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INTERACTION PROFILE FOR: CHLORPYRIFOS, LEAD, MERCURY, AND METHYLMERCURY

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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program (NTP), initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found. The Food Quality Protection Act (FQPA) of 1996 requires that factors to be considered in establishing, modifying, or revoking tolerances for pesticide chemical residues shall include the available information concerning the cumulative effects of substances that have a common mechanism of toxicity, and combined exposure levels to the substance and other related substances. The FQPA requires that the Administrator of the Environmental Protection Agency (EPA) consult with the Secretary of the Department of Health and Human Services (which includes ATSDR) in implementing some of the provisions of the act.

To carry out these legislative mandates, ATSDR's Division of Toxicology (DT) has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists in collaboration with mixtures risk assessors and laboratory scientists have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

The public comment period ends March 31, 2005. Comments should be sent to:

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PEER REVIEW

A peer review panel was assembled for this profile. The panel consisted of the following members:

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All reviewers were selected in conformity with the conditions for peer review specified in CERCLA Section 104(I)(13).

Scientists from ATSDR have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

SUMMARY

Chlorpyrifos, lead, and mercury/methylmercury were chosen as the subject for this interaction profile because of the likelihood of co-exposure and because of concerns about neurological effects in children co-exposed to these chemicals. Chlorpyrifos is an organophosphorus insecticide widely used for agricultural and indoor and outdoor residential applications in the United States. Its use, however, is being phased out. Mercury (metallic and inorganic) and lead are released to the environment from hazardous waste sites and from mining, smelting, and industrial activities. Metallic and inorganic mercury can be transformed by microorganisms into methylmercury, which bioaccumulates in the food chain. For the general population, and particularly for subsistence fishers and hunters, the most important pathway of exposure to mercury is ingestion of methylmercury in foods. Fish (including tuna, a food commonly eaten by children), other seafood, and marine mammals contain the highest concentrations. Lead, present in the environment primarily as divalent lead compounds, also contaminates the environment due to its release from mining and from deteriorating lead paint and its historical use in gasoline.

No pertinent health effects data or physiologically-based pharmacokinetic (PBPK) models were located for the complete mixture. Therefore, as recommended by ATSDR (2001a) guidance, the exposure-based screening assessment of potential health hazards for this mixture depends on an evaluation of the health effects data and mechanistic data for the individual components and on the joint toxic action and mechanistic data for various combinations of the components. This profile discusses and evaluates the evidence for joint toxic action among binary mixtures of these chemicals. The profile also recommends how to incorporate concerns regarding possible interactions or additivity into public health assessments of people who may be exposed to mixtures of these chemicals.

The primary effect of concern for this mixture is neurological, and the sub-population of concern is children. Neurological effects are the critical effects for chlorpyrifos, lead, and methylmercury, and children are known (for lead and methylmercury) or predicted on the basis of animal studies (for chlorpyrifos) to be more sensitive than adults. Although metallic mercury causes neurological effects when inhaled, this route is of concern primarily for occupational exposure. Children may be exposed to metallic mercury if they play with it after finding it in abandoned warehouses or taking it from school laboratories. Broken thermometers and some electrical switches are other sources of metallic mercury. Some absorption of metallic mercury occurs from dental amalgam fillings, probably following volatilization from the fillings. Clear evidence of adverse effects from this pathway of exposure is lacking, as are joint action studies with the other components of this mixture, so this form of mercury is

not considered further in the interaction profile. The critical effect of inorganic mercury is on the kidney, which is not a sensitive target organ for the other components of the mixture.

Recommendations for screening this mixture for potential hazards to public health include estimating the hazard quotients (ratios of exposures to health guidance values) for the individual components. If only one or if none of the components has a hazard quotient that is at least 0.1, no further assessment of the *joint toxic action* is needed because additivity and/or interactions are unlikely to result in significant health hazard. If the hazard quotients for two or more of the mixture components equal or exceed 0.1, the following procedures are recommended. To screen this mixture for potential neurological health hazard, an endpoint-specific hazard index for neurological effects should be estimated for chlorpyrifos, lead, and methylmercury. The weight-of-evidence (WOE) analysis for interactions among these components indicates that joint toxic action is primarily less than additive or additive and, therefore, does not increase the concern for potential health hazard above that indicated by the hazard index. This diminishes the concern for hazard indexes only slightly above one. Confidence in these WOE analyses ranges from medium to medium-low. A separate hazard quotient is recommended to screen for the renal toxicity of inorganic mercury. The WOE analysis concluded that the influence on the renal toxicity of inorganic mercury by chlorpyrifos may be less than additive and by lead may be greater than additive, but confidence in these conclusions was low and, thus, they have little impact on the assessment of potential hazards.

If the neurological hazard index for chlorpyrifos, lead, and methylmercury is significantly greater than 1, or if the hazard quotient for inorganic mercury is greater than 1, then further evaluation is needed (ATSDR 2001a), using biomedical judgment and community-specific health outcome data. Community health concerns should be considered in further evaluations (ATSDR 1992).

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ALA	aminolevulinic acid
ALAD	aminolevulinic acid dehydratase
ALAS	delta-aminolevulinic acid synthetase
ATSDR	Agency for Toxic Substances and Disease Registry
BINWOE	binary weight-of-evidence
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control and Prevention
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act
Cpf	chlorpyrifos
DT	Division of Toxicology
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FQPA	Food Quality Protection Act
GABA	gamma-aminobutyric acid
Hg	mercury
HI	hazard index
IARC	International Agency for Research on Cancer
IEUBK	Integrated Exposure Uptake Biokinetic
ip	intraperitoneal
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
iv	intravenous
kg	kilogram
L	liter
LC_{50}	median lethal concentration (produces desired effect in 50% of the population)
LD_{50}	median lethal dose (produces desired effect in 50% of the population)
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
MeHg	methylmercury
mg	milligram
MRL	Minimal Risk Level
MTD	maximum threshold dose
NHANES	National Health and Nutrition Examination Survey
nM	nanomole
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTP	National Toxicology Program
OP	organophosphorus compound
OPP	Office of Pesticide Programs
PAD	population adjusted dose
2-PAM	pralidoxime
PFC	plaque-forming cells
Pb	lead
pbB	blood lead concentration
PBPK	physiologically based pharmacokinetic
PBPK/PD	physiologically-based pharmacokinetic/pharmacodynamic
ppb	parts per billion
ppm	parts per million
RfC	reference concentration
RfD	reference dose
sc	subcutaneous
TCP	3,5,6-trichloro-2-pyridinol
TTD	target-organ toxicity dose
µg	microgram
µmole	micromole
U.S.	United States
VOC	volatile organic compound
WHO	World Health Organization
WOE	weight-of-evidence
ZPP	zinc protoporphyrin
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to

1. Introduction

The primary purpose of this Interaction Profile for chlorpyrifos, mercury, methylmercury, and lead is to evaluate data on the toxicology of the "whole" mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture Minimal Risk Level (MRL), and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence (WOE) approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Division of Toxicology's (DT) recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.

The chlorpyrifos, lead, mercury/methylmercury mixture was chosen as the subject for this interaction profile based on a concern for neurological effects in children co-exposed to these chemicals. Chlorpyrifos is an organophosphorus insecticide. It is one of the most widely used insecticides for agricultural applications and indoor and outdoor residential applications in the United States (ATSDR 1997; EPA 2000a). Children may have been exposed to chlorpyrifos through the diet, drinking water, and exposure in the home, yard, schools, and parks where the chlorpyrifos may have been applied to lawns or indoor cracks and crevices, and used for whole-building termite treatments. A study of a small number of households in the Lower Rio Grande Valley concluded that indoor dust and air were the primary exposure media for the residents of those households, based on monitoring of those media, as well as outdoor soil and air, food, and a characteristic urinary metabolite of chlorpyrifos (used as a biomarker of exposure) (Buckley et al. 1997). Thus, exposure pathways in this limited study are likely to have been ingestion and

inhalation. A study of urinary pesticide metabolites during the third trimester in 386 pregnant women from East Harlem indicated that exposure to chlorpyrifos was prevalent (42% of the women had detectable levels of the chlorpyrifos metabolite). The study further showed that the exposure was higher than the median in National Health and Nutrition Examination Survey (NHANES) III, did not show seasonal variation, and did not change during the time period of the study (1998–2001) (Berkowitz et al. 2003). The exposure of these women was thought to be primarily indoor, due to household pesticide use and exterminator application, and also dietary. Under the Food Quality Protection Act (FQPA), the Environmental Protection Agency (EPA) and chlorpyrifos registrants have agreed to phase out or reduce many uses of chlorpyrifos that contribute to children's exposure (EPA 2000a, 2002a).

Lead is present in the environment primarily as divalent lead compounds (ATSDR 1999a). Mercury exists in the environment as metallic mercury, inorganic mercury compounds (primarily mercuric), and organic mercury compounds (primarily methylmercury) (ATSDR 1999b). Mercury and lead co-occur in completed exposure pathways at hazardous waste sites, most commonly from soil or water. The exposure pathway of concern from these media is oral.

