. Lead alone did not affect carboxylesterase activity. Carboxylesterase, however, is not known to hydrolyze methyl parathion, which does not contain a carboxylester group. Methyl parathion and other organophosphorus pesticides can be inactivated through covalent binding to carboxylesterases, which also results in inhibition of the enzyme activity. Whether the increased excretion of 4-nitrophenol in lead-treated rats, indicating increased deactivation of methyl parathion, may have been due in part to a direct chemical interaction of lead with these compounds, catalyzing their hydrolysis was not discussed. It would appear that lead treatment was terminated before the methyl parathion and methyl paraoxon were administered, but the interval (if any) was not reported, and lead levels in blood, liver, and other tissues would be expected to have remained elevated. Statistical analyses of the data were not presented.

A similar study in which weanling rats were treated with lead in the diet at up to 200 ppm (approximately 20 mg/kg/day) through sexual maturity and mating, and the dams were continued on the same treatment through gestation and lactation, followed by a single intraperitoneal injection of up to 3.6 mg/kg parathion into the weanling offspring, reported no protective effect of lead on the inhibition of liver carboxyl-esterase activity by parathion (Phillips et al. 1973). Experimental details were reported previously in this section. The doses of lead in this study were much lower than in the study by Hapke et al. (1978).

Gavage administration of 200, 400, or 600 mg/kg/day of lead (from lead acetate) to rat pups on days 3–30 of age, followed 1 day later by a single gavage dose of 750 mg/kg of radiolabeled malathion (a phos-phorodithioate), did not affect the urinary excretion rate of radioactivity from malathion or the types and amounts of urinary metabolites, as compared with non-lead-treated controls (Abd-Elraof et al. 1981). The investigators had hypothesized that the relatively high absorption of lead in young animals (demonstrated in this study by dose-related, greatly elevated lead concentrations in tibia and blood) and lead's inhibition of heme synthesis would lead to a decrease in cytochrome P450 (not tested in this study). The consequence was predicted to be an alteration in malathion metabolism, but no alteration was observed.

Table 1 summarizes the joint action data pertinent to the potential effect of chlorpyrifos on the toxicity and tissue concentrations of lead. Because data for chlorpyrifos were not available, data for similar organophosphorus insecticides are included in Table 1 (and also in Table 2). Simultaneous exposure studies of neurotoxicity and immunotoxicity of lead and a phosphorodithioate insecticide (dimethoate)

suggest that chlorpyrifos may act in a less-than-additive or additive manner with lead. Table 2 summarizes the joint action data pertinent to the effects of lead on the toxicity and tissue concentrations of chlorpyrifos. The data regarding neurotoxicity and immunotoxicity of simultaneous or sequential exposure to lead with other phosphorothioate or with phosophorodithioate insecticides indicate that lead may act in a less-than-additive manner with chlorpyrifos. Pharmacokinetic and chemical interaction studies with other phosphorothioate insecticides indicate that lead may increase the metabolic or chemical inactivation of chlorpyrifos.

**Table 1. Effect of Chlorpyrifos on Toxicity and Tissue Concentrations of Lead**

| Endpoint, species | Duration, route for OP, Pb; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Neurological: electrocortico-grams (mean frequency, ratio of slow to fast waves), evoked potentials, rat offspring (gestational, lactational, and post-weaning exposure) | Intermediate, oral; simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Assuming linear dose response, changes, while greater from mixture than from high-dose single component alone, appeared to be mainly less than additive for electrocorticograms, and mainly additive for evoked potentials; statistical analysis for joint action not performed, some data not shown | Additive or <additive | Nagymajtenyi et al. 1998 Dimethoate, Lead acetate |
| Neurological: electrocortico-grams (mean frequency, ratio of slow to fast waves), evoked potentials, rat (10-weeks old at start) | Intermediate, oral; simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Only two data sets shown; assuming linear dose response, changes in these data sets, while greater from mixture than from high-dose single component alone, appeared to be mainly less than additive | <additive? | Nagymajtenyi et al. 2000b Dimethoate, Lead acetate |
| Immunological: humoral (anti-sheep red blood cell PFC) and cellular (delayed hypersensitivity: footpad thickness), rat | Acute, oral; simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Less inhibition of humoral and cellular immune responses from mixture than from high-dose component alone at same dose as in mixture | <additive | Institoris et al. 1999 Dimethoate, Lead acetate |

OP = organophosphorus compound; Pb = lead; PFC = plaque-forming cells

**Table 2. Effect of Lead on Toxicity and Tissue Concentrations of Chlorpyrifos**

| Endpoint, species | Duration, route for Pb, OP; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Neurological: electrocortico-grams (mean frequency, ratio of slow to fast waves), evoked potentials, rat offspring (gestational, lactational, and post-weaning exposure) | Intermediate, oral; simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Assuming linear dose response, changes, while greater from mixture than from high-dose single component alone, appeared to be mainly less than additive for electrocorticograms, and mainly additive for evoked potentials; statistical analysis for joint action not performed, some data not shown | Additive or <additive | Nagymajtenyi et al. 1998 Lead acetate, Dimethoate |
| Neurological: electrocortico-grams (mean frequency, ratio of slow to fast waves), evoked potentials, rat (10-weeks old at start) | Intermediate, oral; simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Only two data sets shown; Assuming linear dose response, changes in these data sets, while greater from mixture than from high-dose single component alone, appeared to be mainly less than additive | <additive? | Nagymajtenyi et al. 2000b Lead acetate, Dimethoate |
| Neurological: cholinergic signs, rat | Intermediate oral, acute oral; sequential (interval not reported) | Pb pretreatment increased the latency and diminished the duration and severity of acute cholinergic signs following OP | <additive | Hapke et al. 1978 Lead nitrate, Methyl parathion or Methyl paraoxon |
| Neurological: brain and serum cholinesterase activity, rat | Intermediate oral, acute ip; sequential (interval not reported) | Pb pretreatment did not alter OP inhibition of brain or serum cholinesterase (Pb doses much lower than Hapke et al. 1978) | Additive: no effect | Phillips et al. 1973 Lead chloride, Parathion |
| Immunological: humoral (anti-sheep red blood cell PFC) and cellular (delayed hypersensitivity: footpad thickness), rat | Intermediate, oral; simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Less inhibition of humoral and cellular immune responses from mixture than from high-dose component alone at same dose as in mixture | <additive | Institoris et al. 1999 Lead acetate, Dimethoate |
| Body weight, rat | Intermediate, oral; simultaneous; low-dose Pb plus high-dose OP | Low-dose Pb protected against body weight gain depression by high-dose OP | <additive | Institoris et al. 1999 Lead acetate, Dimethoate |
| Death, rat | Intermediate oral, acute ip; sequential (interval not reported) | Pb pretreatment had no effect on mortality from OP | Additive: no effect | Phillips et al. 1973) Lead chloride, Parathion |

**Table 2. Effect of Lead on Toxicity and Tissue Concentrations of Chlorpyrifos (*continued*)**

| Endpoint, species | Duration, route for Pb, OP; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Inactivation of OP, rat | Intermediate oral, acute ip, sequential (interval not reported) | Pb pretreatment increased excretion of inactive products of metabolism or chemical hydrolysis of OP, thus, presumably decreasing the body burden of OP | <additive | Hapke et al. 1978 Lead nitrate, Methyl parathion or Methyl paraoxon |
| Inactivation of OP, rat | Intermediate oral, acute oral; sequential (1 day) | Pb pretreatment did not affect rate of excretion or type or amount to OP metabolites | Additive: no effect | Abd-Elraof et al. 1981 Lead acetate, Malathion |
| Hydrolysis of OP in aqueous solution, pH 5.5–7.2 | *In vitro*; simultaneous | Pb caused hydrolytic inactivation of OP | <additive | Smolen and Stone 1997 Lead nitrate, Methyl chlorpyrifos, Methyl Parathion, or Ronnel |
| Hydrolysis of OP oxon in aqueous solution, pH 4.5–7.3 | *In vitro*; simultaneous | Pb catalyzed hydrolytic inactivation of OP oxon | <additive | Smolen and Stone 1997 Lead nitrate, Methyl chlorpyrifos oxon |

ip = intraperitoneal; OP = organophosphorus compound; Pb = lead; PFC = plaque-forming cells

### 2.2.2 Chlorpyrifos and Mercury or Methylmercury

**Chlorpyrifos and Mercury**

No studies of the joint toxic action of chlorpyrifos and inorganic mercury were located. Only two studies of joint toxic action of other similar organophosphorus insecticides with mercury were found, but neither of these studies is particularly adequate. In addition, studies of a potential chemical interaction of inorganic mercury with similar organophosphorus insecticides are available. These studies are discussed in the following text, and summarized in the tables at the end of the section.

A study of immunotoxicological effects of combined exposure to mercury and dimethoate (Institoris et al. 2001), a phosphorodithioate, reported a possible protective effect of combined exposure on humoral response, but not on cellular immune response, as compared with exposure to each chemical alone. Results, however, were inconsistent across experiments and with regard to dose response, data for cellular immune response were not reported adequately, and statistical comparisons were reported only between treated groups and controls. In this study, 4-week-old male rats were gavaged with non-immunotoxic and immunotoxic doses of mercuric chloride alone (at 0.4 and 3.2 mg/kg/day, equivalent to 0.3 and 2.4 mg Hg/kg/day), dimethoate alone (at 7.04 and 28.2 mg/kg/day), and combination treatments of the high dose of one chemical with the low dose of the other (0.3 mg/kg/day of mercury plus 28.2 mg/kg/day of dimethoate, or 2.4 mg/kg/day of mercury plus 7.04 mg/kg/day of dimethoate) for 28 days. For the combined treatments, the animals were treated first with mercury, followed by dimethoate 30 minutes later. No clinical signs of toxicity were seen, and no gross pathological changes were seen at necropsy. The high dose of each chemical alone and both combination treatments statistically significantly decreased the mean body weights, relative to controls, by about 10% by the end of the study. The high dose of each chemical alone statistically significantly decreased the humoral response (number of anti-sheep red blood cell plaque-forming cells [PFC] per $10^6$ cells and per spleen). The combination treatments appeared to be somewhat protective against the high-dose effect, but statistical comparisons among treated groups were not reported. In addition, in a preliminary series of experiments (reported in the same publication) in which each chemical was tested separately at the low and high dose, along with a control (and low, high, and control groups for an unrelated chemical), dimethoate had no significant effect on the PFC, mercury significantly decreased the number of PFC/$10^6$ cells at the low dose only and the PFC/spleen at the high dose only, and control values across the three experiments varied by more than 2-fold. The variability in results and lack of dose response for mercury greatly reduce confidence in the

findings. A further limitation is that data for effects on cellular immune response (delayed hypersensitivity assayed as footpad swelling) were not presented, but rather were summarized briefly in the text. The investigators stated that high doses of the separate chemicals did not affect this endpoint significantly, although the high dose of dimethoate showed a slight decrease. The combination of high dimethoate and low mercury produced a significant decrease, relative to controls, suggesting a greater response than for high dimethoate alone, but whether this result was statistically significantly different from the result for high dimethoate alone was not reported. Also, the same high dose of dimethoate did cause a significant decrease in this endpoint in the preliminary dose-response experiment. The inconsistency in results (including for controls), lack of dose response for mercury in humoral response experiments, inadequate reporting of data on the cellular immune response, and inadequate reporting of statistical analyses limit the conclusions that can be drawn from this study.

