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INTERACTION PROFILE FOR MIXTURES OF INSECTICIDES:

PYRETHROIDS, ORGANOPHOSPHORUS COMPOUNDS, AND CARBAMATES

Agency for Toxic Substances and Disease Registry
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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, 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 and 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 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 and Human Health Sciences (DTHHS) 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.

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PEER REVIEW

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

1. AMJ Ragas, Ph.D., Dept. of Environ Science, Faculty of Science, Radboud University Nijmegen, Netherlands

2. Richard C. Hertzberg, Ph.D., Emory University, School of Public Health, Biomathematics Consulting, Atlanta, GA, USA

3. Jane Ellen Simons, Ph.D., NHEERL, US Environmental Protection Agency, Raleigh, NC, USA

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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

The purpose of this profile is to investigate the possible joint actions of pyrethroid insecticides, organophosphorus insecticides and carbamate insecticides on neurological end points in humans. In assessing the available information on possible interactions among these chemicals, this profile concludes with recommendations for conducting screening level assessments of public health concerns from joint exposure to mixtures of these chemical classes.

ATSDR recommends that the default assumption of dose-additive joint action at shared targets of toxicity (i.e., effects on neurological end points) be used for screening level assessments of the potential adverse health outcomes from concurrent oral exposures to mixtures of pyrethroids, organophosphorus and carbamate insecticides. The assessments should be accompanied by qualitative descriptions of weight-of-evidence evaluations of available interaction data:

1. greater-than-additive action on neurological end points is possible between certain pyrethroid and organophosphorus insecticides;

2. the available data are inadequate to assess the possible direction of interactions between pyrethroids and carbamates; and

3. limited available data support dose additivity of carbamate and organophosphorus insecticides on neurological end points.

Overall, the evidence is not compelling to move from a dose-additive approach for screening level assessments. The evaluations indicate that greater-than-additive interactions between certain pyrethroid and organophosphorus insecticides are possible, but key findings in mammals come from a study of potentiation of fenvalerate lethality in rats pretreated with certain organophosphorus insecticides (Gaughan et al. 1980). The relevance of these findings to relatively low (nonlethal) environmental concurrent exposure to pyrethroid and organophosphorus insecticides is not well understood.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ATSDR	Agency for Toxic Substances and Disease Registry
BINWOE	binary weight-of-evidence
BPMC	2-sec-butylphenyl *N*-methylcarbamate
ChE	acetylcholinesterase
BMD	benchmark dose
BMDL	benchmark dose limit
BTEX	benzene, toluene, ethylbenzene, and xylene
CYP	cytochrome P450
DEF	S,S,S-tributyl phosphorotrithioate
DEET	N,N-diethyl-m-toluamide
DTHHS	Division of Toxicology and Human Health Sciences
EPA	U.S. Environmental Protection Agency
EPN	*O*-ethyl *O*-(4-nitrophenyl) phenyl phosphonothioate
ERDEM	exposure-related dose estimating model
FQPA	U.S. Food Quality and Protection Act
GABA	gamma-aminobutyric acid
GD	gestation day
HI	hazard index
HQ	hazard quotient
IARC	International Agency Research on Cancer
IR	interactive ratio
IRIS	Integrated Risk Information System
iso-OMPA	tetraisoproylpyrophosphoramide
kg	kilogram
LD	lethal dose
LOAEL	lowest-observed-adverse-effect level
mg	milligram
MOE	margin of exposure
MPMC	3,4-dimethylphenyl *N*-methylcarbamate
MRL	Minimal Risk Level
MTMC	3-methylphenyl *N*-methylcarbamate
NAC	1-naphthyl *N*-methylcarbamate
NOAEL	no-observed-adverse-effect level
NTE	neuropathy target esterase
NTP	National Toxicology Program
OPIDP	organophosphate-induced delayed polyneuropathy
OPP	U.S. EPA Office of Pesticide Programs
PBPK/PD	physiologically based pharmacokinetic/pharmacodynamic
PhAD	photic after discharge
PND	postnatal day
POD	point of departure
ppm	parts per million
RBC	red blood cell
RfD	reference dose
RPF	relative potency factor
TTD	target-organ toxicity dose
U.S.	United States
VGSC	voltage-gated sodium channel
XMC	3,5-dimethylphenyl *N*-methylcarbamate

$>$	greater than
$\geq$	greater than or equal to
$=$	equal to
$<$	less than
$\leq$	less than or equal to

1. Introduction

The primary purpose of this Interaction Profile for Mixtures of Insecticides: Pyrethroids, Organophosphorus Compounds, and Carbamates 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 mixtures of these insecticide classes to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern (i.e., neurological effects), adequacy of the data as the basis for a mixture health guidance value, and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Division of Toxicology and Human Health Sciences (DTHHS) 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.

Interactions between pyrethroid, organophosphorus, and carbamate insecticides are of interest to ATSDR because members of these chemical classes are the most widely used insecticides, and the general population is expected to be exposed to mixtures of members of these insecticide classes from eating food or drinking water with insecticide residues and from the use of insecticides in the home and workplace. The U.S. Environmental Protection Agency (EPA) estimated that the world-wide and U.S. expenditure for insecticides in the year 2012 was U.S. $ 16,023 million (or 25% of the market for all pesticides) and $ 2,184 million (or 25% of the market for all pesticides), respectively, at the producer level (EPA 2007a). Because of the widespread use, these insecticides also end-up at hazardous waste sites. The occurrence of the insecticides at hazardous waste sites based on the ATSDR's database is provided in Appendix E. Exposures at the sites may be higher than those encountered by the general population. In addition, all three routes of exposure may be of concern.

This profile concentrates on neurological effects, as these are the principal and most well-studied toxic effects associated with exposure to individual members of each of these insecticide classes. Appendices A–C to this profile provide background information on the toxicokinetics, health effects, and mechanisms of action of pyrethroid insecticides (Appendix A), organophosphorus insecticides (Appendix B), and carbamate insecticides (Appendix C). Also included in the appendices are descriptions of current ATSDR and EPA health guidelines for members of each insecticide class. As discussed in the appendices, cancer is not an expected health end point of concern for most members of each of these insecticide classes. Likewise, results from standard developmental toxicity and reproductive toxicity tests in animals do not identify developmental toxicity or reproductive toxicity as critical health effects for most members of each class (Appendices A–C). There is a concern for possible neurodevelopmental effects from organophosphorus insecticides based on positive results in a few *in vivo* studies and *in vitro* mechanistic studies (see Appendix B). Available studies that tested these end points, did not clearly establish neurodevelopmental effects as health effects of concern for pyrethroid or carbamate insecticides (see Appendices A and C).

Mechanistic information indicates that pyrethroids induce neurological effects by interfering with voltage-gated sodium channels (VGSC) in nerve cells (see Appendix A), whereas organophosphorus and carbamate insecticides induce neurological effects via inhibition of the enzyme, acetylcholinesterase (ChE), leading to accumulation of acetylcholine at acetylcholine receptors and overstimulation of junctions in central and peripheral nerves (see Appendices B and C). Pyrethroids are manufactured insecticides that are similar in chemical structure to pyrethrins, naturally occurring chemicals found in certain chrysanthemum flowers (Appendix A; ATSDR 2003a). Hundreds of organophosphorus compounds have been synthesized and many commercialized as insecticides, and more than half of insecticides used are organophosphorus compounds (Costa 2008). Carbamate insecticides are structurally diverse derivatives of carbamic acid or N-methyl carbamic acid (Costa 2008). The carbamate insecticides discussed in this profile are all N-methyl carbamate derivatives.

EPA (2006, 2007b) determined that the organophosphorus insecticides and the N-methyl carbamate pesticides represent separate common mechanism of toxicity groups (see Appendices B and C). Although the signs and symptoms of acute high-level exposure to carbamate insecticides are similar to those induced by organophosphorus insecticides, the carbamylated ChE is transiently inhibited, rapidly reversible, and does not undergo the irreversible aging reaction that happens with organophosphorylated ChE (Costa 2008). Thus, cholinergic signs and symptoms of acute carbamate intoxication are generally resolved within a few hours, whereas acute organophosphorus intoxication takes longer to resolve (Costa

2008). To conduct cumulative risk assessments for these two classes of insecticides, EPA's Office of Pesticide Programs (OPP) derived relative potency factors (RPFs) for 33 organophosphorus insecticides based on relative ability to inhibit brain ChE in adult rats exposed for 21 days, compared with the index chemical, methamidophos (EPA 2006; see Appendix B), and RPFs for 10 carbamates based on the ability to inhibit brain ChE in acutely exposed adult rats, compared with the index chemical, oxamyl (EPA 2007b; see Appendix C).

Pyrethroids represent a class of chemicals that rapidly modify the function of nerve cells predominantly by modifying the kinetics of VGSCs, but other diverse targets have been identified, including voltage-gated chloride channels, gamma-aminobutyric acid (GABA)-gated chloride channels, and noradrenaline release and voltage-gated calcium channels (Appendix A; Ray and Fry 2006; Soderlund et al. 2002). EPA (2011b) concluded that pyrethroids share the same mechanism of action, namely interacting with VGSCs in nerve cells. Although different pyrethroids have been demonstrated to interact differentially with different VGSC subunits, all tested pyrethroids alter the kinetics of VGSCs in nerve cells (EPA 2011a, 2011b). In addition, EPA (2011a, 2011b) determined that the weight of evidence supporting mechanisms of action at other channels was "not compelling", relative to action on sodium channels. For a cumulative risk assessment, EPA (2011a) derived RPFs for 15 pyrethroids, based on scores for changing several clinical end points related to alterations in VGSCs in acutely exposed rats, compared with the index pyrethroid, deltamethrin (see Appendix A).

Although members of all of these chemical classes have been detected in air samples and some residential exposure scenarios are expected to involve oral, dermal, and inhalation exposures, the major source of exposure to these classes of insecticides in the general human population is likely to be as residues in food (Appendices A–C). Information regarding mixtures of these chemicals at hazardous waste sites can be found in Appendix E.

Information to prepare this profile was obtained via searches of the literature (conducted in February–July 2009 and updated in February 2013) with a focus on information for members of each insecticide class listed in Table 1. These insecticides were selected because they were either the subject of an ATSDR Toxicological Profile, had a reference dose (RfD) on the EPA Integrated Risk Information System (IRIS 2013), or were included in the EPA OPP cumulative risk assessments for these classes of insecticides (EPA 2006, 2007b, 2011a). Searches were not restricted with regard to toxic end point (even though this profile is focused on neurological end points) or route of exposure.

Table 1. List of Pyrethroid, Organophosphorus, and Carbamate Insecticides That are the Focus of the Literature Search for This Profile

Chemical	CAS Number	RfD on IRIS	ATSDR MRL	OPP RPF
Pyrethroid insecticides				
Allethrin	584-79-2	No	No	Yes
Bifenthrin	82657-04-3	Yes	No	Yes
Cyfluthrin (Baythroid)	68359-37-5	Yes	No	Yes
Cyhalothrin	68085-85-8 or 91465-08-6 (lambda)	Yes	Oral	Yes
Cypermethrin	52315-07-8	Yes	Oral	Yes
Cyphenothrin	39515-40-7	No	No	Yes
Deltamethrin	52918-63-5	No	No	Yes
Esfenvalerate	66230-04-4	No	No	Yes
Fenpropathrin (Danitol)	39515-41-8	Yes	No	Yes
Fenvalerate (Pydrin)	51630-58-1	Yes	No	No
Fluvalinate	69409-94-5	Yes	No	No
Imiprothrin	72963-72-5	No	No	Yes
Permethrin	52645-53-1	Yes	Oral	Yes
Resmethrin	10453-86-8	Yes	No	Yes
Tau-fluvalinate	102851-06-9	No	No	Yes
Tralomethrin	66841-25-6	Yes	No	No
Organophosphorus insecticides				
Acephate	30560-19-1	Yes	No	Yes
Azinphos-methyl (guthion)	86-50-0	No	Inhalation, oral	Yes
Bensulide	741-58-2	No	No	Yes
Chlorethoxyfos	54593-83-8	No	No	Yes
Chlorfenvinphos	470-90-6	No	Oral	No
Chlorpyrifos	2921-88-2	Yes	Oral	Yes
Diazinon	333-41-5	No	Inhalation, oral	Yes
Dichlorvos (DDVP)	62-73-7	Yes	Inhalation, oral	Yes
Dicrotophos (bidrin)	141-66-2	Yes	No	Yes
Dimethoate	60-51-5	Yes	No	Yes
Disulfoton	298-04-4	Yes	Inhalation, oral	Yes
Ethion	563-12-2	Yes	Oral	No
Ethoprop	13194-48-4	No	No	Yes
Fenamiphos	22224-92-6	Yes	No	Yes
Fenthion	55-38-9	No	No	Yes
Fosthiazate	98886-44-3	No	No	Yes
Malathion	121-75-5	Yes	Inhalation, oral	Yes
Methamidophos	10265-92-6	Yes	No	Yes
Methidathion	950-37-8	Yes	No	Yes
Methyl-parathion	298-00-0	Yes	Oral	Yes

Table 1. List of Pyrethroid, Organophosphorus, and Carbamate Insecticides That are the Focus of the Literature Search for This Profile

Chemical	CAS Number	RfD on IRIS	ATSDR MRL	OPP RPF
Mevinphos	7786-34-7	No	No	Yes
Naled	300-76-5	Yes	No	Yes
Omethoate	1113-02-6	No	No	Yes
Oxydemeton-methyl	301-12-2	No	No	Yes
Phorate	298-02-2	No	No	Yes
Phosalone	2310-17-0	Yes	No	Yes
Phosmet	732-11-6	Yes	No	Yes
Phostebupirim	96182-53-5	No	No	Yes
Pirimiphos-methyl	29232-93-7	Yes	No	Yes
Profenofos	41198-08-7	No	No	Yes
Terbufos	13071-79-9	No	No	Yes
Tetrachlorvinphos	961-11-5, 22248-79-9	Yes	No	Yes
Tribufos (merphos oxide)	78-48-8	Yes	No	Yes
Trichlorfon	52-68-6	No	No	Yes
Carbamate insecticides				
Aldicarb	116-06-3	Yes	No	Yes
Aldicarb sulfone	1646-88-4	No	No	Yes
Aldicarb sulfoxide	1646-87-3	No	No	Yes
Carbaryl	63-25-2	Yes	No	Yes
Carbofuran	1563-66-2	Yes	No	Yes
Formetanate hydrochloride	23422-53-9	No	No	Yes
3-Hydroxycarbofuran	16655-82-6	No	No	Yes
5-Hydroxycarbofuran	Not on CHEMIDplus	No	No	Yes
Methiocarb	2032-65-7	No	No	Yes
Methomyl	16752-77-5	Yes	No	Yes
Oxamyl	23135-22-0	Yes	No	Yes
Pirimicarb	23103-98-2	No	No	Yes
Propoxur (Baygon)	114-26-1	Yes	No	Yes
Thiodicarb	59669-26-0	No	No	Yes

ATSDR = Agency for Toxic Substances and Disease Registry; CAS = Chemical Abstracts Service; EPA = U.S. Environmental Protection Agency; IRIS = Integrated Risk Information System; MRL = Minimal Risk Level; OPP = U.S. EPA Office of Pesticide Programs; RfD = reference dose; RPF = relative potency factor

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

2.1 Mixture of Concern

No data were located regarding health or pharmacokinetic end points in humans or animals exposed to mixtures containing at least one of the chemicals from each of the three classes: pyrethroid, organophosphorus, and carbamate insecticides.

No physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models were found for tertiary mixtures of at least one chemical from each of the three classes.

2.2 Component Mixtures

No PBPK/PD models were found for binary mixtures of pyrethroid and organophosphorus insecticides, pyrethroid and carbamate insecticides, or organophosphorus and carbamate insecticides.

This profile is focused on interactions that influence the neurological effects associated with each class of chemicals as discussed in Appendix A (pyrethroid insecticides), Appendix B (organophosphorus insecticides), and Appendix C (carbamate insecticides). The following subsections present information about mixtures of pyrethroid insecticides, mixtures of organophosphorus insecticides, and mixtures of carbamate insecticides, followed by subsections discussing relevant information on the joint toxic action of binary combinations of pyrethroid and organophosphorus insecticides, pyrethroid and carbamate insecticides, and organophosphorus and carbamate insecticides.

2.2.1 MIXTURES OF PYRETHROID INSECTICIDES

PBPK/PD models for mixtures of pyrethroid insecticides are not available.

A PBPK model for a single pyrethroid, deltamethrin, was developed for adult male Sprague-Dawley rats (Mirfazaelian et al. 2006). The model included both flow-limited and diffusion-limited rate equations. Hepatic metabolism accounted for about 78% of the dose. A later model followed-up on the initial work and compared the kinetics and dosimetry in immature and maturing rats (Tornero-Velez et al. 2010). The model predicted doses that would produce equivalent brain concentrations of deltamethrin in 10-, 21-, and 90-day-old rats. Equivalent human age groups were newborns for 11-day-old rats, 3–6-year-old children

for 17-day-old rats, and adults for 90-day-old rats. Doses producing brain concentrations equivalent to those in adults were 3.8-fold lower in 10-day-old rats and 2.5-fold lower in 21-day-old rats, compared with adult rats. In its cumulative risk assessment for pyrethroids, EPA (2011a, 2011c) used these results to support the use of a Food Quality and Protection Act (FQPA) safety factor of 3 to protect children from birth to 6 years old. Godin et al. (2010) improved the original model as to the predictability of tissue concentration data in Long-Evans rats. The rat model was then scaled to humans. The model predicted greater distribution of the insecticide to the brain in humans compared to rats.

Dose additivity adequately explained the joint actions of pyrethroids in mixtures at low individual doses without toxicological effects in studies of motor activity in rats (Starr et al. 2012; Wolansky et al. 2009) and sodium influx in cultured cerebrocortical neurons (Cao et al. 2011). In these studies, observed responses to a mixture of 11 individual pyrethroids (both Type I and Type II: permethrin, bifenthrin, cypermethrin, β-cyfluthrin, deltamethrin, esfenvalerate, tefluthrin, λ-cyhalothrin, fenpropathrin, resmethrin, and S-bioallethrin) were compared with predicted responses using dose-addition models fit to single-chemical data. In the *in vivo* study, response-additivity models, as opposed to dose-additivity models, underestimated the effects on motor activity (Wolansky et al. 2009). The results demonstrate that mixture-induced changes in motor activity after *in vivo* exposure and sodium influx after *in vitro* exposure were explained by a dose-addition model. In conjunction with observations that all tested pyrethroids can alter VGSC function (EPA 2011a, 2011b), the results provide support for the concept that pyrethroids share a common mechanism of acute toxicity involving altered VGSC kinetics leading to altered neurobehavior.

Results from previous studies of exposures to multiple pyrethroids have been interpreted to suggest that joint actions of pyrethroids can be antagonistic or competitive at ion channels and do not lead to effect-additive outcomes (Burr and Ray 2004; Motomura and Narahashi 2001; Ray et al. 2006; Song and Narahashi 1996; Song et al. 1996; Tabarean and Narahashi 1998). End points examined in these studies included sodium currents in single channels (Song and Narahashi 1996) and whole cells (Song et al. 1996), chloride currents in excised membrane patches (Burr and Ray 2004), and *in vivo* hippocampal electrophysiological responses (Ray et al. 2006). However, as pointed out by Wolansky et al. (2009) and Cao et al (2011), these studies did not adequately test the hypothesis of dose additivity due to limitations in study design (e.g., Ray et al. [2006] used lethal dose levels) and/or statistical approach.

In the *in vivo* experiments with Long-Evans adult rats, Wolansky et al. (2009) assessed figure-eight maze activity 2 hours after gavage exposure to a mixture of 11 pyrethroids, or after sequential exposure based

on times of peak effect for the individual pyrethroids (4, 2, or 1 hours before testing). The test mixture contained equipotent relative amounts of each pyrethroid based on ED_{30} values (determined in other experiments). The highest tested doses for each component in the mixture were below the single-chemical thresholds for inducing motor activity effects. When subthreshold (equipotent) doses of the individual components were administered in the mixture (by either exposure protocol), statistically significant decreases in motor activity were found. No statistically significant differences were found between the predicted and empirical fits for data from either exposure protocol, indicating that dose-additivity models were adequately predictive of the observed results.

In a subsequent study of brain concentrations and motor activity, Starr et al. (2012) exposed Long-Evans rats by gavage to a mixture of six pyrethroids at single doses 1.5- and 3.7-fold greater (11.2 and 27.4 mg/kg) than the expected ED_{30}, based on the dose-additive model of Wolansky et al. (2009). The proportion of the pyrethroids in the mixture was reflective of proportions found in indoor floor surface samples from 168 U.S. childcare centers. The mixture contained Type I and II pyrethroids: cypermethrin (II), deltamethrin (II), esfenvalerate (II), cis-permethrin (I), trans-permethrin (I), and β-cyfluthrin (II). Consistent with the concept of dose addition, the observed decreases in motor activity at the times of peak decrease were similar to those predicted by dose-additivity models: 34 versus 40% for the low dose and 67 versus 60% for the high dose, respectively. A relationship between pyrethroid brain concentration and motor activity was described by a sigmoidal E_{max} model.

In the *in vitro* experiments, Cao et al. (2011) assessed sodium influx in cerebrocortical neurons as affected by the 11 individual pyrethroids in the mixture tested by Wolansky et al. (2009), and tested the hypothesis that changes in sodium influx (presumably mediated by VGSCs) induced by a mixture of these pyrethroids would be dose additive. Seven of the 11 individual pyrethroids produced significantly increased sodium influx in a concentration-dependent and tetrodoxin-sensitive manner. Tetrodoxin is a known inhibitor of voltage-gated sodium channels. Cypermethrin and bifenthrin caused only small increases, and resmethrin and permethrin did not change sodium influx within the range of tested concentrations (0.1–30 μM). The action of all of the 11 pyrethroids in the mixture was shown to be predicted by a dose-additive statistical model.

Because the Wolansky et al. (2009) and Cao et al. (2011) studies of the mixtures of 11 pyrethroids were adequately designed to test the hypothesis of dose additivity, the results suggest that it is reasonable to use dose additivity in estimating possible neurotoxic effects in humans from environmental exposure to mixtures of pyrethroids. Support for the predictive utility of dose additivity comes from Starr et al.

(2012). Wolansky et al. (2009), however, did point out several areas of uncertainty associated with such use, including whether dose additivity will predict responses to mixtures with a smaller number of pyrethroids or different ratios and whether differences in exposure would influence dose additivity (e.g., rats were exposed acutely by gavage, whereas humans are likely exposed dermally and via the diet). The recent EPA (2011a) cumulative risk assessment for pyrethroid insecticides is based on the assessment that pyrethroids represent a common mechanism of toxicity group and the assumption of dose additivity. EPA (2011a) concluded that dose addition is a reasonable approach for estimating cumulative risk of exposures to mixtures of pyrethroids and that current data do not provide a sufficient basis to depart from dose additivity.

2.2.2 MIXTURES OF ORGANOPHOSPHORUS INSECTICIDES

PBPK/PD models for the organophosphorus insecticide, chlorpyrifos, in rats and humans have been developed as a starting point in the development of future models than can be used to better understand mixtures of organophosphorus insecticides, mixtures of organophosphorus and carbamate insecticides, and the nature of possible interactions between members of these two classes of ChE-inhibiting insecticides (Timchalk 2006; Timchalk et al. 2002). A limited number of PBPK/PD models for other single organophosphate insecticides or nerve agents have also been developed (see Timchalk et al. 2002 for review). The models reflect the understanding that the balance between activation of most organophosphorus insecticides to reactive intermediates (e.g., formation of chlorpyrifos-oxon via cytochrome P450 [CYP]) and multiple detoxification pathways (e.g., dealkylation or dearylation of parent compounds via CYP isozymes, phosphorylation by parent compounds of B-esterases, and hydrolysis of chlorpyrifos-oxon by A-esterases) is critical to the expression of neurological effects mediated via the inhibition of ChE in nerve tissues. The current understanding of the complexity of this balance indicates that there are multiple potential sites of pharmacokinetic interactions, as well as pharmacodynamic sites of interaction, which can influence toxicological outcomes (i.e., neurological outcomes) with exposure to mixtures of organophosphorus insecticides. As such, knowing about possible interactions at one pharmacokinetic site will not necessarily be predictive of the toxicological outcome.

Based on the above models for individual chemicals, a PBPK model was developed for the binary mixture of chlorpyrifos and diazinon (Timchalk and Poet 2008). Each insecticide inhibited the other's metabolism in *in vitro* experiments in a dose-dependent manner; the pharmacokinetics of the interaction was linear. The interaction reflecting the ChE inhibition was dose-additive and the authors postulated that this type of outcome is most likely to occur in occupational and environmental exposure settings.

To generate data useful for the development of PBPK/PD models for mixtures of organophosphorus insecticides, pharmacokinetic end points (time course of blood levels of parent compounds) and pharmacodynamic end points (ChE activities in plasma, red blood cells [RBCs], and brain) were evaluated in male Sprague-Dawley rats following oral administration of single doses of chlorpyrifos or diazinon alone (0, 15, 30, or 60 mg/kg) or mixtures of chlorpyrifos and diazinon (0, 15/15, 30/30, or 60/60 mg/kg) (Timchalk et al. 2005). At the low dose of the mixture (15/15 mg/kg), pharmacokinetic end points for the components were not influenced, but the high dose (60/60 mg/kg) resulted in increased C_{max} and area under the curve and decreased clearance for both parent compounds. Timchalk et al. (2005) suggested that these results are consistent with dose-dependent competition between chlorpyrifos and diazinon for CYP metabolism and that these pharmacokinetic interactions will not occur at environmentally relevant low doses. Dose-dependent inhibitions of plasma, RBC, and brain ChE were observed with the individual insecticides and the mixture, but statistical determinations of additive, greater-than-additive, or less-than-additive effects on these pharmacodynamic end points were not conducted.