Metallic and inorganic mercury also are released into the environment (primarily into air) from mining, smelting, industrial activities, combustion of fossil fuels, and natural processes (ATSDR 1999b). The metallic and inorganic mercury in air can be deposited to water and soil, where they are transformed by microorganisms into methylmercury, as are metallic and inorganic mercury from hazardous waste sites. Methylmercury bioaccumulates in the food chain, particularly in fish. For the general population, and particularly for subsistence fishers and hunters, the most important pathway of exposure to mercury is ingestion of methylmercury in foods, with fish (including tuna, a food commonly eaten by children), other seafood, and marine mammals containing the highest concentrations. Another source of exposure for the general population is the release of metallic mercury from dental amalgams. Infants can be exposed to inorganic mercury and methylmercury from breast milk, and the developing fetus can be exposed through transplacental transfer of metallic mercury and methylmercury.

Lead also is released into the environment from mining, smelting, and industrial activities (ATSDR 1999a). In addition, children are exposed to lead from deteriorating lead paint, which contaminates soil and house dust with lead, and from the historical use of lead in gasoline, which has contaminated the soil, particularly in urban areas. Lead can be transferred to the fetus through the placenta and to infants through breast milk.

Before evaluating the relevance of joint toxic action data for these chemicals, some understanding of endpoints of concern for oral exposure to this mixture is needed. The endpoints of concern include the critical effects that are the bases for MRLs or other health guidance values, and any other endpoints that may become significant because they are shared targets of toxicity or due to interactions (ATSDR 2001a).

Chlorpyrifos' critical effect, which is the basis of ATSDR (1996) MRLs and EPA (2000c; IRIS 2004) reference doses (RfDs), is neurological, due to inhibition of acetylcholinesterase.

The critical effect for lead is neurological. Although no MRLs have been derived for lead (Pb) (ATSDR 1999a), the Centers for Disease Control and Prevention (CDC 1991) has defined a level of concern for lead exposure in children in terms of a blood lead concentration (PbB) of 10 µg/dL. ATSDR (1999a) suggests the use of media-specific slope factors and site-specific environmental monitoring data to predict media-specific contributions to PbB.

The critical effect of methylmercury also is neurological (ATSDR 1999b). Metallic mercury also causes neurological effects when inhaled, but not when ingested (due to lack of absorption from the gastro-intestinal tract); exposures of concern for inhalation of metallic mercury are generally occupational rather than environmental. The concern from environmental release of metallic mercury is its conversion to methylmercury. Metallic mercury in dental amalgam appears to be absorbed, possibly after volatilization from fillings, but reliable evidence of adverse effects from this source of exposure is lacking. Therefore, methylmercury and inorganic (mercuric) mercury are the forms of mercury discussed further in this interaction profile.

Sensitive subpopulations for chlorpyrifos, lead, and methylmercury are fetuses, infants, and young children. These conclusions are based on human data for lead (ATSDR 1999a) and methylmercury (ATSDR 1999b), or are predicted on the basis of animal studies for chlorpyrifos (ATSDR 1997; EPA 2000b).

Although inorganic mercury can cause neurological effects, these effects are not sensitive effects, but rather are seen primarily at very high acute doses, probably because inorganic mercury does not pass the blood-brain and placental barriers readily, in contrast to methylmercury (ATSDR 1999b). A major concern from environmental release of inorganic mercury, however, is its conversion to methylmercury, which does have neurological effects. The critical effect of inorganic mercury is renal tubular damage (ATSDR 1999b), and also renal glomerular damage, which may be mediated through autoimmune

responses (IRIS 2004). Methylmercury and lead also cause renal damage, but at much higher exposure levels than those that cause neurological effects, and chlorpyrifos is not known to damage the kidney.

Carcinogenic effects are not a particular concern for the components of this mixture. Chlorpyrifos was evaluated for carcinogenicity in 2-year feeding studies in rats, mice, and dogs; results were negative (ATSDR 1997; EPA 2000b). A few lead compounds, mercuric chloride, and methylmercury have produced some evidence of carcinogenicity in animal studies, but the relevance to human exposures has been questioned, and the main concern for these chemicals is noncancer health effects (see appendices for details).

Thus, the primary endpoint of concern for this mixture is neurological, and the subpopulation of concern is developing children. Renal endpoints are also relevant, but are sensitive effects only of inorganic mercury. In addition, the interaction data for renal effects of these chemicals are conflicting, and the available studies are poor models, in terms of route and duration, for human exposure. Accordingly, this interaction profile will focus on the joint toxic action of the mixture on neurological effects. Effects of the other mixture components on the renal toxicity of inorganic mercury also will be assessed.

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 4th 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.

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.

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. 1999) 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 given 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 or 1 mg/kg of methyl paraoxon, reported 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 acetylcholinesterase 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 (≈LD_1) as in mixture; lesions were similar to those caused by Hg alone at ≈LD_{20}	>additive	Schubert et al. 1978 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 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 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 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 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 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 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 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 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 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	Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	–1
?	Indeterminate	0
Quality of the Data		**Weighting**
Mechanistic Understanding		
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur 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.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
Toxicological Significance		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
Modifiers		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05

BINWOE = Direction Factor x Weighting Factor: Ranges from 1 through 0 to +1

*Source: ATSDR 2001a, 2001b

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

BINWOE: <IIIB (-1 x 0.32x 0.71 = -0.23)

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 1999). 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 (-1 x 0.71 x 0.71 = -0.50)

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 (-1 x 0.32 x 0.32 = -0.10)

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 (-1 x 0.71 x 0.32 x 0.79 = -0.18)

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 (+1 x 0.32 x 0.32 = +0.10)

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 (-1 x 0.32 x 0.71 = -0.23)

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 (-1 x 0.32 x 0.32 = -0.10)

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. <u>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</u>. 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: ? (0)

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 (0 x 0.71 x 0.32 = 0)

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 (0 x 0.71 x 0.71 = 0)

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.

3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

As discussed in the introduction, the mixture of chlorpyrifos, lead, mercury (inorganic), and methylmercury was chosen as the subject for this interaction profile because of concerns for neurological effects in developing children (including fetuses and infants) exposed to these chemicals. The exposure scenario of greatest concern for this mixture is intermediate- to chronic-duration low-level oral exposure.

No adequate epidemiological or toxicological studies and no PBPK models are available for this mixture. Recommendations for exposure-based screening for the potential health hazard of this mixture are based on ATSDR (2001a) guidance, and comprise a component-based approach. This approach is used for the components with hazard quotients that equal or exceed 0.1, when at least two of the mixture components fulfill this criterion. Hazard quotients are the ratios of exposure estimates to noncancer health guidance values, such as MRLs. If only one or if none of the mixture components has a hazard quotient of this magnitude, no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard. As discussed by ATSDR (1992, 2001a), the exposure-based assessment of potential health hazard is a screening approach, to be used in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

Because neurological effects are the critical effects of chlorpyrifos, lead, and methylmercury, the recommended approach (ATSDR 2001a) for these components is to estimate an endpoint-specific hazard index (by summing the hazard quotients for these components) for neurological effects. Estimation of hazard quotients for lead is problematic because of the lack of an oral MRL or RfD. Blood lead is a commonly used index of exposure to lead. The use of media-specific slope factors and site-specific environmental monitoring data has been recommended by ATSDR to predict media-specific contributions to blood lead (ATSDR 1999). The predicted contributions from the individual media are summed to yield a total predicted PbB level. The media-specific slope factors were derived from regression analysis of lead concentrations in water, soil, dust, diet, or air and PbBs for various populations. In order to estimate a hazard quotient, the predicted PbB can be divided by the PbB of 10 μg/dL, the level of concern (CDC 1991), an appropriate guidance value adopted as the target-organ toxicity dose (TTD) for neurological effects of lead (Appendix A).

The hazard index is calculated using the guidance values for neurological effects shown in Table 21, or newer values as they become available. This process is shown in the following equation:

$$HI_{NEURO} = \frac{E_{Cpf}}{MRL_{Cpf\,NEURO}} + \frac{E_{Pb}}{CDCPbB_{Pb\,NEURO}} + \frac{E_{MeHg}}{MRL_{MeHg\,NEURO}}$$

where HI_{NEURO} is the hazard index for neurological toxicity, E_{Cpf} is the exposure to chlorpyrifos (as the oral intake in mg/kg/day), MRL_{Cpf} is the intermediate oral MRL for chlorpyrifos (in mg/kg/day), E_{Pb} is the exposure to lead (as the predicted PbB in µg/dL), $CDC_{PbB\,NEURO}$ is the CDC PbB of concern (10 µg/dL), E_{MeHg} is the exposure to methylmercury (as the oral intake in mg Hg/kg/day), and MRL_{MeHg} is the chronic oral MRL for methylmercury (in mg Hg/kg/day). For justification of using an intermediate duration guidance value with a chronic one, see section A.5 and C.5.