A study in calves investigated whether renal damage due to intravenous pretreatment with mercuric chloride (1 mg/kg, equivalent to 0.7 mg Hg/kg) would affect the toxicity of a gavage dose of 120 mg/kg of diazinon, a phosphorothioate insecticide, and administered 5 days later (Abdelsalam and Ford 1987). Pretreatment with mercury resulted in renal damage, as diagnosed by highly increased plasma levels of urea and creatinine, and renal histopathology in the calf that died. Signs of diazinon toxicity, including muscle tremors, ataxia, and increased respiration and defecation, were much greater in the mercury pretreated than in non-mercury calves, and one of three of the mercury-treated calves died, versus none of two of the non-mercury calves. Blood cholinesterase was reduced to a greater extent in the mercury pretreated calves. Brain and other tissues of the calf that died also were found to have reduced acetyl-cholinesterease activity, and reduced carboxylesterase activity was found in its liver. The investigators suggested that the increased toxicity of diazinon in mercury pretreated calves was due to a decreased urinary excretion of the active metabolites of diazinon, but diazoxon, the active metabolite of diazinon, generally is detoxified metabolically rather than excreted directly in the urine, except at very high doses. Urinary excretion of diazinon metabolites was not investigated.

*In vitro* studies with inorganic mercury and other organophosphorus insecticides of the same type as chlorpyrifos (phosphorothioate insecticides) indicate a potential for a chemical interaction between mercury(II) ions and chlorpyrifos. Incubation of methyl parathion with mercury(II) (from mercuric chloride or mercuric nitrate) in buffered solutions resulted in hydrolysis of methyl parathion to p-nitrophenol at pHs in the range of 3.5–7.5 (Wan et al. 1994; Zeinali and Torrents 1998). Similar hydrolyses to phenolic compounds were obtained with other phosphorothioates (fenitrothion, fenthion) and a phosphorodithioate (malathion) during incubation with mercuric chloride at pHs of 5.5–7.5 (Wan

et al. 1994). For chlorpyrifos, analogous hydrolysis by mercury (II) ions would be expected to produce 3,5,6-trichloro-2-pyridinol (and diethyl thiophosphate). These hydrolysis products also are produced during metabolic inactivation and do not have anticholinesterase activity. Concentrations of mercury and phosphorothioates used in these studies were in the ppm range. The concern that prompted the investigations was that sterilization of soil with mercury (II) (as mercuric chloride), which is done in order to study abiotic processes, might be contributing to the degradation of the pesticides under study. Whether co-ingestion of inorganic mercury and phosphorothioate insecticides would result in hydrolysis of the phosphorothioates in the stomach, blood, or tissues was not tested or discussed.

Table 3 provides a summary of the joint action data pertinent to the effects of chlorpyrifos on the toxicity and tissue concentrations of mercury. The limited data from a single study on immunotoxicity of a similar phosphorodithioate insecticide (dimethoate) indicate that chlorpyrifos may act additively or less than additively with mercury. Table 4 provides a summary of the pertinent joint action data for the effects of mercury on the toxicity and tissue concentrations of chlorpyrifos. The studies were conducted with similar phosphorothioate and phosphorodithioate insecticides, and are not consistent regarding direction of interaction across the limited number of endpoints studied. Simultaneous exposure studies of immunotoxicity and of chemical interactions suggest an inhibition of chlorpyrifos toxicity, and a sequential exposure study of organophosphorus neurotoxicity following mercury-induced renal damage suggests potentiation of chlorpyrifos toxicity.

**Table 3. Effect of Chlorpyrifos on Toxicity and Tissue Concentrations of Mercury**

| Endpoint, species | Duration, route for OP, Hg; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Immunological: humoral (anti-sheep red blood cell PFC) and cellular (delayed hypersensitivity: footpad thickness), rat | Intermediate, oral; simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Less inhibition of humoral response from mixture than from high-dose component alone at same dose as in mixture; cellular response data inadequately reported but suggested slightly greater inhibition from mixture than from high-dose component alone; results inconsistent for single chemicals and controls across experiments, and statistical analyses inadequate | <additive for humoral response; indeterminate for cellular response | Institoris et al. 2001 Dimethoate, Mercuric chloride |

Hg = mercury; OP= organophosphorus compound; PFC = plaque-forming cells

**Table 4. Effect of Mercury on Toxicity and Tissue Concentrations of Chlorpyrifos**

| Endpoint, species | Duration, route for Hg, OP; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Neurological: cholinergic signs, blood and brain cholinesterase, calf | Acute iv, acute oral; sequential (5 days) | Renal damage from Hg pretreatment increased the OP-induced cholinergic signs and cholinesterase inhibition in blood and brain | >additive | Abdelsalam and Ford 1987 Mercuric chloride, Diazinon |
| Death, calf | Acute iv, acute oral; sequential (5 days) | One of three calves with renal damage from Hg pretreatment died after subsequent OP, versus none of two calves treated only with OP | >additive | Abdelsalam and Ford 1987 Mercuric chloride, Diazinon |
| Immunological: humoral (anti-sheep red blood cell PFC) and cellular (delayed hypersensitivity: footpad thickness), rat | Intermediate, oral, simultaneous; mixtures=high dose of one component plus low dose of the other and vice versa | Less inhibition of humoral response from mixture than from high-dose component alone at same dose as in mixture; cellular response data inadequately reported but suggested slightly greater inhibition from mixture than from high-dose component alone; results inconsistent for single chemicals and controls across experiments, and statistical analyses inadequate | <additive for humoral response; indeterminate for cellular response | Institoris et al. 2001 Mercuric chloride, Dimethoate |
| Hydrolysis of OP in aqueous solution, pH 3.5–7.5 | Acute, *in vitro*; simultaneous | Hg caused hydrolytic inactivation of OP | <additive | Wan et al. 1994; Zeinali and Torrents 1998 Mercuric chloride or nitrate, Methyl parathion |
| Hydrolysis of OP in aqueous solution, pH 5.5–7.5 | Acute, *in vitro*; simultaneous | Hg caused hydrolytic inactivation of OP | <additive | Wan et al. 1994 Mercuric chloride, Fenitrothion, Fenthion, or Malathion |

Hg = mercury; iv = intravenous; OP= organophosphorus compound; PFC = plaque-forming cells

**Chlorpyrifos and Methylmercury**

No studies of the joint action of methylmercury with chlorpyrifos (or similar organophosphates) in humans or other mammals were located. A sequential study of the joint toxic action of methylmercuric dicyandiamide with parathion (a phosphorothioate) has been performed in quail. Studies of the joint toxic action of methylmercury with chlorpyrifos have been performed in amphipods. Potential direct chemical interactions (i.e., chemical reaction) of methylmercury with chlorpyrifos also have been investigated. These studies are reviewed in the following paragraphs, and the more relevant studies are summarized in the tables at the end of this section.

A potentiation of phosphorothioate lethality was seen in Corturnix quail fed methylmercuric dicyandiamide (morsodren) in the diet for 18 weeks at 0 or 4 ppm methylmercury (3.7 ppm Hg), and then orally dosed with 2, 4, 6, 8, or 10 mg/kg of parathion by an unspecified method, and observed for 48 hours to determine the median lethal dose ($LD_{50}$) (Dieter and Ludke 1975). Additional birds were fasted for 30 minutes, and then orally dosed with a sublethal dose of parathion (1 mg/kg); cholinesterase activity was assayed 60 minutes following parathion dosing. The apparent $LD_{50}$ of parathion was decreased from 5.86 to 4.24 in birds fed methylmercuric dicyandiamide versus those fed control diet. Whether the values were statistically significantly different was not discussed. Plasma and brain cholinesterase, assayed by the Ellman method, were affected by methylmercuric dicyandiamide alone as well as by parathion alone and by the combined treatment. Methylmercury is not known to be a cholinesterase inhibitor. Steevens and Benson (1999) have pointed out that methylmercury interferes with the colorimetric Ellman method assay for cholinesterase, and therefore, evidence of methylmercury inhibition of cholinesterase activity may be artifactual, particularly at concentrations >1 μM methylmercury. Thus, the cholinesterase results in this study may be artifactual, and in any event, did not indicate potentiation of cholinesterase inhibition. Another potential concern for the use of methylmercuric dicyandiamide is that the compound includes the cyanide moiety, which may contribute to its toxicity. Studies addressing this issue were not found through additional searching. Because of the lack of statistical analyses, concerns regarding mercury interference with the cholinesterase assays, and concerns that the cyanide moiety may have influenced toxicity, this study is not considered suitable as the basis for conclusions regarding the joint action of chlorpyrifos and methylmercury, and is not included in the summary tables.

Another study by the same investigators of methylmercuric dicyandiamide pretreatment (0.05–5.0 ppm methylmercury in food) followed by oral administration of parathion (0.5 mg/kg) in Coturnix quail focused on plasma and brain cholinesterase activity, using the same Ellman assay for cholinesterase

(Dieter and Ludke 1978). This study is further limited by lack of concurrent data for brain cholinesterase in methylmercuric dicyandiamide only birds, and also is considered unsuitable as the basis for conclusions regarding chlorpyrifos and methylmercury, and is not included in the summary tables.

A series of studies have investigated the joint toxic action of methylmercury (as methylmercuric chloride) with chlorpyrifos, and the underlying mechanisms in the amphipod *Hyalella azteca* (a 1/4-inch-long, shrimp-like, freshwater crustacean). Both chemicals were tested over a wide range of concentrations during 4-day flow-through exposures to determine concentration-response curves for mortality of juvenile *H. azteca* (Steevens and Benson 2001). Joint action was studied with concentrations of methylmercury ranging from 0.125 to 4 times the median lethal concentration ($LC_{50}$) value (17.8 nM methylmercury, equivalent to 3.57 ppb Hg), together with a constant concentration of chlorpyrifos (0.42 nM, equivalent to 0.147 ppb). The concentration of chlorpyrifos was selected from the linear portion of the response curve (and greater than one standard deviation below the $LC_{50}$). Because the two chemicals appear to have different mechanisms of toxicity, it was expected that the joint toxic action would be independent. The joint action of methylmercury and chlorpyrifos on mortality was additive, however, as judged by the fit of the methylmercury dose-response curve in the presence of chlorpyrifos to the modeled additive dose-response curve. By way of comparison, in the same study, results for other mixtures of chemicals with different mechanisms of action (chlorpyrifos and dieldrin, methylmercury and dieldrin) did fit the modeled independent action dose-response curve.