Greater-than-additive interactions among organophosphorus compounds in inducing lethality have been demonstrated in a number of animal studies (for reviews, see Moser et al. 2005; Padilla 2006). For example, early animal studies showed that there was a marked greater-than-additive interaction between *O*-ethyl *O*-(4-nitrophenyl) phenyl phosphonothioate (EPN) and malathion (Murphy and DuBois 1957; Seume and O'Brien 1960) and between EPN and *O, O,*-dimethyl *S*-(*N*-ethyl carbamoyl)methyl) phosphorodithioate (CL 18706) (Seume and O'Brien 1960). Subsequent research showed that: (1) EPN inhibited the detoxifying hydrolysis of malathion by carboxylesterases; (2) other organophosphorus compounds that inhibited carboxylesterase-mediated detoxification potentiated lethality of other organophosphorus compounds; and (3) the carboxylesterase inhibition by EPN and other organophosphorus compounds does not totally explain the potentiation (see Moser et al. 2005; Padilla 2006).

It is unclear from available research whether greater-than-additive interactions among organophosphorus insecticides may represent special cases only involving certain compounds at doses associated with lethality. An investigation of 43 pairs of organophosphorus insecticides, using a dose-additive experimental design and high dose levels that produced lethality in female rats as the end point, revealed that 21 pairs showed additive effects, 18 pairs showed less-than-additive effects, and only 4 pairs (3/4 of these pairs contained malathion) showed greater-than-additive effects (DuBois 1961, as cited in Padilla

2006). These results suggest that greater-than-additive interactions among organophosphorus insecticides are special cases.

In contrast, a study using a statistical design to evaluate dose additivity, demonstrated greater-than-additive effects on several neurological end points (blood and brain ChE inhibition, motor activity, and gait score, but not in a tail pinch response end point) in male Long-Evans rats orally exposed to either a mixture of five organophosphorus insecticides (chlorpyrifos, diazinon, dimethoate, acephate, and malathion) or a four-component mixture with all of the same insecticides, except malathion (Moser et al. 2005). The relative proportions of the insecticides in the mixtures were similar to those estimated in the U.S. diet. Comparison of predicted (using a dose-additive model based on dose-response relationships for the individual components) and empirical ED_{20} and ED_{50} values for the mixtures on the affected end points indicated that the greater-than-additive effects were small, about 1.2–2.1-fold in magnitude (Moser et al. 2005). In a follow-up study, the organophosphorus insecticides were tested in preweanling rats (Moser et al. 2006). The study used the same chemicals as the previous one in the mixture and the same design (full ray or restricted ray without malathion). Greater-than-additivity (synergism) interaction was reported for neurological end points in preweanling rats. For the full ray mixture, the changes ranged from 2- to 3-fold in magnitude. The departure from additivity was also observed for all but two end points following the treatment with a reduced ray mixture. Thus, the results showing greater-than-additivity can only partially be attributed to the malathion in the mixture.

The EPA (2006) cumulative risk assessment for organophosphorus insecticides is based on the assessment that organophosphorus insecticides represent a common mechanism of toxicity group and the assumption of dose additivity. EPA concluded that dose addition is a reasonable approach for estimating cumulative risk of exposures to mixtures of organophosphorus insecticides and that current data do not provide a sufficient basis to depart from dose additivity. The conclusion was based on an evaluation of the available data, including those illustrating the complexity of biotransformation of organophosphorus insecticides, the recent pharmacokinetic studies by Timchalk et al. (2005) with chlorpyrifos and diazinon, the cases of potentiation of organophosphorus insecticide lethality by certain organophosphorus compounds (e.g., Dubois 1961), and the Moser et al. (2005) report of small (1.2–2-fold), greater-than-additive effects on neurological end points in rats from a four- or five-component mixture of organophosphorus insecticides.

2.2.3 MIXTURES OF CARBAMATE INSECTICIDES

PBPK/PD models for mixtures of carbamate insecticides are not available, but models exist for single carbamates. A PBPK model was developed to characterize ChE inhibition following carbofuran exposure in rats (Zhang et al. 2007). Oral doses of 50 µg/kg and 0.5 mg/kg carbofuran were simulated for the blood and brain ChE activity (exposure-related dose estimating model [ERDEM]). The model parameters were based on the open literature data. Another PBPK model was developed to illustrate the tissue dosimetry of carbaryl and its metabolites and to predict the carbaryl-induced inhibition of cholinesterase inhibition (Nong et al. 2008). In support of the model, kinetic studies (with radioactive tracer) were done in rats exposed orally or intravenously to doses of carbaryl ranging from 0.8 to 9.2 mg/kg.

Data regarding health or pharmacokinetic end points in humans or animals exposed to mixtures of carbamate insecticides are scarce, but there are indications that greater-than-additive effects on ChE inhibition do not occur and that dose additivity is an appropriate approach to assessing the neurological effects of mixtures of N-methyl carbamate insecticides via ChE inhibition.

In an *in vitro* study using a ChE biosensor to measure ChE-inhibiting potencies of three carbamate insecticides (aldicarb, carbaryl, and carbofuran) and binary mixtures (aldicarb + carbofuran, aldicarb + carbaryl), the responses to the mixtures were reported to be less than was predicted from the single compounds, but a statistical analysis was not clearly described in the report (Kok and Hasirici 2004).

EPA scientists characterized dose-response relationships for motor activity and RBC and brain ChE inhibition in adult rats exposed to seven carbamate insecticides alone (carbaryl, carbofuran, formetanate HCl, methiocarb, methomyl, oxamyl, or propoxur) or a seven-component mixture. The composition of the mixture was designed to deliver equipotent contributions to brain ChE inhibition from each of the components and dose levels of the mixture were expected to produce <5, 10, 25, 45, and 60% inhibition of brain ChE based on dose additivity. Portions of the study have been published (McDaniel et al. 2007; Padilla et al. 2007) or presented at scientific meetings (Padilla et al. 2005, 2006), but an abbreviated account of the dose additivity assessment results is currently available only in the EPA (2007b) *Revised N-methyl Carbamate Cumulative Risk Assessment.* Increasing doses of the mixture produced increasing decrements in RBC and brain ChE, as well as in alterations of motor activity. EPA (2007b) presented a figure showing that the 95% confidence intervals for predicted values of brain ChE activities (from a dose-additivity model) overlapped with empirical values, indicating that dose additivity provided an

adequate description of the rat brain ChE inhibition response to the mixture. EPA (2007b) also reported that dose additivity provided adequate predictions of the RBC and motor activity responses to the mixture.

EPA's (2007b) overall conclusion for the N-methyl carbamate cumulative risk assessment was that these chemicals represented a common mechanism of toxicity group and that dose addition is a "reasonable and appropriate approach for estimating the cumulative risk associated with joint exposure to the NMC (*N*-methyl carbamate) common mechanism group."

Mwanza et al. (2012) examined the effects of carbaryl and propoxur, singly and in a 1:1.45 mixture, on brain ChE activity and the duration of photic after discharge (PhAD) of flash-evoked potentials in Long-Evans rats exposed to single doses (0, 3, 10, 45, or 75 mg/kg) or 14 daily doses (0, 3, 10, 30, or 45 mg/kg/day) of the mixture. Acute and repeated exposures to the mixture showed similar dose-response relationships for both PhAD duration and brain ChE. Measured PhAD durations were not significantly different from PhAD durations predicted by a dose-addition model constructed from single-chemical data. Measured brain ChE activities following the repeated exposure scenario were greater than brain ChE activities predicted by a dose addition model by 15.5, 10.6, and 5.8% at the 3, 10, and 30 mg/kg/day dosages, suggesting less-than-additive action. Mwanza et al. (2012) concluded that the results are consistent with minimal concern for non-additive actions between carbamates in human health risk assessment because the observed deviations from dose additivity were small and the dosages used in these studies are greater than anticipated human exposures.

2.2.4 PYRETHROID and ORGANOPHOSPHORUS INSECTICIDES

Intraperitoneal administration of certain organophosphorus compounds to mice has been shown to inhibit liver carboxylesterases that hydrolyze the pyrethroid, trans-permethrin (Gaughan et al. 1980). Effective organophosphorus compounds included profenofos, sulprofos, EPN, and S,S,S-tributyl phosphorotri-thioate (DEF). Other organophosphorus (monocrotophos, azinphosmethyl, methyl parathion, acephate) and carbamate (carbaryl, methomyl, and chlordimeform) insecticides were much less active as inhibitors of mouse liver carboxylase. Intraperitoneal administration of profenophos, EPN, or DEF at 25 mg/kg, 1 hour before intraperitoneal administration of the pyrethroid, fenvalerate, lowered the apparent mouse lethal dose $(LD)_{50}$ value for fenvalerate by more than 25-fold. A similar treatment lowered the apparent mouse LD_{50} value for malathion (an organophosphorus insecticide) by 5–9-fold, depending on the organophosphorus compound, but did not alter the mouse LD_{50} value for trans-permethrin. The results

suggest that certain organophosphorus compounds inhibit carboxylesterases (e.g., EPN, but not acephate) and that this can potentiate the acute lethality of certain pyrethroid insecticides (e.g., fenvalerate, but not trans-permethrin).

Choi et al. (2004) examined the ability of chlorpyrifos (an organophosphorus insecticide), and its major metabolite (chlorpyrifos-oxon) to inhibit the *in vitro* hydrolysis of trans-permethrin by human liver microsomes, presumably via carboxylesterases. Chlorpyrifos did not influence the trans-permethrin hydrolysis activity of human liver microsomes, but chlorpyrifos oxon significantly inhibited trans-permethrin hydrolysis.

Concurrent dermal exposure of male Wistar rats to a commercial formulation of an organophosphorus insecticide, methyl parathion, at nonlethal doses lowered the subcutaneous LD_{50} value for a commercial formulation of permethrin, providing some evidence for a greater-than-additive effect of methyl parathion on the acute lethality of permethrin (Ortiz et al. 1995). LD_{10} and LD_{50} values were determined for dermal exposure to methyl parathion alone (506 and 566 mg methyl parathion/kg, respectively) and subcutaneous exposure to permethrin alone (3,533 and 7,832 mg permethrin/kg respectively), and LD_{50} values for permethrin were determined for co-exposures to subcutaneous doses of permethrin with nonlethal dermal doses of methyl parathion at 380 mg/kg methyl parathion (LD_{50}=7,146 mg permethrin/kg) or 464 mg/kg methyl parathion (LD_{50}=4,981 mg permethrin/kg). The authors proposed that the apparent potentiation of permethrin lethality might have been due to the inhibition by methyl parathion or its reactive metabolite, methyl paraoxon, of detoxifying hydrolysis of permethrin by carboxylesterases.

A greater-than-additive interaction between the pyrethroid insecticide, esfenvalerate, and the organophosphorus insecticide, diazinon, was reported in a 96-hour LC_{50} study of larval fathead minnows in static-renewal tests at test concentrations ranging from 2,000 to 12,000 µg/L for diazinon alone, from 0.1 to 0.3 µg/L esfenvalerate alone, or equitoxic concentrations of the two insecticides at 5, 10, 25, 50, or 100% of their respective published LC_{50} values (Denton et al. 2003). In three replicate tests, the predicted LC_{50} values of the mixture were less than the measured LC_{50} values predicted by additivity (diazinon LC50 in tests 1, 2, and 3: 6.393, 5.048, and 7.969 µg/L; esfenvalerate LC50 values: 0.18, 0.22, and 0.22 µg/L, esfenvalerate and diazinon mixture LC50(%): 24.8, 28.8, 37.9) , indicative of a greater-than-additive interaction. An “interactive ratio” (IR) was calculated as the measured toxic unit of the combination divided by the predicted toxic unit of diazinon plus the predicted toxic unit of esfenvalerate. Deviations in the IR values from 1 were taken as indicators of greater-than-additive action when the IR was >1 and less-than-additive action when the IR was <1. IR values for the three tests were 1.7, 1.4, and

1.4, but no analyses were conducted to assess statistical significance of the deviation in the IR from the additivity value of 1. Following 96-hour exposures between 50 and 1,000 µg/L of diazinon alone or unspecified concentrations of esfenvalerate alone, carboxylesterase activities (hydrolysis of p-nitrophenol) were measured in homogenates of surviving larvae. Diazinon exposure alone was associated with a 46–50% inhibition of carboxylesterase activity, compared with controls, in the concentration range tested, but no effects on carboxylesterase activities were observed from esfenvalerate exposure alone. The results suggest that diazinon inhibition of the detoxifying hydrolysis of esfenvalerate by carboxylesterases played a role in the apparent greater-than-additive interaction.

In another series of studies with fathead minnows and midge larvae, a greater-than-additive interaction between the pyrethroid insecticide, esfenvalerate, and the organophosphorus insecticide, chlorpyrifos, was reported from analyses of tests of exposures to equipotent mixtures at numerous concentrations in both species and a test of the effects of low levels of chlorpyrifos on esfenvalerate toxicity (Belden and Lydy 2006). Organisms were exposed for 96 hours; percentage of fish with decreased mobility (mobility defined as the ability to swim away after gentle probing while maintaining an upright position) or larvae with affected mobility (those that could not perform a figure-eight swimming motion) were the toxic end points, and exposure concentrations were below those associated with lethality. EC_{50} values for exposure of fathead minnows to esfenvalerate or chlorpyrifos alone were 0.4 and 200 µg/L, respectively; for midge larvae, respective EC_{50} values were 0.21 and 0.16 µg/L. Exposure of fathead minnows to the equipotent mixture increased the percentage of fish with decreased mobility with increasing concentrations; empirically determined EC values and 95% confidence intervals occurred at lower concentrations and did not overlap with predicted values based on a concentration-additivity model (i.e., dose addition assuming a common mode of action) or an independent action model (i.e., response addition assuming independent action). Observed EC_{50} values for fathead minnow exposed to the mixtures were 1.5–2.5-fold lower than predicted values from the concentration additivity and independent action models, respectively. For midge larvae exposed to the equipotent mixture, observed EC_{50} values were 1.1–1.5-lower than predicted values from the concentration additivity and independent action models, respectively. Empirical EC_{50} values for fathead minnows exposed to a mixture at various concentrations of esfenvalerate and a fixed concentration of chlorpyrifos (expected to produce less than a 1% response on mobility) were 1.29-fold lower than the EC_{50} value for exposure to esfenvalerate alone. The results from these studies are consistent with a greater-than-additive toxicokinetic interaction between chlorpyrifos and esfenvalerate, which may involve inhibition, by chlorpyrifos oxon, of the detoxifying hydrolysis of esfenvalerate mediated by carboxylesterases. Comparison of the observed and predicted EC_{50} values indicates that the magnitude of the interaction is not large and within a factor of 2.

Joint toxicity of binary mixtures of organophosphates and pyrethroids was also tested in zebrafish (Zhang et al. 2010). Five concentrations of each individual chemical or 50:50 binary combinations were tested in triplicates. Symptoms of fish toxicosis included loss of equilibrium, erratic swimming, fast gill movement, horizontal hanging in water, and trying to avoid contaminated water. Using immovability as the death standard, LC_{50} values were determined for each insecticide alone and for each binary mixture. Combination coefficients for the eight binary mixtures were determined as the ratio of a predicted LC_{50} (based on dose addition) and the observed LC_{50} of the mixture. Ratios <0.57 and >1.75 were taken as evidence for less-than-additive and greater-than-additive actions, respectively. Ratios with intermediate values were taken as evidence of dose additivity. Dose-additive joint actions were indicated for binary combinations of dichlorvos (organophosphate) and permethrin or tetramethrin (pyrethroids) and of phoxim (organophosphate) and permethrin, tetramethrin, or bifenthrin (pyrethroids). Less-than-additive actions were indicated for mixtures of dichlorvos (organophosphate) and etofenprox or bifenthrin (pyrethroids), whereas the mixture of phoxim (organophosphate) and etofenprox (pyrethroids) showed additivity for 24-hour exposure and greater-than-additivity (synergism) at 48, 72, and 96 hours of exposure.

In *in vitro* studies with cultured mouse N2a neuroblastoma and rat C6 glioma cell lines, Flaskos et al. (2007) examined effects of diazinon alone (1 or 10 µM), cypermethrin alone (1 or 10 µM), and an equimolar mixture of diazinon (10 µM) and cypermethrin (10 µM) on numbers of neurite outgrowths in response to cell differentiation stimuli and ChE activities. At the tested concentrations of either agent alone or the mixture (exposure period was 24 hours), no effects on cell viability were observed, compared with controls. Diazinon alone at 10 µM significantly ($p<0.05$) inhibited neurite outgrowth in N2a cells, compared with controls, but diazinon alone did not influence neurite outgrowth in C6 cells. At the tested concentrations, cypermethrin did not alter neurite outgrowth in either N2a or C6 cells, compared with controls. No significant difference was found between the inhibition of neurite outgrowth in N2a cells by 10 µM diazinon and the inhibition produced by the high concentration mixture, indicating that cypermethrin (which did not affect neurite outgrowth) did not influence the inhibitory effect of diazinon on neurite outgrowth. Diazinon (10 µM) significantly inhibited ChE activities in N2a cells after 4 hours of exposure by about 15–20% compared with controls, but no significant effect was apparent at 24 hours, or in diazinon-exposed C6 cells at either time point. Cypermethrin exposure did not significantly alter ChE activities in either cell line at any time point. ChE activities in N2a cells exposed for 4 hours to the high concentration mixture were not significantly different from controls, suggesting that cypermethrin antagonized the slight effect of diazinon on ChE activity. In summary, the results indicate that

cypermethrin did not influence the inhibitory effect of diazinon on neurite outgrowth and provides weak evidence for a possible antagonistic effect of cypermethrin on diazinon anti-ChE activity. Exposure conditions in this study were selected to avoid ChE inhibition, so the test is not an adequate examination of the possible antagonism of diazinon anti-ChE activity by cypermethrin. Other information related to the possible antagonistic effect of cypermethrin on diazinon anti-ChE activity was not located.

In a study of reversibility of ChE inhibition and brain histological changes in female Wistar rats after dermal application of a mixture of chlorpyrifos and cypermethrin for 1 or 4 weeks, serum and rat ChE activities were initially inhibited, but returned to control levels within 2 or 3 weeks after exposure (Latuszynska et al. 2003). Rats were exposed to a mixture of 27.8 mg/cm^2 of chlorpyrifos and 2.7 mg/cm^2 of cypermethrin applied to tail skin under occluded conditions for 4 hours daily for 1 or 4 weeks. Slight histological changes in various regions of the brain were observed when rats were sacrificed 3 weeks after exposure; the changes were described as increased density of the cytoplasm in cells of the cortex cerebri, stratum hippocampi CA 1, hilus area dentatae, thalamus nuclei, and cerebellum. Further information about this study is not provided here, because it provides limited information about dose-response relationships for effects from this binary mixture (only one dose level was applied) and it was not designed to characterize possible interactions between chlorpyrifos and cypermethrin.

In a study of neurobehavioral end points, adult male Sprague-Dawley rats were exposed daily for 30 days to dermal doses of 44.4 mg/kg malathion alone, 40 mg/kg N,N-diethyl-m-toluamide (DEET) alone, 0.13 mg/kg permethrin alone, or binary mixtures or a trinary mixture of the compounds at the same dose levels (Abdel-Rahman et al. 2004). Neurobehavioral end points, examined 24 hours after cessation of exposure, included a beam-walking score, beam walk time, incline plane performance, and grip time. Each of the treatments significantly impaired each neurobehavioral end point; the combination of DEET and permethrin, malathion and permethrin, and the whole mixture of three chemicals resulted in greater effects in decreased performance than after permethrin alone. The design of this study is insufficient to determine joint actions (i.e., additivity or deviations from additivity) of components of the mixtures investigated.

In a study with volunteers, statistical analysis of 24-hour cumulative urinary excretion of pyrethroid metabolites and associated elimination half-lives showed no significant differences after exposure to single doses of 0.01 mg/kg/day deltamethrin alone versus a mixture of 0.01 mg/kg/day deltamethrin plus 0.01 mg/kg/day chlorpyrifos-methyl (Sams and Jones 2011). The results suggest that the ability of

chlorpyrifos oxon to inhibit the hydrolytic metabolism of certain pyrethroids, observed in *in vitro* studies by Choi et al. (2004), did not occur to a sufficient degree to influence excretion profiles of deltamethrin metabolites under the low-dose *in vivo* conditions of this study.

In summary, results from *in vitro* and *in vivo* studies indicate that certain oxon metabolites of organophosphorus insecticides (e.g., chlorpyrifos oxon and diazinon oxon) can inhibit the detoxification of certain pyrethroid insecticides (e.g., trans-permethrin and fenvalerate) via carboxylesterases and that this can potentiate acute pyrethroid lethality in rodents (Choi et al. 2004; Gaughan et al. 1980; Ortiz et al. 1995). Greater-than-additive effects also have been observed on fathead minnow larvae lethality with mixtures of diazinon and esfenvalerate (Denton et al. 2003) and on mobility of fathead minnow and midge larvae with mixtures of chlorpyrifos and esfenvalerate (Belden and Lydy 2006); however, in another study of acute lethality in zebrafish exposed to eight binary mixtures of pyrethroid and organophosphorus insecticides, five mixtures showed evidence for dose additivity, two showed less-than-additive action, and only one showed evidence for greater-than-additive action (Zhang et al. 2010). The aquatic toxicity study results show some evidence for a toxicokinetic interaction consistent with inhibition of carboxylesterases by organophosphorus insecticides leading to a potentiation of pyrethroid toxicity. Comparisons of predicted (i.e., from additivity models) and empirical EC_{50} or LC_{50} values in these aquatic species suggest that the greater-than-additive effects were small, when observed, being <3-fold in magnitude. In volunteers exposed to a mixture with low (0.01 mg/kg) doses of the pyrethroid, deltamethrin, and chlorpyrifos-methyl, no significant effects on the kinetics of urinary excretion of deltamethrin metabolites were found, compared with exposure to deltamethrin alone (Sams and Jones 2011).

Information on possible effects of pyrethroids on organophosphorus insecticide toxicokinetics or toxicity is limited to the report that cypermethrin had no influence on diazinon inhibition of *in vitro* neurite outgrowth in mouse N2a neuroblastoma cells (Flaskos et al. 2007). No strong or consistent evidence was located for an influence of pyrethroid insecticides on organophosphorus insecticide anti-ChE activity. In mouse N2a neuroblastoma cells, exposure to 10 µM diazinon for 4 hours, but not 24 hours, inhibited ChE activity by about 15–20%, but ChE activity was not decreased, compared with controls, following exposure to 10 µM permethrin alone or 10 µM diazinon + 10 µM permethrin for 4 or 24 hours. In rat C6 cells, no statistically significant effects on ChE activities were produced by 4- or 24-hour exposures to 10 µM diazinon alone, 10 µM permethrin alone, or 10 µM diazinon + 10 µM permethrin (Flaskos et al. 2007).

2.2.5 PYRETHROID AND CARBAMATE INSECTICIDES

Carbamate insecticides do not appear to be potent inhibitors of trans-permethrin hydrolysis and are not expected to potentiate acute pyrethroid toxicity via this type of interaction. Intraperitoneal administration of carbaryl, methomyl, or chlordimeform to mice at concentrations up to 16, 4, or 64 mg/kg, respectively, did not decrease liver microsomal activities to hydrolyze trans-permethrin, compared with controls; whereas administration of low intraperitoneal doses (0.65–1.5 mg/kg) of certain organophosphorus compounds (e.g., EPN) produced 50% inhibition of trans-permethrin hydrolytic activity (Guaghan et al. 1980). Mice treated intraperitoneally with the pyrethroid, fenvalerate, at several concentrations up to 1,000 mg/kg did not induce mortalities with or without an intraperitoneal pretreatment with 25 mg/kg of carbaryl, methomyl, or chlordimeform (Guaghan et al. 1980). In contrast, pretreatment by effective organophosphate inhibitors of trans-permethrin hydrolysis caused dramatic changes in mouse intraperitoneal LD_{50} values: from >1,000 mg/kg without pretreatment to 42, 37, or 37 mg/kg with pretreatment with EPN, profenofos, or DEF, respectively (Guaghan et al. 1980). Choi et al. (2004) reported that carbaryl inhibited the hydrolysis of trans-permethrin by human liver microsomes, but kinetic inhibition constants were 2 orders of magnitude higher than those of the potent inhibitor, chlorpyrifos oxon and carbaryl with chlorpyrifos oxon showing greater potency than carbaryl. Complete inhibition of hydrolytic activity by carbaryl required about a 40-fold greater concentration than chlorpyrifos-oxon.

No other studies designed to examine joint actions of pyrethroid and carbamate insecticides on toxicokinetic or toxicological end points were located.

2.2.6 ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

Certain carbamates have been shown to antagonize the neurological effects of organophosphorus nerve gases (see Gordon et al. 2006 and Padilla 2006 for review). The protective action of these carbamates, when given shortly before subsequent challenge with a nerve agent, is thought to involve the temporary reversible inhibition of ChE by the carbamate, thereby protecting against irreversible inhibition of ChE by the organophosphorus nerve agent. Pyridostigmine, mobam, physostigmine, and decarbofuran are carbamates that have been demonstrated to protect guinea pigs, but not rats, from acute poisoning by nerve gases (e.g., soman), with pyridostigmine providing the longest-lasting protective effect (Gordon et al. 1978). These actions have led to the use of pyridostigmine as a prophylactic agent when attack by organophosphorus nerve gases is imminent.

Studies examining other end points with mammalian systems provide evidence for less-than-additive or additive joint action for mixtures of organophosphorus and carbamate insecticides (Bosgra et al. 2009; Carter and Maddux 1974; Gordon et al. 2006; Institoris et al. 2004; Syberg et al.2008). The most well-designed *in vivo* study, which examined pertinent end points of brain ChE and thermoregulation in rats exposed to mixtures of carbaryl and chlorpyrifos in a study design appropriate for testing deviations from dose additivity, found no evidence for greater-than-additive action, but depending on the end point examined and the ratio of the components in the mixture, evidence for additive or less-than-additive action was found (Gordon et al. 2006).

1. Observed percentage inhibitions of *in vitro* ChE activities in human plasma or erythrocytes exposed *in vitro* to mixtures of dichlorvos (an organophosphorus insecticide) + carbaryl or dichlorvos + physostigmine were less than predicted values, based on addition of responses to the components alone (Carter and Maddux 1974).

2. Bosgra et al. (2009) developed a toxicodynamic model of ChE inhibition that incorporated mechanistic differences between inhibition by organophosphorus and carbamate insecticides. The model adequately described data for *in vitro* ChE inhibition by combinations of methamidophos (organophosphorus insecticide) and methomyl (carbamate). ChE inhibition predicted by a dose-addition model were similar to predictions from the toxicodynamic model.

