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.