Studies to determine the joint action of these chemicals on acetylcholinesterase of adult *H. azteca* were performed by the same investigators (Steevens and Benson 1999, 2001). The adult organisms were exposed for 48 hours (with water renewed every 12 hours) to 30, 150, or 350 nM methylmercury alone, or 0.04, 0.14, or 0.4 nM chlorpyrifos alone, or to mixtures of 30 nM methylmercury and 0.04 nM chlorpyrifos, or 150 nM methylmercury and 0.14 nM chlorpyrifos. Chlorpyrifos alone inhibited acetylcholinesterase activity in a statistically significant and dose-related manner at ≥0.14 nM. Methylmercury alone did not affect the acetylcholinesterase activity. The mixture of 150 nM methylmercury (equivalent to 10 ppb Hg) and 0.14 nM (0.049 ppb) chlorpyrifos partially protected against the inhibition of acetylcholinesterase activity, because the inhibition seen with this mixture was statistically significantly less than that seen with 0.14 nM chlorpyrifos alone.

To investigate the mechanisms underlying these apparent discrepant results for the joint action of methylmercury and chlorpyrifos on mortality and cholinesterase activity in *H. azteca*, studies of accumulation and elimination were performed (Steevens and Benson 2001). Adult organisms were

exposed to sublethal concentrations of chlorpyrifos (0.11 nM) and methylmercury (42.4 nM), separately and as a mixture for 144 hours, followed by a transfer to toxicant-free water for 144 hours; water was renewed every 12 hours. At 6 and 12 hours of exposure, accumulation of mercury was statistically significantly higher in the organisms exposed to the mixture than in those exposed to methylmercury alone. By 144 hours of exposure, differences were no longer apparent. Following the transfer to toxicant-free water, mercury concentrations decreased in the organisms exposed to methylmercury alone, but not in those exposed to the mixture, such that after 144 hours, the tissue mercury concentrations in organisms exposed to the mixture were statistically significantly higher than in those exposed to methylmercury alone. (Results were reported as concentrations of "methylmercury," but the analytical method quantitated total mercury.) Chlorpyrifos did not accumulate in the organisms exposed to chlorpyrifos alone or in combination with methylmercury. The relevance of the study to humans is questionable.

The possibility of a chemical interaction (i.e., chemical reaction) between methylmercury and chlorpyrifos was investigated by incubating 0.01 M chlorpyrifos and 0.01 M methylmercury in ethyl acetate or deionized water for 24 hours at 23 °C with slow mixing (Steevens and Benson 1999, 2001). The incubation in water resulted in the formation of a mercury-containing complex that was more polar on thin-layer chromatography than methylmercury or chlorpyrifos. The investigators hypothesized that the methylmercury ion forms a mercury-sulfur bond with chlorpyrifos, followed by hydrolysis of an ester linkage of chlorpyrifos, which would result in a more polar compound and would inactivate chlorpyrifos. Gas-chromatography mass-spectroscopy revealed chlorpyrifos and methylmercury, but not the additional compound seen on thin-layer chromatography. The investigators suggested that the high temperature conditions of gas chromatography may have resulted in degradation of the mercury-sulfur bond. The apparent additive joint action of methylmercury and chlorpyrifos with regard to lethality in *H. azteca* may have been due to the increased accumulation of mercury or methylmercury in the organisms, in combination with the enhanced deactivation of chlorpyrifos by methylmercury, or possibly to the toxicity of the complex.

Table 5 provides a summary of the pertinent joint action data regarding the effects of chlorpyrifos on the toxicity and tissue concentrations of methylmercury. The data indicate that chlorpyrifos may increase the toxicity of methylmercury through chemical interaction. Table 6 provides a summary of the pertinent joint action data for the effects of methylmercury on the toxicity and tissue concentrations of chlorpyrifos. The data indicate that methylmercury may decrease the toxicity of chlorpyrifos through chemical interaction. Although the studies summarized in these tables were well-conducted and organophosphorus

toxicity is similar across species, the applicability of these data in freshwater amphipods to human exposure scenarios may be questionable, because exposure and absorption mechanisms for chlorpyrifos, methylmercury, and the complex formed from chemical interaction, may not be similar. In addition, if the human exposure is multimedia (e.g., methylmercury in fish, chlorpyrifos in dust or fruits and vegetables) chemical interaction would have to occur following absorption.

**Table 5. Effect of Chlorpyrifos on Toxicity and Tissue Concentrations of Methylmercury**

| Endpoint, species | Duration, route for Cpf, MeHg; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Death, *H. azteca*[a] | Acute (4 days), water; simultaneous | Additive influence of Cpf on MeHg, as judging by fit of dose-response curve to modeled additive mortality curve | Additive joint action may result from >additive effect on MeHg and <additive effect on Cpf | Steevens and Benson 2001, Chlorpyrifos, Methylmercuric chloride |
| Hg in whole organism, *H. azteca*[a] | Acute (144 hours), water; simultaneous | Initial rate of accumulation of Hg higher from mixture, but at end of 144 hours exposure Hg same in organisms exposed to mixture or to MeHg alone at same concentration as in mixture; Hg retained in organisms exposed to mixture following end of exposure, but gradually eliminated from organisms exposed to MeHg alone | >additive | Steevens and Benson 2001 Chlorpyrifos, Methylmercuric chloride |
| Chemical interaction in deionized water | Acute (24 hours) *in vitro*; simultaneous | MeHg and Cpf formed a Hg-containing complex that was more polar than starting compounds; complex may contribute to accumulation and retention of Hg in organisms | >additive? | Steevens and Benson 1999, 2001 Chlorpyrifos, Methylmercuric chloride |

[a]a ¼-inch-long freshwater amphipod (shrimp-like crustacean)
Cpf = chlorpyrifos; Hg = mercury; MeHg = methylmercury

**Table 6. Effect of Methylmercury on Toxicity and Tissue Concentrations of Chlorpyrifos**

| Endpoint, species | Duration, route for MeHg, Cpf; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Neurological (acetylcholinesterase activity), *H. azteca*[a] | Acute (8 hours), water; simultaneous | Less inhibition of acetylcholin-esterase from mixture than from Cpf alone at same concentration as in mixture | <additive | Steevens and Benson 1999, 2001 Methylmercuric chloride, Chlorpyrifos |
| Death, *H. azteca*[a] | Acute (4 days), water; simultaneous | Apparent additive joint action, as judged by fit of dose-response curve to modeled additive curve | Additive joint action may result from >additive effect on MeHg and <additive effect on Cpf | Steevens and Benson 2001 Methylmercuric chloride, Chlorpyrifos |
| Cpf in whole organism, *H. azteca*[a] | Acute (144 hours), water; simultaneous | Cpf did not accumulate in organisms exposed to Cpf alone or the mixture | indeterminate | Steevens and Benson 2001 Methylmercuric chloride, Chlorpyrifos |
| Chemical interaction in deionized water | Acute (24 hours), *in vitro*; simultaneous | MeHg and Cpf formed an Hg-containing complex that was more polar than starting compounds, suggesting inactivation of Cpf through hydrolysis | <additive? | Steevens and Benson 1999, 2001 Methylmercuric chloride, Chlorpyrifos |

[a]a ¼-inch-long freshwater amphipod (shrimp-like crustacean)
Cpf = chlorpyrifos; Hg = mercury; MeHg = methylmercury

### 2.2.3 Lead and Mercury or Methylmercury

**Lead and Mercury**

Studies of the joint toxic action of inorganic mercury and lead include simultaneous and sequential injection studies of lethality and renal toxicity in mice and rats, and a single study of tissue distribution of mercury following simultaneous or sequential oral administration of lead and mercury to mice. These studies are reviewed in the following text, and summarized in the tables at the end of this section.

The acute lethality and renal toxicity of combinations of mercury (as mercuric chloride) and lead (as lead acetate) were studied following virtually simultaneous intravenous injection into adult rats (Shubert et al. 1978). The lead-mercury mixtures apparently formed a precipitate when mixed, and therefore, the components were injected separately: lead first, followed immediately by mercury. Mercury was reported to act synergistically with lead on lethality when the dose of mercury was held constant at 4.8 µmole Hg/kg and the lead dose was varied to determine the lead dose response in the presence of mercury. The mercury dose was said to be near the $LD_1$, but actually was slightly greater than the $LD_{20}$ of 4.5 µmole Hg/kg, which, in separate experiments, resulted in massive acute tubular necrosis (see next paragraph). The $LD_{50}$ for lead in the presence of mercury was 18.15 µmole/kg, versus 477.6 µmole/kg in the absence of mercury, but the results in the presence of mercury were "not statistically significant" (the basis for this conclusion was not explained). Conversely, co-administration of lead at a dose of 241.7 µmole Pb/kg (slightly less than the $LD_1$) in combination with varying doses of mercury, was protective against lethality. The $LD_{50}$ for mercury in the presence of lead was 243.8 µmole/kg, versus 5.35 µmole/kg in the absence of lead. Although these results suggest a marked potentiation of lead lethality by mercury and a marked inhibition of mercury lethality by lead following an intravenous co-injection, interpretation of these results is problematic, given the lack of detail regarding statistical analyses and the asymmetrical study design (use of a $>LD_{20}$ dose of mercury plus increasing doses of lead to determine lead $LD_{50}$ in the presence of mercury; use of a $<LD_1$ dose of lead plus increasing doses of mercury to determine mercury $LD_{50}$ in the presence of lead).

Additional experiments in this study focused on renal and testicular histopathology (Schubert et al. 1978). Adult rats were injected intravenously with vehicle alone, 4.5 µmole Hg/kg (approximately the $LD_{20}$), 12.7 µmole Pb/kg (<1/20 of the $LD_1$), or 1.7 µmole Hg/kg together with 12.7 µmole Pb/kg. Mercury alone at 4.5 µmole/kg caused massive acute tubular necrosis on day 4; no renal lesions were seen in the

lead-alone group, and the mercury-plus-lead group had the same renal lesions as the mercury-alone group. There were no testicular lesions in any group. Because of the severe renal lesions caused by mercury, additional studies of renal toxicity were performed with a lower dose of mercury and a higher dose of lead. Additional groups of adult rats were injected intravenously with vehicle alone, 1.7 μmole Hg/kg ($<LD_1$), 296 μmole Pb/kg ($LD_1$), or 1.7 μmole Hg/kg together with 296 μmole Pb/kg. The mercury-alone group had no renal lesions. The lead-alone group had minimal renal changes (increased number of sloughed necrotic tubular epithelial cells in the lumens of the straight tubules and slightly increased number of mitotic figures in tubular epithelial cells, relative to controls). The mercury-plus-lead group had moderate acute tubular necrosis (necrosis and sloughing of tubular epithelial cells, flattening of remaining and regenerating epithelium, tubular dilation, and tubular casts). The results were considered indicative of synergism by the investigators.

