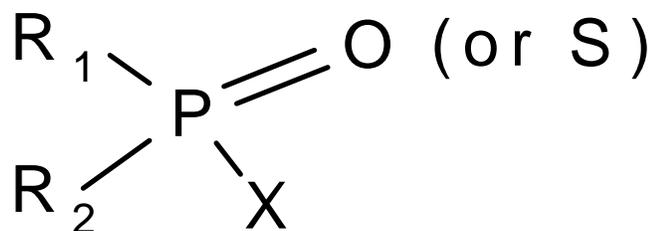


US EPA ARCHIVE DOCUMENT

***Preliminary Cumulative
Hazard and Dose-Response Assessment for
Organophosphorus Pesticides:***

***Determination of Relative Potency
and Points of Departure
for Cholinesterase Inhibition***



Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460

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PREFACE

The Food Quality Protection Act (FQPA) of 1996 requires the USEPA to consider the cumulative effect to human health that can result from exposure to pesticides and other substances that have a common mechanism of toxicity. Over the last two years, the Office of Pesticide Programs (OPP) has presented to the FIFRA Scientific Advisory Panel (SAP) for comment proposed guidance, tools and methodologies for conducting cumulative risk assessments. In September 2000, a case study on 24 organophosphorus pesticides was presented to the SAP to demonstrate hazard assessment methods for determining cumulative risk.

In order to accomplish the reassessment of 66% of pesticide tolerances by August 3, 2002 as required by the FQPA, the Agency needs to complete the tolerance reassessment for the organophosphorus pesticides by performing a cumulative risk assessment for this group. Therefore, the Agency presently intends to complete the preliminary cumulative risk assessment for the organophosphorus pesticides by December 2001. Toward this end, the Agency presently has considered the SAP written comments on the approach that was outlined in the September 27, 2000 document entitled "Endpoint Selection and Determination of Relative Potency in Cumulative Hazard and Dose-response Assessment: A Pilot Study of Organophosphorus Pesticide Chemicals" (<http://www.epa.gov/scipoly/sap/2000/index.htm>). The current document has revised the relative potency factor analysis by incorporating the recommendations of the SAP.

The purpose of the current review is to seek comment from the SAP on whether the revised hazard and dose-response methodology on the organophosphorus pesticides has adequately addressed the September 2000 SAP comments.

EXECUTIVE SUMMARY

The Food Quality Protection Act (FQPA) of 1996 requires the USEPA to consider the cumulative effect to human health that can result from exposure to pesticides and other substances. Toward this end, the Agency has incorporated the SAP written comments on the approach to cumulative hazard and dose-response for the organophosphate pesticides (OPs) that was outlined in the September 27, 2000 document entitled "Endpoint Selection and Determination of Relative Potency in Cumulative Hazard and Dose-response Assessment: A Pilot Study of Organophosphorus Pesticide Chemicals" (<http://www.epa.gov/scipoly/sap/2000/index.htm>). There were 25 OP pesticides included in the current analysis which are listed in Table 1. Nine of the OPs have residential/nonoccupational exposure. Ethoprop, fenthion, chlorpyrifos-methyl, and dicrotophos were identified as appropriate for inclusion after the completion of this assessment and will also be included in the cumulative risk assessment. The relative potency of these four OPs will be evaluated using the same methodology described for the 25 pesticides.

Before the cumulative risk of OPs can be estimated, the relative toxic potency of each OP must be determined using a uniform basis of comparison, by using to the extent possible, the same common response derived from comparable measurement methodology, species, and sex for all the exposure routes of interest. OPs exert neurotoxicity by binding to and phosphorylation of the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems. Because peripheral cholinesterase measures were not widely available, this analysis focused on plasma, red blood cell (RBC), and brain cholinesterase inhibition for which laboratory animal data were widely available. Specifically, cholinesterase activity data derived were evaluated from male and female rats from subchronic and chronic guideline toxicity studies for the oral, dermal, and inhalation routes. It was necessary to use dermal toxicity studies in rabbit for four chemicals with residential/nonoccupational exposure potential because dermal toxicity study data in rats were not available. Of the nine chemicals with residential/nonoccupational exposure, a dermal toxicity study was not available for dichlorvos and inhalation toxicity studies were not available for bensulide and tetrachlorvinphos. It has not yet been determined whether or how residential/nonoccupational exposure potential will be quantitatively assessed in the Preliminary Cumulative Risk Assessment of OPs for these chemicals where route-specific data were not available.