Table 21. MRLs and TTDs for Intermediate and Chronic Oral Exposure to Chemicals of Concern[a]

	Chemical			
Endpoint	**Chlorpyrifos mg/kg/day**	**Lead PbB µg/dL**	**Mercury (inorganic) mg Hg/kg/day**	**Methylmercury mg Hg/kg/day**
Neurological	0.003[b]	10[c]	NA	$3x1^{-4}$[d]
Renal	NA	NA	0.002[e]	NA

[a]See Appendices A, B, and C for details.
[b]Intermediate oral MRL for chlorpyrifos.
[c]CDC (1991) PbB level of concern, adopted as TTD.
[d]Chronic oral MRL for methylmercury.
[e]Intermediate oral MRL for mercury (inorganic).
NA = not applicable (see text)

Renal endpoints are also relevant to this mixture, but are sensitive effects only of inorganic mercury. Chlorpyrifos does not affect the kidney, and lead affects the kidney at much higher exposure levels than for neurological endpoints in humans. For methylmercury, although some studies in animals provide evidence of renal effects, the MRL for methylmercury is based on human epidemiological studies showing neurological effects, and the human studies do not provide dose-response data for renal effects specific to methylmercury. In addition, the joint action data pertinent to renal effects of these chemicals do not indicate that renal effects of the mixture would become significant due to interactions. Accordingly, the calculation of an endpoint-specific hazard index for renal effects is not recommended. Rather, a separate hazard quotient is recommended for assessing the potential hazard from renal effects of

inorganic mercury. The primary concern for inorganic mercury released to the environment is the conversion of inorganic mercury to methylmercury, which is bioaccumulated in the food chain, particularly fish, and readily absorbed by humans who ingest contaminated organisms.

If the hazard index for neurological effects exceeds 1, it provides preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of components on that endpoint (ATSDR 2001a). Similar preliminary conclusions apply if the hazard quotient for inorganic mercury's renal effects exceeds 1. The impact of interactions from the WOE analysis also is considered.

The BINWOE predictions for joint toxic action on neurological effects are predominantly less than additive (for lead, mercury, and methylmercury's influence on chlorpyrifos neurotoxicity; and for chlorpyrifos' influence on lead neurotoxicity), with confidence ratings generally in the medium to medium low range. As reflected in several scores, using interaction data for chemical surrogates (instead of chlorpyrifos) increased the uncertainty in interaction assessments. Two BINWOEs are additive (for methylmercury on lead neurotoxicity with medium confidence, and lead on methylmercury neurotoxicity with medium low confidence), and only one is greater than additive (chlorpyrifos on methylmercury neurotoxicity with low confidence). Thus, the predicted impact of interactions on the potential neurological hazard of this mixture is to decrease the hazard, but confidence in this conclusion is only medium to medium low. Therefore, a hazard index for neurological effects that is less than 1 or that is only slightly greater than 1 may not be of concern, but a hazard index that more markedly exceeds 1 will still indicate preliminary evidence of a mixture health hazard. It should be further noted that uncertainty regarding the actual mechanisms of neurodevelopmental toxicity contributes to the lower confidence in the conclusions as they are applied to developing children. The BINWOE predictions for joint toxic action on the renal toxicity of mercury do not significantly alter the conclusions that would be reached from the hazard quotient alone, because the BINWOE for chlorpyrifos on mercury is less than additive with low confidence and for lead on mercury is greater than additive, but also with low confidence.

If this screening procedure indicates preliminary evidence of a mixture health hazard, additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2001a). The additional evaluation includes biomedical judgment, assessment of community-specific health outcome data, and consideration of community health concerns (ATSDR 1992).

Table 22. Matrix of BINWOE Determinations for Intermediate or Chronic Simultaneous Exposure to Chemicals of Concern

	ON TOXICITY OF				
EFFECT OF		Chlorpyrifos	Lead	Mercury	Methylmercury
	Chlorpyrifos		<IIIB (-0.23) n	<IIIC (-0.10) r	>IIIC (+0.10) n
	Lead	<IIB (-0.50) n		>IIIC (+0.10) r	=IIIC (0) n
	Mercury	<IIC (-0.23) n	? (0) n		—
	Methylmercury	<IIIB (-0.23) n	=IIIC (0) n	—	

r = reproductive, n = neurological,

The BINWOE determinations were explained in Section 2.3.

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

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

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) and likely direction (0.71);
III: mechanistic data do not clearly indicate direction of interaction (0.32).

TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).

4. Conclusions

A component-based approach is recommended for the exposure-based screening assessment of potential hazards to public health from exposure to this mixture. The recommendations include the estimation of a hazard index for the neurological effects of the chlorpyrifos, lead, and methylmercury components of this mixture. The subpopulation of greatest concern for neurological effects of this mixture includes infants, young children, and fetuses. In addition, a separate hazard quotient is to be estimated for the renal effects of inorganic mercury. This approach is appropriate when the hazard quotients of at least two of the components equal or exceed 0.1 (ATSDR 2001a). The WOE evaluation of interactions indicates that the overall impact of interactions among the components of the mixture on the additivity assumption (hazard index) for neurological effects is to decrease the predicted hazard. Thus, the hazard index may overestimate the degree of hazard, such that a hazard index only slightly greater than 1 may not require further evaluation. Confidence in this conclusion, as reflected in the BINWOE scores, is medium to medium low. Predictions of the impact of chlorpyrifos and lead on the renal toxicity of inorganic mercury are that chlorpyrifos may have a less-than-additive influence and lead may have a greater-than-additive influence, but confidence in both these conclusions is low. When the screening criteria are exceeded (hazard index significantly greater than 1 for neurological effects of chlorpyrifos, lead, and methylmercury; hazard quotient greater than 1 for renal effects of inorganic mercury), further evaluation is needed (ATSDR 2001a), using biomedical judgment and community-specific health outcome data, and taking into account community health concerns (ATSDR 1992).

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Appendix A: Background Information for Chlorpyrifos

Chlorpyrifos is an organophosphorus insecticide. It belongs to the phosphorothioate (also called phosphorothionate) group, composed of organophosphorus compounds that contain the P=S substructure. Other organophosphorus insecticides in this group include diazinon, methyl parathion, parathion, fenthion, and fenitrothion. These compounds require metabolic activation to their oxon analogs (compounds in which the =S is replaced by =O) for anticholinesterase activity. The structures of chlorpyrifos and its toxic metabolite, chlorpyrifos oxon, are provided in Appendix D.

As of 2000, chlorpyrifos was one of the most widely used organophosphorus insecticides in the United States, for both agricultural and residential purposes. Registered uses included food crops, turf and ornamental plants, indoor pest control (including crack and crevice treatment), termite control, mosquito control, and pet collars. It was registered for use in a wide variety of buildings, including residential, commercial, schools, daycare centers, restaurants, hospitals, hotels, and food manufacturing plants (EPA 2000c). Many uses of chlorpyrifos are being phased out (see Section A.4).

A study of urinary pesticide metabolites during the third trimester in 386 pregnant women from East Harlem indicated that exposure to chlorpyrifos was prevalent (42% of the women had detectable levels of the chlorpyrifos metabolite), was higher than the median in NHANES III, did not show seasonal variation, and did not change during the time period of the study (1998–2001) (Berkowitz et al. 2003). The exposure of these women was thought to be primarily indoor, due to household use and pesticide exterminator application, and also dietary. A study of nine homes and 18 adult residents, 2/home, conducted in the Lower Rio Grande Valley concluded that indoor dust and air were the primary exposure media for the residents of those households, based on monitoring of those media, as well as outdoor soil and air, food, and a characteristic urinary metabolite of chlorpyrifos (as a biomarker of exposure) (Buckley et al. 1997).

A.1 Toxicokinetics

Chlorpyrifos is known to be absorbed through the respiratory tract in humans and animals, but quantitative estimates were not available (ATSDR 1997; FAO/WHO 1999).

Chlorpyrifos is readily absorbed from the gastrointestinal tract. Based on the percent of administered dose excreted in the urine, absorption of chlorpyrifos in humans was about 70% in a single-dose oral study (Nolan et al. 1984), and in rats ranged from 84–90% in single-dose gavage studies (ATSDR 1997; EPA 2000b).

Dermal absorption of chlorpyrifos (in dipropylene glycol methyl ether) in humans was about 1.3% of the administered dose within 48 hours (Nolan et al. 1984). Dermal absorption in animals was much higher, but results were confounded by dermal irritation, and even blistering, which compromised the integrity of the skin.

The main features of chlorpyrifos metabolism (ATSDR 1997; Buratti et al. 2003; FAO/WHO 1999; Tang et al. 2001) are:

- activation of chlorpyrifos by cytochrome P450 oxidative desulfuration of the P=S moiety to P=O, resulting in the toxic intermediate, chlorpyrifos oxon;
- detoxification by cytochrome P450 dearylation of chlorpyrifos, resulting in 3,5,6-trichloro-2-pyridinol, and diethyl thiophosphate;
- detoxification by A-esterases (including paraoxonases) that hydrolyze the phosphate ester bonds of chlorpyrifos and chlorpyrifos oxon to form 3,5,6-trichloro-2-pyridinol, and also diethyl thiophosphate (from chlorpyrifos), or diethylphosphate (from chlorpyrifos oxon); and
- formation of glucuronide and sulfate conjugates of 3,5,6-trichloro-2-pyridinol (TCP).

Metabolic activation occurs predominantly in the liver. Detoxification occurs predominantly in liver and plasma (ATSDR 1997; FAO/WHO 1999).

Metabolism of chlorpyrifos is rapid and extensive; the parent compound and the oxon are not detected or are found only in trace concentrations in blood or urine, except following very high exposures. The metabolite TCP is the principal form found in the circulation (ATSDR 1997; FAO/WHO 1999). The elimination half-life for this metabolite in humans following oral or dermal exposure was approximately 27 hours (Nolan et al. 1984). Chlorpyrifos metabolites are excreted primarily in the urine (ATSDR 1997; FAO/WHO 1999).