A comparison of the consequences of simultaneous and sequential oral administration of lead (as lead nitrate) and mercury (as mercuric chloride) to young adult male mice (20–25 g body weight) on the tissue distribution of mercury (Sin et al. 1985) provides data by a route more relevant to anticipated human exposures, but no information on health endpoints. The mice were gavaged with 25, 50, 100, or 200 μg of lead (approximately 1.1, 2.2, 4.4, or 8.9 mg Pb/kg) followed immediately by a gavage dose of 200 μg of mercury (approximately 8.9 mg Hg/kg), and were killed 24 hours later for analysis of the mercury content of the kidneys, liver, and spleen. The same doses of lead (except the highest dose was deleted) and mercury were also administered by gavage in a sequential manner, with lead given 24 hours before mercury, and the mice killed 24 hours after the mercury treatment. In the simultaneous exposure experiment, there were no significant differences in mercury concentration in kidney or liver between the lead and the no-lead mercury-treated groups. Mercury concentrations in spleen, however, increased with increasing dose of lead, and were statistically significantly higher than the no-lead group at all but the lowest dose of lead. The mercury in the spleen was found primarily in the lumens of the veins and in the phagocytic cells in the red pulp. The investigators hypothesized that binding of co-administered lead and mercury to the sulfhydryl groups of the erythrocytes caused more damage than did the metals administered sequentially. The damaged erythrocytes would then be removed by the spleen, with a consequent increase in spleenic concentrations of mercury associated with these erythrocytes. In the sequential experiment, however, renal concentrations of mercury were statistically significantly decreased by the previous oral administration of the two higher doses of lead; spleen concentrations of mercury were not significantly affected by lead. In an additional sequential experiment, a higher dose of lead (200 μg or 8.9 mg/kg) was administered intravenously 24 hours before oral administration of mercury (8.9 mg/kg), and the mice were killed 24 hours after mercury administration. Blood concentrations of

mercury were similar in the lead and no-lead groups. The investigators stated that there was no significant difference in the total amount of mercury from the four tissue samples in the lead and no-lead groups. The meaning of this statement is not clear—it could mean that the sum of the concentrations in kidneys, liver, spleen, and blood were not different across the two groups (which appears to be true). Total mercury per tissue (e.g., total Hg/liver) was not reported in the paper. The renal concentration of mercury was decreased and the spleen concentration of mercury was increased in the lead-pretreated group as compared with the no-lead group.

In a sequential exposure study in female mice, lead acetate was administered by intravenous injection at a dose of 5 mg Pb/kg 48 hours prior to an intraperitoneal injection of mercuric chloride at a dose of 6 mg/kg (equivalent to 4.4 mg Hg/kg) (Ewald and Calabrese 2001). Blood urea nitrogen (BUN) concentrations were monitored as an index of kidney damage. Lead alone did not affect BUN; mercury alone caused an approximately 4-fold increase in mean BUN over controls, while the lead pretreatment followed by mercury resulted in only an approximately 2-fold increase in BUN. Thus, the results indicate that lead pretreatment partially protected against mercury-induced renal damage.

In a sequential exposure study in mice, administration of relatively low intraperitoneal doses of mercury (0.45 mg Hg/kg, as mercuric chloride) or lead (10 mg Pb/kg, as lead nitrate) to mice followed 2 days later by a challenge intraperitoneal dose of mercury (4.5 mg Hg/kg, about 70–80% of the lethal dose) resulted in a notable decrease in mortality (20% mortality from either pretreatment versus 90% mortality from no pretreatment) (Yoshikawa and Hisayoshi 1982). The same low-dose mercury pretreatment followed 2 days later by a challenge intraperitoneal dose of lead (60 mg Pb/kg) had no effect upon mortality (70% with or without pretreatment). The same low-dose lead pretreatment, however, completely prevented subsequent mortality from the challenge intraperitoneal dose of lead. A potential mechanism for protection against mercury toxicity by lead pretreatment, but no protection against lead toxicity by mercury pretreatment, is that both lead and mercury induce metallothionein, but only mercury binds to metallothionein. Metallothionein may protect against the acute lethality of metals by sequestering the bound metal and preventing its binding to critical cellular constituents. This mechanism, however, does not explain why lead pretreatment protected against the lethality of a challenge dose of lead.

Similar results were obtained with regard to a protective effects of low-dose lead or mercury pretreatment on the acute lethality of a challenge dose of mercury or lead in another study in mice (Garber and Wei 1972). Lead nitrate (20 mg/kg, equivalent to 12.5 mg Pb/kg) or vehicle was injected intraperitoneally followed 4 days later by an intraperitoneal injection of a challenge dose of mercuric chloride (8 mg/kg,

equivalent to 5.9 mg Hg/kg). Mortality was statistically significantly lower in the lead-pretreated group (4/10 versus 9/10 in non-pretreated). Conversely, pretreatment with low-dose mercuric chloride (0.8 mg/kg, equivalent to 0.59 mg Hg/kg), followed 4 days later by a challenge dose of lead nitrate (200 mg/kg, equivalent to 125 mg Pb/kg), did not significantly alter mortality.

Table 7 summarizes data pertinent to the effect of lead on the toxicity and tissue concentrations of mercury. Most of the studies indicate that lead may inhibit the acute lethality and renal toxicity of mercury. A single acute oral simultaneous exposure study found that lead did not affect the distribution of mercury to the kidney, but did not investigate toxicity. Table 8 summarizes pertinent data regarding the effect of mercury on the toxicity and tissue concentrations of lead. These data also all were obtained from injection studies, and results were mixed; greater than additive for mortality and renal lesions in a simultaneous intravenous study, and no effect of mercury pretreatment on lead mortality in two sequential intraperitoneal studies. The toxicity studies in these tables all were conducted by injection, which bypasses possible interactions at the level of pharmacokinetic mechanisms, particularly absorption. An additional limitation is that they all were acute in duration.

**Table 7. Effect of Lead on Toxicity and Tissue Concentrations of Mercury**

| Endpoint, species | Duration, route for Pb, Hg: sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Renal (tubular necrosis), rat | Acute, iv; simultaneous. | Severity of lesions much greater following mixture than from single chemicals at same dose ($\approx LD_1$) as in mixture; lesions were similar to those caused by Hg alone at $\approx LD_{20}$ | >additive | Schubert et al. 1978<br>Lead acetate, Mercuric chloride |
| Renal: BUN, mouse | Acute iv, acute ip; sequential (48 hours) | BUN 2-fold lower in Pb-pretreated Hg group than in Hg-alone group, and not affected by Pb alone | <additive | Ewald and Calabrese 2001<br>Lead acetate, Mercuric chloride |
| Death, rat | Acute, iv; simultaneous. | $LD_{50}$ for Hg in presence of $<LD_1$ of Pb increased almost 50-fold, indicating greatly decreased lethality of Hg | <additive | Shubert et al. 1978<br>Lead acetate, Mercuric chloride |
| Death, mouse | Acute, ip; sequential (48 hours) | Decreased mortality from Hg in Pb-pretreated group (2/10 versus 9/10 in non-pretreated) | <additive | Yoshikawa and Hisayoshi 1982<br>Lead nitrate, Mercuric chloride |
| Death, mouse | Acute, ip; sequential (4 days) | Decreased mortality from Hg in Pb-pretreated group (4/10 versus 9/10 in non-pretreated) | <additive | Garber and Wei 1972<br>Lead nitrate, Mercuric chloride |
| Renal: Hg levels, mouse | Acute, oral; simultaneous | No difference in renal Hg for Hg alone or Hg+Pb groups | Additive: no effect | Sin et al. 1985<br>Lead nitrate, Mercuric chloride |
| Renal: Hg levels, mouse | Acute oral or iv, acute oral; sequential (24 hours) | Renal Hg lower in Pb-pretreated Hg group than in Hg-alone group | <additive | Sin et al. 1985<br>Lead nitrate, Mercuric chloride |

BUN = blood urea nitrogen; Hg = mercury; ip = intraperitoneal; iv = intravenous; Pb = lead

**Table 8. Effect of Mercury on Toxicity and Tissue Concentrations of Lead**

| Endpoint, species | Duration, route for Hg, Pb; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Renal (tubular necrosis), rat | Acute, iv–iv; simultaneous | Severity of lesions much greater following mixture than from single chemicals at same dose (≈$LD_1$) as in mixture; lesions were similar to those caused by Hg alone at ≈$LD_{20}$ | >additive? | Schubert et al. 1978 Mercuric chloride, Lead acetate |
| Death, rat | Acute, iv–iv; simultaneous | In presence of ≈$LD_{20}$ dose of Hg, $LD_{50}$ for Pb decreased almost 50-fold, indicating greatly increased lethality of Pb | >additive | Shubert et al. 1978 Mercuric chloride, Lead acetate |
| Death, mouse | Acute, ip–ip; sequential (48 hours) | Mortality from Pb in Hg-pretreated group was not changed compared with non-pretreated group | Additive: no effect | Yoshikawa and Hisayoshi 1982 Mercuric chloride, Lead nitrate |
| Death, mouse | Acute, ip–ip; sequential (4 days) | Mortality from Pb in Hg-pretreated group was not altered compared with non-pretreated group | Additive: no effect | Garber and Wei 1972 Mercuric chloride, Lead nitrate |

Hg = mercury; ip = intraperitoneal; iv = intravenous; Pb = lead

**Lead and Methylmercury**

Studies of the joint action of lead and methylmercury include a simultaneous exposure study of developmental toxicity in mice, simultaneous oral exposure studies of joint toxic action and tissue distribution in Pekin ducks, and sequential studies of the effect of lead pretreatment on the lethality and tissue distribution of mercury from methylmercury in rats. These studies are discussed in the following text and summarized in the tables at the end of this section.

In a study of developmental toxicity, pregnant mice were injected subcutaneously with lead nitrate (25 mg/kg, equivalent to 15.6 mg Pb/kg) and/or gavaged with methylmercuric chloride (12.5 mg/kg, equivalent to 10 mg Hg/kg) on day 10 of gestation (Belles et al. 2002). Methylmercury alone was associated with a slight but statistically significant increase in maternal deaths (1/12 versus 0/10 for controls). The mixture was associated with a significantly greater number of maternal deaths (3/14) than the mercury-alone group. Lead alone did not result in litters without fetuses or maternal deaths. Other effects in the dams gavaged with the mixture were increased absolute and relative liver weights and increased absolute kidney weights; neither lead nor methylmercury alone affected these endpoints. Average fetal body weight/litter was statistically significantly decreased, relative to controls, to a similar extent by mercury alone and the mixture, and not by lead alone. In addition, the incidences of cleft palate and of some skeletal defects were statistically significantly increased to a similar extent in the mercury-alone group and the mixture group, but not in the lead-alone group, relative to controls. These fetal data indicate that lead did not influence methylmercury fetotoxicity. Concentrations of lead in placenta and of mercury in placenta and fetus did not differ significantly between the mixture group and the group that received the metal alone. Lead was not detectible in the fetus. Greater maternal toxicity resulted from the mixture than the individual chemicals, but the mode of joint action cannot be further determined because the total dose of metals was higher in the mixture group, and because of the lack of response to one or both chemicals when tested alone. Fetal toxicity (reduced fetal weight and increased incidence of cleft palate and skeletal defects) appeared to be attributable only to methylmercury. Thus, it appears that lead did not affect the fetal toxicity of methylmercury, as measured by the usual fetal endpoints. This study, however, does not provide information about sensitive neurological endpoints.