OPP has elected to use data reflecting steady state conditions to estimate relative potencies for the OPs in the interest of producing relative potency factors (RPFs) that are reproducible and reflect less uncertainty due to rapidly changing, time-sensitive measures of cholinesterase.

A preliminary review determined that numerous oral studies with comparable methodologies were available and suitable for dose-response analysis. In order to determine chemical potency, a hierarchical methodology was used to determine the overall potency of each OP. First the potency of separate cholinesterase measurements taken at 21 days or more on study was determined. Each individual cholinesterase measurement was fit to the following exponential function using generalized least squares regression: $y = B + (A - B) \times e^{-m \times \text{dose}}$. In this equation, **y** is cholinesterase activity, **dose** is the dose of OP, in mg/kg/day, **m** is the dose scale factor and is the measure of absolute potency, **A** is background cholinesterase activity, and **B** is the y-asymptote. Both **y** and **dose** were extracted from the oral toxicity studies. **The absolute potency was quantified by the dose scale factor, m.** Confidence limits and standard errors for the estimates of A, B, and m were also calculated. The next step in potency determination was the averaging of single cholinesterase measurements *within* a study. Finally, the *overall* average potency was calculated by averaging the within-study potencies. **The overall average absolute potency was used in the calculation of RPFs.** Technical details of the statistical methods used are described in the appendix of this document.

As opposed to selecting a single point or single study, the large majority of the 1496 cholinesterase data sets collected from 69 studies were utilized in the dose-response assessment. These datasets represent cholinesterase activity measured in plasma (516 data sets), RBC (524 data sets), and brain (213 data sets from whole brain and 243 from separate anatomical sections should total 456) from male and female rats orally exposed to each of the 25 OPs from 21 to > 700 days. On average there were 59 ± 33 data sets per chemical ranging from 24 for tetrachlorvinphos to 106 for acephate. At least one data set was available for each of the 25 chemicals in each compartment in both sexes. A total of 1312 data sets were analyzed. The cholinesterase data were adequately fit to the exponential model for 83% of the datasets; potency from only six of 1312 datasets could not be estimated. The potency estimates in most of the data sets with inadequate goodness-of-fit were comparable to potency estimates for data sets with adequate fit. Absolute potencies ranged from 0.002 for tetrachlorvinphos to 3.08 for terbufos. Overall, there was good concordance between average absolute potency values calculated from cholinesterase activity measured in brain, RBC, and plasma between male and female rats for each chemical, although a few exceptions were noted. Potency values were generally consistent across time for the majority of chemical-sex-compartment combinations for all of the 25 OPs; most chemicals appeared to reach steady state by 21 or 28 days of exposure in both sexes and all three compartments. It was determined that the RPFs derived from data on **inhibition of cholinesterase activity in male rat RBC should be used to estimate potential cumulative risk.**

The dose-response analysis for the oral studies was performed using a computer program, called OPCumRisk which is freely available to the public.

OPCumRisk was developed at Office of Research and Development's (ORD) National Health and Environmental Effects Laboratory (NHEERL) specifically for use in the OP dose-response assessment.

Relative potency for OPs with dermal and inhalation exposure potential through residential/nonoccupational exposure pathways was determined using no-observed-adverse-effect-levels (NOAELs) for RBC cholinesterase inhibition because of limited data availability. The NOAEL was defined as the lowest dose where a maximum 10-15% RBC cholinesterase inhibition (compared to control) occurred. Even though dermal and inhalation RPFs were based on NOAELs whereas the oral RPFs were based on potency values estimated from dose-response modeling, the relative rank order of the potency for the residential chemicals with route specific data was comparable to the order of the oral potencies.