3. In volunteers exposed to a mixture with low doses of 0.02 mg/kg pirimicarb (a carbamate) and 0.01 mg/kg/day chlorpyrifos-methyl (an organophosphorus insecticide), no significant effects on the kinetics of urinary excretion of pirimicarb metabolites were found, compared with exposure to the carbamate alone (Sams and Jones 2011). This study also examined the influence of chlorpyrifos-methyl on the kinetics of urinary excretion of metabolites of a pyrethroid, deltamethrin.

4. Toxicity of a mixture of dimethoate (organophosphate) and pirimicarb (carbamate) was tested in a *Daphnia magna* immobilization experiment (Syberg et al. 2008). Dose addition was confirmed for the mixture when compared with results of the individual chemicals.

5. Several neurological end points, plaque-forming spleen cell counts, and body and liver weights were examined in male Wistar rats exposed 5 days/week for 6 weeks to oral doses of methyl parathion alone at 0.218 or 0.872 mg/kg/day (1/100 or 1/25 of LD_{50} value), propoxur (a

carbamate also known as Baygon™) alone at 0.851 or 8.51 mg/kg/day (1/100 or 1/10 of LD_{50} value), or mixtures of 0.218 mg/kg/day methyl parathion + 8.51 mg/kg/day propoxur or 0.872 mg/kg/day methyl parathion + 0.851 mg/kg/day propoxur (Institoris et al. 2004). The mixtures were formulated to assess whether the low dose of the organophosphorus insecticide would alter the responses to the high dose of the carbamate and vice versa. Neurological end points included open field behavior, auditory startle response, rotarod performance, somatosensory and auditory brain evoked potentials, and peripheral nerve conduction velocity. The mixture of low-dose methyl parathion and high-dose propoxur did not influence any end point to a degree that was significantly ($p<0.05$) different from the effect of the high dose of propoxur, with the exception of increased relative liver weight, decreased acoustic startle response score, and decreased plaque-forming spleen cell counts. Responses to the mixture of high-dose methyl parathion and low-dose propoxur were not significantly different from responses to the high dose of methyl parathion alone. The results from this study provide no evidence for a gross interaction between methyl parathion and carbaryl on the end points measured, but the design of the study precludes determination of additive, greater-than-additive, or less-than-additive joint action for this binary mixture.

6. Body temperature regulation and inhibition of plasma and brain ChE were measured in adult male Long-Evans rats exposed by gavage to chlorpyrifos alone (0, 10, 20, 30, or 50 mg/kg), or carbaryl alone (0, 25, 50, 75, or 150 mg/kg), and the temperature index was quantified (Gordon et al. 2006). Also tested were doses of mixtures with chlorpyrifos: carbaryl ratios of 2:1 (0, 7, 14, 21, 28, or 35 mg/kg) or 1:1 (0, 5, 12, 22, 32, or 42 mg/kg) on the temperature index, which were expected to be equally spaced above and below the threshold for induction of hypothermia, using an assumption of dose additivity. For the hypothermia end point, a less-than-additive (i.e., antagonistic) response was observed with the 2:1 mixture, but an additive response was observed with the 1:1 mixture. For brain ChE inhibition, the 2:1 mixture showed additive effects, but the 1:1 mixture showed less-than-additive effects. For plasma ChE inhibition, the 2:1 and 1:1 mixture showed less-than-additive effects. No evidence for greater-than-additive interaction was found. Based on previously observed correlations between hypothermic effects of anti-ChE agents and inhibition of ChE and physiological understanding that organophosphorus or carbamate exposure produces hypothermia in rodents primarily by stimulation of muscarinic cholinergic receptors, the direction of the joint action was expected to be the same for the hypothermia end point and the ChE end points. However, the results indicate that demonstration

of dose additive or less-than-additive joint action between chlorpyrifos and carbaryl depended on the ratio of the compounds in the mixture, as well as the end point.

In contrast to the expectations from the previously described studies that less-than-additive or additive interactions may occur with mixtures of organophosphorus and carbamate insecticides, certain organophosphorus insecticides (at subtoxic exposure levels) have been demonstrated to potentiate the acute toxicity of certain carbamate insecticides, but the degree of potentiation has been shown to vary with different combinations of organophosphorus and carbamate insecticides (Gupta and Dettbarn 1993; Keplinger and Deichmann 1967; Takahashi et al. 1987).

1. In an early study of joint action of components of equitoxic binary mixtures of one of several organophosphorus insecticides with carbaryl, the ratios of predicted rat oral LD_{50} values (based on response to the individual compounds alone and assuming dose additivity) to the observed LD_{50} values were: 1.82 for malathion + carbaryl; 1.58 for delnav + carbaryl; 1.58 for V-C 13 + carbaryl; 1.58 for parathion + carbaryl; 1.3 for diazinon + carbaryl; and 1.05 for trithion + carbaryl (Keplinger and Deichmann 1967). The results indicate that the magnitude of the apparent greater-than-additive interaction was not large and within a 2-fold factor for the investigated organophosphorus and carbamate insecticide mixtures.

2. Pretreatment of mice with low levels of the organophosphorus insecticides, fenitrothion or fenthion, markedly lowered the LD_{50} values for 2-sec-butylphenyl N-methylcarbamate (BPMC) (Miyaoka et al. 1984; Takahashi et al. 1984; Tsuda et al. 1984). In a subsequent study, pretreatment with low levels (about 1/20 of LD_{50} values) of any one of several organophosphate insecticides with a thiophosphorus (P=S) functional group (cyanophos, fenitrothion, and malathion) potentiated the acute lethality of BPMC (Takahashi et al. 1987). In contrast, pretreatment with dichlorvos (which contains a P=O functional group) did not potentiate the acute lethality of BPMC (Takahashi et al. 1987). Pretreatment with fenitrothion (1/24 of LD_{50} value) potentiated the acute lethality of five carbamate insecticides to varying degrees, with BPMC showing the greatest degree of potentiation. The ratios of the non-pretreated LD_{50}/pretreated LD_{50} values were 4.9 for BPMC, 2.5 for MTMC (3-methylphenyl *N*-methylcarbamate), 1.8 for NAC (1-naphthyl *N*-methylcarbamate), 1.7 for XMC (3,5-dimethylphenyl *N*-methylcarbamate), and 1.4 for MPMC (3,4-dimethylphenyl *N*-methylcarbamate). The potentiation was initially thought to involve inhibition of CYP-mediated metabolism of BPMC (leading to increased time of exposure to BPMC), based on a correlation with increased plasma levels of BPMC in mice

pretreated with fenitrothion or fenthion, but the degree of potentiation for other tested carbamates was not correlated with degrees of increase of plasma carbamate concentrations induced by the organophosphorus agent (Takahashi et al. 1987). Pretreatment with a well-known nonorganophosphorus inhibitor of CYP oxygenases, SKF 525-A, increased plasma levels of BPMC to the same degree as fenitrothion, but potentiation of toxicity by SKF 525-A was much less than that by fenitrothion. The results indicate that the organophosphorus-induced increase in plasma BPMC concentrations, mediated by inhibition of CYP by the organophosphorus agent, did not fully explain the potentiation of carbamate acute lethality by organophosphorus insecticides. The magnitude of the greater-than-additive interaction was greatest for the fenitrothion:BPMC combination (about 5-fold), and ranged from about 1.2- to 2.5-fold for the other four fenitrothion:carbamate mixtures.

3. *In vitro* metabolism of carbaryl mediated by CYP isozymes in human liver microsomes was markedly inhibited by the presence of chlorpyrifos (Tang et al. 2002). The most affected pathway was methyl hydroxylation catalyzed mostly by CYP2B6, the isozyme expected to be most active in forming chlorpyrifos oxon from chlorpyrifos.

4. Intraperitoneal administration of rats with 1 mg/kg of tetraisoproylpyrophosphoramide (iso-OMPA) alone or 0.1 mg/kg aldicarb alone produced no cholinergic signs of toxicity and inhibited cortical brain ChE activities to about 95% (not significant, $p>0.05$) or 70% ($p<0.05$) of control values, respectively (Gupta and Dettbarn 1993). When this dose of iso-OMPA was administered 1 hour before 0.1 mg/kg aldicarb, rats displayed distinct cholinergic signs of toxicity and cortical brain ChE activities of about 10% of control values. Carboxylesterase activities in cortical brain tissues were about 75% of control values in rats administered iso-OMPA or aldicarb alone, and were about 50% of control values in rats exposed to iso-OMPA + aldicarb. The apparent potentiation of aldicarb toxicity by iso-OMPA was correlated with the increased combined inhibitory effect of the two compounds on carboxylesterase, which is involved in detoxification of aldicarb and other carbamates.

Studies of ChE inhibition (the shared toxicity target of carbamates and organophosphorus insecticides) in salmon have shown that binary mixtures of organophosphorus and carbamate insecticides act in an additive joint manner to inhibit ChE *in vitro*, but with 96-hour *in vivo* exposures to binary mixtures, greater-than-additive action on brain ChE inhibition can occur with some mixtures at low concentrations that would be expected (based on the assumption of dose additivity) to produce 10% ChE inhibition.

(Laetz et al. 2009; Scholz et al. 2006). With increasing exposure concentrations, increasing numbers of tested binary mixtures of organophosphorus and carbamate insecticides showed greater-than-additive action (Laetz et al. 2009).

1. Scholz et al. (2006) provided evidence for additive joint action of organophosphorus and carbamate insecticides on inhibition of *in vitro* ChE activities extracted from olfactory nerves of adult Chinook salmon (2–3 years of age) by examining the effects of two carbamates alone at several concentrations (carbaryl and carbofuran), several metabolites of organophosphorus insecticides alone at several concentrations (oxons of diazinon, chlorpyrifos, and malathion), and all possible binary mixtures at two concentrations. For each insecticide pair, mixtures were formulated at equitoxic concentrations expected to produce 25 or 50% ChE inhibition based on the assumption of dose addition. Observed ChE inhibition activities for each of the binary mixtures appeared to cluster tightly around a regression for ChE inhibition as a component-based dose-addition function of normalized EC_{50} values. No obvious deviations above or below the regression for any of the binary mixtures were apparent; deviations above or below the regression would indicate less-than-additive or greater-than-additive interaction, respectively.

2. Laetz et al. (2009) provided evidence of additive or greater-than-additive actions on brain ChE with *in vivo* exposures of juvenile salmon (4–7 months of age) to all possible binary mixtures of two carbamate (carbaryl and carbofuran) and three organophosphorus insecticides (diazinon, malathion, and chlorpyrifos). Brain ChE activities were measured following 96-hour exposure (on a 24-hour static renewal schedule) to each of the seven insecticides alone, at 4–7 sublethal concentrations producing varying degrees of ChE inhibition, and to all possible binary mixtures at concentrations predicted (based on an assumption of dose additivity) to produce inhibitions of 10, 29, and 50%. With exposure to the low level binary mixtures, 4 of the 10 binary mixtures displayed significantly ($p<0.05$) greater inhibition than predicted (10%) and the other 6 displayed inhibition not significantly different from predicted. The mixtures displaying greater-than-additive joint action were carbofuran + chlorpyrifos (about 25% inhibition), carbofuran + diazinon (about 30%), diazinon + chlorpyrifos (about 30%), and diazinon + malathion (>90%). At this low exposure level, two of the six carbamate + organophosphorus mixtures showed greater-than-additive action. The number of combinations showing significant deviations from additivity increased with increasing exposure concentrations, with pairings of organophosphorus insecticides showing the greatest degree of synergism (especially those with malathion). With high level exposure (expected to produce 50% ChE inhibition), all tested mixtures showed

significant greater-than-additive action: carbaryl + carbofuran (60%), carbaryl + chlorpyrifos (60%), carbofuran + chlorpyrifos (60%), carbaryl + diazinon (60%), carbofuran + malathion (70%), carbaryl + malathion (80%), carbofuran + diazinon (90%), diazinon + chlorpyrifos (70%), malathion + chlorpyrifos (>90%), and diazinon + malathion (>90%).

In summary, the available data examining action of mixtures of carbamate and organophosphorus insecticides presents a tableaux of conflicting results for which there are only some mechanistic explanations. Dose-additive or less-than-additive joint actions on brain ChE and associated thermoregulatory end points were observed in a well-designed study of rats orally exposed to mixtures of carbaryl and chlorpyrifos, depending on the end point and relative proportion of components in the mixture (Gordon et al. 2006). Deviations from additivity are not explained by current mechanistic understanding, but they were not large for brain ChE activities. In volunteers, no evidence was found for an influence of chlorpyrifos-methyl on the elimination kinetics of pirimicarb metabolites (Sams and Jones 2011). Greater-than-additive actions were observed when rodents were pretreated with subtoxic levels of certain organophosphorus insecticides (e.g., fenitrothion, fenthion, iso-OMPA) leading to the potentiation of the acute lethality of oral doses of carbamates (Gupta and Dettbarn 1993; Keplinger and Deichmann 1967; Takahashi et al. 1987). The potentiation was associated with inhibition of CYP-mediated metabolism of the carbamates by certain organophosphorus agents, but inhibition of CYP monooxygenase activity alone was insufficient to explain the potentiation (Takahashi et al. 1987). Greater-than-additive action on brain ChE activities in juvenile salmon was observed for two of six mixtures of carbamate and organophosphorus insecticides at low concentrations expected to inhibit ChE by about 10% (Laetz et al. 2009). The number of tested mixtures showing greater-than-additive action increased with increasing concentrations; at concentrations predicted by dose addition to inhibit brain ChE by 50%, all six tested binary mixtures showed significantly greater than expected inhibition, ranging from about 60 to 90% inhibition (Laetz et al. 2009). The greater-than-additive interaction is not fully understood, but is not expected to involve direct interaction at the active site of ChE; additive joint action of binary mixtures of organophosphorus and carbamate insecticides has been demonstrated for inhibition of ChE in *in vitro* systems (Scholz et al. 2006). Possible sites of interaction leading to this greater-than-additive action include alteration of the activities of the numerous CYP and carboxylesterase isozymes involved in the biotransformation of these types of insecticides. The protective actions of certain carbamates against the acute lethality of organophosphorus nerve gases appear to be special cases that may have limited pertinence to the assessment of environmental mixtures of carbamate and organophosphorus insecticides.

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

No studies were located that examined health effects in humans or animals exposed to three-component mixtures representing all three pesticide classes (pyrethroid insecticides, organophosphate insecticides, and carbamate insecticides), precluding the derivation of Minimal Risk Levels (MRLs) for three component mixtures of these classes of insecticides. While there are PBPK/PD models for some of the insecticides under consideration in this profile, PBPK/PD "interaction" models for chemicals from all classes of concern were not located.

As discussed in Appendices A, B, and C, neurological effects are the principal and most well-studied toxic effects associated with exposure to individual members of each of these insecticide classes. As discussed in the appendices, cancer is not an expected health end point of concern for most members of each of these insecticide classes. Likewise, results from standard developmental toxicity and reproductive toxicity tests in animals do not identify developmental toxicity or reproductive toxicity as critical health effects for many members of each class (Appendix A–C). There is a concern for possible neurodevelopmental effects from organophosphorus insecticides based on positive results in a few *in vivo* studies and *in vitro* mechanistic studies (see Appendix B). Available data do not clearly establish neurodevelopmental effects as health effects of concern for pyrethroid or carbamate insecticides (see Appendices A and C). On the basis of these observations, target toxicity doses (TTDs) were not developed in this profile, and recommendations are made for assessing health risks for neurological effects only from these insecticide classes.

In the absence of studies that examine relevant end points and describe dose-response relationships following oral exposures to mixtures that contain chemicals from these three chemical classes (e.g., in food), component-based approaches to assessing their joint action that assume dose additivity for neurological effects appear to be reasonable for practical public health concerns (e.g., the hazard index [HI] approach). Given the overlap in toxicity targets of these chemicals, such approaches are preferable, from a public health protection perspective, to approaches that would assess hazards of the individual classes separately.

For each of these chemical classes, it is recommended that hazard quotients (HQs) for neurological effects be calculated using appropriate index-chemical equivalent doses and provisional oral MRLs for index chemicals. Index-chemical equivalent doses would be calculated from measured levels in environmental media, exposure models, and EPA-derived RPFs listed in Appendices A–C. The recommended

provisional oral MRLs are based on the EPA-derived points of departure (PODs) and appropriate uncertainty factors (see Chapter 3). For screening-level assessments, HQs for neurological effects from each of the three classes would then be added (under an assumption of dose additivity) to calculate the HI for neurological effects from pyrethroid, carbamate, and organophosphorus insecticides (see Chapter 3).

It is recognized that the assumption of dose additivity in the last step is not supported by mechanistic information, indicating that these classes of chemicals do not share a common mechanism of toxicity. However, the approach is viewed as reasonable and practical for screening-level assessments if available data on the possible joint actions of pairs of the chemical classes of concern are evaluated. As discussed in Section 2.2, "interaction" PBPK/PD models for pairs of chemicals, or sets of three chemicals, from the three classes are not available. Using the classification scheme summarized in Table 2 and ATSDR (2004a), Tables 3 through 8 describe binary weight-of-evidence determinations (BINWOEs) for the pairs of the three chemical classes of concern. The conclusions presented in these tables were based on the evaluations of results from the available interaction literature presented in Section 2.2. A summary of the BINWOEs is presented in Table 9. The BINWOEs focus on simultaneous oral exposure, as this is the exposure scenario of most interest for public health concerns for the subject classes of insecticides and their mixtures. A summary discussion of the BINWOEs follows this paragraph and precedes the descriptive tables.

Acute neurological effects are expected from all three classes of insecticides through different modes of action: (1) alteration of kinetics of VGSCs in neurons by pyrethroids, predominantly via parent compounds; (2) irreversible ChE inhibition by organophosphorus insecticides or their metabolites; and (3) reversible ChE inhibition by carbamate insecticides, predominantly via parent compounds.

As discussed in Tables 3 and 4, greater-than-additive action is possible between certain pyrethroid and organophosphorus insecticides based on observations of greater-than-additive joint action on lethality and nonlethal end points in aquatic species exposed to diazinon + esfenvalerate or chlorpyrifos + esfenvalerate (Belden and Lydy 2006; Denton et al. 2003), a small decrease in the acute intraperitoneal LD_{50} value for permethrin in rats pretreated with methyl parathion at nonlethal doses (Ortiz et al. 1995), and a substantial decrease in the intraperitoneal LD_{50} value for fenvalerate, but not trans-permethrin, in mice pretreated with profenofos, EPN, or DEF (Gaughan et al. 1980). The BINWOE scores for the possible greater-than-additive effect of certain organophosphorus insecticides on pyrethroids (III.C.ii) indicates that: (1) mechanisms underlying the possible interaction are not well characterized and are not consistently demonstrated across studies or chemicals (III); (2) the toxicological significance is unclear

(C – evidence come from aquatic species and mammalian studies of lethal doses); and (3) the evidence comes from routes of exposure other than oral administration (ii). The uncertainty of greater-than-additive actions occurring in humans exposed to mixtures of pyrethroids and organophosphorus insecticides in the environment is emphasized by the observation that the elimination kinetics of metabolites of deltamethrin were not significantly influenced in humans exposed to a mixture of deltamethrin and chlorpyrifos-methyl at low doses (0.01 mg/kg each), compared with exposure to deltamethrin alone (Sams and Jones 2011).

As discussed in Tables 5 and 6, the direction of possible interactions between pyrethroid and carbamate insecticides cannot be predicted due to the lack of appropriate data.

As discussed in Tables 7 and 8, the available evidence supports using additive joint action for screening-level assessments of neurological effects from mixtures of carbamates and organophosphorus insecticides. The BINWOE scores (= III.C.1.a.1.) reflect evidence for dose additivity in a well-designed study of brain ChE and thermoregulatory end points in rats exposed to single oral doses of a mixture of carbaryl and chlorpyrifos and evidence for small deviations from additivity (less than additive) without adequate mechanistic explanation (Gordon et al. 2006). The toxicological significance would be clearer with similar findings for mixtures with other members of the two classes of insecticides. Evidence for greater-than-additive action on brain ChE was found in an *in vivo* fish study (Laetz et al. 2009), but the results in rats are taken to be more relevant to human oral exposure scenarios.

On the basis of the existing data as summarized in the BINWOE tables, the evidence to move from a dose-additive approach to screening-level assessments of neurological hazards from mixtures of pyrethroids, carbamates, and organophosphorus insecticides is not compelling. ATSDR recommends that the default assumption of dose-additive joint action at shared targets of toxicity (i.e., effects on neurological end points) be employed to assess potential adverse health outcomes associated with concurrent oral exposures to pyrethroid, organophosphorus, and carbamate insecticides with qualitative descriptions of the possible impact of the BINWOE assessments on the resultant hazard assessment.

Table 2. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

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

Source: ATSDR 2004a

Table 3. Effect of Pyrethroids on Organophosphorus Insecticides
BINWOE: > III.C.ii

Direction of Interaction – No strong or consistent evidence for an influence of pyrethroids on the anti-ChE activity of organophosphorus insecticides is available, but this issue is poorly studied. Greater-than-additive action is possible between certain pyrethroid and organophosphorus insecticides based on observations of greater-than-additive joint action on lethality and nonlethal end points in aquatic species exposed to diazinon + esfenvalerate or chlorpyrifos + esfenvalerate (Belden and Lydy 2006; Denton et al. 2003), a small decrease in the acute intraperitoneal LD_{50} value for permethrin in rats pretreated with methyl parathion at nonlethal doses (Ortiz et al. 1995), and a substantial decrease in the intraperitoneal LD_{50} value for fenvalerate, but not trans-permethrin, in mice pretreated with profenofos, EPN, or DEF (Gaughan et al. 1980). Mechanistic understanding of this joint action is poor, but a mechanism that has received attention is the inhibition of detoxifying metabolism of pyrethroids by organophosphorus agents. Available data indicate that not all members of these insecticide classes will interact to produce greater-than-additive action on toxicological end points. For example, pretreatment with profenofos did not alter the acute mouse LD_{50} value for trans-permethrin (Gaughan et al. 1980). The magnitude of possible greater-than-additive effects is uncertain; effects were <3-fold for the cases of diazinon + esfenvalerate and chlorpyrifos + esfenvalerate in aquatic species and methyl parathion + permethrin in rats, but substantial (about 25-fold) for profenofos, EPN, or DEF potentiation of fenvalerate.

Mechanistic Understanding – Acute neurological effects are expected from both classes of insecticides through different mechanisms of action—irreversible ChE inhibition by organophosphorus agents or their metabolites and alteration orpf ion channel kinetics by pyrethroids, predominantly via parent compounds. Effects on biotransformations of these insecticides have received attention as possible sites of interactions. Greater-than-additive action between certain organophosphorus (e.g., EPN and DEF, but not methyl parathion) and certain pyrethroid insecticides (e.g., fenvalerate, but not trans-permethrin) on acute lethality end points in mice was not strictly associated with the ability of organophosphorus agents to inhibit hydrolysis of pyrethroids via carboxylesterases, suggesting that other detoxification routes, such as CYP monooxygenases, may be more important than hydrolysis for some pyrethroids (e.g., trans-permethrin) (Gaughan et al. 1980). *In vitro* hydrolysis of trans-permethrin by human liver microsomes is inhibited by chlorpyrifos-oxon or carbaryl, with chlorpyrifos-oxon showing 40-fold greater inhibiting activity than carbaryl (Choi et al. 2004), but studies examining possible effects of pyrethroids on metabolism of organophosphorus insecticides were not located. The complexity of biotransformations of organophosphorus and pyrethroid insecticides (multiple toxifying and detoxifying mechanisms can act on members of both classes of insecticides) precludes prediction of the direction of the interaction based on observation of interaction at one biotransformation step.

Toxicologic Significance – The possible influence of pyrethroids on the anti-ChE activity of organophosphorus insecticides is poorly studied. Mouse N2a cells exposed for 4 hours to 10 µM diazinon showed 15–20% inhibited ChE activity, but no ChE inhibition, compared with controls, following exposure to10 µM diazinon + 10 µM permethrin (Flaskos et al. 2007). This apparent antagonism was not found in rat C6 cells exposed to 10 µM diazinon + 10 µM permethrin versus 10 µM diazinon alone (Flaskos et al. 2007). Greater-than-additive action was demonstrated on fathead minnow larvae lethality with mixtures of diazinon + esfenvalerate (Denton et al. 2003) and on mobility of fat head minnows or midge larvae exposed to mixtures of chlorpyrifos + esfenvalerate (Belden and Lydy 2006). In both cases, the magnitude of the greater-than-additive effect was <3-fold.

Additional Uncertainties – Available studies have not explored the possible route dependency of interactions between organophosphorus and pyrethroid insecticides; available *in vivo* mammalian data utilized dermal and intraperitoneal routes and not the oral route of concern for this profile.

Table 4. Effect of Organophosphorus Insecticides on Pyrethroids

BINWOE: > III.C.ii

Direction of Interaction – Greater-than-additive action is possible between certain organophosphorus and pyrethroid insecticides based on observations of greater-than-additive joint action on lethality and nonlethal end points in aquatic species exposed to diazinon + esfenvalerate or chlorpyrifos + esfenvalerate (Belden and Lydy 2006; Denton et al. 2003), a small decrease in the acute intraperitoneal LD_{50} value for permethrin in rats pretreated with methyl parathion at nonlethal doses (Ortiz et al. 1995), and a substantial decrease in the intraperitoneal LD_{50} value for fenvalerate, but not trans-permethrin, in mice pretreated with profenofos, EPN, or DEF (Gaughan et al. 1980). Mechanistic understanding of this joint action is poor, but proposed mechanisms include inhibition of detoxifying metabolism of pyrethroids by certain organophosphorus agents. Available data indicate that not all members of these insecticide classes will interact to produce greater-than-additive action on toxicological end points. For example, pretreatment with profenofos did not alter the acute mouse LD_{50} value for trans-permethrin (Gaughan et al. 1980). The magnitude of possible greater-than-additive effects is uncertain; effects were <3-fold for the cases of diazinon + esfenvalerate and chlorpyrifos + esfenvalerate in aquatic species and methyl parathion + permethrin in rats, but substantial (about 25-fold) for profenofos, EPN, or DEF potentiation of fenvalerate.