In humans, the cytochrome P450 isozymes that activate chlorpyrifos are CYP1A2 and CYP2B6 at low chlorpyrifos concentrations (environmentally relevant) (Buratti et al. 2003), and CYP3A4 and also

CYP2B6 at higher concentrations (Burati et al. 2003; Tang et al. 2001). Isozymes involved in dearylation of chlorpyrifos are reported to be CYP2C19 and CYP3A4 (Tang et al. 2001).

A.2 Health Effects

The principal toxic effect of chlorpyrifos in humans, experimental animals, and insects is acetylcholinesterase inhibition. Acetylcholine is a neurotransmitter in the central and peripheral neurons. Inhibition of acetylcholinesterase, the enzyme that breaks down and terminates the action of acetylcholine, results in the accumulation of acetylcholine at acetylcholine receptors leading to continued stimulation.

In humans and experimental animals, the accumulation of acetylcholine results in cholinergic responses in the peripheral (muscarinic and nicotinic) and central nervous system and neuromuscular junctions. These cholinergic responses, seen in severe acetylcholinesterase inhibition, include excessive glandular secretions (salivation, lacrimation, rhinitis), miosis, bronchoconstriction, vasodilation, hypotension, diarrhea, nausea, vomiting, urinary incontinence, and bradycardia associated with muscarinic receptor stimulation. Tachycardia, mydriasis (dilation of the pupil), muscle fasciculations, cramping, twitching, muscle weakness, muscle paralysis, and hypertension are associated with nicotinic receptor stimulation. Central nervous system toxicity includes respiratory depression, anxiety, insomnia, headache, apathy, drowsiness, dizziness, loss of concentration, confusion, tremors, convulsions, and coma. These effects usually appear within a few minutes to 24 hours after exposure, depending on the extent and route of exposure. In nonfatal exposures, the effects are usually transient, with rapid and complete recovery following cessation of exposure. Recovery from chlorpyrifos poisoning results from increased availability of active acetylcholinesterase either from synthesis of new enzyme, the spontaneous hydrolysis of the enzyme-phosphate ester complex, or treatment with atropine, a competitive antagonist of acetylcholine at muscarinic and central nervous system receptors, and with pralidoxime (2-PAM), a drug that regenerates inhibited acetylcholinesterase enzyme by displacing the diethylphosphoester bond that chlorpyrifos oxon forms at the active site (Aaron and Howland 1998; ATSDR 1997).

Transient, delayed polyneuropathy has been reported in humans in case reports of acute- or intermediate-duration exposure to chlorpyrifos; these reports did not adequately characterize exposure (ATSDR 1997; FAO/WHO 1999). Chlorpyrifos has been tested for organophosphate-induced delayed neurotoxicity in chickens; results were negative in these oral studies except at doses 4–6 times the LD_{50}, which required aggressive antidotal treatment (ATSDR 1996).

Acetylcholinesterase activity is also present in erythrocytes where it is known as erythrocyte acetyl-cholinesterase. Both forms of acetylcholinesterase are produced by the same gene and are kinetically identical. In *in vitro* assays, erythrocyte and neural acetylcholinesterase are inhibited to roughly the same extent by exposure to diazinon and many other organophosphorus compounds with insecticidal activity; measurement of erythrocyte acetylcholinesterase can be used as a surrogate indicator of the extent of inhibition of neural acetylcholinesterase (ATSDR 1996, 1997).

A cholinesterase capable of hydrolyzing acetylcholine and butrylcholine is produced by the liver and circulates in the blood. This enzyme, referred to as serum cholinesterase, plasma cholinesterase, pseudo-cholinesterase, or butyrylcholinesterase, is also inhibited by chlorpyrifos and is often used as a marker for exposure (ATSDR 1997). This enzyme is present in some nonneural cells in the central and peripheral nervous systems as well as in plasma and serum, the liver, and other organs. Its physiologic function is not known, but is hypothesized to be the hydrolysis of esters ingested from plants (Lefkowitz et al. 1996). Plasma cholinesterases are also inhibited by organophosphate compounds through irreversible binding; this binding can act as a detoxification mechanism as it affords some protection to acetylcholinesterase in the nervous system (Parkinson 1996; Taylor 1996). In general, this enzyme is inhibited at lower levels of organophosphate exposure than required to inhibit neural or erythrocyte acetylcholinesterase. For chlorpyrifos, plasma cholinesterase is inhibited earlier in the time course of events following a single dose or exposure, followed by acetylcholinesterase (ATSDR 1997).

Developing children (including infants and fetuses) are predicted to be more sensitive than adults to the neurotoxicity of chlorpyrifos, based on studies in animals (ATSDR 1997; EPA 2000b).

Chlorpyrifos was evaluated for carcinogenicity in 2-year feeding studies in rats, mice, and dogs; results were negative (ATSDR 1997; EPA 2000b).

A.3 Mechanisms of Action

Chlorpyrifos and chlorpyrifos oxon inhibit acetylcholinesterase by reacting with the active site to form a stable dialkylphosphorylated enzyme that cannot hydrolyze acetylcholine. Chlorpyrifos oxon, the active metabolic intermediate of chlorpyrifos, is much more potent than chlorpyrifos in inhibiting acetylcholinesterase (ATSDR 1997; FAO/WHO 1999).

A.4 Health Guidelines

ATSDR (1997) did not derive inhalation MRLs for chlorpyrifos because of the lack of suitable information for any exposure duration.

ATSDR (1997) derived acute and intermediate oral MRLs of 0.003 mg/kg/day for chlorpyrifos based on a no-observed-adverse-effect level (NOAEL) of 0.03 mg/kg/day for plasma cholinesterase inhibition in adult male volunteers who ingested chlorpyrifos by capsule for 20 days (Coulston et al. 1972). An uncertainty factor of 10 was used for human variability. The lowest-observed-adverse-effect level (LOAEL) (65% mean decrease in plasma cholinesterase; and symptoms possibly associated with exposure in one of four volunteers) was 0.1 mg/kg/day for 9 days.

ATSDR (1997) derived a chronic oral MRL of 0.001 mg/kg/day based on a NOAEL of 0.1 mg/kg/day for plasma, erythrocyte, and brain cholinesterase inhibition in rats fed chlorpyrifos in their diet for 2 years. Plasma and erythrocyte cholinesterase were inhibited at 1 and 3 mg/kg/day, and brain cholinesterase was inhibited at 3 mg/kg/day. An uncertainty factor of 100 was used to extrapolate from animals to humans and to account for human variability.

EPA (IRIS 2004) derived a chronic RfD for chlorpyrifos based on the human study that ATSDR (1997) also used for oral MRL derivation (referenced as Dow Chemical Company 1972 by IRIS 2004). EPA applied an uncertainty factor of 10 to the no-observed-effect level (NOEL) of 0.03 mg/kg/day, resulting in a chronic oral RfD of 0.003 mg/kg/day. This RfD was verified in 1986.

More recently, the EPA (2000c) Office of Pesticide Programs (OPP) reevaluated the use of the human data as the basis for its acute and chronic RfDs because of a joint Science Advisory Panel/Science Advisory Board meeting in December 1998 that discussed issues regarding the scientific and ethical concerns for human toxicity testing. There was a concern that a regulatory decision cannot be based on a human study until a formal decision has been made concerning the ethical aspects of this use (EPA 2000c). Since the ethics decision had not yet been made, and as part of the reevaluations conducted for reregistration and under the FQPA, the EPA (2000c) OPP has reevaluated the human and animal data, concluding that the human data provided useful information that can be used as supportive data, and derived new RfDs based on the animal data. An acute RfD of 0.005 mg/kg/day, based on plasma cholinesterase inhibition in an acute oral study in rats, and a chronic RfD of $3x10^{-4}$ mg/kg/day, based on the weight of evidence for plasma and erythrocyte cholinesterase inhibition from five oral studies in dogs

and rats, were derived. Although not on in the Integrated Risk Information System (IRIS), these derivations include a consideration of toxicological and mechanistic data that have become available since the RfD on IRIS was derived (by OPP). These newer RfDs have been subjected to extensive review, including public comment, and are available online (EPA 2000c). The FQPA safety factor of 10 (EPA 2003) was applied to these RfDs to estimate population adjusted doses (PADs) for children and females 13–50 when assessing dietary (food + drinking water) exposures, resulting in a acute PAD of $5x10^{-4}$ mg/kg/day and a chronic PAD of $3x10^{-5}$ mg/kg/day (EPA 2000c).

In June of 2000, EPA announced an agreement with chlorpyrifos registrants to eliminate certain uses of this pesticide (EPA 2000a, 2002a). Uses on foods frequently eaten by children (apples, grapes, tomatoes), uses by homeowners (except for ant baits in child resistant containers), and uses in settings such as schools and parks where children may be exposed, are being canceled, or phased out, or limited to minimize exposure. Residential uses by licensed applicators are being phased out or limited to lower application concentrations or rates. Reduced application rates for other agricultural uses and golf courses also are being instituted to protect workers and wildlife.

The National Toxicology Program (NTP) (2003) and the International Agency for Research on Cancer (IARC) (2003) do not include chlorpyrifos in their listings. The EPA (2002b) Office of Water classified chlorpyrifos in Cancer Group D (n*ot classifiable as to human carcinogenicity*).