For the cholinesterase-inhibiting OP pesticides, the ideal index chemical should exhibit high quality dose-response data in plasma, RBC, and brain for both sexes of a single species for all exposure routes of interest. It was preferred to use one index chemical for all of the exposure routes of interest in order to express cumulative risk for all pathways using the same index chemical exposure equivalents. **Methamidophos was selected as the index chemical for the Preliminary OP Cumulative Risk Assessment.** In the dose-response analysis of the oral studies, average absolute potency values for methamidophos were *comparable for all three cholinesterase measures in both sexes*. Quality dose-response data were also available for the dermal and inhalation routes for calculation of route-specific points of departure (PoDs) for extrapolation of cumulative risk. The benchmark dose where cholinesterase activity is reduced 10% compared to background activity (BMD_{10}) and the lower 95% confidence interval on the BMD_{10} ($BMDL$) were also calculated for the available RBC cholinesterase measurements in male rats for the oral, dermal, and inhalation routes (USEPA 2000b). The average BMD_{10} s for each route of exposure were calculated and are expected to be used as for the PoDs in cumulative risk assessment of OPs. These values are 0.09 mg/kg/day, 1.21 mg/kg/day, and 0.0046 mg/L for the oral, dermal, and inhalation routes, respectively. The analogous values in female RBC, plasma and brain and also for male plasma and brain for all three routes were similar to the male RBC average BMD_{10} values.

In summary, relative potency factors of 25 OPs for the oral route of exposure as well as nine chemicals with residential/nonoccupational exposure have been determined in addition to endpoints needed for the extrapolation of cumulative risk to humans. The exponential function performed well in the determination of potency for cholinesterase inhibition for the oral studies. The use of NOAELs for potency determination for the dermal and inhalation routes, although not the preferred method, was adequate and generated reasonable estimates.

LIST OF ABBREVIATIONS

| | |
|-------------------------|---|
| A | Estimate of A (background cholinesterase activity) |
| ACh | Acetylcholine |
| B | Estimate of B (y-asymptote) |
| B/A | Ratio of estimate of B/estimate of A |
| BMD₁₀ | Benchmark dose with 10% reduction in cholinesterase activity compared to the background |
| BMDL | Lower 95% confidence limit on the BMD ₁₀ |
| chel | Cholinesterase activity (usually in international units) |
| CL | Confidence limit |
| D | Number of days on study |
| DER | Data evaluation record |
| Duplicate | Duplicate animals (i.e., satellite group, recovery animals, etc) |
| Duplicatewhole | Whole brain from duplicate animals |
| F | Female |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| FQPA | Food Quality Protection Act |
| GOF | Model goodness-of-fit |
| HED | Health Effects Division |
| ILSI | International Life Sciences Institute |
| LOAEL | Lowest-Observed-Adverse-Effect Level |
| m | Estimate of absolute potency for a single cholinesterase measurement |
| M | Male |
| main | Main study animals |
| Max. | Maximum value |
| Min. | Minimum value |
| MRID # | MRID study identification number |
| N | Sample size |
| NA | Not available |
| NHEERL | National Health and Environmental Effects Laboratory |
| NOAEL | No-Observed-Adverse-Effect Level |
| OP | Organophosphate pesticide |
| OPP | Office of Pesticide Programs |
| ORD | Office of Research and Development |
| PDP | USDA's Pesticide Data Program |
| PoD | Point of Departure |
| RBC | Red blood cells |
| RfD | Reference Dose |
| RSI | Risk Sciences Institute |
| RPF | Relative Potency Factor |

SAP
whole
1st Qu.
3rd Qu.

Scientific Advisory Panel
Whole brain from the main study animals
First quartile
Third quartile

I. INTRODUCTION

In 1996, passage of the Food Quality Protection Act (FQPA)¹ imposed upon the Office of Pesticide Programs (OPP) the requirement to consider potential human health risks from all pathways of dietary and non-dietary exposures to more than one pesticide acting through a common mechanism of toxicity. On June 30, 2000, OPP issued guidance for conducting cumulative risk assessment for public comment (USEPA, 2000a). Prior to that FR notice, in September, 1999, OPP presented the hazard and dose-response components of its cumulative guidance document for review by the FIFRA Science Advisory Panel (FIFRA SAP, 2000a). Shortly afterwards in December, 1999, OPP presented to the SAP for comment the exposure and risk characterization elements of the guidance, as well as an early case study on the dietary risk of three pesticides (FIFRA SAP, 2000b).