Mechanistic Understanding – Acute neurological effects are expected from both classes of insecticides through different mechanisms of action—irreversible ChE inhibition by organophosphorus agents or their metabolites and alteration of ion channel kinetics by pyrethroids, predominantly via parent compounds. Effects on biotransformations of these insecticides have received attention as possible sites of interactions. Greater-than-additive action between certain organophosphorus (e.g., EPN and DEF, but not methyl parathion) and certain pyrethroid insecticides (e.g., fenvalerate, but not trans-permethrin) on acute lethality end points in mice was not strictly associated with the ability of organophosphorus agents to inhibit hydrolysis of pyrethroids via carboxylesterases, suggesting that other detoxification routes, such as CYP monooxygenases, may be more important than hydrolysis for some pyrethroids (e.g., trans-permethrin) (Gaughan et al. 1980). *In vitro* hydrolysis of trans-permethrin by human liver microsomes is inhibited by chlorpyrifos-oxon or carbaryl, with chlorpyrifos-oxon showing 40-fold greater inhibiting activity than carbaryl (Choi et al. 2004). The complexity of biotransformations of organophosphorus and pyrethroid insecticides (multiple toxifying and detoxifying mechanisms can act on members of both classes of insecticides) precludes prediction of the direction of the interaction based on observation of interaction at one biotransformation step.

Toxicologic Significance – No data were located on the possible influence of organophosphorus insecticides on the ability of pyrethroids to alter the kinetics of nervous tissue ion channels. Greater-than-additive action was demonstrated on fathead minnow lethality with mixtures of diazinon + esfenvalerate (Denton et al. 2003) and on mobility of fathead minnows or midge larvae exposed to mixtures of chlorpyrifos + esfenvalerate (Belden and Lydy 2006). In both cases, the magnitude of the greater-than-additive effect was <3-fold. Dermal doses of methyl parathion (MP), which were below the LD_{10} of 506 mg MP/kg, lowered the subcutaneous LD_{50} value for permethrin in rats by 9% (380 mg MP/kg) and 39% (464 mg MP/kg) (Ortiz et al. 1995). Pretreatment (intraperitoneal injection) of mice with certain organophosphorus agents (profenofos, EPN, DEF), but not others (sulprofos, monocrotophos, azinphosmethyl, methyl parathion, acephate), lowered the intraperitoneal LD_{50} value for fenvalerate by about 25-fold, but had no effect on trans-permethrin toxicity (Gaughan et al. 1980).

Additional Uncertainties – Available studies have not explored the possible route dependency of interactions between organophosphorus and pyrethroid insecticides; available *in vivo* animal data utilized dermal and intraperitoneal routes and not the oral route of concern for this profile. Uncertainty associated with the possible occurrence of greater-than-additive action is highlighted by the observation that elimination kinetics of metabolites of deltamethrin were not significantly influenced in humans exposed to a mixture of deltamethrin and chlorpyrifos-methyl, compared with exposure to deltamethrin alone (Sams and Jones 2011).

Table 5. Effect of Pyrethroids on Carbamates

BINWOE: ?

Direction of Interaction – The direction of possible interactions cannot be predicted because there are no relevant *in vivo* or *in vitro* data examining joint action on pertinent neurological end points or other toxicological end points, and the available mechanistic understanding for carbamates and pyrethroids does not support reliable projections of possible interactions.

Mechanistic Understanding – Acute neurological effects are expected from both classes of insecticides through different mechanisms of action—reversible ChE inhibition by carbamates predominantly via parent compounds and alteration of ion channel kinetics by pyrethroids, predominantly via parent compounds. Effects on biotransformations of these insecticides have received limited attention as possible sites of interactions (e.g., effects of carbamates on pyrethroid metabolism), but no data were located on the possible influence of pyrethroids on the metabolism of carbamates.

Toxicologic Significance – No studies were located on the possible influence of pyrethroids on the anti-ChE activities of carbamates.

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

Table 6. Effect of Carbamates on Pyrethroids

BINWOE: ?

Direction of Interaction – The direction of possible interactions cannot be predicted because there are no relevant *in vivo* or *in vitro* data examining joint action on pertinent neurological end points or other toxicological end points, and the available mechanistic understanding for carbamates and pyrethroids does not support reliable projections of possible interactions.

Mechanistic Understanding – Acute neurological effects are expected from both classes of insecticides through different mechanisms of action—reversible ChE inhibition by carbamates predominantly via parent compounds and alteration of ion channel kinetics by pyrethroids, predominantly via parent compounds. Effects on biotransformations of these insecticides have received limited attention as possible sites of interactions. *In vitro* hydrolysis of trans-permethrin by human liver microsomes is inhibited by carbaryl (Choi et al. 2004), but intraperitoneal administration of 16, 4, or 64 mg/kg carbaryl, methomyl, or chlordimeform to mice did not influence liver microsomal activities for hydrolysis of trans-permethrin (Guaghan et al. 1980). Carbamate insecticides do not appear to be potent inhibitors of detoxifying hydrolysis of pyrethroid insecticides. The complexity of biotransformations of carbamate and pyrethroid insecticides (multiple detoxifying mechanisms [i.e., CYP oxidation and hydrolysis via carboxylesterases] can act on members of both classes of insecticides) precludes prediction of the direction of the interaction based on observation of interaction at one biotransformation step.

Toxicologic Significance – No data were located on the possible influence of carbamate insecticides on the ability of pyrethroids to alter the kinetics of nervous tissue ion channels. Pretreatment (intraperitoneal) of mice with 25 mg/kg of carbaryl, methomyl, or chlordimeform did not potentiate the acute lethality of fenvalerate (Guaghan et al. 1980). No other studies designed to examine joint actions of carbamate and pyrethroid insecticides on toxicological end points were located.

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

Table 7. Effect of Carbamates on Organophosphorus Insecticides
BINWOE: = III.C.1.a.i.

Direction of Interaction – Additive or less-than-additive joint actions on ChE and associated thermoregulatory end points were observed in a well-designed study of rats orally exposed to mixtures of carbaryl and chlorpyrifos, depending on the end point and relative proportion of components in the mixture (Gordon et al. 2006). For example, additive action on brain ChE was evident with 2:1 mixtures of chlorpyrifos:carbaryl and less-than-additive with 1:1 mixtures. The deviation from dose additivity on brain ChE with the 1:1 mixture was about 30%. The 1:1 mixture showed no significant deviation from additivity on a hypothermia index, but the 2:1 mixture showed less-than-additive action. Because the deviations from additivity were not large on brain ChE, and the study involved oral exposure of a mammalian species to nonlethal doses, the results support using dose-additive joint action for screening-level assessments of neurological effects from mixtures of carbamates and organophosphorus insecticides.

In vivo fish studies provide evidence for greater-than-additive action on brain ChE by mixtures of carbamates and organophosphorus insecticides (Laetz et al. 2009), but the results in rats are taken to be more relevant to humans. The protective action of certain carbamates (e.g., pyridostigmine) against the acute lethality of organophosphorus nerve gases is taken to be of limited relevance to comparatively low level concurrent dietary exposures of humans to mixtures of insecticides of these classes.

Mechanistic Understanding – Acute neurological effects are expected from both classes of insecticides through similar, but not identical, mechanisms of action at the enzymatic active site of ChE: irreversible ChE inhibition by organophosphorus insecticides and their metabolites, and reversible ChE inhibition by carbamates predominantly via parent compounds. Additive joint action on brain ChE is a reasonable assumption based on the rat results from Gordon et al. (2006), but mechanistic information is inadequate to explain the variability in deviations from dose additivity in this study.

Dose addition provided an adequate explanation of the action of binary mixtures of carbaryl, carbofuran, and oxons of organophosphorus insecticides (diazinon, chlorpyrifos, malathion) on *in vitro* ChE activities from adult salmon nerve tissues (Scholz et al. 2006), but greater-than-additive actions on brain ChE in juvenile salmon were observed with *in vivo* 96-hour exposures to all possible binary mixtures of the same insecticides (Laetz et al. 2009). The mechanistic nature of this greater-than-additive action is poorly understood, but the possible sites of interaction include alteration of the activities of the numerous CYP and carboxylesterase isozymes involved in the biotransformation of these types of insecticides (Gupta and Dettbarn 1993; Takahashi et al. 1987; Tang et al. 2002). The complexity of biotransformations of carbamate and organophosphorus insecticides (e.g., multiple detoxifying mechanisms can act on members of both classes of insecticides) precludes prediction of the direction of the interaction based on observations of interaction at one biotransformation step.

Toxicologic Significance and Modifiers – The toxicological significance is unclear (C). Although evidence for additive joint action was demonstrated in one *in vivo* (a) study (Gordon et al. 2006) for appropriate end points (brain ChE and thermoregulatory index) in orally exposed (i) rats for the anticipated duration and sequence (1–acute and concurrent), small deviations from additivity were observed that were dependent on end point and relative proportions of components in the mixture, and binary mixtures of only a single member of each class were studied.

Additional Uncertainties – Available studies have not explored the possible route dependency of interactions between carbamate and organophosphorus insecticides.

Table 8. Effect of Organophosphorus Insecticides on Carbamates
BINWOE: = III.C.1.a.i.

Direction of Interaction – Additive or less-than-additive joint actions on ChE and associated thermoregulatory end points were observed in a well-designed study of rats orally exposed to mixtures of carbaryl and chlorpyrifos, depending on the end point and relative proportion of components in the mixture (Gordon et al. 2006). For example, additive action on brain ChE was evident with 2:1 mixtures of chlorpyrifos:carbaryl and less-than-additive with 1:1 mixtures. The deviation from dose additivity on brain ChE with the 1:1 mixture was about 30%. The 1:1 mixture showed no significant deviation from additivity on a hypothermia index, but the 2:1 mixture showed less-than-additive action. Because the deviations from additivity were not large on brain ChE, and the study involved oral exposure of a mammalian species to nonlethal doses, the results support using dose-additive joint action for screening-level assessments of neurological effects from mixtures of carbamates and organophosphorus insecticides. In volunteers, no evidence was found for an influence of chlorpyrifos-methyl on the elimination kinetics of pirimicarb metabolites (Sams and Jones 2011).

In vivo fish studies provide evidence for greater-than-additive action on brain ChE by mixtures of carbamates and organophosphorus insecticides (Laetz et al. 2009), but the results in rats are taken to be more relevant to humans. Pretreatment of rodents by certain organophosphorus insecticides has been reported to potentiate the acute lethality of certain carbamates (Gupta and Dettbarn 1993; Keplinger and Deichmann 1967; Takahashi et al. 1987), but these studies were inadequately designed to test a hypothesis of dose additivity for concurrent exposure to mixtures of carbamates and organophosphorus insecticides at nonlethal doses.

Mechanistic Understanding – Acute neurological effects are expected from both classes of insecticides through similar, but not identical, mechanisms of action at the enzymatic active site of ChE: irreversible ChE inhibition by organophosphorus insecticides and their metabolites, and reversible ChE inhibition by carbamates predominantly via parent compounds. Additive joint action on brain ChE is a reasonable assumption based on the rat results from Gordon et al. (2006), but mechanistic information is inadequate to explain the variability in deviations from dose additivity in this study.

Dose addition provided an adequate explanation of the action of binary mixtures of carbaryl, carbofuran, and oxons of organophosphorus insecticides (diazinon, chlorpyrifos, malathion) on *in vitro* ChE activities from adult salmon nerve tissues (Scholz et al. 2006), but greater-than-additive actions on brain ChE in juvenile salmon were observed with *in vivo* 96-hour exposures to all possible binary mixtures of the same insecticides (Laetz et al. 2009). The mechanistic nature of this greater-than-additive action is poorly understood, but the possible sites of interaction include alteration of the activities of the numerous CYP and carboxylesterase isozymes involved in the biotransformation of these types of insecticides (Gupta and Dettbarn 1993; Takahashi et al. 1987; Tang et al. 2002). The complexity of biotransformations of carbamate and organophosphorus insecticides (e.g., multiple detoxifying mechanisms can act on members of both classes of insecticides) precludes prediction of the direction of the interaction based on observations of interaction at one biotransformation step.

Toxicologic Significance – The toxicological significance is unclear (C). Although evidence for additive joint action was demonstrated in one *in vivo* (a) study (Gordon et al. 2006) for appropriate end points (brain ChE and thermoregulatory index) in orally exposed (i) rats for the anticipated duration and sequence (1–acute and concurrent), small deviations from additivity were observed that were dependent on end point and relative proportions of components in the mixture, and binary mixtures of only a single member of each class were studied.

Additional Uncertainties – Available studies have not explored the possible route dependency of interactions between carbamate and organophosphorus insecticides.

Table 9. Matrix of BINWOE Determinations for Repeated Simultaneous Oral Exposure to Insecticide Classes of Concern

		ON THE TOXICITY OF		
		Pyrethroids	Organophosphorus Insecticides	Carbamates
EFFECT OF	Pyrethroids		**> III.C.ii**	**?**
	Organophosphorus Insecticides	**> III.C.ii**		**= III.C.1.a.i.**
	Carbamates	**?**	**= III.C.1.a.i.**	

BINWOE scheme condensed from ATSDR (2004a):

DIRECTION: = additive; > greater than additive; < less than additive; ? indeterminate

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

TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant end point;
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals;
C: toxicologic significance of interaction is unclear.

MODIFYING FACTORS:
1: anticipated exposure duration and sequence;
2: different exposure duration or sequence;
a: in vivo data;
b: in vitro data;
i: anticipated route of exposure;
ii: different route of exposure.

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

To conduct exposure-based assessments of possible neurologic health hazards from oral exposures to mixtures of members of all three insecticide classes (pyrethroid, organophosphorus, and carbamate insecticides), ATSDR recommends the use of a component-based approach, because there are no direct data available to characterize health hazards (and dose-response relationships) from exposure. In addition, "interaction" PBPK/PD models have not yet been developed that would predict appropriate target doses of the components.

Recommendations focus on oral exposure because it is the predominant route of exposure for the general population to these insecticide classes. In the EPA cumulative risk assessments for organophosphorus insecticides (EPA 2006), carbamate insecticides (EPA 2007b), and pyrethroids (EPA 2011a), analysis of exposure estimates from food, water, and residential use (the latter pathway involved oral, dermal, and inhalation exposures) indicated that exposures through the food pathway were greater than the residential use and drinking water pathways for the general population. For carbamates, food exposure estimates were greater than the residential pathway estimates (which were dominated by dermal exposures from lawn uses of carbaryl), which were greater than exposures through drinking water (EPA 2007b). For organophosphorus insecticides, exposure estimates from the food pathway were substantially greater than exposure estimates from drinking water, which were substantially greater than exposure estimates from residential use pathways (EPA 2006). Similarly, the general population's exposure to pyrethroid insecticides is expected to be primarily from food sources, especially fruits and vegetables (ATSDR 2003a; EPA 2011a).

As discussed by ATSDR (1992, 2004a), the exposure-based assessment of potential health hazard is a screening approach, to be used in conjunction with evaluation of community-specific health outcome data, consideration of community health concerns, and biomedical judgment, to assess the degree of public health hazard presented by mixtures of substances released into the environment. In a component-based approach for noncancer health effects: (1) joint additive actions of the components on shared targets of toxicity are assumed; (2) oral intakes are calculated based on measured concentrations of the components in media of concern; (3) intakes are divided by MRLs or TTDs; and (4) resulting HQs are summed to arrive at a HI.

TTDs are developed for an end point of concern when the critical effect levels for those effects are higher than those associated with the most sensitive end point. When the most sensitive end point is the effect of concern, the MRL is used as the reference toxicity benchmark for estimating the effect-specific HI (ATSDR 2004a). For this assessment, TTDs were not developed for any of the insecticide classes because neurological effects from short-term exposures are the most clearly established health effect of concern for each class, and other end points are not clearly established as shared toxicity targets by most members of each class (see Appendices A–C).

ATSDR recognizes that EPA's RPF approaches for organophosphorus insecticides (EPA 2006), carbamate insecticides (EPA 2007b), and pyrethroids (EPA 2011a) represent more comprehensive and up-to-date approaches for assessing neurological hazards from these insecticide classes, compared with the use of ATSDR's MRLs or EPA RfDs listed on IRIS. RPFs for 33 organophosphorus insecticides were developed versus intermediate oral ATSDR MRLs for 9 members of this class (see Appendix B). RPFs for 15 pyrethroids were developed versus acute oral MRLs for 3 pyrethroids and intermediate oral MRLs for 2 pyrethroids (see Appendix A). RPFs for 13 carbamates were developed versus no ATSDR MRLs for any carbamates (see Appendix C). As described herein, ATSDR recommends the use of provisional oral MRLs for the index chemicals (methamidophos for organophosphorus insecticides, deltamethrin for pyrethroids, and oxamyl for the carbamates) and RPFs for other members of each class to assess neurological effects from organophosphorus, pyrethroid, and carbamate insecticides.

For the assessment of organophosphorus insecticides, concentrations of organophosphorus residues in the media of concern should be converted to methamidophos equivalents via multiplication by the pertinent RPF (see Table B-4 in Appendix B) and summed to arrive at exposure levels that can be converted to oral intakes (in units of mg/kg/day). The estimated intake is then divided by the provisional oral MRL for methamidophos to arrive at a HQ for exposure to methamidophos equivalents. To derive a provisional oral MRL for methamidophos, the $BMDL_{10}$ value for 10% inhibition of brain ChE in rats in the principal study, 0.07 mg/kg/day (see Table B-5 in Appendix B), is selected as the POD and divided by a total uncertainty factor of 100 (10 to account for extrapolation from rats to humans and 10 for human variability) as follows:

$$\text{Provisional oral MRL for methamidophos} = \text{POD} \div \text{uncertainty factor} = 0.07 \text{ mg/kg/day} \div 100 = 0.0007 \text{ mg/kg/day}$$

For the assessment of carbamate insecticides, concentrations of carbamate residues in the media of concern should be converted to oxamyl equivalents via multiplication by the pertinent RPF (the

unadjusted "Oral RPF" values in Table C-2 in Appendix C) and summed to arrive at exposure levels that can be converted to oral intakes (in units of mg/kg/day). The estimated intake is then divided by the provisional oral MRL for oxamyl to arrive at a HQ for exposure to oxamyl equivalents. To derive a provisional oral MRL for oxamyl, the $BMDL_{10}$ value for 10% inhibition of brain ChE in rats in the principal study, 0.18 mg/kg/day, is selected as the POD (see Table C-3 in Appendix C) and divided by a total uncertainty factor of 100 (10 to account for extrapolation from rats to humans and 10 for human variability) as follows:

Provisional oral MRL for oxamyl = POD ÷ uncertainty factor =
0.18 mg/kg/day ÷ 100 = 0.0018 mg/kg/day

In the EPA (2007b) carbamate cumulative risk assessment, EPA developed chemical-specific "interspecies factors" for aldicarb (factor = 2), aldicarb sulfone (factor = 2), aldicarb sulfoxide (factor = 2), methomyl (factor = 5), and oxamyl (factor = 3) to replace the default interspecies uncertainty factor of 10 (see Table C-1 in Appendix C). These factors were calculated as ratios of rat and human BMD_{10} values for ChE inhibition, when they were available. Since this type of data is not available for all 13 carbamates assessed by EPA (2007b), and the provisional MRL for oxamyl will be used to assess a cumulative HQ for all carbamates with RPFs, the default factor of 10 was used in the derivation of the provisional oral MRL for oxamyl.

For the assessment of pyrethroid insecticides, concentrations of pyrethroid residues in the media of concern should be converted to deltamethrin equivalents via multiplication by the pertinent RPF (see Table A-1 in Appendix A) and summed to arrive at exposure levels that can be converted to oral intakes (in units of mg/kg/day). The estimated intake is then divided by the provisional oral MRL for deltamethrin to arrive at a HQ for exposure to deltamethrin equivalents. To derive a provisional oral MRL for deltamethrins, the $BMDL_{20}$ value for 20% increase in incidence for scores >4 on a composite neurological score of body temperature, tremors, clonic convulsions, salivation, and altered mobility, 11 mg/kg/day (Table A-1 in Appendix A), is selected as the POD and divided by a total uncertainty factor of 100 (10 to account for extrapolation from rats to humans and 10 for human variability) as follows:

Provisional oral MRL for deltamethrin = POD ÷ uncertainty factor =
11 mg/kg/day ÷ 100 = 0.11 mg/kg/day

As specified by the FQPA, EPA (2006, 2007b, 2011a) used additional FQPA factors to provide additional protection of infants and children in its cumulative risk assessments for organophosphorus, carbamate,

and pyrethroid insecticides. The default FQPA factor of 10 was replaced with chemical-specific FQPA factors for 9 of the 13 carbamates assessed (see Table C-2 in Appendix C) and for 10 of the 33 organophosphorus insecticides assessed (see Table B-4 in Appendix B). For pyrethroids, the default 10 safety factor was replaced with a 3 safety factor for children from birth to <6 years of age (see Appendix A.2). Therefore, to provide additional protection for infants and children, the concentrations of the individual carbamates, organophosphorus, or pyrethroid insecticides in the media of concern could be multiplied by the chemical-specific RPF values and appropriate FQPA factors before summing and converting to intakes of index chemical equivalents.

ATSDR recommends the calculation of a screening-level HI for assessing neurological effects from oral exposures to mixtures of pyrethroid, organophosphorus, and carbamate insecticides under the assumption of dose additivity. The HI for neurological effects from oral exposure to a mixture of pyrethroid, organophosphorus, and carbamate insecticides would be calculated as follows:

$$HI_{Neurologic} = \frac{E_{oxamyl\ equiv}}{MRL_{oxamyl}} + \frac{E_{methamidophos\ equiv}}{MRL_{methamidophos}} + \frac{E_{deltamethrin}}{MRL_{deltamethrin}}$$

where E = estimated oral intake in units of mg/kg/day. Modification of the intakes with FQPA factors can provide HIs providing additional protection for infants and children.

Preliminary evidence that the exposure to the mixture may constitute a hazard is provided when the HI exceeds 1. In practice, concern for the possibility of a health hazard increases with increasing value of the HI above 1.

The addition of HQs assumes that less-than-additive (e.g., antagonistic or inhibitory) or greater-than-additive (e.g., synergistic or potentiating) interactions do not occur among the components of the mixture. As discussed in Sections 2.2 and 2.3, greater-than-additive action on neurological end points is possible between certain pyrethroid and organophosphorus insecticides (Tables 3 and 4), the available data are inadequate to assess the possible direction of interactions between pyrethroids and carbamates (Tables 5 and 6), and available data support dose additivity of carbamate and organophosphorus insecticides on neurological end points (Tables 7 and 8). Overall, the evidence is not compelling to move from a dose-additive approach. ATSDR recommends that screening-level assessments of neurological hazard using the HI approach be accompanied by qualitative descriptions of these evaluations of the available interaction data. The evaluations indicate that evidence is available for greater-than-additive interactions

between certain pyrethroid and organophosphorus insecticides, but key findings come from a study of potentiation of fenvalerate lethality in rats pretreated with certain organophosphorus insecticides (Gaughan et al. 1980). The relevance of these findings to relatively low (nonlethal) environmental concurrent exposure to pyrethroid and organophosphorus insecticides is not well understood. No evidence of a toxicokinetic interaction was found in a study of elimination kinetics of pyrethroid metabolites in volunteers exposed to a mixture of low doses (0.01 mg/kg) of deltamethrin and chlorpyrifos-methyl (Sams and Jones 2011). These findings reflect the uncertainty that greater-than-additive joint actions between pyrethroid and organophosphorus insecticides may occur in humans exposed to levels of these insecticides in food.

Data Needs for Assessing Joint Toxic Actions of Pyrethroid, Organophosphorus, and Carbamate Insecticides. Although there are PBPK models for some individual chemicals within these three classes of insecticides, there are no "interaction" PBPK models like those that exist for benzene, toluene, ethylbenzene, and xylene (BTEX) and certain other volatile organic chemicals (e.g., see ATSDR Interaction Profile for BTEX; ATSDR 2004b). Before such models can be developed, pharmacokinetic or pharmacodynamic points of interactions between members of the subject classes must first be identified. To date, no common points of pharmacokinetic or pharmacodynamic interaction have been clearly identified, other than ChE inhibition for carbamate and organophosphorus insecticides. Possible points of interaction including various steps in biotransformation, but the understanding of the complexity of biotransformations for these insecticide classes is too limited to identify key interaction events. With the identification of a common point of pharmacokinetic or pharmacodynamic interaction, it would be possible to design the additional studies needed to develop an "interaction" PBPK model for members of these insecticide classes. Following identification of common points of pharmacokinetic interaction, *in vivo* studies could be conducted to examine the kinetics of internal concentrations of the parent chemicals of concern and their metabolites following co-exposure, comparing results to exposure to each component alone.

Neurodevelopmental effects from exposure to insecticides is of concern to public health because of the likelihood of exposure to mixtures of insecticides in food, but adequate research to establish these types of neurological effects as hazards is not available for most members of each of the subject classes of insecticides (see Appendices A–C). With the possible future identification of neurodevelopment effects as hazards from several members in each class, additional research on possible interactions of these insecticides and the impact of the interactions on neurodevelopmental end points would help to decrease uncertainties in the current approach to assessing only short-term neurological human health hazards.

4. Conclusions

ATSDR recommends a component-based HI approach that assumes dose-additive joint toxic action for preliminary assessment of possible neurological health hazards from oral exposure to mixtures of pyrethroid, organophosphorus, and carbamate insecticides. No studies were located that examined neurological end points following exposure to any mixtures of members of all three of these insecticide classes, thereby precluding the derivation of any "whole mixture" MRLs. Acute neurological effects are expected from all three classes of insecticides albeit through different mechanisms of action: (1) alteration of VGSCs by pyrethroids, predominantly via parent compounds; (2) irreversible ChE inhibition by organophosphorus insecticides or their metabolites; and (3) reversible ChE inhibition by carbamate insecticides, predominantly via parent compounds. The common general toxicity target shared by all members of each of these insecticide classes supports the use of a component-based HI approach as a reasonable and practical strategy for addressing public health concerns.

On the basis of the existing data presented in Section 2.2 and summarized and evaluated in the BINWOE tables presented in Section 2.3, ATSDR recommends that the default assumption of dose-additive action at shared targets of toxicity (i.e., effects on neurological end points) be used for screening-level assessments of the potential adverse neurological outcomes from concurrent oral exposures to mixtures of pyrethroid, organophosphorus and carbamate insecticides. For each insecticide class, ATSDR recommends a RPF approach using RPFs derived by the EPA OPP (EPA 2006, 2007b, 2011b) and provisional oral MRLs for index chemicals of each class (Section 3). The HI for neurological effects would be calculated as the sum of class-specific HQs of estimated intakes of index chemical divided by provisional oral MRLs for the index chemical. When the screening assessment indicates a potential hazard (concern increases as the HI increases beyond a value of 1), further evaluation is needed including 1) further refined cumulative risk assessment methods, 2) the use of biomedical judgment, 3) community-specific health outcome data, and 4) taking into account community health concerns.