A.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

The relevant endpoint for chlorpyrifos in this mixture is neurological. The intermediate oral MRL of 0.003 mg/kg/day for chlorpyrifos is based on neurological effects in humans. Chlorpyrifos is not known to be a cumulative or persistent toxin, so this MRL is appropriate for the screening level assessment of neurological effects of intermediate to chronic oral exposure to chlorpyrifos. Derivation of this MRL was described in the Section A.4.

Summary (TTD for Chlorpyrifos)

MRL_{NEURO} = 0.003 mg/kg/day

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Appendix B: Background Information for Lead

Lead is present in the environment primarily as divalent lead compounds. Contamination of the environment was ubiquitous, even in residential areas, due to the use of leaded gasoline and lead paint. Both of these uses have been phased out, but lead paint remains a problem in residences, and lead from paint and gasoline remains in soil and household dust. Other sources of lead emissions or exposure include mining, smelting, industrial activities, and hazardous waste sites (ATSDR 1999a).

B.1 Toxicokinetics

Gastrointestinal absorption of soluble lead salts in adult humans can be high during fasting (40–50%), but is about 3–15% when ingested with food. On the basis of dietary balance studies, gastrointestinal absorption of lead in children appears to be higher and may account for 40–50% of the ingested dose. Studies in animals also provide evidence that gastrointestinal absorption of lead is much higher in younger organisms. Absorption is strongly affected by nutritional status, with higher absorption of lead in children who are iron deficient. Calcium deficiency also may increase lead absorption, based on studies in children. Co-administration of calcium with lead decreases lead absorption in adults, and in animal studies. Vitamin D administration has been shown to enhance lead absorption in animal studies. The distribution of lead appears similar across routes of exposure. Initially, lead is distributed to the blood plasma and soft tissues, but under steady-state conditions, 99% of the lead in blood is found in the erythrocyte, where much of it is bound to hemoglobin. Lead accumulates in blood, such that bone lead accounts for approximately 73% of the body burden in children, increasing to 94% in adults. Inorganic lead is not known to be metabolized, but lead ions are complexed by macromolecules. Unabsorbed lead is excreted in the feces; absorbed lead that is not retained is excreted through the urine and bile (ATSDR 1999a).

B.2 Health Effects

The effects of lead are similar across inhalation and oral routes of exposure. Lead has been shown to affect virtually every organ and system in the body in both humans and animals. The most sensitive effects of lead appear to be neurological (particularly in children), hematological, and cardiovascular.

Epidemiological studies provide evidence for an association between prenatal and postnatal exposure to lead and adverse effects on neurodevelopment in infants and young children, and support the use of PbB as an index of toxicological effect. The neurological effects included impaired cognitive ability and IQ deficits in children. On the basis of several meta-analyses, it appears that a highly significant IQ decrement of 1–3 points is associated with a change in PbB from 10 to 20 µg/dL. In addition, associations between biomarkers of lead exposure and increased problem behavior in the classroom have been reported (ATSDR 1999a; Marlowe et al. 1985). In adult humans, slowing of nerve conduction velocity occurs at PbBs of 30 µg/dL; peripheral nerve function appears to be affected in children at similar PbBs. Oral studies in animals support the human evidence regarding neurobehavioral toxicity of lead to infants and children from prenatal and postnatal exposure. In animals, lead has been shown to alter a number of neurotransmitter systems including dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid systems (ATSDR 1999a).

Lead interferes with the synthesis of heme, resulting in accumulation of aminolevulinic acid (ALA) in tissues and elevated excretion of ALA in urine, elevation of zinc protoporphyrin in erythrocyte, reductions in blood hemoglobin, and in a hypochromic, normocytic anemia at higher levels of exposure. Many epidemiological studies have found increases in blood pressure to be associated with increases in PbB. The contribution of lead, as compared with other factors, is relatively small, and whether the observed associations represent causality is controversial. Animal data demonstrate that oral exposure to lead increases blood pressure. At higher levels of exposure in humans, lead produces cardiac lesions and electrocardiographic abnormalities. Chronic nephropathy in humans is associated with PbB levels of 40–>100 µg/dL. Oral exposure of animals to lead causes renal damage; histopathology is similar in humans and animals and includes intranuclear inclusion bodies, swollen mitochondria, and tubular damage. Adverse effects on the testes and sperm have been seen in occupationally exposed men with PbBs of 40–50 µg/dL, and the more recent literature suggest that PbB concentrations <40 µg/dL also may be associated with adverse effects on sperm counts and morphology (ATSDR 1999a).

B.3 Mechanisms of Action

Lead can affect virtually every organ or system in the body through mechanisms that involve fundamental biochemical processes. These mechanisms include the ability of lead to inhibit or mimic the action of calcium and to interact with proteins. In the interaction with proteins, lead binds with virtually every available functional group, including sulfhydryl, amine, phosphate, and carboxyl groups, with sulfhydryl

having the highest affinity. In its binding with sulfhydryl groups, lead may interfere with the activity of zinc metalloenzymes, as zinc binds to a sulfhydryl group at the active site. Lead also binds to metallothionein, a sulfhydryl-rich protein, but does not appear to displace cadmium or zinc. Metallothionein is induced by cadmium, zinc, and arsenic, but apparently not by lead, although metallothionein sequesters lead in the cell. Another lead-binding protein is an acidic, carboxyl-rich protein found in the kidney and brain (ATSDR 1999a).

Lead interferes with heme synthesis by altering the activity of several mitochondrial and cytosolic enzymes. One of the most sensitive hematological effects is inhibition of the cytosolic enzyme aminolevulinic acid dehydratase (ALAD), with no threshold apparent through the lowest PbB levels (3 µg/dL). Lead's inhibition of ALAD occurs through binding of lead to vicinal sulfhydryls at the active site of ALAD, where zinc is normally bound to a single sulfhydryl. Lead stimulates the mitochondrial enzyme delta-aminolevulinic acid synthetase (ALAS), through feedback derepression, with a threshold in human leukocytes at a PbB of about 40 µg/dL. As a result of the inhibition of ALAD and stimulation of ALAS, ALA accumulates in blood, urine, and soft tissues, including brain. ALA is structurally similar to gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. ALA appears to act as a GABA agonist at the presynaptic GABA receptors, causing negative-feedback inhibition of GABA release. In addition, ALA undergoes autooxidation, generating free radicals that may contribute to toxicity, and ALA promotes oxyhemoglobin oxidation. At relatively high levels of lead exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Decreases in tissue heme pools can have deleterious effects throughout the body, not only because heme is a constituent of hemoglobin, but also because heme is a prosthetic group of cytochrome P450 and the cytochromes of cellular energetics (ATSDR 1999a; EPA 1986). Lead inhibits the insertion of iron into protoporphyrin by the mitochondrial enzyme ferrochelatase, possibly through binding of lead to the sulfhydryl groups of the active site or indirectly through disruption of mitochondrial structure. Inhibition of ferrochelatase results in elevation of zinc protoporphyrin (ZPP) in erythrocytes; ZPP is a sensitive indicator of lead exposure, occurring in children at PbBs of about 25 µg/dL. Effects on heme synthesis are not restricted to the erythrocyte. A number of studies suggest that lead-impaired heme production itself may be a factor in lead's neurotoxicity (ATSDR 1999a). Other potential mechanisms of neurotoxicity include lead acting as a calcium agonist in a number of processes (ATSDR 1999a), and lead inhibition of receptor binding to the NMDA receptor channel, which does not appear to occur at the zinc allosteric site and is relatively insensitive (Lasley and Gilbert 1999).

Mechanisms by which lead might affect blood pressure include effects on several hormonal and neural regulatory systems, changes in vascular smooth muscle reactivity, cardiac muscle contractility, changes in cell membrane cation transport systems, and possible effects on vascular endothelial cells (ATSDR 1999a).

Lead has been shown to interfere with the DNA binding properties of zinc-finger regions of transcription factors, and this interference could potentially elicit multiple responses, but consequences have not yet been defined (Zawia et al. 2000).

B.4 Health Guidelines

ATSDR (1999a) has not derived MRLs for lead. ATSDR (1999a) has suggested the use of media-specific slope factors and site-specific environmental monitoring data to predict media-specific contributions to blood lead. The predicted contributions from the individual media are summed to yield a total predicted PbB level. The media-specific slope factors were derived from regression analysis of lead concentrations in water, soil, dust, diet, or air and PbBs for various populations.

The CDC determined in 1991 that blood lead levels of >10 µg/dL are to be considered elevated (ATSDR 1999a; CDC 1991).

EPA (IRIS 2004) has not developed a reference concentration (RfC) or RfD for lead. EPA stated that it would be inappropriate to develop an RfD for inorganic lead (and lead compounds) because some of the health effects occur at PbBs so low as to be essentially without a threshold. Instead, EPA defines lead risk as the probability of exceeding a PbB of concern (i.e., 10 µg/dL) in children (EPA 1994a) or in fetuses (EPA 1996). This approach is supported by human epidemiological studies that have associated PbBs exceeding 10 µg/dL with impairment or delays in neurobehavioral development and other effects on children (e.g., blood enzymes). EPA estimates lead risk in children using the Integrated Exposure Uptake Biokinetic (IEUBK) model (EPA 1994b). This model translates estimates of site-specific exposure concentrations into estimates of the probability that children's blood leads will exceed a PbB of concern.