In a series of studies in female Pekin ducks, methylmercury (8 mg/kg feed methylmercuric chloride) and/or lead (80 mg/kg feed lead acetate) were administered in the diet for 12–13 weeks, and kidney and liver endpoints were studied (Jordan et al. 1990; Prasada Rao et al. 1989a, 1989b). The authors pointed out that dietary concentrations of calcium, which were optimal for egg production, may have antagonized

lead absorption. In the kidney, lead alone was associated with dense bodies in the cytoplasm of the proximal tubular cells, mercury alone caused lipid infiltration and cytoplasmic vacuolation and dense body accumulation in the proximal tubular cells, and the mixture resulted in similar effects as for methylmercury alone, and in addition, in the collecting ducts, loss of apical cytoplasm and some tubular degeneration (Prasada Rao et al. 1989a). The authors considered the effects to be consistent with additivity. Electron microscopy of the kidneys revealed a thickening of the glomerular basement membrane in the three treated groups that was statistically significantly different from controls in the lead-alone and the mixture groups (Prasada Rao et al. 1989b). The thickness of the glomerular membrane was not significantly different, however, among the three treated groups. Other ultrastructural changes were similar across the treated groups and included mitochondrial swelling, and increases in lysosomal bodies and vacuoles, with the changes somewhat more prevalent or severe in the mixture group. The exact mode of joint toxic action cannot be determined given the study design and the mostly descriptive data. Assuming linearity of dose response, the results may be consistent with less than additivity or with additivity.

Additional findings of interest in the kidney (Prasada Rao et al. 1989a) were that all three treatments increased the metallothionein concentration to the same extent. Metallothionein induction did not appear to be saturated, because cadmium alone in the same experiment induced metallothionein to a much greater extent. The renal concentration of mercury was the same in the mercury-alone group as in the mixture group. The mean renal concentration of lead in the mixture group was approximately twice that in the lead-alone group, but the values were not statistically significantly different. In the liver (Jordan et al. 1990), the metallothionein concentration was increased to the same extent by each chemical alone and by the mixture. The livers were not examined histopathologically.

The effect of pretreatment with lead on the acute lethality of methylmercury has been studied in rats (Congiu et al. 1979). Lead nitrate was injected intravenously in a dose of 0 or 20.7 mg Pb/kg, followed 24 hours later by methylmercuric chloride, administered by gavage at doses of 34.6, 39.6, and 44.6 mg Hg/kg (corresponding to the theoretical $LD_{25}$, $LD_{50}$, and $LD_{75}$ for methylmercuric chloride alone). Controls received the lead pretreatment, followed 24 hours later by the corn oil vehicle. Lead pretreatment was associated with an apparent increase in lethality at all three dose levels of mercury. Lead alone was not lethal. Although the authors did not perform statistical analyses, the increased mortality was statistically significant (by Fisher Exact test) at the 34.6 and 39.6 mg Hg/kg doses in the lead-pretreated animals as compared with non-pretreated animals.

The effect of pretreatment with lead on the tissue distribution of mercury from methylmercury also was studied in rats (Congiu et al. 1979). Lead nitrate (or saline vehicle) was injected intravenously in a dose of 0 or 20.7 mg Pb/kg, followed 24 hours later by methylmercuric chloride, administered by gavage at a dose of 34.6 mg Hg/kg. This dose of methylmercuric chloride was the $LD_{25}$. Lead pretreatment statistically significantly increased the concentration of mercury in the kidney, but not in the liver, at 6 and 24 hours after methylmercuric chloride treatment.

Table 9 summarizes data pertinent to the effect of lead on the toxicity and tissue concentrations of methylmercury. For many of the studies, the study designs (particularly the dosing scheme, which gives a higher *total* chemical dose from the mixture than from the components tested separately) and the lack of statistical analyses preclude definitive conclusions. Nevertheless, the data generally suggest an additive or less-than-additive influence of lead on mercury, with the exception of an acute sequential lethality study in which lead was injected intravenously. Table 10 summarizes data pertinent to the effects of methylmercury on the toxicity and tissue concentrations of lead, and includes the same simultaneous exposure studies as in Table 9. Again, the results suggest additive or less-than-additive joint action.

**Table 9. Effect of Lead on Toxicity and Tissue Concentrations of Methylmercury**

| Endpoint, species | Duration, route for Pb, MeHg; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Developmental: fetal weight, cleft palate, skeletal defects, mouse | Acute sc, acute oral; simultaneous | Fetotoxic effects were the same in MeHg and MeHg+Pb groups; no fetal effects in Pb group; dose of each chemical in mixture same as when given alone | Additive: no effect | Belles et al. 2002 Lead nitrate, Methylmercuric chloride |
| Renal: proximal tubular and glomerular damage, ducks | Intermediate, oral; simultaneous | Histopathological and ultrastructural changes in proximal tubules of mixture group somewhat more severe than for each chemical alone at same dose as in mixture, thickening of glomerular membrane same in mixture and single chemical groups | Additive and <additive? | Prasad Rao et al. 1989a, 1989b Lead acetate, Methylmercuric chloride |
| Renal: weight, mouse | Acute sc, acute oral; simultaneous | Increased absolute (but not relative) kidney weight from mixture, but not from either chemical alone at same dose as in mixture | Additive or >additive? | Belles et al. 2002 Lead nitrate, Methylmercuric chloride |
| Hepatic: weight, mouse | Acute sc, acute oral; simultaneous | Increased absolute and relative liver weight from mixture, but not from either chemical alone at same dose as in mixture | Additive or >additive? | Belles et al. 2002 Lead nitrate, Methylmercuric chloride |
| Renal and hepatic: metallothionein, duck | Intermediate, oral; simultaneous | Metallothionein increased to same extent with the mixture as with each chemical alone at same dose as in mixture | <additive?: for metallothionein induction | Jordan et al. 1990; Prasad Rao et al. 1989a Lead acetate, Methylmercuric chloride |
| Death, mouse, pregnant | Acute sc, acute oral; simultaneous | Maternal mortality slightly but significantly greater in MeHg+Pb (3/14) than in MeHg (1/12 group), and in both these groups relative to controls (0/10); no maternal deaths in Pb group; dose of each chemical in mixture same as when given alone | Additive or > additive? | Belles et al. 2002 Lead nitrate, Methylmercuric chloride |
| Death, rat | Acute iv, acute oral; sequential (24 hours) | Increased lethality from Hg in Pb-pretreated group, versus Hg alone | >additive | Congiu et al. 1979 Lead nitrate, Methylmercuric chloride |
| Placental and fetal Hg levels, mouse | Acute sc, acute oral, simultaneous | No difference in placental and fetal Hg between mixture group and MeHg-alone group | Additive: no effect | Belles et al. 2002 Lead nitrate, Methylmercuric chloride |

**Table 9. Effect of Lead on Toxicity and Tissue Concentrations of Methylmercury (*continued*)**

| Endpoint, species | Duration, route for Pb, MeHg; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Renal: Hg levels, duck | Intermediate, oral; simultaneous | Renal Hg same for mixture as for MeHg alone at same dose as in mixture | Additive: no effect | Prasada Rao et al. 1989a<br>Lead acetate, Methylmercuric chloride |
| Renal: Hg levels, rat | Acute iv, acute oral; sequential (24 hours) | Increased renal Hg from MeHg in Pb-pretreated group | >additive | Congiu et al. 1979<br>Lead nitrate, Methylmercuric chloride |
| Liver: Hg levels, rat | Acute iv, acute oral; sequential (24 hours) | No effect on liver Hg from MeHg in Pb-pretreated group versus MeHg-alone group | Additive: no effect | Congiu et al. 1979<br>Lead nitrate, Methylmercuric chloride |

Hg = mercury; iv = intravenous; MeHg = methylmercury; Pb = lead; sc = subcutaneous

**Table 10. Effect of Methylmercury on Toxicity and Tissue Concentrations of Lead**

| Endpoint, species | Duration, route for MeHg, Pb; sequence (interval) | Results | Conclusions | Reference, chemicals |
|---|---|---|---|---|
| Renal: proximal tubular and glomerular damage, ducks | Intermediate, oral; simultaneous | Histopathological and ultrastructural changes in proximal tubules of mixture group somewhat more severe than for each chemical alone at same dose as in mixture, thickening of glomerular membrane same in mixture and single chemical groups | Additive and <additive? | Prasad Rao et al. 1989a, 1989b Methylmercuric chloride, Lead acetate |
| Renal: weight, mouse | Acute oral, acute sc; simultaneous | Increased absolute (but not relative) kidney weight from mixture, but not from either chemical alone at same dose as in mixture | Additive? | Belles et al. 2002 Methylmercuric chloride, Lead nitrate |
| Hepatic: weight, mouse | Acute oral, acute sc; simultaneous | Increased absolute and relative liver weight from mixture, but not from either chemical alone at same dose as in mixture | Additive? | Belles et al. 2002 Methylmercuric chloride, Lead nitrate |
| Renal and hepatic: metallothionein, duck | Intermediate, oral; simultaneous | Metallothionein increased to same extent with the mixture as with each chemical alone at same dose as in mixture | <additive?: for metallothionein induction | Jordan et al. 1990; Prasad Rao et al. 1989a Methylmercuric chloride, Lead acetate |
| Death, mouse, pregnant | Acute oral, acute sc; simultaneous | Maternal mortality slightly but significantly greater in MeHg+Pb than in MeHg group, and in both these groups relative to controls; no maternal deaths in Pb group; dose of each chemical in mixture same as when given alone | Additive? | Belles et al. 2002 Methylmercuric chloride, Lead nitrate |
| Placental and fetal: Pb levels, mouse | Acute oral, acute sc; simultaneous | No difference in placental Pb between mixture group and Pb-alone group; Pb not detectible in fetuses of either group | Additive: no effect | Belles et al. 2002 Methylmercuric chloride, Lead nitrate |
| Renal: Pb levels, duck | Intermediate, oral; simultaneous | Renal Pb twice as high for mixture as for Pb alone at same dose as in mixture, but not statistically different | Additive (no effect) or >additive? | Prasada Rao et al. 1989a Methylmercuric chloride, Lead acetate |

MeHg = methylmercury; Pb = lead; sc = subcutaneous

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

The chlorpyrifos, lead, mercury, and methylmercury mixture is of concern because children may be co-exposed to these chemicals in their indoor and outdoor environments and through their diet. Exposure of the developing fetus to chlorpyrifos, lead, and methylmercury occurs through transplacental transfer, and infants can be exposed to all four chemicals through breast milk. The expected durations of exposures are primarily intermediate to chronic. No epidemiological or toxicological studies of the complete mixture are available. No PBPK models are available for the complete mixture or for any of the submixtures. Some information and studies are available for binary mixtures of the components, but they are not adequate to support a quantitative assessment of interactions. Therefore, the WOE approach is appropriate (ATSDR 2001a, 2001b) to predict the potential impact of interactions. This approach involves determining, for each binary mixture, the weight of evidence for the influence of one component on the toxicity of the other, and vice versa.

The binary weight-of-evidence (BINWOE) classification scheme is summarized in Figure 1. This figure gives a general idea of the approach, which rates confidence in the predicted direction of interaction according to the quality of the data. The direction of interaction is predicted from the available mechanistic and toxicological data. The quality of the data, as it pertains to prediction of direction of interaction, is classified by the main data quality factors for *mechanistic understanding* and *toxicological significance*. If concerns regarding the applicability of the data are not completely addressed under the main data quality factors, they can be addressed by the use of the *modifiers*. More detailed guidance is given in ATSDR guidance documents (ATSDR 2001a, 2001b). Rationales for the BINWOE determinations are presented in the tables at the end of this section. The BINWOE determinations are presented for the binary mixtures in the same order as these mixtures were considered in Section 2.2.