ATSDR recommends that screening-level assessments of neurological hazard using the HI approach be accompanied by qualitative descriptions of weight-of-evidence evaluations of available interaction data:

1. greater-than-additive action on neurological end points is possible between certain pyrethroid and organophosphorus insecticides;

2. the available data are inadequate to assess the possible direction of interactions between pyrethroids and carbamates; and

3. limited available data support dose additivity of carbamate and organophosphorus insecticides on neurological end points.

Overall, the evidence is not compelling to move from a dose-additive approach. The evaluations indicate that greater-than-additive interactions between certain pyrethroid and organophosphorus insecticides are possible, but key findings come from a study of potentiation of fenvalerate lethality in rats pretreated with certain organophosphorus insecticides (Gaughan et al. 1980). The relevance of these findings to relatively low (nonlethal) environmental concurrent exposure to pyrethroid and organophosphorus insecticides is not well understood.

Table 10. Interactions/Mixtures Terminology

Interaction	When the effect of a mixture is different from the expectation of additivity based on the dose-response relationships of the individual components.
Additivity	When the effect of the mixture can be estimated from the sum of the exposure levels (weighted for potency in dose or concentration additivity) or the probabilities of effect (response additivity) of the individual components.
No apparent influence	When a component that is not toxic to a particular biological system does not influence the toxicity of a second component on that system.
Synergism	When the effect of a mixture is greater than that estimated by additivity. Synergism is defined in the context of the definition of no interaction, which is usually dose additivity or response additivity. The use of “greater-than-additive” is preferred over the use of the term synergism.
Potentiation	When a component that is not toxic to a particular biological system increases the effect of a second chemical on that system.
Antagonism	When the effect of a mixture is less than that estimated by additivity. Antagonism is defined in the context of the definition of no interaction, which is usually dose additivity or response additivity. The use of “less-than-additive” is preferred over the use of the term antagonism.
Inhibition	When a component that does not have a toxic effect on a particular biological system decreases the apparent effect of a second chemical on that organ system.
Masking	When the components produce opposite or functionally competing effects on the same biological system, and diminish the effects of each other, or one overrides the effect of the other.

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Appendix A: Background Information for Pyrethroids

Pyrethroids are manufactured insecticides that are similar in chemical structure to pyrethrins, naturally occurring chemicals found in certain chrysanthemum flowers (ATSDR 2003a). The pyrethroids are more photostable than pyrethrins and are widely used in agriculture and in medical and veterinary products (EPA 2011a). In the 1990s, permethrin was the most frequently used pyrethroid in U.S. agricultural crop production, representing about 40% of the amount of pyrethroids applied to U.S. crops in this period (about 2.5 million pounds total pyrethroids per year; ATSDR 2003a). It has been estimated that pyrethroids account for about 25% of the recent global insecticide market (Costa 2008; Soderlund et al. 2002).

A.1 Toxicokinetics

Based on studies in humans and animals, pyrethroids are expected to be absorbed via the oral, inhalation, and dermal routes of exposure (ATSDR 2003a). Following absorption, pyrethroids are rapidly and widely distributed to tissues, including central and peripheral nerve tissues (ATSDR 2003a). Biotransformation of pyrethroids involves the hydrolytic cleavage of the central ester bond catalyzed by carboxylesterases to yield carboxylic acid derivatives and phenoxybenzoic acid derivatives and oxidation of alcohol groups by CYP oxygenases (ATSDR 2003a; Costa 2008). For most pyrethroids, hydrolytic and oxidative metabolic transformations represent detoxification processes. Inhibitors of CYP and carboxylesterases enhance the toxicity of pyrethroids, and piperonyl butoxide (a CYP inhibitor) consequently is a component of many commercial pyrethroid formulations (Costa 2008). Results from animal studies indicate that pyrethroids are eliminated from the body within 4–12 days following oral exposure via urinary excretion of metabolites and fecal excretion of parent compounds and metabolites (ATSDR 2003a).

A.2 Health Effects

Neurological effects are the principal effects associated with exposure to pyrethroids (ATSDR 2003a; EPA 2011a). Pyrethroid insecticides act by interfering with the function of voltage-gated sodium channels (VGSCs) in nerve cells, producing whole-body tremors associated with coma or seizures and death in mammals with acute exposure to high doses (ATSDR 2003a; EPA 2011a). Modification of the kinetics of VGSC activation and inactivation are thought to underlie acute clinical signs of pyrethroid neurotoxicity (EPA 2011a, 2011b). At nonlethal doses of pyrethroids, acute- and intermediate-duration studies with animals have reported signs of neurological impairment including increased excitability and

aggressiveness, decreased grip strength and motor activity, and altered gait in rats given single doses of pyrethroids or repeatedly exposed to permethrin in the diet, and diarrhea in dogs given cyhalothrin in gelatin capsules daily for 26 weeks (ATSDR 2003a). Two types of pyrethroids are recognized based on syndromes of toxic signs observed in acutely exposed rats (ATSDR 2003a; EPA 2011a; Ray and Fry 2006; Soderlund et al. 2002). Both types have an acid and an alcohol structural moiety. Type II pyrethroids typically have a cyano substituent attached to the alpha carbon of the alcohol moiety, Type I pyrethroids do not (EPA 2011a). Type I pyrethroids (also known as "T" pyrethroids; e.g., bifenthrin, permethrin, resmethrin) produce marked behavioral arousal, aggressive sparring, increased startle response, and tremors progressing from fine to whole-body tremors and prostration (ATSDR 2003a; EPA 2011a; Ray and Fry 2006; Soderlund et al. 2002). Type II pyrethroids (also known as "CS" pyrethroids; e.g., cyfluthrin, cyhalothrin, cypermethrin, fenvalerate, fluvalinate, tralomethrin) produce salivation and coarse tremors progressing to choreoathetosis and clonic seizures. Some pyrethroids (cyphenothrin, flucythrinate, esfenvalerate, and fenpropathrin) produce a mixture of the signs assigned to the two syndromes (ATSDR 2003a; EPA 2011a; Ray and Fry 2006; Soderlund et al. 2002).

Standard developmental toxicity and reproductive toxicity tests in animals orally exposed to pyrethroids have found no consistent evidence for developmental toxicity or reproductive toxicity (ATSDR 2003a; EPA 2011a). EPA (2011a) reported the absence of prenatal sensitivity in 76 guideline studies submitted to EPA for 24 pyrethroids. ATSDR (2003a) evaluated developmental toxicity tests with permethrin and resmethrin and reproductive toxicity tests with cyhalothrin, cypermethrin, and resmethrin. Neurodevelopmental effects have not been consistently associated with oral exposure of rodents orally exposed to pyrethroids (bioallethrin or deltamethrin) during neonatal stages of development (ATSDR 2003a; Costa 2008). However, in its cumulative risk assessment for pyrethroids (see next paragraph), EPA (2011a, 2011c) used a 3X safety factor for childhood exposures from birth to <6 years of age based on: (1) rat PBPK model predictions of a 3-fold increase in pyrethroid concentrations in juvenile brains, compared with adult brains; (2) similar *in vivo* and *in vitro* pharmacodynamic responses to pyrethroids in

juvenile and adult rats; and (3) evidence that rat VGSCs are more sensitive to pyrethroids than homologous human isoforms.

The EPA (2011a) OPP conducted a cumulative risk assessment for 16 pyrethrin/pyrethroid insecticides. The assessment used:

1. 24-hour exposure estimates for the general human population to 13–15 pyrethroids by three exposure pathways (food, water, and residential use);

2. a relative potency approach using neurobehavioral data in rats exposed to multiple gavage dose levels of each of 16 pyrethroids (including deltamethrin, the index chemical) to estimate doses expected to be without risk for acute neurobehavioral effects in humans;

3. a margin-of-exposure (MOE) analysis using a target MOE of 100 for adults (10 each for inter- and intra-species variability) and 300 for children (3 for a FQPA safety factor and 10 each for inter- and intra-species variability) and a POD for deltamethrin of 11 mg/kg/day.

The POD was the $BMDL_{20}$ for incidence of rats with a composite score for acute clinical signs of neurological impairment based on measures of body temperature, tremors, clonic convulsions, salivation, and mobility. The EPA (2011a, 2011c) determined that the 10X FQPA safety factor would be reduced to 1X for all populations >6 years of age, including women of child-bearing age, based on the absence of pre-natal sensitivity in 76 guideline studies submitted to EPA for 24 pyrethroids. EPA retained a 3X safety factor for exposures from birth to <6 years of age, based on the reasons described in the previous paragraph.

Relative potencies derived by EPA (2011a) for pyrethroids are listed in Table A-1. Deltamethrin was selected as the index chemical, because it had one of the most robust databases of guideline and literature toxicity studies among the evaluated pyrethroids, and it was tested with three dose levels in the principal studies (Herberth 2010, as cited in EPA 2011a; Weiner et al. 2009), compared with two dose levels for the other candidate index chemical, permethrin (EPA 2011a). For food and water dietary exposure to deltamethrin equivalents, the MOE for the general population was 4,700, indicating no risk of concern. Analyses for age-related subgroups indicated that the MOE of highest concern, 2,000, was for infants <1 year old, but this value is also indicative of no risk of concern. For residential use of pyrethroid

insecticides involving dermal and inhalation exposure, MOEs ranged for 3,000 to 240,000 for dermal exposures and from 130,000 to 10,000,000 for inhalation exposures.

The EPA IRIS files (IRIS 2013) present abbreviated summaries of results from unpublished animal toxicity tests and derived oral RfDs for 10 pyrethroids, but these files do not reflect the updated toxicity assessment conducted by the U.S. EPA OPP (EPA 2011a).

Table A-1. Relative Potency Estimates for Pyrethroids Included in the U.S. EPA (2011a) Screening-Level Cumulative Risk Assessment

Pyrethroid	Oral BMD_{20}	Oral RPF[b,c]
Allethrin	135	0.11
Bifenthrin	14.3	1.01
Cyfluthrin	12.6	1.15
Lambda-cyhalothrin	8.9	1.63
Cyphenothrin	100[a]	0.15
Cypermethrin	76.3	0.19
Deltamethrin[d]	**14.5**	**1.0**
Esfenvalerate	40.5	0.36
Fenpropathrin	29	0.50
Tau-fluvalinate	14.5	1.0
Imiprothrin	750[a]	0.02
Permethrin	156	0.09
Prallethrin	150[a]	0.1
Pyrethrins	800[a]	0.02
Resmethrin	291	0.05

[a]Values estimated from studies other than the principal study. All other BMD_{20} values were estimated using benchmark dose (BMD) analyses of incidence data from the principal study of gavage-exposed rats. The incidences were for composite scores >4 on a 4–12 composite score based on measures of body temperature, tremors, clonic convulsions, salivation, and mobility.
[b]RPF = BMD_{20} of index chemical (deltamethrin) / BMD_{20} of subject chemical. For example, allethrin RPF = 14.5/135 = 0.11.
[c]RPFs for dermal and inhalation exposures were based on oral BMDs. Five percent absorption values were applied to dermal assessments, and no adjustments were made when assessing inhalation exposures.
[d]Chosen as the index chemical because it had the best dose-response data among candidate pyrethroids with robust databases. The point of departure (POD) in the cumulative risk assessment was the $BMDL_{20}$ value of 11 mg/kg/day for deltamethrin.

A.3 Mechanisms of Action

***Neurotoxicity*.** Although mammals have been estimated to be about 3 orders of magnitude less sensitive to pyrethroids than insects (due to faster metabolism, higher body temperatures, and lower sensitivity of mammal, compared with insect, ion channel sites), the mode of action for pyrethroid neurotoxicity is expected to be the same in insects and mammals (Ray and Fry 2006). Pyrethroids reversibly slow the closing of sodium channel gates in nerve cells during the depolarizing phase of an action potential (ATSDR 2003a; Costa 2008). This ability of pyrethroids has been proposed to involve pyrethroid stimulation of protein phosphorylation (Ray and Fry 2006). Other molecular target sites have been identified that may play roles in pyrethroid neurotoxicity including voltage-gated chloride channels, GABA-gated chloride channels, noradrenaline release, and voltage-gated calcium channels (Ray and Fry 2006; Soderlund et al. 2002), but sodium channels appear to be the major target (Ray and Fry 2006). The neuropotency of pyrethroids is influenced by the presence or absence of a cyano group in the alpha position of the central ester bond (Type II pyrethroids contain a cyano group) and stereochemical

orientation (e.g., the 1R conformation is more potent than the 1S conformation in Type I pyrethroids) (ATSDR 2003a; Costa 2008; EPA 2011a, 2011b). Type I ("T") and II ("CS") pyrethroids have different effects on the kinetics of the sodium channel opening and closing; these differences have been proposed to be the basis of the differences observed in the "T" and "CS" syndromes of toxic signs (Ray and Fry 2006). Type II pyrethroids cause a prolonged open state of the sodium channel, compared with Type I pyrethroids (EPA 2011a, 2011b).

The identification of multiple molecular target sites for various pyrethroids and different effects on VGSCs by Type I and II pyrethroids has led to uncertainty about whether pyrethroids represent a common mechanism group of chemicals (Ray and Fry 2006; Soderlund et al. 2002). However, all tested pyrethroids have been shown to affect the function of VGSCs (EPA 2011a, 2011b), and results from studies of motor activity in rats (Wolansky et al. 2009) and sodium influx in cultured cerebrocortical neurons (Cao et al. 2011) showed that actions of a mixture of 11 pyrethroids (that included both Type I and II pyrethroids) were consistent with a statistical model of dose additivity. Demonstration of dose additivity is consistent with a common mechanism of action. Based on these and other findings, EPA (2011a, 2011b) concluded that all pyrethroids represent a common mechanism group of structurally related chemicals that modify the kinetics of VGSCs leading to altered neuronal excitability and two syndromes of clinical signs of neurotoxicity.

For most pyrethroids, metabolic transformations represent detoxification processes; inhibition of CYP- and carboxylesterase-mediated metabolism is expected to enhance the toxicity of pyrethroids (Costa 2008). Evidence for a few pyrethroids indicates that neurotoxic action in mammals may involve metabolites, but this evidence appears to be presently equivocal and in need of further confirmation (Ray and Fry 2006). Neonatal rats have been reported to be 4–17 times more sensitive than adult rats to the acute neurotoxicity of permethrin or cypermethrin (Ray and Fry 2006). Potential explanations for this age-related susceptibility include lesser metabolic capacities in neonates and the existence of a specific type of sodium channel in neonatal rats showing a higher binding affinity for saxitoxin (Ray and Fry 2006). EPA (2011a, 2011c) concluded that the increased sensitivity of neonates to pyrethroids was attributable to increased pyrethroid concentrations in juvenile brains, compared with adult brains.

***Neurodevelopmental Toxicity*.** Possible associations between oral exposure to pyrethroids and neurodevelopmental effects are not clearly established. One group of investigators reported that oral exposure of mice to bioallethrin or deltamethrin during early postnatal exposure (postnatal days [PNDs] 6–10) caused increases in spontaneous activity behavior at 4 months of age with doses in the

range of 0.21–0.7 mg/kg, and decreased spontaneous activity at 4 months of age with a neonatal exposure to a higher dose level of 42 mg/kg; however, other investigators could not duplicate the findings of the first group of investigators (ATSDR 2003a). Shafer et al. (2005) concluded that current evidence is inadequate to establish neurodevelopmental toxicity as a possible human hazard associated with exposure to pyrethroids (Shafer et al. 2005). Similarly, Ray and Fry (2006) concluded that observations of developmental neurotoxic effects in animals repeatedly exposed to pyrethroids during neonatal periods are in need of confirmation. However, EPA (2011a, 2011c) concluded, in its cumulative risk assessment for pyrethroids, that there was sufficient evidence of increased juvenile sensitivity (due to pharmacokinetic differences leading to increased brain concentrations in juveniles compared with adults) to warrant using a 3 safety factor for children <6 years old.

***Other Effects*.** Mode-of-action information on other effects found in studies of individual pyrethroids (e.g., decreased body weight, diarrhea, and decreased pup survival; see ATSDR 2003a) was not located. Some of these effects (e.g., decreased body weight, diarrhea) may be related to the actions of pyrethroids on the sodium channels of nerve cells; the mode of action for decreased pup survival and liver hypertrophy is unknown.

A.4 Health Guidelines

For oral exposure, ATSDR (2003a) derived acute-duration MRLs for *permethrin*, *cypermethrin*, and *cyhalothrin* and intermediate-duration MRLs for *permethrin* and *cyhalothrin*. No chronic-duration oral MRLs were derived for any pyrethroids, but, because no clinical signs of neurotoxicity were observed in a 2-year study of rats exposed to 1,000 ppm *permethrin* in the diet (at estimated doses of 40 mg/kg/day), ATSDR (2003a) expected that the intermediate-duration oral MRL for permethrin would be protective for a chronic duration of exposure.

ATSDR (2003a) derived an MRL of 0.1 mg/kg/day for acute-duration oral exposure to *permethrin* based on a no-observed-adverse-effect level (NOAEL) of 25 mg/kg and a lowest-observed-adverse-effect level (LOAEL) of 75 mg/kg for neurological impairment in rats given single doses of the chemical (increased excitability and aggressiveness, abnormal motor movement, and decreased grip strength and motor activity). This derivation used an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR (2003a) derived an MRL of 0.02 mg/kg/day for acute-duration oral exposure to *cypermethrin* based on a LOAEL of 20 mg/kg for neurological impairment in rats given single doses of the chemical (altered gait and decreased motor activity). This derivation used an uncertainty factor of 1,000 (10 for the lack of a NOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

ATSDR (2003a) derived an MRL of 0.01 mg/kg/day for acute-duration oral exposure to *cyhalothrin* based on a NOAEL of 1 mg/kg/day and a LOAEL of 2.5 mg/kg/day for diarrhea in dogs given the chemical daily in gelatin capsules for 26 weeks. This derivation used an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR (2003a) derived an MRL of 0.2 mg/kg/day for intermediate-duration oral exposure to *permethrin* based on a NOAEL of 15.5 mg/kg/day and a LOAEL of 91.5 mg/kg/day for neurological impairment (hindlimb splay) in rats given permethrin in the diet for 13 weeks. This derivation used an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR (2003a) derived an MRL of 0.01 mg/kg/day for intermediate-duration oral exposure to *cyhalothrin* based on a NOAEL of 1 mg/kg/day and a LOAEL of 2.5 mg/kg/day for diarrhea in dogs given the chemical daily in gelatin capsules for 26 weeks. As with the acute-duration MRL for cyhalothrin, this derivation used an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

In a cumulative risk assessment for pyrethroid insecticides, the U.S. EPA OPP (EPA 2011a) selected deltamethrin as the index chemical in a relative potency approach that derived relative potency factors for 14 additional pyrethroids and a $BMDL_{20}$ POD of 11 mg/kg/day for deltamethrin. The RPF values (listed in Table A-1) were derived using benchmark dose (BMD) analyses of incidence data from the principal studies of gavage exposed rats (Herberth 2010; Weiner et al. 2009). The incidences were for composite scores >4 on a 4–12 scale, based on measures of body temperature, tremors, clonic convulsions, salivation, and mobility. RfDs for 10 pyrethroids are still listed on EPA IRIS (IRIS 2013), but these values do not represent the most up-to-date pyrethroid assessment by EPA (i.e., EPA 2011a). The 2011 EPA relative potency assessment has the advantage of being predominantly based on data collected from a single laboratory, obviating the uncertainty from interlaboratory variability associated with subjectivity in behavioral assessments and pharmacokinetic differences from different gavage vehicles and volumes.

The International Agency for Research on Cancer (IARC 2012) classified deltamethrin, fenvalerate, and permethrin in Cancer Group 3—*Not classifiable as to its carcinogenicity to humans*. IARC (2012) has not evaluated the carcinogenic potential of other pyrethroids. The National Toxicology Program (NTP 2011), the EPA OPP (EPA 2011a), and the EPA IRIS program (IRIS 2013) have not formally evaluated the evidence for the human carcinogenic potential of pyrethroid insecticides.

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

The most clearly established end points of concern for pyrethroids are neurological effects mediated via the slowing of sodium channels in nerve cells during action potentials (ATSDR 2003a; EPA 2011a, 2011b). In the absence of mode-of-action data to indicate otherwise, other effects (which are the critical effects for ATSDR MRLs or EPA RfDs listed on IRIS, such as diarrhea, decreased body weight gain, decreased pup survival, and increased liver weight) are assumed to occur via the same mode of action. Thus, TTD values for other effects were not derived for the pyrethroid pesticides of concern. Neurodevelopmental TTD values for pyrethroids were not developed due the equivocal nature of evidence for associations between pyrethroids and neurodevelopmental effects. Results from standard developmental toxicity and reproductive toxicity tests with animals orally exposed to the 10 pyrethroids listed on EPA's IRIS do not identify reproductive effects (e.g., effects on fertility) or standard developmental effects (e.g., developmental delays, malformations) as health hazards of concern from exposure to most pyrethroids, with the possible exception of resmethrin. More recently, EPA's OPP reported that no prenatal sensitivity was observed in 76 guideline studies submitted for 24 pyrethroids (EPA 2011a).

A.6 References

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Appendix B: Background Information for Organophosphorus Insecticides

Organophosphorus compounds have been synthesized and developed as insecticides beginning in the 1940s and continuing to the present time (Costa 2008). Hundreds of organophosphorus compounds have been synthesized and commercialized as insecticides, and more than half of insecticides used are organophosphorus compounds (Costa 2008).

B.1 Toxicokinetics

Most organophosphorus insecticides are expected to be absorbed following inhalation, oral, or dermal exposures, distributed by the blood to various tissues including nervous system tissues, rapidly metabolized, and eliminated as metabolites in the urine (the principal route of elimination) and feces without significant accumulation in tissues (ATSDR 1995, 1997a, 1997b, 1997c, 2000, 2001, 2003, 2008a, 2008b). Many organophosphorus insecticides are metabolized, via CYP enzymes, to metabolites more potent than the parent compound in inhibiting ChE, the principal mode of action by which these compounds cause neurological effects: diazinon to diazoxon (ATSDR 2008a); guthion to gutoxon (ATSDR 2008b); ethion to monoxon (ATSDR 2000); methyl parathion to methyl paraoxon (ATSDR 2001); malathion to malaoxon (ATSDR 2003); disulfoton to disulfoton sulfoxide, disulfoton sulfone, demeton S-sulfoxide, or demeton S-sulfone (ATSDR 1995); and chlorpyrifos to chlorpyrifos-oxon (ATSDR 1997b). For these organophosphorus insecticides, further metabolism of the potent ChE-inhibiting initial metabolites to other, often more polar, metabolites represent detoxification processes. Examples of detoxification processes include CYP-mediated dealkylation or dearylation of the parent compound, hydrolysis of oxon intermediates by A-esterases, and hydrolysis via reaction with B-esterases, such as carboxylesterases and butyrylcholinesterase (Costa 2008). Thus, the balance between the kinetics of bioactivation and detoxification is critical in the expression of the neurotoxicity of these chemicals. For direct-acting organophosphorus insecticides, metabolism represents a detoxification process. Examples of direct acting organophosphorus insecticides include chlorfenvinphos, which is metabolized by CYP monooxygenases, esterases, and glutathione S-transferases (ATSDR 1997a), and dichlorvos, which is metabolized by esterases and glutathione S-transferases (ATSDR 1997c). The extent of potential reactivation of organophosphate-inhibited acetylcholinesterase decreases with time, a phenomenon called aging. Aging is due to dealkylation of the alkoxyl group of the residue bound to the enzyme. The rate of ageing is proportional to the electron-donating capacity of the alkyl group (ATSDR 2008a, 2008b, 2003).

B.2 Health Effects

The critical and most well-studied effect of organophosphorus insecticides is the inhibition of ChE, which results in accumulation of acetylcholine at acetylcholine receptors and overstimulation of nerve junctions in the peripheral and central nervous systems (ATSDR 1995, 1997a, 1997b, 1997c, 2000, 2001, 2003, 2008a, 2008b). Acute exposure to high doses of organophosphorus insecticides causes severe ChE inhibition associated with cholinergic signs and symptoms including lacrimation, perspiration, miosis, diarrhea, nausea, and vomiting, accompanied with cramps or muscle weakness, drowsiness, fatigue, mental confusion, convulsions, or coma. Numerous animal studies and limited controlled-exposure human studies have identified acute and repeated exposure levels (via oral, inhalation, dermal, or parenteral routes) resulting in inhibition of plasma, RBC, and/or brain ChE (ATSDR 1995, 1997a, 1997b, 1997c, 2000, 2001, 2003, 2008a, 2008b). Inhibition of RBC or brain ChE in the 20–59% range is generally considered to be a "less serious" adverse neurological effect not associated with the gross signs and symptoms of serious neurological dysfunction resulting from high dose levels (ATSDR 1995, 1997a, 1997b, 1997c, 2000, 2001, 2003, 2008a, 2008b).

The critical nature of ChE inhibition for acute, intermediate, and chronic exposure to organophosphate insecticides is reflected in the critical effects for ATSDR MRLs for organophosphorus insecticides, which are listed in Table B-1. Inhibition of either plasma, RBC, or brain ChE is the critical effect for the majority of ATSDR's inhalation MRLs (9/10) and oral MRLs (21/25) for organophosphorus insecticides (Table B-1). The exceptions include:

- The intermediate inhalation MRL for malathion, which is based on nasal and larynx lesions in rats exposed for 13 weeks at an exposure level (100 mg/m^3) that did not significantly inhibit RBC ChE (ATSDR 2003; Table B-1). Decreased RBC ChE activity was observed at the next highest exposure level (450 mg/m^3) in the principal study, and decreased RBC or brain ChE was the critical effect for the other MRLs for malathion (acute inhalation, intermediate oral, and chronic oral).

- The intermediate oral MRL for methyl parathion, which is based on nerve function deficits in rat dams given daily gavage doses on gestation days (GDs) 5–15 and extending through PNDs 1–28 (ATSDR 2001; Table B-1).