NTP (2001) has determined that lead acetate and lead phosphate can reasonably be *anticipated to be human carcinogens*, based on sufficient evidence of carcinogenicity in experimental animals. NTP (2001) considered lead chromate as one of the "Chromium Hexavalent Compounds." IARC (1987) has

determined that the animal data are sufficient to classify lead and some lead compounds as *possibly carcinogenic to humans* (Group 2B). EPA (IRIS 2004) classified lead in Group B2 (*probable human carcinogen*). EPA did not develop an oral slope factor for lead because of the many uncertainties, some of which may be unique to lead. An EPA inhalation unit risk also is not available for lead (IRIS 2004). American Conference of Governmental Industrial Hygienists (ACGIH 2003) classified lead and certain inorganic lead compounds as A3 carcinogens (*confirmed animal carcinogen with unknown relevance to humans*). Lead chromate, assessed on the basis of both lead and chromate, was classified by ACGIH (2003) as an A2 carcinogen—carcinogenic in animals at doses considered relevant to worker exposure, but with insufficient epidemiological data to confirm risk to humans.

B.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

A TTD for chronic oral exposure to lead was derived for the primary endpoint of concern for this mixture, i.e., neurological effects in the fetus, infant, and young child from exposure to chlorpyrifos, lead, and methylmercury. Relevant endpoints for another metal mixture, which is the subject of a separate interaction profile, also included hematological, renal, cardiovascular, and testicular. For the sake of completeness, the TTDs derived for those endpoints are retained in this Appendix, but are not recommended for use with the present mixture. The chronic oral TTDs for lead were derived using the methods described in ATSDR (2001a, 2001b). Because ATSDR's approach to the assessment of lead uses media-specific slope factors and site-specific contributions to PbB, the TTDs for lead are derived based on PbB as well (see rationale in Chapter 3 of this profile). The derivations are based on data provided in ATSDR (1999a), and particularly Section 2.2.1 (Effects in Humans Based on Blood Lead [PbB] Levels), Section 2.5 (Relevance to Public Health), and Section 2.7 (Biomarkers of Exposure and Effect). The derivation methods used similar reasoning as for the CDC and EPA levels of concern (see neurological effects).

Neurological Effects

A large number of epidemiological studies and case reports indicate that exposure to lead causes neurological effects. Slowing of nerve conduction velocity is associated with PbBs of 30 µg/dL in children and adults. Of greater concern are the inverse linear relationships between IQ and other neurobehavioral measures in children at PbBs extending down through 10 µg/dL or possibly lower. Children appear to be more sensitive to the neurobehavioral toxicity of lead than are adults. Limited data

suggest an association between decreased neurobehavioral performance and PbB in aging subjects at relatively low PbBs, indicating that the elderly may be another sensitive population. Although results of the epidemiological studies in children are not entirely consistent, several meta-analyses have indicated that a highly significant IQ decrement of 1–3 points is associated with a change in PbB from 10 to 20 µg/dL in children (IPCS 1995; Needleman and Gatsonis 1990; Pocock et al. 1994; Schwartz 1994). The CDC (1991) determined that blood lead levels of >10 µg/dL are to be considered elevated in children, based largely on concern for the effects of low-level lead exposure on the central nervous system. EPA defines lead risk as the probability of exceeding a PbB of concern (10 µg/dL) in children or fetuses (EPA 1994a, 1996). The CDC level of concern for lead of 10 µg/dL is adopted as the TTD for neurological effects (TTD_{NEURO}).

Renal Effects

Chronic nephropathy is associated with PbB levels of 40–>100 µg/dL in humans exposed to lead occupationally. There are some indications of renal damage in a study of children whose mean PbB was 34.2 µg/dL (increased N-acetyl-β-D-glucosaminidase activity in urine, a sensitive indicator) (Verberk et al. 1996). The value for children, supported by the occupational data, and rounded to 34 µg/dL, is taken as the TTD for renal effects (TTD_{RENAL}).

Cardiovascular Effects

At higher levels of exposure, lead produces cardiac lesions and electrocardiographic abnormalities in humans. Many epidemiological studies have reported an association between increases in blood pressure and increases in PbB. The contribution of lead, as compared with other factors, is relatively small, and whether the associations indicate causality is controversial. Animal data demonstrate that oral exposure to lead increases blood pressure ATSDR (1999a). The correlation between PbB and blood pressure is apparent at relatively low PbBs extending through 10 µg/dL (e.g., Schwartz 1995). Therefore, the CDC level of concern, 10 µg/dL, is adopted as the TTD for cardiovascular effects (TTD_{CARDIO}).

Hematological Effects

Lead interferes with the synthesis of heme. The consequence at higher levels of exposure is a hypochromic, normocytic anemia. The most sensitive indicator of effect on heme synthesis is the inhibition of ALAD. ALAD activity is inversely correlated with PbB through the lowest levels of PbB in

the general population. Even in the absence of detectable effects on hemoglobin levels, there is concern that effects on heme synthesis may have far-reach impacts, particularly on children (ATSDR 1999a). Accordingly, the CDC PbB of concern, 10 µg/dL (CDC 1991), is selected as the TTD for hematological effects (TTD_{HEMATO}).

Testicular Effects

Adverse effects of the testes and sperm have been reported in occupationally exposed men with PbBs of 40–50 µg/dL in some studies, but not in others, and are well-established at higher levels of exposure (PbBs 66 µg/dL) (ATSDR 1999a). The point of departure for increased risk of below normal sperm and total sperm count was 40 µg/dL (Alexander et al. 1996). This value is selected as the TTD for testicular effects (TTD_{TESTIC}).

Summary (TTDs for Lead)

TTD_{NEURO} = 10 µg/dL PbB = CDC level of concern
TTD_{RENAL} = 34 µg/dL PbB
TTD_{CARDIO} = 10 µg/dL PbB
TTD_{HEMATO} = 10 µg/dL PbB
TTD_{TESTIC} = 40 µg/dL PbB

Only the TTD_{NEURO} is used in this interaction profile. As explained previously, the other TTDs were derived for endpoints of concern for joint toxic action of a different mixture, which is the subject of a separate interaction profile.

B.6 References

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Appendix C: Background Information for Mercury and Methylmercury

Mercury exists in the environment as metallic mercury (also called elemental mercury), inorganic mercury compounds (primarily mercuric), and organic mercury compounds (primarily methylmercury). The structure of methylmercury is shown in Appendix D. Metallic and inorganic mercury released into air from mining, smelting, industrial activities, combustion of fossil fuels, and natural processes can be deposited to water and soil, where the mercury is transformed by microorganisms into methylmercury, which bioaccumulates in the food chain, particularly in fish. For the general population, the most important pathway of exposure to mercury is ingestion of methylmercury in foods, with fish, other seafood, and marine mammals containing the highest concentrations (ATSDR 1999b). Another source of exposure for the general population is intake of metallic mercury from dental amalgams. Infants can be exposed to inorganic mercury and methylmercury from breast milk, and the developing fetus can be exposed through transplacental transfer of metallic mercury and methylmercury (ATSDR 1999b). For residents near mercury-contaminated hazardous waste sites, the following information provides insight into important routes of exposure. Exposure analysis of residents near an abandoned industrial site that had produced various inorganic and organic mercury compounds (and was not located near drinking water sources) indicated that the children were exposed to mercury primarily though soil and dust ingestion (Nublien et al. 1995).

C.1 Toxicokinetics

In humans, approximately 15% of a trace oral dose of inorganic mercury (mercuric nitrate) was absorbed through the gastrointestinal tract (ATSDR 1999b). Qualitative information indicates that ingested mercuric chloride and mercuric sulfide also were absorbed through the gastrointestinal tract of humans. Studies in animals indicate gastrointestinal absorption of inorganic mercury is in the 10–30% range, and depends on intestinal pH, compound dissociation, and other factors. Qualitative evidence indicates that the absorption of mercuric sulfide may be less than that of mercuric chloride. Absorption of inorganic mercury tended to be higher in young animals than in adults. Following absorption from the gastrointestinal tract, inorganic mercury distributes to the liver and kidneys, with the highest concentrations in the kidneys. Although concentrations in brain are substantially lower, mercury was retained longer in brain than in other tissues.

Metallic mercury is volatile and is readily absorbed (approximately 70–80%) through the respiratory tract, and because of its lipophilic nature, it crosses the blood-brain and placental barriers (ATSDR 1999b). Retention of mercury from metallic mercury exposure is longest in the brain, based on data from humans. Absorption of metallic mercury through the gastrointestinal tract, however, is negligible (ATSDR 1999b).

Results from studies with humans and laboratory animals indicate that methylmercury and its salts (e.g., methylmercuric chloride and methylmercuric nitrate) are readily and completely absorbed by the gastro-intestinal tract, but quantitative information on absorption of methylmercury by the respiratory tract is not available (ATSDR 1999b). Absorbed methylmercury is widely distributed among tissues, with the kidney showing the highest accumulation of mercury. Mercury from methylmercury can also accumulate in the brain and fetus due to methylmercury's abilities to penetrate the blood-brain and placental barriers and its conversion in the brain and fetus to the inorganic divalent cation (ATSDR 1999b). Excretion of methylmercury and other organic forms of mercury is thought to occur predominantly in the feces through biliary excretion.