As discussed in the introduction to this interaction profile, and further detailed for each chemical in the appendices, the endpoint of particular interest for BINWOE determination is neurological, and the subpopulations of greatest concern are fetuses, infants, and young children. In addition, the influence of the other mixture components on the renal toxicity of inorganic mercury is assessed.

The predicted directions of interaction, presented in the same order as in the BINWOE rationale tables at the end of this section, are as follows:

- chlorpyrifos on lead neurological toxicity—less than additive with medium low confidence;
- lead on chlorpyrifos neurological toxicity—less than additive with medium confidence;
- chlorpyrifos on mercury renal toxicity—less than additive with low confidence;
- mercury on chlorpyrifos neurological toxicity—less than additive with medium low confidence;
- chlorpyrifos on methylmercury neurological toxicity—greater than additive with low confidence;
- methylmercury on chlorpyrifos neurological toxicity—less than additive with medium low confidence;
- lead on mercury renal toxicity—greater than additive with low confidence;
- mercury on lead neurological toxicity—indeterminate;
- lead on methylmercury neurological toxicity—additive with medium low confidence; and
- methylmercury on lead neurological toxicity—additive with medium confidence.

**Figure 1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions***

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

* Adapted from: ATSDR 2001a, 2001b

Table 11. Effect of **Chlorpyrifos** on **Lead**: Neurological Toxicity

**BINWOE: <IIIB**

*Direction of Interaction* - The direction of interaction is expected to be less than additive, based on the data suggesting lead has less-than-additive or additive joint action with dimethoate in electrophysiological studies (Nagymajtenyi et al. 1998, 2000b), and supporting evidence from studies with dimethoate on immunotoxicity and body weight endpoints (Institoris et al. 1999).

*Mechanistic Understanding* - Joint action data relevant to mechanisms of a potential influence of chlorpyrifos (or other similar organophosphorous insecticide) on lead toxicity were not located. Chlorpyrifos is a phosphorothioate organophosphorus insecticide that is metabolically activated through oxidative desulfuration to chlorpyrifos oxon by cytochrome P450. Chlorpyrifos oxon binds to acetylcholinesterase, inhibiting its ability to hydrolyze the neurotransmitter acetylcholine. The resulting accumulation of acetylcholine at the nerve endings causes continual neurological stimulation. Lead also is a neurotoxin, with potential mechanisms of action that include acting as a calcium agonist in a number of processes, and altering neurotransmitter systems including dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid systems (ATSDR 2005). Thus, it is conceivable that lead and chlorpyrifos together might have a greater impact on neurological functioning than either chemical alone, but the mode of joint action is unclear. The appropriate rating for mechanistic understanding is III.

*Toxicological Significance* - In a rat neurodevelopmental study of simultaneous oral exposure to lead and dimethoate (a phosphorodithioate) in which the dams were treated by gavage during gestation and lactation, followed by direct treatment of the offspring for 8 weeks, the joint toxic action of these agents on electrocorticograms and evoked potentials appeared to be additive or less than additive (Nagymajtenyi et al. 1998). The study design and lack of rigorous statistical analysis preclude more definitive conclusions, and there were no effects on brain cholinesterase or clinical signs. A similar study in rats treated starting as young adults for 4–12 weeks with lead and dimethoate reported similar results, with apparent less-than-additive activity in the two data examples provided (Nagymajtenyi et al. 2000b). In a study of immunotoxicity, gavage treatment of 4-week-old rats with lead and dimethoate for a 28-day period protected against the inhibition of humoral and cellular immune response seen with either chemical alone (Institoris 1999). Thus, data regarding the influence of chlorpyrifos on lead toxicity provides some evidence of less-than-additive joint action in studies with a similar organophosphorus insecticide, and the neurological effects data are toxicologically relevant. Because of the use of data for a similar chemical as the basis for the prediction of less than additive, a rating of B is appropriate.

Table 12. Effect of **Lead** on **Chlorpyrifos**: Neurological Toxicity

**BINWOE: <IIB**

*Direction of Interaction* - The direction of interaction is expected to be less than additive, based on the data suggesting that lead protects against cholinergic toxicity of methyl parathion (Hapke et al. 1978) and has less-than-additive or additive joint action with dimethoate in electrophysiological studies (Nagymajtenyi et al. 1998, 2000b), together with the evidence that lead can catalyze the hydrolysis of similar phosphorothioates, including methyl chlorpyrifos, to inactive compounds (Smolen and Stone 1997), and supporting evidence from studies with dimethoate on immunotoxicity and body weight endpoints (Institoris et al. 1999).

*Mechanistic Understanding* - Chlorpyrifos is a phosphorothioate organophosphorus insecticide that is metabolically activated through oxidative desulfuration to chlorpyrifos oxon by cytochrome P450. Chlorpyrifos oxon binds to acetylcholinesterase, inhibiting its ability to hydrolyze the neurotransmitter acetylcholine. The resulting accumulation of acetylcholine at the nerve endings causes continual neurological stimulation. The related phosphorothioate methyl chlorpyrifos and its oxon are hydrolyzed to non-cholinesterase-inhibiting compounds by lead *in vitro* at pHs in the range of about 4.5–7.3 (Smolen and Stone 1997). Other related phosphorothioates, methyl parathion and ronnel, also are hydrolyzed to inactive compounds by lead *in vitro* (Smolen and Stone 1997). This mechanism, if it occurs with chlorpyrifos and chlorpyrifos oxon *in vivo*, would be protective against the toxicity of chlorpyrifos. In addition, oral pretreatment of young adult rats for 3 months with lead in their drinking water, followed by a single oral dose of methyl parathion or methyl paraoxon, resulted in increased urinary excretion of a organophosphorus breakdown product that is inactive in cholinesterase inhibition (Hapke et al. 1978). A study involving gavage pretreatment of 3-day-old rats with lead for 4 weeks, followed by a gavage dose of a less similar organophosphorus insecticide, the phosphorodithioate malathion, did not detect any differences in urinary excretion of breakdown products (Abd-Elraof et al. 1981). Placing greater confidence in the studies with the phosphorothioates leads to the conclusion that lead may inhibit the toxicity of chlorpyrifos through a chemical interaction leading to increased break down of chlorpyrifos to compounds that are not cholinesterase inhibitors. Because this mechanism of interaction is inferred from similar chemicals, a rating of II is chosen for mechanistic understanding.

*Toxicological Significance* - In a rat neurodevelopmental study of simultaneous oral exposure to lead and dimethoate (a phosphorodithioate) in which the dams were treated by gavage during gestation and lactation, followed by direct treatment of the offspring for 8 weeks, the joint toxic action of these agents on electrocorticograms and evoked potentials appeared to be additive or less than additive (Nagymajtenyi et al. 1998). The study design and lack of rigorous statistical analysis preclude more definitive conclusions, and there were no effects on brain cholinesterase or clinical signs. A similar study in rats treated starting as young adults for 4–12 weeks with lead and dimethoate reported similar results, with apparent less-than-additive activity in the two data examples provided (Nagymajtenyi et al. 2000b). Pretreatment of young adult rats for 3 months with lead in their drinking water, followed by a single oral dose of methyl parathion (phosphorothioate) ameliorated the acute signs of cholinesterase inhibition due to the insecticide (Hapke et al. 1978). In a study of immunotoxicity, gavage treatment of 4-week-old rats with lead and dimethoate for a 28-day period protected against the inhibition of humoral and cellular immune response seen with either chemical alone (Institoris 1999). In addition, lead protected against depressed body weight resulting from dimethoate exposure. Thus, the weight of evidence for toxicological significance supports a prediction of less than additive, and is given a rating of B to reflect evidence from similar chemicals.

Table 13. Effect of **Chlorpyrifos** on **Mercury**: Renal Toxicity

**BINWOE: <IIIC**

*Direction of Interaction* - The direction of interaction is predicted to be less than additive, based on results for a related organophosphorus insecticide administered orally with mercuric chloride to rats in an intermediate-duration study of immunotoxicity (Institoris et al. 1999), in which joint toxic action appeared less than additive for humoral response, and was indeterminate for cellular response.

*Mechanistic Understanding* - Chlorpyrifos is a phosphorothioate organophosphorus insecticide that is metabolically activated through oxidative desulfuration to chlorpyrifos oxon by cytochrome P450. Chlorpyrifos oxon binds to acetylcholinesterase, inhibiting its ability to hydrolyze the neurotransmitter acetylcholine. The resulting accumulation of acetylcholine at the nerve endings causes continual neurological stimulation. Chlorpyrifos is not known to affect the kidneys. Inorganic mercury's critical effect is renal damage (ATSDR 1999) or renal damage mediated through autoimmune effects (IRIS 2004). Neurological or neurodevelopmental effects of mercury are far less sensitive, presumably because inorganic (mercuric) mercury does not readily pass the blood-brain or placental barriers. Mechanisms whereby chlorpyrifos could affect mercury toxicity are not known. Therefore, mechanistic understanding does not lead to a prediction of interaction direction, leading to a classification of III.

*Toxicological Significance* - Joint toxic action studies of chlorpyrifos and mercury were not available. Some relevant information can be extracted from a study of the joint toxic action of mercuric chloride with dimethoate, a phosphorodithioate organophosphorus insecticide that, like chlorpyrifos, is activated through metabolic desulfuration and produces neurological effects through acetylcholinesterase inhibition. This study focused on effects of intermediate duration, oral (gavage) administration of dimethoate and mercuric chloride to rats on indices of humoral and cellular immune response (Institoris et al. 1999). Results suggested that the mixtures (high dose of mercury component with low dose of dimethoate and vice versa) were less inhibitory to humoral response than either high-dose component alone at the same dose as in the mixture. Cellular response data were not reported adequately, but suggested slightly greater inhibition from the mixture than from either high-dose component alone. Whether the greater inhibition of cellular response reflects additivity, or less than or greater than additivity, cannot be even tentatively determined because the data were incompletely reported. Inconsistent results across experiments within the study and inadequate reporting of statistical analyses and of some of the data limit the confidence in this study. The direction of interaction appeared to be less than additive for humoral response. For cellular response, the direction cannot be determined, but the results give no strong indication of potentiation or synergism, and may be consistent with additive or less-than-additive joint action. The weight of evidence weakly supports less than additivity. Immunological findings are relevant to the critical effect of inorganic mercury, because sensitive renal effects may be mediated through an autoimmune mechanism. The appropriate classification, given the ambiguity in the data, and the use of data for a related chemical, is C.

Table 14. Effect of **Mercury** on **Chlorpyrifos**: Neurological Toxicity

**BINWOE: <IIC**

*Direction of Interaction* - The direction is predicted to be less than additive, based on mechanistic data that indicate mercury may catalyze the hydrolytic inactivation of chlorpyrifos (Wan et al. 1994; Zeinali and Torrents 1998).