- The chronic oral MRL for methyl parathion, which is based on decreased hematocrit and RBC counts in rats exposed to methyl parathion in the diet for 2 years at a dose level (0.25 mg/kg/day) that did not significantly decrease RBC or brain ChE (ATSDR 2001; Table B-1). In the principal study, decreased RBC and brain ChE activities were reported in rats exposed to the next highest dose level of methyl parathion (2.5 mg/kg/day, Table B-1).

Table B-1. Critical Effects and PODs for ATSDR MRLS for Organophosphorus Insecticides

Chemical	Critical effect	POD		Species and exposure	Other NOAELs (N) and LOAELs (L) for neurological or developmental effects (mg/kg/day or mg/m^3 for N and L)
		NOAEL	LOAEL		
Acute inhalation exposure (mg/m^3)					
Dichlorvos, acute MRL	↓ RBC ChE	1.81 (0.2 ppm)	4.34 (0.48 ppm)	Rats, continuous exposure, 3 days	Fetal body weight and number of live fetuses, mice or rabbits; N=0.44 (7 hours/day, GDs 6–15 or 6–10); L=ND.
Disulfoton, acute MRL	↓ RBC ChE	0.5	1.8	Rats, 4 hours/day, 5 days/week, 5 days	
Guthion, acute MRL	↓ RBC ChE	1.24	4.72	Rats, 6 hours/day, 5 days/week, 2 weeks	
Malathion, acute MRL	↓ RBC ChE	65	123	Rabbits, 6 hours	Nasal and eye irritation, volunteers, 2-hour exposure, N=21; L=85; no effect on plasma or RBC ChE.
Intermediate inhalation exposure (mg/m^3)					
Dichlorvos, intermediate MRL	↓ RBC ChE	0.27 (0.03 ppm)	1.26 (0.14 ppm)	Rats, 23 hours/day, GDs 1–20	
Diazinon, intermediate MRL	↓ RBC ChE	1.57	11.6	Rats, 6 hours/day, 5 days/week, 3 weeks	
Disulfoton, intermediate MRL	↓ RBC ChE and lethargy	0.02	0.1	Rats, 6 hours/day, 5 days/week, 3 weeks	↓ RBC ChE, rats, 6 hours/day, 5 days/week, 13 weeks, N=0.16; L=1.4.
Guthion, intermediate MRL	↓ RBC ChE	1.24	4.72	Rats, 6 hours/day, 5 days/week, 12 weeks	

Table B-1. Critical Effects and PODs for ATSDR MRLS for Organophosphorus Insecticides

Chemical	Critical effect	POD		Species and exposure	Other NOAELs (N) and LOAELs (L) for neurological or developmental effects (mg/kg/day or mg/m^3 for N and L)
		NOAEL	LOAEL		
Malathion, intermediate MRL	Nasal and larynx lesions	NI	100	Rats, 6 hours/day, 5 days/week, 13 weeks	↓ RBC ChE, rats, 6 hours/day, 5 days/week, 13 weeks, N=100; L=450. ↓ RBC ChE, volunteers, 2 hours/day, 42 days, N=85; L=NI.
Chronic inhalation exposure (mg/m^3)					
Dichlorvos, chronic MRL	↓ RBC ChE	0.05 (0.006 ppm)	0.54 (0.06 ppm)	Rats, 23 hours/day, 2 years	
Acute oral exposure (mg/kg/day)					
Chlorfenvinphos, acute MRL	↓ RBC ChE	NI	2.4	Rats, in diet for 10 days	↓ brain ChE, rats, single gavage dose, N =1; L=2.
Chlorpyrifos, acute MRL	↓ plasma ChE	0.03	0.10	Humans, daily capsules for 9–20 days	↓ RBC ChE, rat dams, gavage on GDs 6–15; N=0.1; L=1.
Diazinon, acute MRL	↓ RBC ChE	0.6	1.2	Rats, in diet for 12 days	Deficits in neurobehavioral tests, mouse offspring, gavage GDs 1–18, N=NI; L=0.18.
Dichlorvos, acute MRL	↓ brain ChE	NI	4	Rats, gavage, 1time/day for 14 days	Decreased fetal body weight and number of live fetuses, mice or rabbits; N=60 (mouse), 5 (rabbit); L=ND.
Disulfoton, acute MRL	↓ RBC ChE	0.1	0.3	Rat dams, gavage GDs 6–15	Delayed ossification, rat fetuses, gavage GDs 6–15, N=0.3; L=1.
Ethion, acute MRL	↓ RBC and brain ChE	0.06	0.71	Dogs, in diet for 90 days	Delayed ossification, rat fetuses, gavage GDs 6–15, N=0.6, L=2.5. Fused sterna, rabbit fetuses, gavage GDs 6–18, N=2.4, L=9.6
Guthion, acute MRL	↓ RBC and brain ChE	1	2	Rat dams, gavage GDs 6–15	Misaligned sternebrae, mouse fetuses, gavage GDs 6–15, N=2.5, L=5.
Intermediate oral exposure (mg/kg/day)					
Chlorfenvinphos, intermediate MRL	↓ immune responses	NI	1.5	Mice, gavage for 90 days	↓ RBC ChE, rats, in diet for 12 weeks, N=3; L=10.

Table B-1. Critical Effects and PODs for ATSDR MRLS for Organophosphorus Insecticides

Chemical	Critical effect	POD		Species and exposure	Other NOAELs (N) and LOAELs (L) for neurological or developmental effects (mg/kg/day or mg/m^3 for N and L)
		NOAEL	LOAEL		
Chlorpyrifos, intermediate MRL	↓ plasma ChE	0.03	0.1	Humans, daily capsules for 9–20 days	↓ RBC ChE, rat dams, gavage on GDs 6–15; N=0.1; L=1.
Diazinon, intermediate MRL	↓ RBC ChE	0.18	0.27	Rats, in diet for 42 days	↓ RBC and brain ChE, dogs, in diet for 13 weeks; N=0.75, L=5.6. Deficits in neurobehavioral tests, mouse offspring, gavage GDs 1–18, N=NI, L=0.18.
Dichlorvos, intermediate MRL	↓ RBC ChE	0.033	NI	Humans, 3 times/day for 21 days	
Disulfoton, intermediate MRL	↓ brain ChE in F1a pups	0.009	0.03	Rats, in diet for 2 generations	↓ RBC ChE, adult rats, in diet for 6 months, N=0.03, L=0.07.
Ethion, intermediate MRL	↓ RBC and brain ChE	0.06	0.71	Dogs, in diet for 90 days	
Guthion, intermediate MRL	↓ RBC ChE	0.15	0.69	Dogs, in diet for 26 weeks	↓ RBC, rats, in diet 14 weeks pre-mating through postpartum day 5 or 28, F0 dams at postpartum day 5 or 28: N=NI; L=0.55; F1 pups at postpartum day 5 or 28: N=1.5, L=4.9. Gross neuromuscular incoordination and decreased fetal survival, rat offspring, gavage GD 6 through postpartum day 21, N=2.5, L=5.
Malathion, intermediate MRL	↓ RBC ChE	0.24	0.34	Humans, daily capsules for 32–56 days	↓ brain ChE on PND 21, rat offspring, gavage on GDs 6–13; N=NI; L 138.

Table B-1. Critical Effects and PODs for ATSDR MRLS for Organophosphorus Insecticides

Chemical	Critical effect	POD		Species and exposure	Other NOAELs (N) and LOAELs (L) for neurological or developmental effects (mg/kg/day or mg/m^3 for N and L)
		NOAEL	LOAEL		
Methyl parathion, intermediate MRL	Nerve function deficits	NI	0.22	Rats, gavage GDs 5–15 through PND 28 via dams, followed by gavage from weaning through 11–12 weeks of age	Nerve function deficits, rat offspring, gavage GDs 5–15 through PND 28 via dams only, N=0.88, L=NI. ↓ RBC and brain ChE, mouse, gavage 28 days, N=1; L=3. ↓ RBC and brain ChE, dogs, in diet 13 weeks, N=0.3; L=3.
Chronic oral exposure (mg/kg/day)					
Chlorfenvinphos, chronic MRL	↓ RBC ChE	NI	10	Rats, in diet for 2 years	↓ RBC ChE, dogs, in diet for 2 years, N=2; L=10.
Chlorpyrifos, chronic MRL	↓ RBC ChE	0.1	1	Rats, in diet for 2 years	↓ RBC ChE, dogs, in diet for 1 year; N=0.1; L=1.
Diazinon, chronic MRL	↓ RBC ChE	0.065	5.5	Rats, in diet for 97 weeks	↓ RBC and brain ChE, dogs, in diet for 52 weeks, N=0.017, L=4.6. Deficits in neurobehavioral tests, mouse offspring, gavage GDs 1–18, N=NI, L=0.18.
Dichlorvos, chronic MRL	↓ RBC and brain ChE	0.05	1	Dogs, 1 time/day, 52 weeks	
Disulfoton, chronic MRL	↓ RBC ChE	NI	0.06	Rats, in diet for 1.5–2 years	↓ RBC and brain ChE, dogs, in diet for 2 years, N=0.03, L=0.14.
Ethion, chronic MRL	↓ RBC and brain ChE	0.06	0.71	Dogs, in diet for 90 days	↓ RBC or brain ChE, rats or mice, in diet for 2 years, N=2 (rats) or 1.2 (mice), L=NI.
Guthion, chronic MRL	↓ RBC and brain ChE	0.15	0.69	Dogs, in diet for 52 weeks	↓ RBC and brain ChE, rats, in diet 2 years, N=0.75, L=2.3.
Malathion, chronic MRL	↓ RBC ChE	2 (males) 3 (females)	29 (males) 35 (females)	Rats, in diet for 2 years	
Methyl parathion, chronic MRL	Decreased hematocrit and RBC counts	0.025	0.25	Rats, in diet for 2 years	↓ RBC and brain ChE, rats, in diet 2 years, N=0.25; L=2.5.

Table B-1. Critical Effects and PODs for ATSDR MRLS for Organophosphorus Insecticides

Chemical	Critical effect	POD		Species and exposure	Other NOAELs (N) and LOAELs (L) for neurological or developmental effects (mg/kg/day or mg/m^3 for N and L)
		NOAEL	LOAEL		

ATSDR = Agency for Toxic Substances and Disease Registry; ChE = acetylcholinesterase; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; NI = not identified; NOAEL = no-observed-adverse-effect level; PND = postnatal day; POD = point of departure; RBC = red blood cell

Sources: Sources: ATSDR 1995, 1997a, 1997b, 1997c, 2000, 2001, 2003, 2008a, 2008b

- The intermediate oral MRL for chlorfenvinphos, which is based on decreased immune response in mice exposed to 1.5 mg/kg/day chlorfenvinphos for 90 days (ATSDR 1997a; Table B-1). Decreased RBC and brain ChE activities were observed in rats exposed to chlorfenvinphos in the diet for 12 weeks at 10 mg/kg/day, but not at 3 mg/kg/day (Table B-1). Decreased RBC ChE activity is the critical effect for the acute and chronic oral MRLs for chlorfenvinphos (Table B-1).

There is concern for the possible neurodevelopmental toxicity of organophosphorus insecticides, but most have not been evaluated for neurodevelopmental effects. As reviewed by EPA (2006), ChE has been suspected to play roles in the development of the neural system involving cell adhesion during neurite growth, but inhibition of the acetylcholine esterase activity by organophosphorus insecticides does not appear to be closely correlated with inhibition of neurite growth—some organophosphate insecticides inhibit neurite outgrowth *in vitro*, but others do not (EPA 2006). Many organophosphorus insecticides have been evaluated in standard developmental toxicity tests, but most have not been evaluated for neurodevelopmental effects. Concern for the neurodevelopmental effects of organophosphorus insecticides in general is raised by positive results found in a few studies that have been conducted. For example, offspring of mice exposed to 0.18 or 9 mg/kg/day diazinon on GDs 1–18 showed developmental delays and abnormal neuromuscular endurance and coordination (Spyker and Avery 1977). Similarly, offspring of rats exposed to 5 mg/kg/day guthion on GD 6 through PND 21 (weaning) showed gross neuromuscular incoordination and reduced survival; these effects were not observed in offspring of rats exposed to 2.5 mg/kg/day (Short et al. 1980). Another example is the finding that male rats showed electrophysiological changes in neural tissues following oral exposure to 0.22 mg/kg/day methyl parathion during GDs 5–15 (via the dam), lactational days 2–28 (via the dam), and PNDs 23–84, a dose below those inhibiting ChE in adult rats, but when exposure did not include PNDs 23–84, no effects were found, even at a dose level of 0.88 mg/kg/day (Desi et al. 1998).

There is also concern for the possible increased susceptibility of children, compared with adults, to the ChE inhibiting activity of organophosphorus insecticides, but the relative sensitivities of early life and adult stages of development have been assessed only for a few of these chemicals. In its cumulative risk assessment for organophosphorus insecticides, EPA (2006) applied a FQPA 10 safety factor to RPFs to provide protection for the possible susceptibility of infants to prenatal and postnatal toxicity, except for 13 insecticides with appropriate data evaluating ChE inhibition in juvenile and adult rats. These data were used to reduce the FQPA safety factor for 10 of these insecticides (see Section B.4 for more details). In concurrence with this recommendation, Costa (2008) concluded that there is evidence to indicate that young animals are more sensitive to acute toxicity from organophosphorus insecticides and the increased sensitivity appears to be due to lower detoxification abilities of young animals, compared with mature animals. Illustrating this conclusion, Slotkin (2006) cited published evidence that the LD_{50} value for chlorpyrifos in 1-day-old rats was about 10- and 100-fold less than LD_{50} values in 1-week-old and adult rats, respectively.

Exposure to certain organophosphorus insecticides has also been associated with two other neurological conditions that do not appear to involve ChE (Costa 2008). The "intermediate syndrome" has been described in 20–50% of acute organophosphorus poisoning cases with distinguishing features that develop one to several days after poisoning and can often resolve within 15 days (Costa 2008). Distinguishing features include weakness of respiratory, neck, and proximal limb muscles that can lead to mortality due to respiratory paralysis and associated complications. The mechanism of this syndrome is unknown, although it does not appear to involve ChE inhibition (Costa 2008).

"Organophosphate-induced delayed polyneuropathy" (OPIDP) is characterized by a progressive set of signs and symptoms associated with axonal degeneration starting with initial tingling of the hands and feet and progressing to sensory loss, muscle weakness of the lower and upper extremities, and ataxia (Costa 2008). Although initial phosphorylation of a nerve tissue esterase (neuropathy target esterase, NTE), followed by an aging reaction, are thought to be key events in the mode of action for OPIDP, events linking these effects on NTE to axonal degeneration have not been characterized. Organophosphorus agents that inhibit NTE, but do not age, do not cause OPIDP (Costa 2008). Commercial organophosphorus insecticides must undergo specific testing ensuring that cholinergic potency is much higher than potency in producing OPIDP, so this effect is not expected with general public or occupational exposures from the use of most organophosphorus insecticides. Nevertheless, symptoms characteristic of OPIDP have been described in several cases of high level poisoning from

certain organophosphorus insecticides including chlorpyrifos, dichlorvos, isofenphos, methamidophos, mipafox, trichlorfon, and trichlornat (Lotti and Moretto 2005).

B.3 Mechanisms of Action

Neurotoxicity. The principal and common mode of action by which organophosphorus insecticides cause neurological effects is through inhibition of ChE. The organophosphorus insecticides, and in some cases their metabolites, form stable phophorylated complexes with the active sites of ChE, preventing the enzymatic catalysis of the hydrolyzation of acetylcholine at nerve endings and nerve junctions and leading to excessive nerve stimulation and receptor desensitization (ATSDR 2008a, 2008b). Muscarinic effects from organophosphorus insecticides involve postganglionic parasympathetic nerve stimulation in end organs (e.g., heart, blood vessels, and secretory glands) and include miosis; excessive salivation, lacrimation, and rhinitis; vomiting; and diarrhea (ATSDR 2008a). Nicotinic effects from acetylcholine accumulation at skeletal muscle junctions and sympathetic preganglionic nerve endings include muscular fasciculations, weakness, tachycardia, and hypertension (ATSDR 2008a). Central nervous system effects from nerve stimulation in the cerebral cortex, hippocampus, and extrapyramidal motor system include respiratory depression, restlessness, mental confusion, drowsiness, ataxia, and coma (ATSDR 2008a).

For organophosphorus insecticides that are metabolized to more potent ChE inhibitors via CYP enzymes (e.g., diazinon is metabolized to diazoxon), coexposure to substances that induce or inhibit CYP enzymes may influence the toxic response to the parent insecticide as determined by the balance of the kinetics of activation and detoxification mechanisms (ATSDR 2008a). Inhibitors of enzymes involved in the metabolism of direct acting organophosphorus insecticides (e.g., dichlorvos and chlorfenvinphos) would be expected to enhance or prolong neurotoxic effects from these chemicals (ATSDR 1997a, 1997c).

Modes of action by which organophosphorus produce non-ChE-mediated neurological effects are poorly characterized. The “intermediate syndrome” of weakness of respiratory, neck, and proximal limb muscles has been hypothesized to result from nicotinic receptor desensitization due to chronic cholinergic stimulation, but molecular mechanisms are unknown (Costa 2008). OPIDP has been clearly associated with high-level exposure to certain organophosphorus agents that phosphorylate and “age” NTE, but events occurring between NTE aging and axonal degeneration are unknown (Costa 2008).

Neurodevelopmental Toxicity. Animal studies involving gestational exposure to some organophosphorus insecticides have noted mild delays in skeletal development at maternally toxic exposure levels, but most

organophosphorus insecticides have not been evaluated for neurodevelopmental effects. In a recent review, Costa (2008) concluded that accumulating evidence from rodent studies in recent years indicates that perinatal exposure to organophosphorus insecticides can affect various cellular processes (e.g., neuronal survival, neurite outgrowth) and non-cholinergic pathways (e.g., serotoninergic synaptic functions), and cause various behavioral abnormalities. Slotkin (2006) recently reviewed evidence, predominantly from *in vitro* studies, that chlorpyrifos or its reactive metabolite, chlorpyrifos oxon, can influence neurodevelopment via multiple mechanisms, including: (1) alteration of acetylcholine concentrations leading to overstimulation of cholinergic receptors involved in signaling cascades important in neural development; (2) direct effects on expression and function with serotonin receptors involved in neural developmental signaling cascades; (3) direct effects on expression and function of signaling intermediates, such as adenylyl cyclase, and on expression of nuclear transcription factor; and (4) indirect effects, through generation of oxidative stress, on signaling pathways controlling cell replication, differentiation, growth, or apoptosis.

B.4 Health Guidelines

ATSDR Guidelines Based on Neurotoxicity As discussed in the previous section, decreased RBC or brain ChE activity is the critical effect for the majority of ATSDR MRLs for organophosphorus insecticides. Table B-2 lists the MRLs, PODs, and uncertainty factors used in the derivations. For inhalation exposure scenarios, MRLs ranged from 0.006 to 0.2 mg/m^3 for acute duration and from 0.0002 to 0.01 mg/m^3 for intermediate duration (Table B-2). Only one chronic inhalation MRL was derived (0.0005 mg/m^3 for dichlorvos). For oral exposure scenarios, MRLs ranged from 0.001 to 0.01 mg/kg/day for acute duration, from 0.00009 to 0.02 mg/kg/day for intermediate duration, and from 0.00006 to 0.02 mg/kg/day for chronic duration.

EPA Guidelines Based on Neurotoxicity. Although EPA lists chronic oral RfDs for 17 organophosphorus insecticides on IRIS (IRIS 2013), these assessments are less up to date than the EPA OPP RPFs used in the cumulative risk assessment for organophosphorus insecticides (EPA 2006). Organophosphorus insecticides were determined to represent a common mechanism group based on similar structural characteristics and shared ability to inhibit ChE at the active site. Methamidophos was selected as the index chemical in a RPF approach that derived RPFs for 33 organophosphorus insecticides, based on adult rat brain ChE inhibition data, submitted to EPA under the pesticide registration program (EPA 2006). Based on an analysis of available data, EPA (2006) concluded that the time to peak ChE inhibition, the persistence of action following acute exposure, and the duration of

exposure required to reach a steady-state level of ChE is influenced by a number of toxicokinetic and toxicodynamic factors and that RPFs should be based on rat studies of duration of ≥21 days (when steady-state levels of ChE inhibition are expected) in order to develop RPFs that are reproducible and applicable to human exposure scenarios of concern.

Table B-2. ATSDR MRLS for Organophosphorus Insecticides: PODs and Uncertainty Factors

Chemical	POD		MRL	Uncertainty factor[a]
	NOAEL	LOAEL		
Acute inhalation exposure (mg/m^3)				
Dichlorvos	1.81 (0.2 ppm)	4.34 (0.48 ppm)	0.02	100 (10 AH, 10 HV)
Disulfoton	0.5	1.8	0.006	30 (3 AH, 10 HV); applied to duration-adjusted NOAEL
Guthion	1.24	4.72	0.02	30 (3 AH, 10 HV): applied to NOAEL(HEC), 0.5 mg/m^3
Malathion	65	123	0.2	100 (10 AH, 10 HV); applied to duration-adjusted NOAEL
Intermediate inhalation exposure (mg/m^3)				
Dichlorvos	0.27 (0.03 ppm)	1.26 (0.14 ppm)	0.003	100 (10 AH, 10 HV)
Diazinon	1.57	11.6	0.01	30 (3 AH, 10 HV); applied to duration-adjusted NOAEL
Disulfoton	0.02	0.1	0.0002	30 (3 AH, 10 HV); applied to duration-adjusted NOAEL
Guthion	1.24	4.72	0.01	30 (3 AH, 10 HV); applied to NOAEL(human equivalent concentration), 0.37 mg/m^3
Malathion	NI[b]	100	0.02	1,000 (10 LN, 10 AH, 10 HV); applied to duration-adjusted LOAEL
Chronic inhalation exposure (mg/m^3)				
Dichlorvos	0.05 (0.006 ppm)	0.54 (0.06 ppm)	0.0005	100 (10 AH, 10 HV)
Acute oral exposure (mg/kg/day)				
Chlorfenvinphos	NI[b]	2.4	0.002	1,000 (10 LN, 10 AH, 10 HV)
Chlorpyrifos	0.03	0.10	0.003	10 HV
Diazinon	0.6	1.2	0.006	100 (10 AH, 10 HV)
Dichlorvos	NI[b]	4	0.004	100 (10 AH, 10 HV)
Disulfoton	0.1	0.3	0.001	100 (10 AH, 10 HV)
Ethion	0.06	0.71	0.002	30 (3 AH, 10 HV)
Guthion	1	2	0.01	100 (10 AH,10 HV); applied to BMDL, 1 mg/kg/day
Intermediate oral exposure (mg/kg/day)				
Chlorfenvinphos	NI[b]	1.5	0.002	1,000 (10 LN, 10 AH, 10 HV)
Chlorpyrifos	0.03	0.1	0.003	10 HV
Diazinon	0.18	0.27	0.002	100 (10 AH, 10 HV)
Dichlorvos	0.033	NI[b]	0.003	10 HV
Disulfoton	0.009	0.03	0.00009	100 (10 AH, 10 HV)
Ethion	0.06	0.71	0.002	30 (3 AH, 10 HV)
Guthion	0.15	0.69	0.003	100 (10 AH, 10 HV); applied to BMDL, 0.29 mg/kg/day

Table B-2. ATSDR MRLS for Organophosphorus Insecticides: PODs and Uncertainty Factors

Chemical	POD		MRL	Uncertainty factor[a]
	NOAEL	LOAEL		
Malathion	0.24	0.34	0.02	10 HV
Methyl parathion	NI[b]	0.22	0.0007	300 (3 LN,10 AH, 10 HV)
Chronic oral exposure (mg/kg/day)				
Chlorfenvinphos	NI[b]	10	0.0007	1,000 (10 LN, 10 AH, 10 HV)
Chlorpyrifos	0.1	1	0.001	100 (10 AH, 10HV)
Diazinon	0.065	5.5	0.0007	100 (10 AH,10 HV)
Dichlorvos	0.05	1	0.0005	100 (10 AH, 10 HV)
Disulfoton	NI[b]	0.06	0.00006	1,000 (10 LN, 10 AH, 10 HV)
Ethion	0.06	0.71	0.0004	150 (3 AH,10 HV, 5 for long-term effects and possible susceptibility of children)
Guthion	0.15	0.69	0.003	100 (10 AH, 10 HV); applied to BMDL, 0.30 mg/kg/day
Malathion	2	29	0.02	100 (10 AH, 10 HV)
Methyl parathion	0.025	0.25	0.0003	100 (10 AH, 10 HV)

ATSDR = Agency for Toxic Substances and Disease Registry; BMDL = benchmark dose limit; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; NI=not identified; NOAEL = no-observed-adverse-effect level; POD = point of departure

[a]Uncertainty factor abbreviations: AH for animal to human extrapolation; HV for human variability; LN for LOAEL to NOAEL extrapolation; SCC for subchronic to chronic extrapolation.

Sources: ATSDR 1995, 1997a, 1997b, 1997c, 2000, 2001, 2003, 2008a, 2008b

The RPF values (listed in Table B-3) were derived using BMD analyses of brain ChE activity data for adult female rats (female rats were generally more sensitive than male); the central tendency estimate of the dose of the index chemical resulting in 10% ChE inhibition (e.g., BMD_{10}) was divided by BMD_{10} values for the other chemicals to derive the unadjusted RPFs. For estimating risks for children, the RPFs were adjusted by multiplication by the default FQPA 10 factor or replaced with chemical-specific FQPA factors, when appropriate data were available to compare juvenile and adult susceptibility to ChE inhibition (see Table B-3). Methamidophos was selected as the index chemical, because it had the highest quality dose-response data and the most robust database for all three exposure routes of interest (oral, dermal, inhalation) among the evaluated organophosphorus insecticides. Route-specific BMD_{10} values for female rats exposed to the index chemical were selected as the POD for the cumulative risk assessments and are listed (along with $BMDL_{10}$ values) in Table B-4.