Studies with animals indicate that methylmercury, but not inorganic mercury, can be reabsorbed from the gall bladder and the intestine, resulting in a biliary-hepatic cycle that contributes to longer clearance half-times for methylmercury compared with inorganic mercury (ATSDR 1999b). Intestinal flora and various mammalian tissues can produce the divalent mercury ion from methylmercury presumably via hydroxyl radicals produced by cytochrome P450 reductase (ATSDR 1999b). Inorganic mercury enters an oxidation-reduction equilibrium between itself, mercurous mercury (Hg^{+}), and metallic mercury (Hg^{0}) (ATSDR 1999b).

C.2 Health Effects

The nervous system is one of the primary sites of toxicity in humans and animals following exposure to metallic mercury, methylmercury, or inorganic salts of mercury (ATSDR 1999b). Neurological and behavioral disorders (including hand tremors, emotional lability, and performance deficits in tests of cognitive and motor function) have been observed in humans following inhalation of metallic mercury vapor, ingestion or dermal application of medicinal products containing inorganic mercurous salts, and ingestion of seafood contaminated with methylmercury. A single case study of lethal ingestion of mercuric chloride reported neurological symptoms and brain lesions. Animal studies have demonstrated changes in neurobehavioral function, morphology of neurological tissues, and brain neurochemistry

following inhalation exposure to metallic mercury or oral exposure to methylmercury. Data for neurological effects of inorganic mercuric mercury salts are limited, and whether associated with oral dosing is uncertain. Effects on neurological development ranging from delays in motor and verbal development to severe brain damage have been observed in children of human mothers orally exposed to organic forms of mercury, including methylmercury (ATSDR 1999b). Animal studies provide confirmatory evidence that neurological development of the fetus can be impaired by inhalation exposure of the dams to metallic mercury or oral exposure to methylmercury (ATSDR 1999b). Effects on neurological development appear to occur at much lower doses of methylmercury than those producing other effects discussed below (ATSDR 1999b). Neurological effects may be the most sensitive effects of inhalation exposure to metallic mercury (ATSDR 1999b).

The kidney is another major site of mercury toxicity. Degeneration or necrosis of the proximal convoluted tubules has been observed in humans and animals exposed to metallic mercury, inorganic mercury, or methylmercury (ATSDR 1999b). Renal damage is a sensitive effect, however, only for inorganic mercury. In the absence of renal tubular degeneration, exposure to inorganic mercury has been associated in several human cases and certain genetically disposed animals (New Zealand rabbits, Brown Norway rats, and certain strains of mice) with a toxic glomerular response (proteinuria, deposition of immune material in the renal mesangium and glomerular blood vessels, and minimal glomerular cell hyperplasia) that is thought to involve mercury-induced autoimmunity through a stimulation of the humoral and cellular immune systems and systemic autoimmunity (ATSDR 1999b; Hultman and Enestrom 1992; Hultman et al. 1994; IRIS 2004). Studies demonstrating an association of this type of autoimmune response with exposure to methylmercury were not located (ATSDR 1999b).

Immunosuppressive effects have also been associated with mercury exposure including decreased T-cell reactivity and decreased B cell levels in peripheral blood of mercury-exposed humans, increased susceptibility of mercury-exposed animals to infectious agents, and decreased natural killer cell activity in the spleen and blood of methylmercury-exposed rats (ATSDR 1999b; Hultman and Enestrom 1992; Ilback 1991; Ilback et al. 1991).

Effects on male and female reproductive organs or functions associated with mercury exposure include decreased sperm motility in male monkeys orally exposed to methylmercury, decreased spermatogenesis and degeneration of seminiferous tubules in male mice after prolonged oral exposure to methylmercury, impaired spermatogenesis and infertility in male rats and mice following parenteral administration of

methylmercury, and increased abortions, increased resorptions, or decreased implantations in female monkeys, guinea pigs, and mice orally exposed to methylmercury (ATSDR 1999b).

C.3 Mechanisms of Action

The high-affinity binding activity of divalent mercuric ion to thiol compounds or sulfhydryl groups of proteins is thought to be a central molecular mode involved in the various toxic actions of inorganic mercury and methylmercury (see ATSDR 1999b for review). The greater potency of methylmercury in producing toxic effects, relative to mercuric salts, is thought to be due to differences in dispositional processes, including gastrointestinal absorption and hepato-biliary recycling, leading to longer retention times and higher doses of the mercuric ion at sites of toxicity.

Mercury-induced damage to neurological or renal tissues has been postulated to involve oxidative stress damage from mercury-induced depletion of reduced glutathione levels, depolarization of mitochondrial inner membranes leading to hydrogen peroxide formation, and depleted levels of reduced pyridine nucleotides (ATSDR 1999b). It has been further postulated that neurons are particularly sensitive to mercury because of their low endogenous glutathione content or their inefficient glutathione reduction activity (ATSDR 1999b).

Postulates regarding methylmercury's mechanism of action on the developing nervous system include inhibitory effects of methylmercury on mitosis through impairment of microtubule assembly, methyl-mercury and inorganic mercury inhibition of enzymes such as protein kinase C, and inhibition of transport mechanisms in developing brain cells (ATSDR 1999b).

Molecular and cellular events underlying the immunosuppressive effects of mercury such as increased susceptibility to infectious agents are unclear, but Shenker et al. (1993) showed that methylmercury or mercuric chloride inhibited the mitogenic responses of cultured human T or B cells at concentrations that were about 10-fold lower than those that caused cytotoxicity, and that methylmercury was more potent than mercuric chloride. These authors postulated that immunosuppression involves inhibition by mercury of early stages in the response of these cells to mitogens. The genetically-controlled autoimmunity response to mercury that leads to glomerulonephropathy has been proposed to involve mercury disruption of the balance of helper and suppressor cells within the immunoregulatory network, but the molecular and cellular events that lead to glomerular immune-complex deposits have not been elucidated (ATSDR

1999b). Hultman et al. (1994) showed that, in a genetically susceptible mouse strain, prolonged exposure to inorganic mercury caused glomerular immune-complex deposits as well as stimulation of humoral immunity (increased levels of IgM and IgG1), cellular immunity (increased expression of class II molecules and increased mitogen-induced proliferation of T and B cells), and systemic autoimmunity (increased autoantibodies against the nucleolus).

C.4 Health Guidelines

Data were inadequate for derivation of acute- or intermediate-duration inhalation MRLs for metallic mercury, or for any duration inhalation MRL for inorganic mercury or for methylmercury (ATSDR 1999b).

ATSDR (1999b) derived a chronic inhalation MRL for metallic mercury vapor of $2x10^{-4}$ mg/m^3 based on a LOAEL in occupationally exposed humans of 0.026 mg/m^3 for neurological effects (equivalent continuous exposure concentration =0.0062 mg/m^3, after adjusting for 8/24 hours/day and 5 days/week). An uncertainty factor of 30 (10 for human variability and 3 for minimal-effect LOAEL) was used.

ATSDR (1999b) did not derive oral MRLs for metallic mercury due to the lack of data. Oral exposure to metallic mercury is expected to present little health risk, because it is so poorly absorbed through the health gastrointestinal tract.

ATSDR (1999b) derived an acute-duration oral MRL for inorganic mercury of 0.007 mg Hg/kg/day, based on a NOAEL of 0.93 mg Hg/kg/day (5 days/week) for renal effects in rats administered mercuric chloride for 2 weeks. The NOAEL dose was duration-adjusted and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). The LOAEL was 1.9 mg/kg/day, 5 days/week.

ATSDR (1999b) derived an intermediate-duration oral MRL for inorganic mercury of 0.002 mg Hg/kg/day, based on a NOAEL of 0.23 mg Hg/kg/day for renal effects in rats administered mercuric chloride days/week for 6 months.

ATSDR did not derive a chronic oral MRL for inorganic mercury due to inadequate data.

ATSDR (1999b) did not derive acute- or intermediate-duration oral MRLs for methylmercury due to the absence of data or the lack of sufficient information regarding exposure levels associated with observed effects.

ATSDR (1999b) derived a chronic oral MRL of $3x10^{-4}$ mg Hg/kg/day for methylmercury based on observations of no adverse effects in a 66-month evaluation of neurobehavioral development in children who were conceived, born, and resided on the Seychelles Islands and were members of an isolated population that consumed a high quantity and variety of ocean fish containing methylmercury. A NOAEL for methylmercury of 0.0013 mg Hg/kg/day was calculated based on an average level of mercury in maternal hair, 15.3 ppm, from a group (n=95) of the most highly exposed mothers. The NOAEL was divided by a factor of 4.5 to arrive at the MRL. The factor of 4.5 was the sum of an uncertainty factor of 3 (1.5 to address variability in hair-to-blood ratios among women and fetuses in the U.S. population plus 1.5 to address any additional sources of human variability in response to methylmercury) and a modifying factor of 1.5 to address uncertainty regarding the sensitivity of the neurobehavioral tests used in the available report of the Seychelles Islands cohort study.

EPA (IRIS 2004) developed an inhalation RfC of $3x10^{-4}$ mg/m^3 for metallic mercury based on a LOAEL of 0.025 mg/m^3 for 8-hour occupational exposure (converted to LOAEL of 0.009 mg/m^3 for continuous exposure), and using and uncertainty factor of 30 (10 for human variability and the use of a LOAEL, and 3 for database deficiencies, particularly the lack of developmental and reproductive studies). Inhalation RfCs have not been developed by EPA for inorganic mercury (mercuric chloride) or for methylmercury.