*Mechanistic Understanding* - Chlorpyrifos is a phosphorothioate organophosphorus insecticide that is metabolically activated through oxidative desulfuration to chlorpyrifos oxon by cytochrome P450. Chlorpyrifos oxon binds to acetylcholinesterase, inhibiting its ability to hydrolyze the neurotransmitter acetylcholine. The resulting accumulation of acetylcholine at the nerve endings causes continual neurological stimulation. Inorganic mercury's critical effect is renal damage (ATSDR 1999) or renal damage mediated through autoimmune effects (IRIS 2004). Neurological or neurodevelopmental effects of inorganic (mercuric) mercury are far less sensitive, presumably because mercuric mercury does not readily pass the blood-brain or placental barriers. Inorganic (mercuric) mercury reacts in aqueous solutions (pH 3.5–7.5) with other phosphorothioate organophosphorus insecticides (methyl parathion, fenitrothion, fenthion) and with phosphorodithioate organophosphorus insecticide (malathion) to catalyze hydrolytic inactivation of these compounds (Wan et al. 1994; Zeinali and Torrents 1998). This type of reaction would be protective against the toxicity of chlorpyrifos, but whether it occurs in other media, or in the body following co-exposure to mercury and chlorpyrifos, is not known. Because understanding of a potential mechanism of interaction comes from studies with related chemicals, a rating of II for mechanistic understanding is appropriate.

*Toxicological Significance* - Joint toxic action studies of mercury and chlorpyrifos were not available. Studies with diazinon (phosphorothioate) and dimethoate (phosphorodithioate) provide limited information on interactions of mercury with similar chemicals. Diazinon and dimethoate are organophosphorus insecticides that, like chlorpyrifos, are activated through metabolic desulfuration and produce neurological effects through acetylcholinesterase inhibition. A study in calves that were injected intravenously with mercuric chloride at a dose sufficient to cause renal damage, and 5 days later dosed orally with diazinon, reported an increased severity of cholinergic signs and decreased cholinesterase activity of blood and brain in the mercury-pretreated calves as compared with non-pretreated calves (Abdelsalam and Ford 1987). Very few calves (n=2–3/group) were studied, and the induction of severe kidney damage by an intravenous mercury pretreatment is of questionable relevance to environmental exposure. The other study of joint toxic action focused on effects of intermediate duration, oral (gavage) administration of dimethoate and mercuric chloride to rats on indices of humoral and cellular immune response (Institoris et al. 1999). Results suggested that the mixtures (high dose of mercury component with low dose of dimethoate and vice versa) were less inhibitory to humoral response than either high-dose component alone at the same dose as in the mixture. Cellular response data were not reported adequately, but suggested slightly greater inhibition from the mixture than from either high-dose component alone. Whether the greater inhibition of cellular response reflects additivity, or less than or greater than additivity, cannot be even tentatively determined because the data were incompletely reported. Inconsistent results across the experiments in this study, inadequate reporting of statistical analyses, and lack of corroborating information that dimethoate (or chlorpyrifos) are immunotoxic, limit the confidence that can be placed in this study. In addition, the relevance of the study to the neurotoxicity of chlorpyrifos is uncertain. Thus, the available information on joint toxic action are not consistent, appear to be of marginal relevance, and are not suitable as the basis for a conclusion. The mechanistic data regarding mercury-catalyzed hydrolytic inactivation of a related compound suggest that mercury may have a protective effect against chlorpyrifos neurotoxicity, but because of the ambiguous joint toxic action data, the appropriate classification is C.

Table 15. Effect of **Chlorpyrifos** on **Methylmercury**: Neurotoxicity

**BINWOE: >IIIC**

*Direction of Interaction* - The direction of interaction is expected to be greater than additive, based on a more rapid initial uptake and longer retention of mercury in the amphipod *H. azteca* (a small freshwater crustacean) following exposure to chlorpyrifos and methylmercury than to methylmercury alone (Steevens and Benson 1999, 2001). Acute lethality data fit an additive model, but this result appeared due to an increase in mercury accumulation and a decrease in chlorpyrifos toxicity.

*Mechanistic Understanding* - Chlorpyrifos and methylmercury, incubated in aqueous solution, formed a Hg-containing complex that was more polar than the starting compound, suggesting inactivation of chlorpyrifos through hydrolysis (Steevens and Benson 1999, 2001). When *H. azteca* were exposed to chlorpyrifos and methylmercury in water, the initial rate of mercury uptake was higher than for methylmercury alone. Following transfer to contaminant-free water, mercury was retained in the organisms exposed to the mixture, but gradually was eliminated from organisms exposed to methylmercury alone (Steevens and Benson 2001). Thus, chemical interaction could result in faster absorption and greater retention of mercury, leading to a potentiation of methylmercury toxicity. Because the understanding of these mechanisms is incomplete, and is for aquatic organisms absorbing the chemicals from the water they live in (a scenario that may not be a good model for human exposure and absorption), a classification of III is appropriate.

*Toxicological Significance* - Chlorpyrifos and methylmercury contributed to mortality of *H. azteca* in an additive manner (Steevens and Benson 2001). This finding appeared to be the result of an increase in methylmercury toxicity and a decrease in chlorpyrifos toxicity, due to chemical interaction to form a mercury-containing complex that was more polar than the starting compounds, with consequent greater accumulation/retention of mercury in the organisms, but decreased acetylcholinesterase inhibition (Steevens and Benson 1999, 2000). The toxicological significance is uncertain, because the conclusion is based in part on mercury absorption and retention, as well as the inference that mercury toxicity must be increasing in order for the mixture to behave additively when chlorpyrifos toxicity is decreasing. In addition, the absorption/retention of mercury observed in aquatic crustaceans exposed to these compounds or their chemical interaction product in their aqueous environment may not be a good model for human exposure and absorption/retention, and it is uncertain whether the retained mercury was in a form that would be neurotoxic. Therefore, the appropriate rating for toxicological significance is C.

Table 16. Effect of **Methylmercury** on **Chlorpyrifos**: Neurotoxicity

**BINWOE: <IIIB**

*Direction of Interaction* - The direction of interaction is expected to be less than additive, based on a decreased acetylcholinesterase inhibition in the amphipod *H. azteca* (a small freshwater crustacean) following exposure to chlorpyrifos and methylmercury as compared with chlorpyrifos alone, which appears to be the result of a chemical interaction between methylmercury and chlorpyrifos to form a mercury-containing complex containing a hydrolyzed residue of chlorpyrifos (Steevens and Benson 1999, 2001). Acute lethality appeared additive, but this result appeared due to an increase in mercury accumulation and a decrease in chlorpyrifos toxicity.

*Mechanistic Understanding* - Chlorpyrifos is a phosphorothioate organophosphorus insecticide that is metabolically activated through oxidative desulfuration to chlorpyrifos oxon by cytochrome P450. Chlorpyrifos oxon binds to acetylcholinesterase, inhibiting its ability to hydrolyze the neurotransmitter acetylcholine. The resulting accumulation of acetylcholine at the nerve endings causes continual neurological stimulation. Chlorpyrifos has the same mechanism of toxicity across a broad range of animal species. Chlorpyrifos and its oxon can be inactivated by hydrolysis. Chlorpyrifos and methylmercury, incubated in aqueous solution, formed an Hg-containing complex that was more polar than the starting compound, suggesting the chlorpyrifos moiety had been hydrolyzed (Steevens and Benson 1999, 2001). Further identification and characterization of the complex was unsuccessful, but toxicological findings showed decreased acetylcholinesterase inhibition from exposure of *H. azteca* to the mixture than to chlorpyrifos alone. Thus, understanding of the mechanism is incomplete, but suggestive of inhibition of chlorpyrifos neurotoxicity by methylmercury. Therefore, a classification of III is appropriate.

*Toxicological Significance* - Chlorpyrifos and methylmercury contributed to mortality of *H. azteca* in an additive manner (Steevens and Benson 2001). This finding appeared to be the result of an increase in methylmercury toxicity and a decrease in chlorpyrifos toxicity, due to chemical interaction to form a mercury-containing complex that was more polar than the starting compounds, suggesting hydrolysis of the chlorpyrifos moiety. The accumulation/retention of mercury in the organisms exposed to the mixture was greater than in those exposed to methylmercury alone, but acetylcholinesterase inhibition was less severe in organisms exposed to the mixture than in those exposed to chlorpyrifos alone due to the apparent hydrolytic inactivation of chlorpyrifos (Steevens and Benson 1999, 2000). Thus, co-exposure to methylmercury inhibited the toxicity of chlorpyrifos. Acetylcholinesterase inhibition in crustaceans is toxicologically relevant to humans. Whether the chemical interaction leading to chlorpyrifos inactivation seen in aqueous solution, and the outcome in crustaceans exposed to these compounds or their chemical interaction product in their aqueous environment prior to absorption, is relevant to human exposure scenarios is unclear. Therefore, an appropriate classification is B.

Table 17. Effect of **Lead** on **Mercury**: Renal Toxicity

**BINWOE: >IIIC**

*Direction of Interaction* - The direction is predicted to be greater than additive, based on an acute, simultaneous intravenous injection study of renal tubular necrosis in rats (Schubert et al. 1978). The database, consisting largely of injection studies, does not support an unambiguous prediction, and is of questionable relevance to the exposure scenario of concern; therefore, confidence is low.

*Mechanistic Understanding* - Inorganic mercury's critical effect is renal damage (ATSDR 1999) or renal damage mediated through autoimmune effects (IRIS 2004). Neurological or neurodevelopmental effects of mercury are far less sensitive, presumably because inorganic (mercuric) mercury does not readily pass the blood-brain or placental barriers. The critical effect of lead is neurological; lead also can cause renal damage, but this is not a sensitive effect of lead. Mechanisms of joint toxic action of lead and mercury on the kidney are not known. An acute oral simultaneous exposure study in mice detected no difference in renal mercury concentrations for mice exposed to lead and mercuric chloride as compared with mercuric chloride alone at the same dose as in the mixture (Sin et al. 1985). Similar, but sequential administration of lead followed 24 hours later by mercury decreased renal mercury concentrations in mice (Sin et al. 1985). A mechanism suggested (though not investigated) for a protective effect of lead against subsequent challenge with mercury is that lead induces but does not bind to metallothionein, which then may bind mercury and sequester it (Yoshikawa and Hisayoshi 1982). This mechanism may help to explain the results of the sequential injection studies, but may not be relevant to simultaneous exposure, as indicated by a lack of effect of lead administered orally and simultaneously with mercury on the distribution of mercury to the kidney. In addition, relevance to long-term simultaneous exposure is questionable. Therefore, a classification of III is selected for mechanistic understanding.