In September and December, 2000, OPP presented to the SAP a pilot analysis of 24 organophosphorus (OP) pesticides in which OPP defined methods and parameters that should be considered in estimating cumulative risk from exposure to common mechanism pesticides and in evaluating multiple pathways of exposure (USEPA 2000c; FIFRA SAP 2001a). In their review of the hazard and dose-response component of the OP pilot, the SAP offered several recommendations. In particular, the SAP suggested that there would be much greater confidence in the determination of each chemical's toxic potency if it were derived from several relatively consistent studies rather than a single study as used in the September, 2000 analysis on OPs. The SAP also recommended that the Agency reevaluate the selection of the probit model for determining the relative potencies. They specifically suggested that the Agency consider Michaelis-Menton kinetics or an exponential model as potential alternative methods (FIFRA SAP, 2001a). The Panel further commented on the careful selection of an index chemical with "the best and most complete data for the common endpoint(s)" to minimize the uncertainties in the estimation of cumulative risk.

The current cumulative hazard and dose-response assessment has incorporated the recommendations and comments of the SAP. This evaluation represents a collaborative effort between the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) and the Office of Research and Development's (ORD) National Health and Environmental Effects Laboratory (NHEERL).

¹For details see *The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FDCA) As Amended by the Food Quality Protection Act (FQPA) of August 3, 1996; U.S. Environmental Protection Agency, Office of Pesticide Programs, document # 730L97001, March, 1997.*

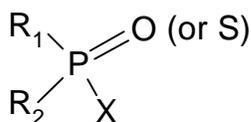
II. IDENTIFICATION OF CHEMICALS THAT SHARE A COMMON MECHANISM OF TOXICITY

The identification of a candidate group of pesticides and other substances that cause a common toxic effect by a common mechanism is the first step in the cumulative risk assessment process. Because organophosphorus pesticides have been assigned priority for tolerance reassessment, OPP has considered data for identifying whether these pesticides cause common toxic effects by common mechanisms of toxicity under EPA's *Guidance For Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity* (USEPA, 1999a). The inhibition of acetylcholinesterase has been a focal point given that most organophosphorus pesticides cause this response as their critical and common effect. The physiological action of acetylcholinesterase is to hydrolyze the neurotransmitter acetylcholine (ACh) so that the activation of the cholinergic receptor is transient. When acetylcholinesterase is inhibited, acetylcholine accumulates and cholinergic toxicity results due to continuous stimulation of cholinergic receptors throughout the central and peripheral nervous systems which innervate virtually every organ in the body.

Several years ago, OPP asked the Risk Sciences Institute (RSI) of the International Life Sciences Institute (ILSI) to convene an expert panel to address whether organophosphorus pesticides act by a common mechanism of toxicity (Miles et al., 1998). This panel applied several basic principles to evaluate the potential for two or more organophosphorus pesticides to act by the same mechanism: 1) do they cause the same critical effect(s), 2) do they act on the same molecular target at the same target tissue, and 3) do they act by the same biochemical mechanism of action perhaps because they share a common toxic intermediate? The RSI panel focused on acetylcholinesterase inhibition as a scientifically accepted mechanism of action for the cholinesterase inhibiting phosphorus pesticides and found that these principles were met. The workgroup concluded that the cholinesterase-inhibiting OP pesticides should be considered to act by a common mechanism of toxicity. In March 1998, OPP presented its draft document on a common mechanism of toxicity for the organophosphorus pesticides for comment by the FIFRA SAP (see <http://www.epa.gov/scipoly/sap/1998/march/comec.htm>). The SAP agreed with OPP that acetylcholinesterase inhibition provides a sufficient basis for determining that a common mechanism exists for the purpose of grouping the OP pesticides. Following consideration of the ILSI report and the SAP comments, OPP developed the final policy document that identifies a common mechanism of toxicity for organophosphorus pesticides: *"Policy On A Common Mechanism of Toxicity: The Organophosphate Pesticides"* (USEPA, 1999b).