EPA (2006) evaluated cumulative risks for various U.S. regions from organophosphorus pesticides in food, residential and recreational use, and drinking water. The residential pathways comprised oral, dermal, and inhalation exposures. In accordance with the 1996 FQPA, the RPF/MOE approach used RPF values, which were adjusted with chemical-specific or default uncertainty factors (FQPA factors) to protect infants and children. Concentrations of organophosphorus residues in appropriate media (e.g., food, drinking water) were multiplied by appropriate FQPA-adjusted RPF values (see Table B-3) and summed to arrive at methamidophos-equivalent concentrations, which were then used in exposure models to estimate methamidophos-equivalent intakes (in units of mg/kg/day) for the various exposure scenarios investigated. The exposure estimates for food were based on residue monitoring data collected by the U.S. Department of Agriculture's Pesticide Data Program supplemented with information from the U.S. Food and Drug Administration's Surveillance Monitoring Programs and Total Diet Study. Drinking water and residential exposures were estimated with probabilistic modeling approaches which were focused on U.S. regions where organophosphorus insecticide use was high and drinking water vulnerability was high. The exposure assessments indicated that exposure to organophosphorus insecticides from foods represents the dominant exposure pathway for the general population.

Table B-3. EPA RPFs for Oral, Dermal and Inhalation Exposures to Organophosphorus Insecticides, Based on Data for Brain ChE Inhibition in Female Rats and FQPA Factors used to Adjust the RPFs in Cumulative Risk Assessments

Chemical	Oral RPF	Dermal RPF	Inhalation RPF	FQPA factor[a]
Acephate	0.08	0.0025	0.208	1
Azinphos-methyl	0.1	ND[1]	ND	4.5
Bensulide	0.003	0.0015	ND	10
Chlorethoxyfos	0.13	ND	ND	10
Chlorpyrifos	0.06	ND	ND	1
Chlorpyrifos-methyl	0.005	ND	ND	10
Diazinon	0.01	ND	ND	10
DDVP (dichlorvos)	0.03	ND	0.677	1
Dicrotophos	1.91	ND	ND	1.7
Dimethoate	0.32	ND	ND	1
Disulfoton	1.26	0.47	6.596	2.2
Ethoprop	0.06	ND	ND	10
Fenamiphos	0.04	1.5	0.315	10
Fenthion	0.33	0.015	ND	10
Fosthiazate	0.07	ND	ND	2.6
Malathion	0.0003	0.015	0.003	10
Methamidophos	1	1	1	2
Methidathion	0.32	ND	ND	10
Methyl-parathion	0.12	ND	ND	10
Mevinphos	0.76	ND	ND	10
Naled	0.08	0.075	0.82	10
Omethoate	0.93	ND	ND	10
Oxydemeton-methyl	0.86	ND	ND	1
Phorate	0.39	ND	ND	10
Phosalone	0.01	ND	ND	10
Phosmet	0.02	ND	ND	10
Phosteburpirim	0.22	ND	ND	10
Pirimiphos-methyl	0.04	ND	ND	10
Profenofos	0.004	ND	ND	10
Terbufos	0.85	ND	ND	6.5
Tetrachlorvinphos	0.001	0.00075	ND	10
Tribufos	0.02	ND	ND	10
Trichlorfon	0.003	0.0075	0.087	10

[a]The default FQPA 10 factor was replaced with chemical-specific FQPA factors, when appropriate data were available to compare juvenile and adult susceptibility to ChE inhibition. In the EPA cumulative risk assessments, route-specific RPFs were adjusted by multiplying by the appropriate FQPA factor.
[b]ND = not determined due to lack of pertinent data.

ChE = acetylcholinesterase; EPA = U.S. Environmental Protection Agency; FQPA = U.S. Food Quality and Protection Act; RPF = relative potency factor

Source: EPA 2006

Table B-4. Oral, Dermal, and Inhalation BMD_{10} and $BMDL_{10}$ Values for Adult Female Rat Brain ChE Inhibition by Methamidophos, the Index Chemical for the EPA Cumulative Risk Assessment for Organophosphorus Insecticides

Endpoint	Oral (mg/kg/day)	Dermal (mg/kg/day)	Inhalation (mg/kg/day)
BMD_{10}	0.08	2.12	0.39
$BMDL_{10}$	0.07	1.77	0.21

BMD = benchmark dose; BMDL = benchmark dose limit; ChE = acetylcholinesterase EPA = U.S. Environmental Protection Agency

Source: EPA 2006

MOE values for the various age group and exposure scenario combinations were calculated by dividing the appropriate methamidophos POD (e.g., BMD_{10} = 0.08 mg/kg/day for oral exposure, see Table 5) by the appropriate methamidophos-equivalent intake estimate. The target MOE for the cumulative risk assessment was 100 to account for uncertainty in extrapolating from rats to humans (10) and uncertainty related to human variability in susceptibility to organophosphorus insecticides (10). EPA (2006) evaluated the available toxicity studies with human subjects and determined that none provided appropriate data to adjust the default inter-species or intra-species uncertainty factors. MOE values <100 were taken as values requiring some mitigation action; those >100 were assessed to be without the need for mitigation. The combined analysis of cumulative risk for three expected pathways (food + water + residential use) indicated combined MOEs at the 99.9^{th} percentile (of exposure) that were approximately >100 for all populations using 21-day average results. In this analysis, the food pathway dominated the combined MOEs. MOE values calculated for 95, 99^{th} and 99.9^{th} percentiles of 21-day average food intakes for children and adults are listed in Table B-5.

Table B-5. MOE Values for 95^{th}, 99^{th}, and 99.9% Percentiles of Cumulative 21-Day Average Intakes of Organophosphorus Insecticides in Food for U.S. Children and Adults

Age group	95^{th} percentile	99^{th} percentile	99.9^{th} percentile
Children, 1–2 years old	550	250	110
Children, 3–5 years old	670	300	99
Adults, 20–49 years old	820	610	280

MOE = margin of exposure

Source: U.S. EPA 2006

Cancer Guidelines. Most organophosphorus insecticides have not been evaluated for cancer. Among the 17 organophosphorus insecticides assessed on the EPA IRIS (IRIS 2013), only three have been assessed for carcinogenicity: acephate was classified in Cancer Group C—*possible human carcinogen*; dichlorvos was classified in Cancer Group B2—*probable human carcinogen*; and methidathion was classified in Cancer Group C—*possible human carcinogen*. Oral cancer slope factors were developed for acephate (0.0087 per mg/kg/day) and dichlorvos (0.29 per mg/kg/day). No oral slope factor was developed for methidathion.

NTP (2011) has not assessed any organophosphorus insecticides for carcinogenicity.

IARC (2012) has assessed the possible carcinogenicity of five organophosphorus insecticides. Dichlorvos was classified in Cancer Group 2B—*possibly carcinogenic to humans*. Parathion, malathion, methyl parathion, and tetrachlorvinphos were classified in Cancer Group 3—*not classifiable as to its carcinogenicity to humans*.

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

The most clearly established end points of concern for organophosphorus insecticides are neurological effects mediated via the inhibition of ChE. Inhibition of plasma, RBC, or brain ChE is the critical effect for the majority of ATSDR's inhalation MRLs (9/10) and oral MRLs (23/25), regardless of duration of exposure. Based on results from *in vivo* animal tests for a few organophosphorus agents and *in vitro* mechanistic studies, there is concern for the possible neurodevelopmental toxicity of organophosphorus insecticides, but there are inadequate data to describe possible dose-response relationships for neurodevelopmental toxicity of most organophosphorus insecticides (Costa 2008; EPA 2006). Due to the lack of appropriate dose-response data for effects other than ChE inhibition for most organophosphorus insecticides, TTDs were not developed.

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Appendix C: Background Information for Carbamates

Carbamate insecticides are structurally diverse derivatives of carbamic acid or N-methyl carbamic acid, which display a range of potencies in inducing toxic effects in mammals (Costa 2008). The carbamate insecticides discussed in this document (aldicarb, carbaryl, carbofuran, methomyl, oxamyl, and propoxur) are derivatives of N-methyl carbamic acid (EPA 2007b). Like organophosphorus insecticides, their principal and most well-studied effect in insects and mammals is neurological dysfunction mediated through inhibition of ChE (Baron 1991; Costa 2008). The differences in potencies of carbamate insecticides in producing toxic effects in mammals have been associated to a limited degree with structural differences influencing water solubility (Costa 2008). For example, aldicarb, with a water solubility of 6 g/L, is much more potent than carbaryl and carbofuran, with water solubilities of about 0.7 g/L; acute oral LD_{50} values for rats are 0.8, 10, and 400 mg/kg for aldicarb, carbaryl, and carbofuran, respectively (Costa 2008).

C.1 Toxicokinetics

Carbamate insecticides are expected to be absorbed following inhalation, oral, and dermal exposures, and skin penetration is increased by organic solvents and emulsifiers present in commercial preparations (Costa 2008). Results from animal studies indicate that, after absorption, carbamates are rapidly distributed by the blood to tissues and organs, metabolized to water soluble products, and rapidly excreted, predominantly in urine with no bioaccumulation in tissues (Baron 1991). Carbamate insecticides inhibit ChE directly, and most metabolites of carbamate insecticides from pathways involving oxidation and hydrolysis do not inhibit ChE (Costa 2008). Aldicarb is an example of a carbamate, however, with metabolites (sulfoxide and sulfone derivatives) that are potent ChE inhibitors (Costa 2008).

C.2 Health Effects

The principal and most well-studied effect of carbamate insecticides is neurological effects mediated by the inhibition of ChE (Costa 2008). EPA (2007b) determined that the N-methyl carbamate pesticides represent a common mechanism group based on similar structural characteristics and shared ability to inhibit ChE at the active enzymatic site.

The signs and symptoms of acute high-level exposure to carbamate insecticides are similar to those induced by organophosphorus insecticides: miosis, urination, lacrimation, diarrhea, muscle fasciculation, and central nervous system effects such as dizziness, mental confusion, depression of respiratory centers, and coma (Costa 2008). However, the carbamylated ChE is transiently inhibited, rapidly reversible, and

does not undergo the irreversible aging reaction, which happens with organophosphorylated ChE (Costa 2008). Thus, cholinergic signs and symptoms of acute carbamate intoxication are generally resolved within a few hours (Costa 2008). Results from subchronic and chronic animal toxicity studies with carbamate insecticides have identified a number of effects including signs of ChE inhibition, kidney, liver or spleen effects, and decreased body weight gain, but ChE inhibition is a sensitive effect for all studied carbamate insecticides (EPA 2007b).

Results from developmental toxicity, developmental neurotoxicity, and reproductive toxicity studies with several carbamates (aldicarb, carbofuran, methoymyl, oxamyl, and baygon) provide no evidence for effects on reproductive performance from these chemicals and show that fetotoxic effects, such as decreased fetal body weight or delayed ossification of sternebrae, only occurred at doses equal to or greater than those inducing maternal toxicity (see Section C.4). EPA (2007b) compared NOAELs and LOAELs and BMDs from developmental neurotoxicity studies and ChE inhibition studies in animals and reported that, for three carbamates with appropriate data (aldicarb, carbaryl, and carbofuran), ChE inhibition was 10–100-fold more sensitive than developmental neurotoxic effects. As a result, in its cumulative risk assessment for N-methyl carbamates (see Section C.4), EPA (2007b) used ChE inhibition as the end point of concern, as well as the end point of concern for evaluating the FQPA 10X safety factor for infants and children.

C.3 Mechanisms of Action

Neurotoxicity. The principal and common mode of action by which carbamates induce neurological effects in insects and mammals is inhibition of ChE (Baron 1991; Costa 2008). Carbamates rapidly bind to the active site of ChE, but the inhibition of enzyme function is transient and reversible because the reactivation of the carbamylated enzyme is rapid and no “aging” of the active site occurs, such as that which occurs with organophosphorus insecticides (Baron 1991; Costa 2008). EPA (2007b) determined that the N-methyl carbamate pesticides represent a common mechanism group based on similar structural characteristics and shared ability to inhibit ChE by carbamylation of the serine hydroxyl group located in the active site of the enzyme.

Even though some carbamates have been shown to inhibit NTE, they are not expected to cause delayed peripheral neuropathy such as that caused by organophosphorus insecticides, because the carbamylated enzyme does not age (Costa 2008).

Other Effects. Results from chronic exposure animal studies with a few carbamate insecticides have identified a few effects that occur at chronic administered dose levels similar to or slightly lower than those associated with ChE inhibition. The mechanism(s) by which these carbamate insecticides induce effects other than ChE inhibition is unknown.

C.4 Health Guidelines

ATSDR has not prepared toxicological profile for any of the carbamate insecticides discussed in this document.

The EPA IRIS (IRIS 2013) lists chronic oral RfDs for aldicarb, carbaryl, carbofuran, methomyl, oxamyl, and baygon (also known as propoxur), but these assessments are not as up-to-date as the EPA cumulative risk assessment for N-methyl carbamates (EPA 2007b).

EPA (2007b) determined that N-methyl carbamate insecticides represent a common mechanism group based on similar structural characteristics and shared ability to inhibit ChE at the active enzymatic site. A multi-chemical, multi-pathway PBPK/PD model could not be developed for the cumulative risk assessment because appropriate pharmacokinetic data for model development were only available for one carbamate insecticide, carbaryl (EPA 2007b). Based on an analysis of available data, including data collected by EPA and data submitted for registration, EPA (2007b) concluded that acute ChE inhibition, measured at the peak time of effect, was the most sensitive effect from exposure to carbamates and thus, the pertinent effect of concern. A component-based RPF approach, assuming dose additivity, was used in the cumulative risk assessment. RPFs for 10 carbamates (and several carbamate metabolites—aldicarb sulfone, aldicarb sulfoxide, and 3- and 5-hydroxy carbofuran) were developed based on brain ChE inhibition data for adult rats (Table C-1). The rat brain ChE inhibition data were modeled with a dose-time response model to estimate BMD_{10} values (doses at which ChE was inhibited by 10%), and the RPF values were calculated by dividing the BMD_{10} value for the subject carbamate by the BMD_{10} value for the index carbamate, oxamyl (EPA 2007b). Oxamyl was chosen as the index chemical, because oxamyl, compared with the other nine carbamates, had the most robust database for all three pertinent routes of exposure (oral, dermal, inhalation).

Table C-1. EPA RPFs for Oral, Dermal, and Inhalation Exposure to Carbamate Insecticides Based on Rat Brain ChE Inhibition

Chemical	Oral RPF	Dermal RPF	Inhalation RPF
Aldicarb	4	ND[b]	ND
Aldicarb sulfone	3.44	ND	ND
Aldicarb sulfoxide	3.68	ND	ND
Carbaryl	0.15	0.71	0.51
Carbofuran	2.4	ND	ND
3- and 5-hydroxycarbofuran	2.4	ND	ND
Formetanate HCL	2.18	ND	ND
Methiocarb	0.18	0.09	0.62
Methomyl	0.67	ND	ND
Oxamyl	1	1	1
Primicarb	0.02	ND	ND
Propoxur	0.11	0.03	0.18
Thiodicarb	0.89	ND	ND

[a]Values for aldicarb sulfone and aldicarb sulfoxide were calculated based on molecular weight conversions from aldicarb assuming equipotency to aldicarb. 3- and 5-Hydroxycarbofuran were assumed to be equipotent to carbofuran.
[b]ND = not derived due to lack of data.

ChE = acetylcholinesterase; EPA = U.S. Environmental Protection Agency; RPF = relative potency factor

Source: EPA 2007b

EPA (2007b) incorporated uncertainty and extrapolation factors into the cumulative risk assessment for carbamate insecticides in two ways:

1. Adjustment of the RPF: Chemical-specific information was evaluated, when available, to determine chemical-specific inter-species uncertainty factors (animal to human extrapolation) and FQPA factors to arrive at adjusted RPF values for children and adults (Table C-2). Chemical-specific FQPA factors were calculated, when appropriate data were available, by dividing the adult BMD_{10} by the pup BMD_{10} for ChE inhibition; in the absence of appropriate data, the default value of 10 was used. Chemical-specific interspecies uncertainty factors were calculated similarly when appropriate data were available to compare human BMD_{10} values for ChE inhibition with rat BMD_{10} values.

2. Incorporation into the Target MOE: A default uncertainty factor of 10 for intrahuman variability was taken as the target MOE for each of the carbamate insecticides. The PODs, used in the

cumulative risk assessments to compare against exposure estimates, were the route-specific rat $BMDL_{10}$ values for brain ChE inhibition shown in Table C-3.

Table C-2. EPA Adjusted Oral RPFs for Children and Adults Based on Inter-Species and FQPA Factors

Chemical	Oral RPF	Interspecies factor	FQPA factor for children	Adjusted RPF children	Adjusted RPF adult
Aldicarb	4	2	2	16	8
Aldicarb sulfone	3.44	2	2	13.8	6.9
Aldicarb sulfoxide	3.68	2	2	14.7	7.4
Carbaryl	0.15	10	1.8	2.7	1.5
Carbofuran	2.4	10	2.75	66	24
3- and 5-hydroxycarbofuran	2.4	10	2.75	66	24
Formetanate HCL	2.18	10	2.03	44	22
Methiocarb	0.18	10	10	18	1.8
Methomyl	0.67	5	3.05	10	3.3
Oxamyl	1	3	3.48	10	3
Primicarb	0.02	10	10	2	0.2
Propoxur	0.11	10	10	11	1.1
Thiodicarb	0.89	10	10	89	8.9

EPA = U.S. Environmental Protection Agency; FQPA = U.S. Food Quality and Protection Act; RPF = relative potency factors

Source: EPA 2007b

Table C-3. Oral, Dermal, and Inhalation BMD_{10} and $BMDL_{10}$ Values for Rat Brain ChE Inhibition by Oxamyl, the Index Chemical for the EPA Cumulative Risk Assessment for N-Methyl Carbamates

End point	Oral	Dermal	Inhalation
BMD_{10}	0.24 mg/kg	34.91 mg/kg	0.0047 mg/L
$BMDL_{10}$	0.18 mg/kg	17.05 mg/kg	0.0038 mg/L (converted to 0.66 mg/kg)

BMD = benchmark dose; BMDL = benchmark dose limit; ChE = acetylcholinesterase; EPA = U.S. Environmental Protection Agency

Source: EPA 2007b

EPA (2007b) conducted route-specific cumulative risk assessments for adult and children exposures to N-methyl carbamate insecticides by incorporating the RPF values into a MOE approach applied to food, water, and residential exposure pathways. The residential pathways comprised oral, dermal, and inhalation exposures. Concentrations of carbamate residues in appropriate media (e.g., food, drinking water) were multiplied by appropriate interspecies- and FQPA-adjusted RPF values (Table C-2) and summed to arrive at oxamyl-equivalent concentrations, which were then used in exposure models to estimate oxamyl equivalent intakes (in units of mg/kg) for the various exposure scenarios investigated. MOE values were calculated by dividing the appropriate oxamyl POD (e.g., the oral rat $BMDL_{10}$—Table C-3—for oral exposure scenarios) by the estimated oxamyl equivalent intake. MOE values <10 were taken as values requiring some mitigation action; those >10 were assessed to be without the need for mitigation. EPA also determined total MOE values for combined estimates of food, water, and residential exposure scenarios, showing that the food pathway was the dominant exposure pathway for the general population.

EPA (IRIS 2013) classified aldicarb in Cancer Group D—*not classifiable as to human carcinogenicity*, based on no human carcinogenicity data and inadequate carcinogenicity data in animal studies. EPA (2007b) reviewed studies that found no statistically significant increases in tumor incidence in mice or rats in 2-year feeding studies or in mice in a skin painting study. EPA (IRIS 2013) noted that there were significant trends in pituitary tumors in female rats and fibrosarcomas in male mice in the feeding studies.

EPA (IRIS 2013) concluded that the assays were inadequate to assess the carcinogenicity of aldicarb, because maximum tolerated doses were not included in the assays. EPA (IRIS 2013) has not formally assessed the carcinogenicity of any other carbamate insecticide.

NTP (2011) has not assessed the possible carcinogenicity of carbamate insecticides.

IARC (2012) classified aldicarb and carbaryl in cancer Group 3—n*ot classifiable as to carcinogenicity to humans*, but has not assessed other carbamates for possible carcinogenicity.

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

The most clearly established end points of concern for carbamate insecticides are neurological effects mediated via the inhibition of ChE. ATSDR has not derived MRLs for any of the carbamate insecticides, but EPA (2007b) concluded that acute ChE inhibition, measured at the peak time of effect, was the most sensitive effect from exposure to carbamates and thus, the pertinent effect of concern in its cumulative

risk assessment for N-methyl carbamates. Due to the lack of appropriate and readily available dose-response data for effects other than ChE inhibition for most carbamate insecticides, TTDs were not developed.

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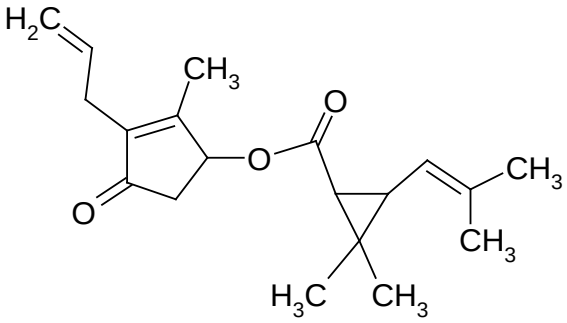
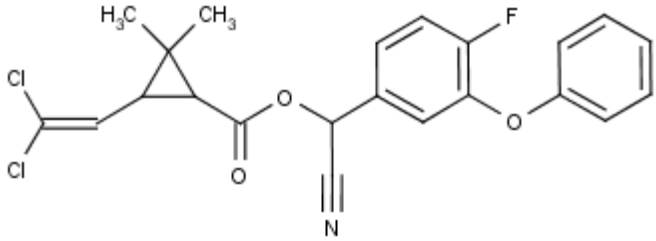
Appendix D: Chemical Structures of Mixture Components

Pyrethroid Insecticides

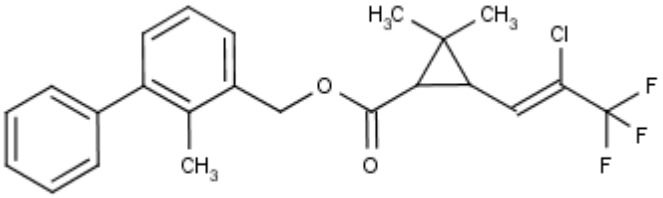
Allethrin



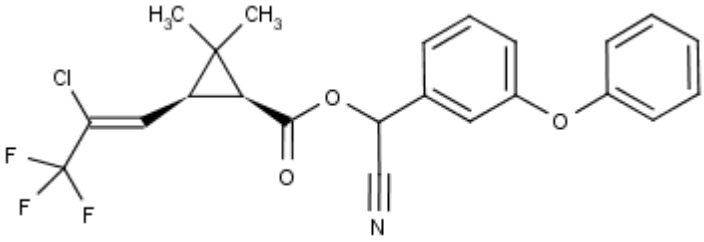
Cyfluthrin (Baythroid)



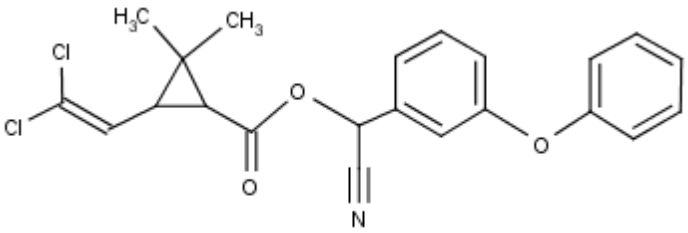
Bifenthrin



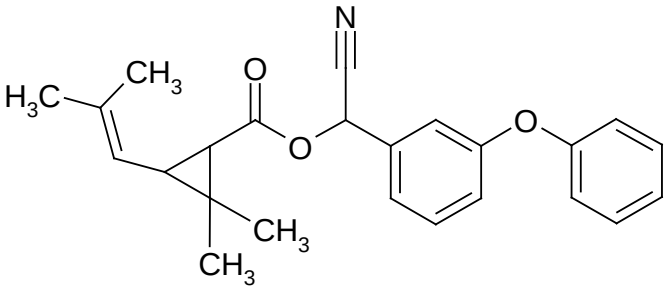
Cyhalothrin



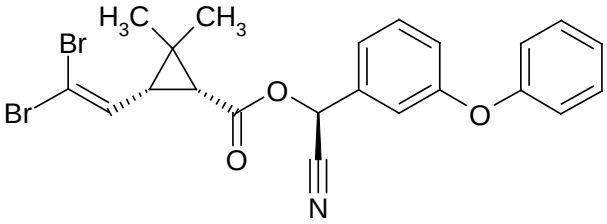
Cypermethrin



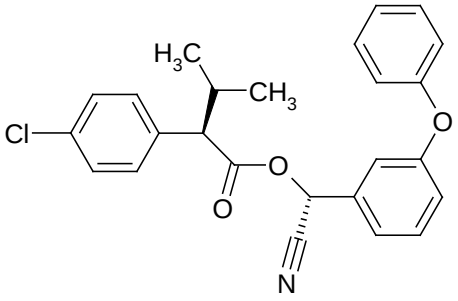
Cyphenothrin



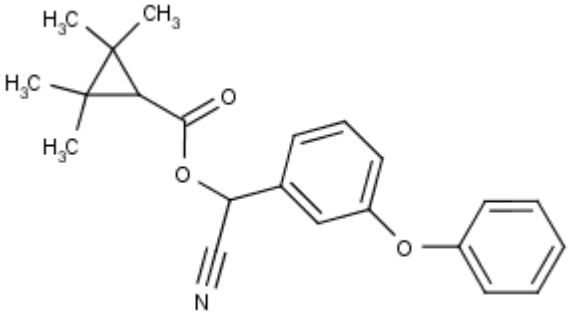
Deltamethrin



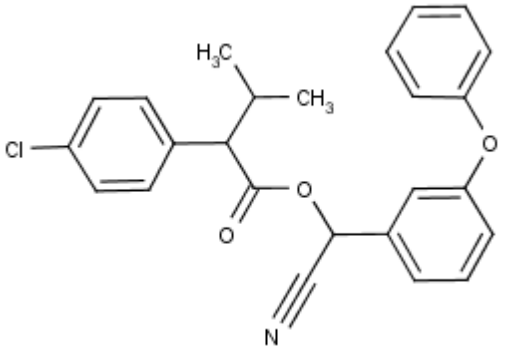
Esfenvalerate



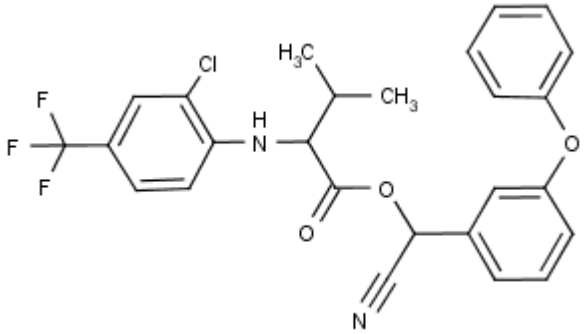
Fenpropathrin (Danitol)