*Toxicological Significance* - An acute, simultaneous intravenous injection study in rats, using approximate $LD_1$ doses of lead acetate and mercuric chloride, singly and combined, reported acute renal tubular necrosis following the mixture, whereas mercury alone caused no renal lesions and lead alone caused minimal renal tubular changes (Schubert et al. 1978). The lesions in the mixture group were similar to those seen from a higher intravenous dose of mercury alone. In the same simultaneous injection study, determination of the intravenous $LD_{50}$ for mercury in the presence of a constant intravenous dose ($<LD_1$) of lead indicated a greatly decreased lethal potency of mercury in the presence of lead. Sequential injection studies in mice, however, have provided evidence of a protective effect of lead pretreatment on the renal toxicity and lethality of mercury. A decrease in renal toxicity (assessed by BUN) was observed in mice injected intravenously with lead acetate 48 hours prior to an intraperitoneal injection of mercuric chloride, as compared with mercuric chloride alone (Ewald and Calabrese 2001). Two acute sequential intraperitoneal injection studies have reported decreased mortality from mercuric mercury due to lead pretreatment of mice (Garber and Wei 1972; Yoshikawa and Hisayoshi 1982). Thus, the data are ambiguous. The relevance of the injection route-single dose data and of lethality data are questionable to intermediate or chronic oral exposure, because injection bypasses potential interactions during absorption from the gastrointestinal tract, and single acute doses do not allow induction and other processes to reach steady state. In predicting the direction of interaction, greater weight is given to the simultaneous intravenous injection study of renal toxicity than to sequential studies and lethality studies. Accordingly, the direction is predicted to be greater than additive. Because of the ambiguity in the data, and the concerns regarding the relevance of acute intravenous data to intermediate or chronic oral exposure, the appropriate classification is C.

Table 18. Effect of **Mercury** on **Lead**: Neurotoxicity

**BINWOE: ?**

*Direction of Interaction* - The direction cannot be predicted from the available data, which are of questionable relevance to lead neurotoxicity.

*Mechanistic Understanding* - Inorganic mercury's critical effect is renal damage (ATSDR 1999) or renal damage mediated through autoimmune effects (IRIS 2004). Neurological or neurodevelopmental effects of mercury are far less sensitive, presumably because inorganic (mercuric) mercury does not readily pass the blood-brain or placental barriers. The critical effect of lead is neurological; lead also can cause renal damage, but this is not a sensitive effect of lead. Mechanisms relevant to the effect of mercury on lead's neurotoxicity are not known. Studies of mercury's potential effect on the distribution of lead to the brain or other organs were not located. Therefore, mechanistic understanding does not lead to a prediction of interaction direction.

*Toxicological Significance* - An acute, simultaneous intravenous injection study in rats, using approximate $LD_1$ doses of lead acetate and mercuric chloride, singly and combined, reported acute renal tubular necrosis following the mixture, whereas mercury alone caused no renal lesions and lead alone caused minimal renal tubular changes (Schubert et al. 1978). The lesions in the mixture group, however, were similar to those seen from a higher intravenous dose of mercury alone. In the same simultaneous injection study, determination of the intravenous $LD_{50}$ for lead in the presence of a constant intravenous dose ($LD_{20}$) of mercury, indicated a greatly increased lethal potency of lead in the presence of toxic doses of mercury. In two studies, acute, sequential injection of mercuric mercury followed by lead into mice indicated that mercury pretreatment had no effect on the lethality of a subsequent challenge dose of lead (Garber and Wei 1972; Yoshikawa and Hisayoshi 1982). The data are conflicting, and their toxicological relevance to the influence of mercury on lead's neurological toxicity is questionable. Thus, the available data do not support the prediction of direction of interaction.

Table 19. Effect of **Lead** on **Methylmercury**: Neurological Toxicity

**BINWOE: =IIIC**

*Direction of Interaction* - The direction is predicted to be additive based on a lack of influence of lead on the distribution of methylmercury to the placenta and fetus in a simultaneous acute exposure study in pregnant mice (Belles et al. 2002), and the absence of strong indications of deviations from additivity on toxicity endpoints in simultaneous exposure studies in mice and ducks (Belles et al. 2002; Prasada Rao et al. 1989a, 1989b). A sequential acute study in rats, however, suggested potentiation of methylmercury lethality by lead pretreatment (Congiu et al. 1979), lessening confidence in the assessment of direction from the toxicity data.

*Mechanistic Understanding* - Both lead and methylmercury are neurotoxic. Mechanisms of neurotoxicity for both chemicals are complex and not fully understood. Tissue distribution studies indicated that simultaneous subcutaneous injection of lead and oral administration of methylmercury to mice on day 10 of gestation did not affect the distribution of mercury to the placenta or fetus. Simultaneous intermediate-duration oral exposure of Pekin ducks to lead acetate and methylmercuric chloride in their diet increased renal and hepatic metallothionein to the same extent for the mixture as for each chemical alone at the same dose as in the mixture (Jordan et al. 1990; Prasad Rao et al. 1989a). Lead did not affect the mercury concentration in the liver or kidney (Jordan et al. 1990; Prasad Rao et al. 1989a). Administration of lead by intravenous injection followed 24 hours later by oral methylmercury to rats did not affect distribution of mercury to the liver, but increased renal mercury concentrations as compared with rats given methylmercury alone (Congiu et al. 1979). Distribution to the brain was not investigated in any of these studies. The lack of influence of lead on placental and fetal concentrations of mercury (from methylmercury) provides some mechanistic evidence of a lack of effect of lead on methylmercury that may be relevant to developmental neurotoxicity. An appropriate rating for mechanistic understanding is III.

*Toxicological Significance* - Simultaneous treatment of pregnant mice with a subcutaneous injection of lead nitrate and gavage administration of methylmercuric chloride on day 10 of gestation resulted in slightly but significantly more maternal deaths (3/14) than methylmercury alone (1/12) at the same dose as in the mixture (Belles et al. 2002). No maternal deaths occurred from lead alone at the same dose as in the mixture had no effects. Liver and kidney weights were increased by the mixture but not by either chemical alone. Fetotoxic effects (decreased fetal weight, increased cleft palate and skeletal defects) were the same in the mixture and methylmercury-alone groups and did not occur in the lead-alone group. These results do not provide definitive information regarding the mode of joint action, and in general, indicate little or no effect of lead on the toxicity of methylmercury. Neurobehavioral endpoints were not investigated. Simultaneous intermediate-duration oral exposure of Pekin ducks to lead acetate and methylmercuric chloride in their diet produced somewhat more marked histopathological and ultrastructural changes in the renal proximal tubules than either chemical alone at the same dose as in the mixture, but thickening of the glomerular membrane was the same for the mixture and the individual chemicals (Prasada Rao et al. 1989a, 1989b). Lead injected intravenously into rats 24 hours before oral administration of a challenge dose of methylmercury resulted in higher mortality than methylmercury alone at the same dose (Congiu et al. 1979). The most relevant information, from the simultaneous exposure studies in mice and ducks, does not indicate strong potentiation or synergism, and may be consistent with additivity, given that the total chemical dose in the mixture groups is higher than in the corresponding single chemical groups. Neurobehavioral effects on the fetus, infant, or young child are the critical effects of methylmercury; tissue analyses during the developmental toxicity study in mice showed no effect of lead on placental or fetal concentrations of mercury from methylmercury. Therefore, the direction of joint action is predicted to be additive, but the lack of joint action data for the endpoint of concern and ambiguity in the data reduce the toxicological significance rating to C.

Table 20. Effect of **Methylmercury** on **Lead**: Neurological Toxicity

**BINWOE: =IIIC**

*Direction of Interaction* - The direction is predicted to be additive based on a lack of influence of methylmercury on the distribution of lead to the placenta and fetus in a simultaneous acute exposure study in pregnant mice (Belles et al. 2002), and the absence of strong indications of deviations from additivity on toxicity endpoints in simultaneous exposure studies in mice and ducks (Belles et al. 2002; Prasada Rao et al. 1989a, 1989b).

*Mechanistic Understanding* - Both lead and methylmercury are neurotoxic. Mechanisms of neurotoxicity for both chemicals are complex and not fully understood. Tissue distribution studies indicated that simultaneous subcutaneous injection of lead and oral administration of methylmercury to mice on day 10 of gestation did not affect the distribution of lead to the placenta, and did not increase distribution of lead to detectible levels in the fetus. Simultaneous intermediate-duration oral exposure of Pekin ducks to lead acetate and methylmercuric chloride in their diet increased renal and hepatic metallothionein to the same extent for the mixture as for each chemical alone at the same dose as in the mixture (Jordan et al. 1990; Prasad Rao et al. 1989a). Methylmercury did not significantly affect the lead concentration in the kidney (Prasad Rao et al. 1989a). Distribution to the brain was not investigated in any of these studies. The lack of influence of methylmercury on placental and fetal concentrations of lead provides some mechanistic evidence of a lack of effect of lead on methylmercury that may be relevant to developmental neurotoxicity. An appropriate rating for mechanistic understanding is III.

*Toxicological Significance* - Simultaneous treatment of pregnant mice with a subcutaneous injection of lead nitrate and gavage administration of methylmercuric chloride on day 10 of gestation resulted in slightly but significantly more maternal deaths (3/14) than methylmercury alone (1/12) at the same dose as in the mixture (Belles et al. 2002). No maternal deaths occurred from lead alone at the same dose as in the mixture. Liver and kidney weights were increased by the mixture, but not by either chemical alone. Fetotoxic effects (decreased fetal weight, increased cleft palate and skeletal defects) were the same in the mixture and methylmercury-alone groups and did not occur in the lead-alone group. These results do not provide definitive information regarding the mode of joint action, and in general, indicate little or no effect of methylmercury on the toxicity of lead. Neurobehavioral endpoints were not investigated. Simultaneous intermediate-duration oral exposure of Pekin ducks to lead acetate and methylmercuric chloride in their diet produced somewhat more marked histopathological and ultrastructural changes in the kidneys than either chemical alone at the same dose as in the mixture, but thickening of the glomerular membrane was the same for the mixture and the individual chemicals (Prasada Rao et al. 1989a, 1989b). The most relevant information, from the simultaneous exposure studies in mice and ducks, does not indicate strong potentiation or synergism, and may be consistent with additivity, given that the total chemical dose in the mixture groups is higher than in the corresponding single chemical groups. Neurobehavioral effects on the fetus, infant, and young child are the critical effects of lead; tissue analyses during the developmental toxicity study in mice showed no effect of mercury on placental or fetal concentrations of lead. Therefore, the direction of joint action is predicted to be additive, but the lack of joint action data for the endpoint of concern reduces the toxicological significance rating to C.

## 2.4 Recommendations for Data Needs

The mixture of chlorpyrifos, lead, and mercury/methylmercury was chosen as the subject of this interaction profile because co-exposure to components of this mixture is likely, and because of concerns for its potential neurological impact on the developing fetus, infant, and young child. Neither *in vivo* data from human or animal studies nor *in vitro* data examining the toxicity of the chlorpyrifos, lead, mercury, and methylmercury are available. In addition, no pertinent studies are available for the three-component sub-mixture of particular concern for neurological effects (chlorpyrifos, lead, and methylmercury) in the fetus, infant, and young child. Similarly, PBPK models describing the behavior of the mixture or the three- or two-component sub-mixtures are not available. In the absence of data for the complete mixture, a component-based approach was recommended. However, mechanistic or toxicological data pertinent to the influence of inorganic mercury on lead's neurological toxicity are lacking. Data for some of the other binary mixtures are ambiguous or of limited relevance to the endpoints of concern or to likely exposure scenarios, leading to low (IIIC) or medium low (IIIB and IIC) confidence ratings for some of the predictions of interactions, as detailed in the BINWOE classifications derived in the previous section, and summarized in the BINWOE matrix in Chapter 3. It should be further noted that some BINWOEs pertinent to Chlorpyrifos are derived by analogy to other pesticides with similar mechanism of action. More interaction studies are needed to properly evaluate this mixture.