EPA (IRIS 2004) has not developed a chronic oral RfD for metallic mercury.

EPA (IRIS 2004) derived a chronic oral RfD for inorganic mercury (mercuric chloride) of $3x10^{-4}$ mg Hg/kg/day, based on a LOAELs for autoimmune effects (mercuric mercury induced autoimmune glomerulonephritis) in rat subchronic oral and subcutaneous studies, and back-calculated from a drinking water equivalent level of 0.010 mg/L recommended by a panel of mercury experts, following intensive review and workshop discussions of the entire inorganic mercury database, including the rat LOAELs and limited human tissue data. Data from Brown Norway rats were chosen because this strain is considered a good surrogate for mercury-induced kidney damage in sensitive humans. An uncertainty factor of 1,000 was applied in this derivation (10 for extrapolation from a LOAEL to a NOAEL, 10 for the use of subchronic studies, and a combined 10 for interspecies and intraspecies extrapolation).

EPA (IRIS 2004) derived a chronic oral RfD of $1x10^{-4}$ mg/kg/day for methylmercury based on an estimated NOAEL of 0.857–1.472 µg/kg/day (maternal intake of methylmercury during pregnancy based on estimated NOAEL cord blood range of 46–79 ppb) for neuropsychological effects in the offspring at 7 years of age in a longitudinal developmental study of about 900 mother-infant pairs from a fish-eating population in the Faroe Islands (Grandjean et al. 1997). The NOAEL was estimated by a benchmark dose approach as the 95% lower confidence limit for a daily dietary intake associated with 5% incidence for the above neurological effects. It is supported by similar studies of mother-infant pairs in the Seychelles islands and in New Zealand. The NOAEL was divided by an uncertainty factor of 10 to derive the RfD (3 for human variability and uncertainty in estimating an ingested mercury dose from cord-blood mercury data and 3 for human variability and uncertainty in pharmacodynamics).

EPA (IRIS 2004) classified metallic mercury in Group D (*not classifiable as to human carcinogenicity*) based on inadequate human and animal data, and limited and equivocal findings from genotoxicity tests.

EPA (IRIS 2004) classified inorganic mercury (mercuric chloride) in Group C (*possible human carcinogen*) based on the absence of data in humans and on limited evidence of carcinogenicity in rats and mice. The evidence in animals was considered limited because: the relevance of the observed forestomach papillomas in rats in one oral study of mercuric chloride is of questionable relevance to humans because there was no evidence of progression to malignancy; the relevance of an increase in thyroid tumors in male rats in the same study also is questionable because these tumors are generally considered to be secondary to hyperplasia; the doses in this study exceeded the maximum threshold dose (MTD) for male rats; and evidence for renal adenomas and adenocarcinomas in male mice in another oral study of mercuric chloride study was equivocal. Genotoxicity assays of mercuric chloride gave mixed results.

EPA (IRIS 2004) classified methylmercury in Group C (*possible human* carcinogen) based on inadequate data in humans and limited evidence of carcinogenicity in animals. The animal evidence was judged to be limited because: methylmercury-induced tumors (kidney tumors, from oral exposure) were observed at a single site, in a single species and in a single sex; the tumors were observed only in the presence of profound nephrotoxicity; several nonpositive oral cancer bioassays have also been reported; and the evidence that methylmercury is genotoxic is equivocal. Quantitative estimates of cancer risk from oral exposure were not derived based on evidence that methylmercury exerts its carcinogenic effects only at high doses above a maximum tolerated dose and that systemic noncancer effects on the nervous system

would be seen at methylmercury exposure levels lower that those required to produce kidney damage and subsequent kidney tumor development. Pertinent inhalation data were not available.

C.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

An intermediate oral MRL is available for inorganic (mercuric) mercury, based on renal effects, and can be used for the screening level assessment recommended in this interaction profile.

A chronic oral MRL is available for methylmercury, based on neurological effects, the primary endpoint of concern for this mixture.

TTDs for oral exposure to methylmercury were derived previously for endpoints relevant to a different mixture that is the subject of other interaction profiles. The previously derived TTDs are retained in this appendix only for the sake of completeness. The methods used for deriving TTDs are described in ATSDR (2001a). The derivations are based on data provided in ATSDR (1999b), and in particular, the oral Levels of Significant Exposure (LSE) table. Where the data were inadequate to derive a chronic oral TTD for a given endpoint, the chronic oral MRL is recommended as a conservative alternative that is protective of human health.

Renal Effects (Inorganic Mercury)

ATSDR (1999b) derived an intermediate oral MRL for inorganic (mercuric) mercury was based on a NOAEL of 0.23 mg Hg/kg/day for renal effects in rats administered mercuric chloride days/week for 6 months. The duration of the study was viewed as ideal, because it was long enough to detect long-term effects and short enough not to mix them with renal effects of aging rats. This MRL is suitable for the screening level assessment for intermediate to chronic oral exposure recommended in this interaction profile.

Immunological Effects (Methylmercury)

Although immunosuppression is a known toxic endpoint for mercury, quantitative dose-response information for methylmercury is limited. Ilback (1991) reported a LOAEL of 0.5 mg Hg/kg/day for decreased thymus weight and cell number and reduced natural killer cell activity in mice fed methyl-mercury in the diet for 12 weeks. No other data were located regarding oral exposure to organic mercury (ATSDR 1999b). Because a free-standing intermediate LOAEL with no supporting data is not a suitable

basis for TTD derivation, the chronic oral MRL of $3x10^{-4}$ mg Hg/kg/day is adopted as TTD_{IMMUNO} for methylmercury. Using the chronic oral MRL as the TTD is protective of human health.

Neurological Effects (Methylmercury)

The neurological effects of methylmercury are well documented in humans and animals, and are the critical effects of methylmercury (ATSDR 1999b). The chronic oral MRL of $3x10^{-4}$ mg Hg/kg/day developed by ATSDR (1999b) is based on neurological effects in the population of particular concern for this mixture (developing children). Therefore, a TTD is not needed for this endpoint.

Reproductive Effects (Methylmercury)

Studies of the reproductive effects of methylmercury are described in ATSDR (1999b). The lowest reliable LOAEL for reproductive effects was 0.06 mg Hg/kg/day, which produced an increased rate of reproductive failure due to decreased conceptions and increased early abortions and stillbirths in female monkeys treated with methylmercury for 4 months (Burbacher et al. 1988). The NOAEL in this study was 0.04 mg Hg/kg/day. Although there was a report of sperm effects in male monkeys exposed to 0.025 or 0.035 mg Hg/kg/day by gavage for 20 weeks (Mohamed et al. 1987), this study was not considered reliable by ATSDR (1999b). Chronic studies in rodents reported testicular lesions (tubular atrophy) and decreased spermatogenesis at approximately 0.7 mg Hg/kg/day, with NOAEL values of roughly 0.1 mg Hg/kg/day in both sexes (Hirano et al. 1986; Mitsumori et al. 1990; Verschuuren et al. 1976). The 4-month monkey study is a suitable basis for a TTD. Application of an uncertainty factor of 100 (10 for extrapolation from monkeys to humans and 10 to protect sensitive individuals) to the NOAEL of 0.04 mg Hg/kg/day yields a TTD_{repro} of $4x10^{-4}$ mg Hg/kg/day, which is only slightly higher than the chronic oral MRL of $3x10^{-4}$ mg Hg/kg/day.

Summary (TTDs for inorganic mercury)

MRL_{RENAL} = 0.002 mg Hg/kg/day

Summary (TTDs for methylmercury)

MRL_{NEURO} = $3x10^{-4}$ mg Hg/kg/day ($3x10^{-4}$ mg/kg/day)
TTD_{IMMUNO} = $3x10^{-4}$ mg Hg/kg/day ($3x10^{-4}$ mg/kg/day)
TTD_{REPRO} = $4x10^{-4}$ mg Hg/kg/day ($4x10^{-4}$ mg/kg/day)

For methylmercury, only the MRL_{NEURO} is used in this interaction profile. As explained previously, the other TTDs were derived for endpoints of concern for joint toxic action of a different mixture, which is the subject of a separate interaction profile.

C.6 References

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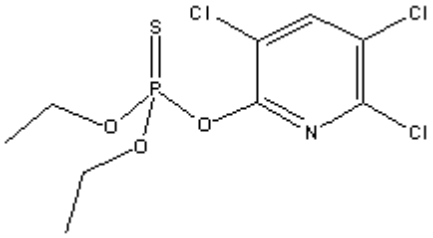
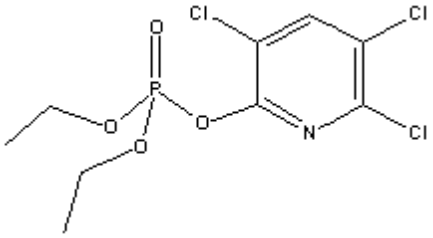
Appendix D: Chemical Structures of Organic Mixture Components



Chlorpyrifos
CAS No. 2921-88-2



Chlorpyrifos Oxon
CAS No. 5598-15-2

$H_3C—Hg^+$

Methylmercury
CAS No. 22967-92-6