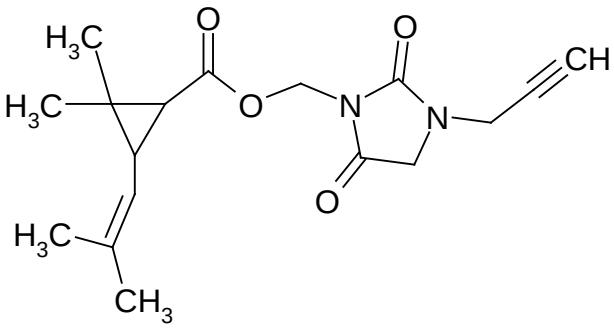
Fenvalerate (Pydrin)



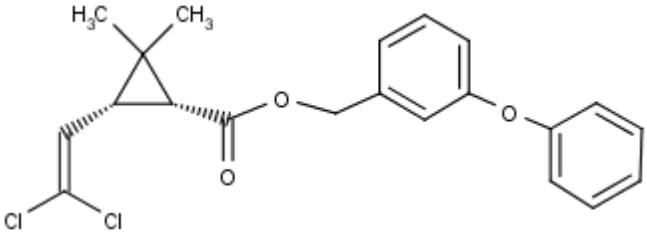
Fluvalinate



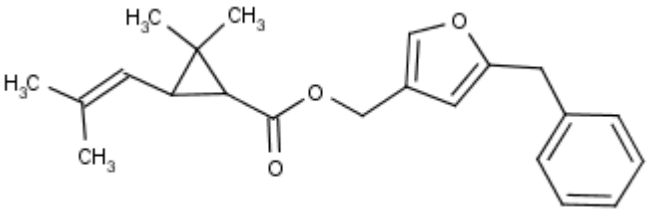
Imiprothrin



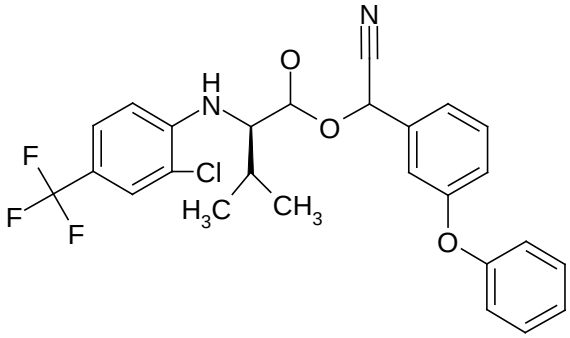
Permethrin



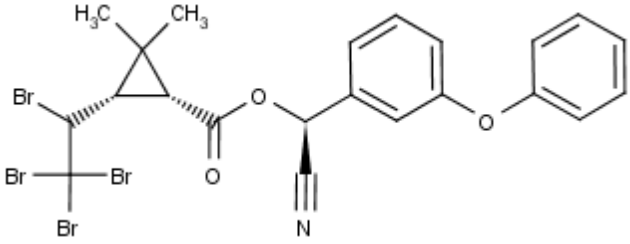
Resmethrin



Tau-fluvalinate

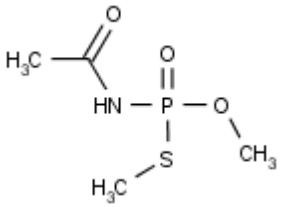


Tralomethrin

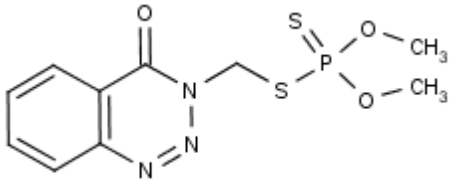


Organophosphorus insecticides

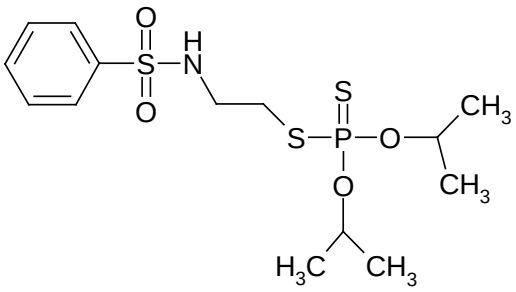
Acephate



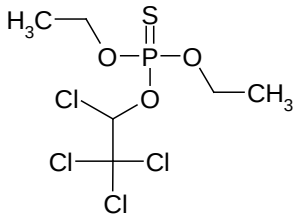
Azinphos-methyl



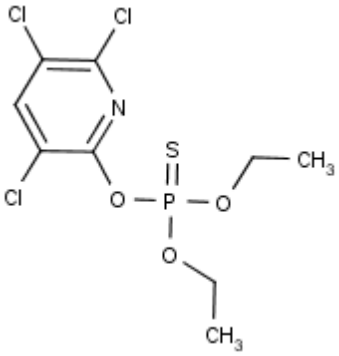
Bensulide



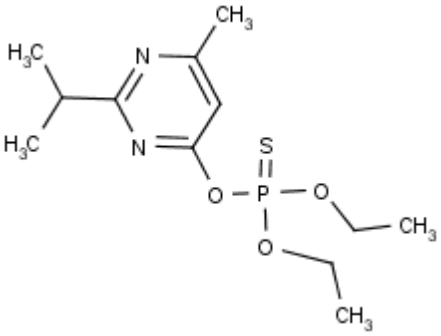
Chlorethoxyfos



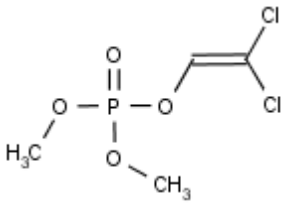
Chlorpyrifos



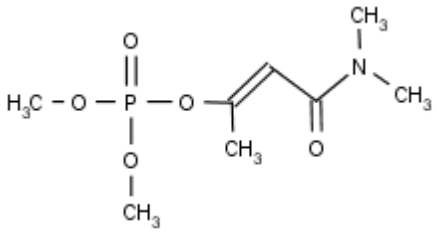
Diazinon



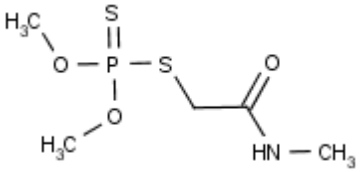
Dichlorvos



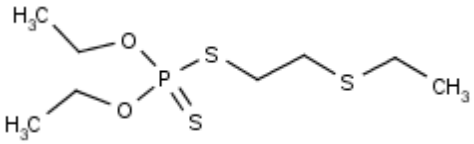
Dicrotophos



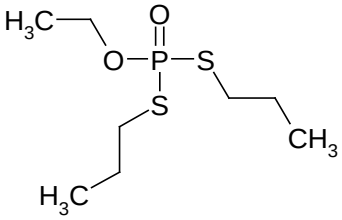
Dimethoate



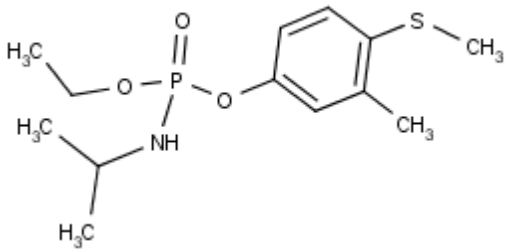
Disulfoton



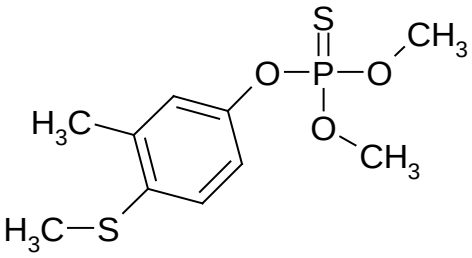
Ethoprop



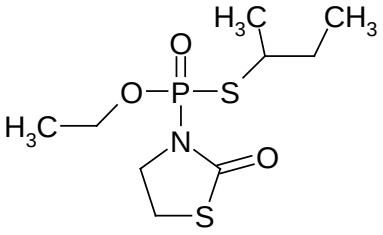
Fenamiphos



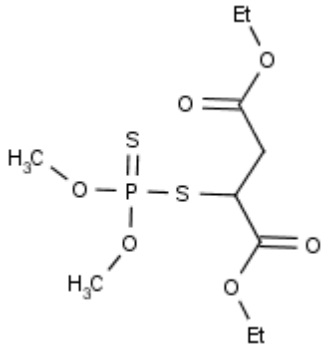
Fenthion



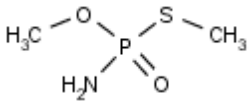
Fosthiazate



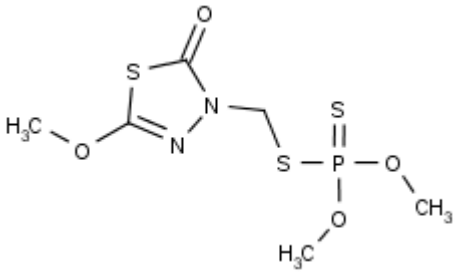
Malathion



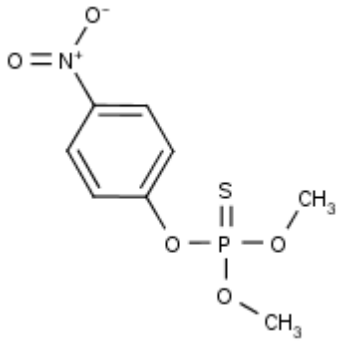
Methamidophos



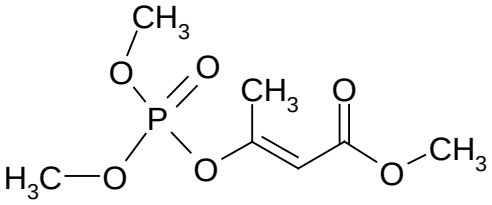
Methidathion



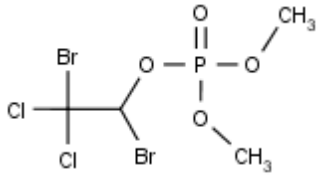
Methyl-parathion



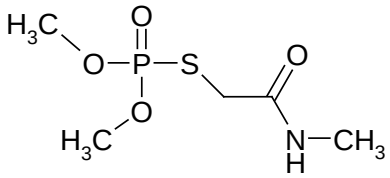
Mevinphos



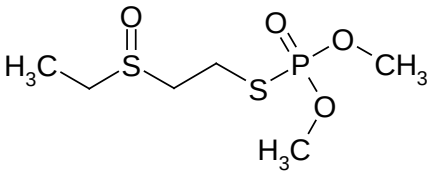
Naled



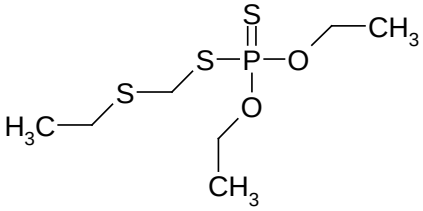
Omethoate



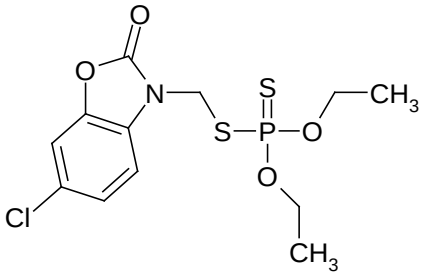
Oxydemeton-methyl



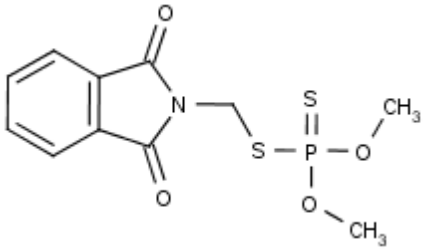
Phorate



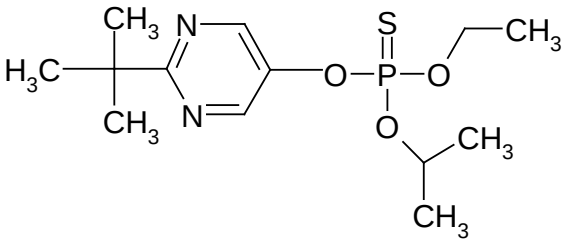
Phosalone



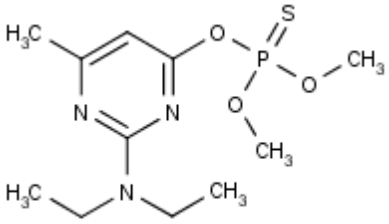
Phosmet



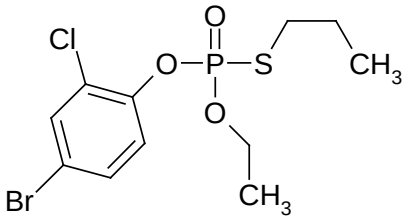
Phostebupirim



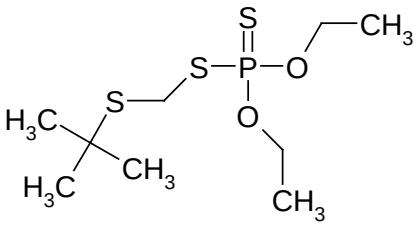
Pirimiphos-methyl



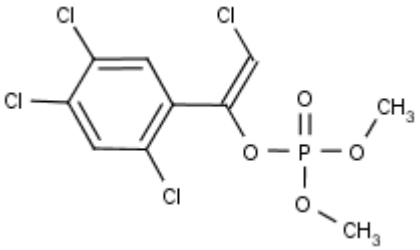
Profenofos



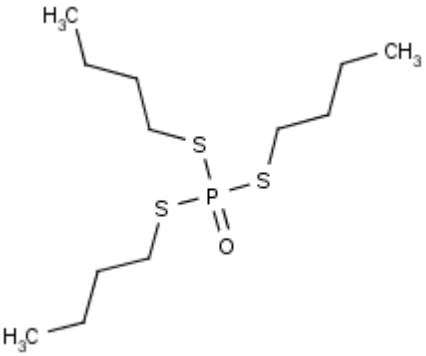
Terbufos



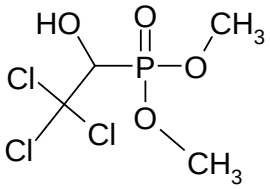
Tetrachlorvinphos



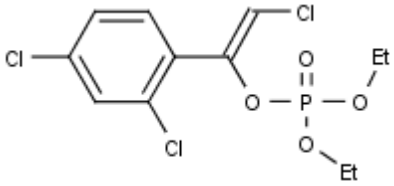
Tribufos (merphos oxide)



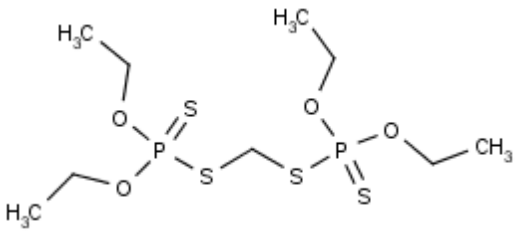
Trichlorfon



Chlorfenvinphos

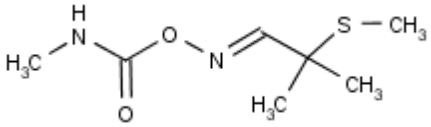


Ethion

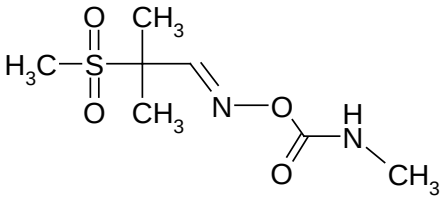


Carbamate insecticides

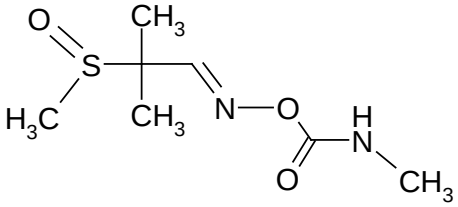
Aldicarb



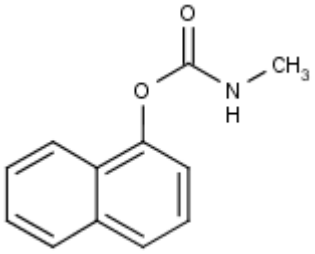
Alidcarb sulfone



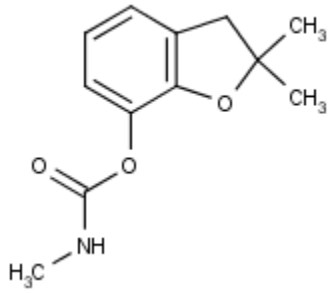
Aldicarb sulfoxide



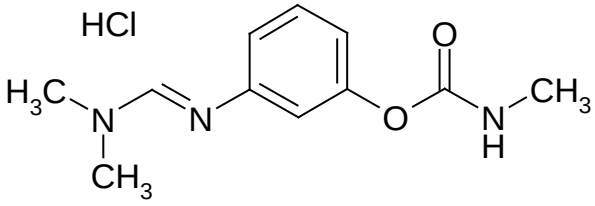
Carbaryl



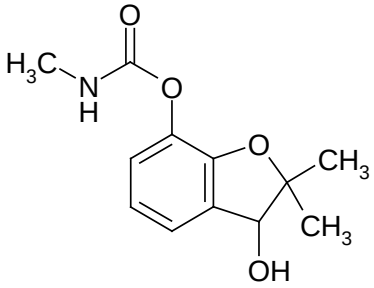
Carbofuran



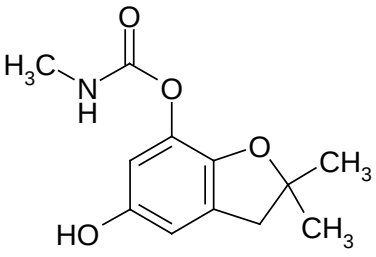
Formetanate hydrochloride



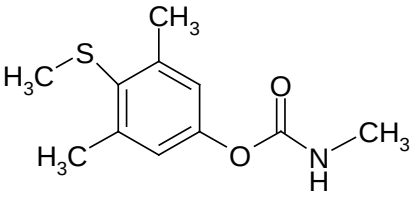
3-Hydroxycarbofuran



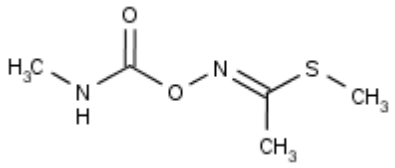
5-Hydroxycarbofuran



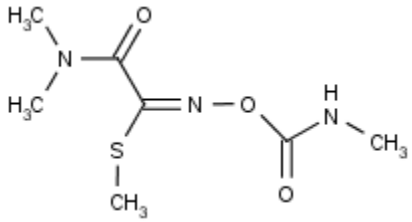
Methiocarb



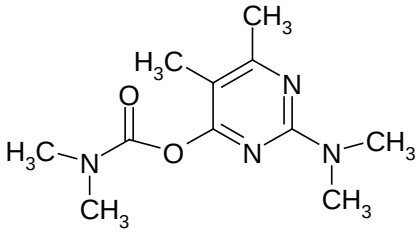
Methomyl



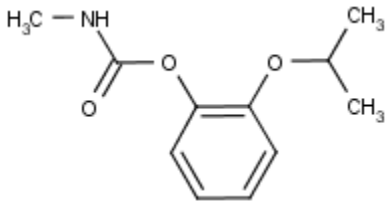
Oxamyl



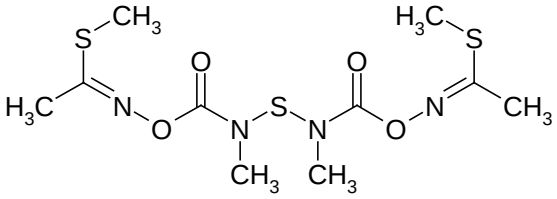
Pirimicarb



Propoxur (Baygon)



Thiodicarb



Appendix E: Mixtures of Insecticides at Hazardous Waste Sites

Table E-1. Sites with Two of the Types of Pesticides[a]

Site Event Key[b]	Type	Contaminant	CAS Number
AZR000041764	Organophosphorus	Chlorpyrifos	2921-88-2
	Pyrethroid	Permethrin	52645-53-1
CAD000629998	Carbamate	Carbaryl	63-25-2
		Carbofuran	1563-66-2
	Organophosphorus	Diazinon	333-41-5
		Dimethoate	60-51-5
		Disulfoton	298-04-4
		Ethion	563-12-2
		Guthion	86-50-0
		Malathion	121-75-5
		Methyl parathion	298-00-0
		Naled	300-76-5
		S,S,S-Tributyl phosphorotrithioate	78-48-8
CAD071530380	Carbamate	Carbaryl	63-25-2
		Methomyl	16752-77-5
		Propoxur	114-26-1
	Organophosphorus	Diazinon	333-41-5
		Disulfoton	298-04-4
CAXCR9P200000004	Carbamate	Carbaryl	63-25-2
		Carbofuran	1563-66-2
	Organophosphorus	Chlorpyrifos	2921-88-2
		Diazinon	333-41-5
		Disulfoton	298-04-4
		Malathion	121-75-5
GAD033478389	Carbamate	Carbofuran	1563-66-2
	Organophosphorus	Methamidophos	10265-92-6
		Methyl parathion	298-00-0
GAD991275686	Carbamate	Carbaryl	63-25-2
	Organophosphorus	Methyl parathion	298-00-0
MAXCR1#MA0000047	Carbamate	Carbaryl	63-25-2
	Organophosphorus	Chlorpyrifos	2921-88-2
MD2210020036	Carbamate	Carbaryl	63-25-2
		Propoxur	114-26-1
	Organophosphorus	Chlorpyrifos	2921-88-2
MN8570024275	Carbamate	Propoxur	114-26-1
	Organophosphorus	Diazinon	333-41-5
		Malathion	121-75-5

Table E-1. Sites with Two of the Types of Pesticides[a]

Site Event Key[b]	Type	Contaminant	CAS Number
MOD000830554	Carbamate	Carbaryl	63-25-2
	Organophosphorus	Diazinon	333-41-5
		Disulfoton	298-04-4
NCD980557656	Carbamate	Carbaryl	63-25-2
		Carbofuran	1563-66-2
	Organophosphorus	Malathion	121-75-5
NVXCRA7220000001	Carbamate	Carbofuran	1563-66-2
	Organophosphorus	Chlorpyrifos	2921-88-2
		Diazinon	333-41-5
		Malathion	121-75-5
TXD007349863	Carbamate	Carbaryl	63-25-2
		Propoxur	114-26-1
	Organophosphorus	Chlorpyrifos	2921-88-2
		Diazinon	333-41-5
		Dichlorvos	62-73-7
		Dimethoate	60-51-5
		Malathion	121-75-5
		Methyl parathion	298-00-0
TXN000605514	Carbamate	Carbaryl	63-25-2
	Organophosphorus	Diazinon	333-41-5
TXXCR6#TX0000006	Carbamate	Carbaryl	63-25-2
	Organophosphorus	Acephate	30560-19-1
TXXCR6#TX0000012	Carbamate	Propoxur	114-26-1
	Organophosphorus	Acephate	30560-19-1
WAD000643577	Carbamate	Carbaryl	63-25-2
	Organophosphorus	Diazinon	333-41-5
		Ethion	563-12-2
		Malathion	121-75-5
		Methyl parathion	298-00-0

[a]Information from the ATSDR HazDat and Sequoia database (through FY2007).
[b]Site IDs that are 12 characters long are for sites (usually defined by EPA). Site IDs that are 16 characters long are for events (like emergency events; always defined by ATSDR).

Table E-2. Sites with All Three Types of Pesticides[a]

Site Event Key[b]	Type	Substance	CAS Number
AZD980735666	Carbamate	Carbaryl	63-25-2
		Methomyl	16752-77-5
	Organophosphorus	Diazinon	333-41-5
		Dimethoate	60-51-5
		Guthion	86-50-0
		Malathion	121-75-5
		Methyl parathion	298-00-0
	Pyrethroid	Permethrin	52645-53-1
CO5210020769	Carbamate	Methomyl	16752-77-5
	Organophosphorus	Chlorfenvinphos	470-90-6
		Dichlorvos	62-73-7
		Dicrotophos	141-66-2
		Malathion	121-75-5
		Methyl parathion	298-00-0
		Naled	300-76-5
		Stirofos	22248-79-9
	Pyrethroid	Fenvalerate	51630-58-1
LAD981057706	Carbamate	Aldicarb	116-06-3
		Carbofuran	1563-66-2
		Oxamyl	23135-22-0
	Organophosphorus	Chlorpyrifos	2921-88-2
		Diazinon	333-41-5
		Dimethoate	60-51-5
		Disulfoton	298-04-4
		Guthion	86-50-0
		Malathion	121-75-5
		Methyl parathion	298-00-0
		Naled	300-76-5
	Pyrethroid	Permethrin	52645-53-1
NVXCRA7220000002	Carbamate	Carbaryl	63-25-2
		Propoxur	114-26-1
	Organophosphorus	Chlorpyrifos	2921-88-2
		Diazinon	333-41-5
		Malathion	121-75-5
		Methyl parathion	298-00-0
	Pyrethroid	Cyfluthrin	68359-37-5
		Cypermethrin	52315-07-8
		Permethrin	52645-53-1

Table E-2. Sites with All Three Types of Pesticides[a]

Site Event Key[b]	Type	Substance	CAS Number
NVXCRA7220000004	Carbamate	Carbaryl	63-25-2
		Propoxur	114-26-1
	Organophosphorus	Chlorpyrifos	2921-88-2
		Diazinon	333-41-5
		Malathion	121-75-5
		Methyl parathion	298-00-0
	Pyrethroid	Cyfluthrin	68359-37-5
		Cypermethrin	52315-07-8
		Permethrin	52645-53-1

[a]Information from the ATSDR HazDat database (through FY2007).
[b]Site IDs that are 12 characters long are for sites (usually defined by EPA). Site IDs that are 16 characters long are for events (like emergency events; always defined by ATSDR).