III. SELECTION OF ORGANOPHOSPHORUS PESTICIDES

The 25 OP pesticides included in this analysis for determination of their potency to inhibit cholinesterase are listed in Table 1. Organophosphorus pesticides are characterized by the general formula:



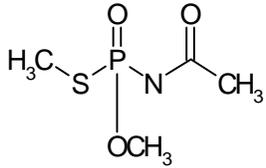
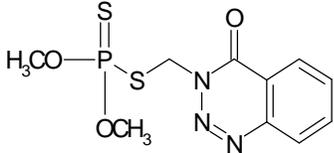
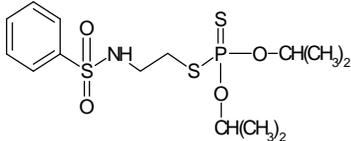
where X is a leaving group of variable structure and R₁ and R₂ are alkoxy, amino, thioalkyl or other substituents.

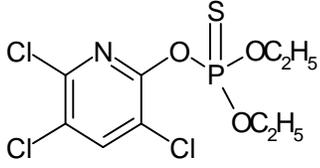
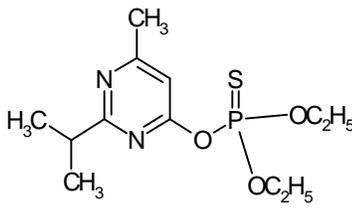
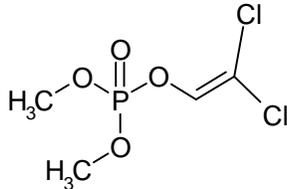
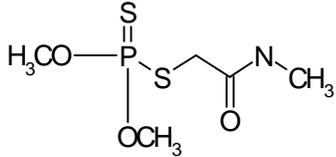
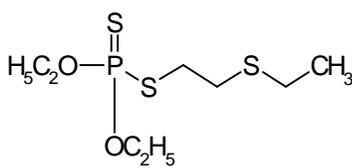
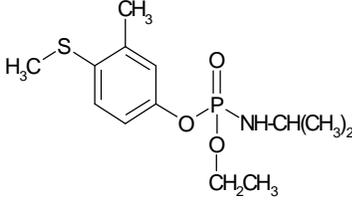
Organophosphorus pesticides with a P = O group are direct acting cholinesterase inhibitors, whereas those with a P = S group are relatively poor cholinesterase inhibitors until they undergo metabolic activation by cytochromes P450 or flavin monooxygenases to the oxon form (i.e., with a P = O group). Organophosphorus pesticides vary in their *in vivo* potency due to their structural, steric, and hydrophobicity properties, as well as differences in tissue distribution and detoxification and other toxicokinetic characteristics.

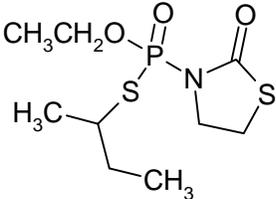
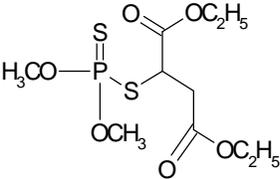
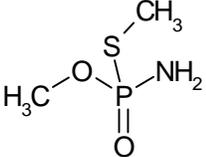
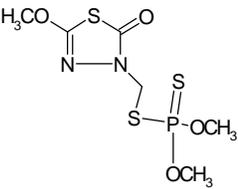
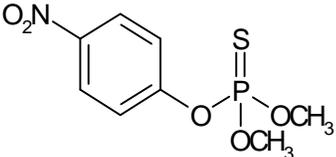
Currently, there are 37 organophosphorus pesticides registered by EPA. Twenty-three of 25 (Table 1) pesticides included in this analysis were selected based on their detection in USDA's Pesticide Data Program (PDP) for monitoring data of commonly eaten children foods such as fruits, vegetables, and milk, collected between the years 1994 and 1999 as well as their potential for human exposure through residential or non-occupational uses and/or drinking water. Two additional pesticides, bensulide and fosthiazate have not been tested in PDP. Bensulide does not pose a dietary concern (i.e., the dietary risk [food only] is less than 1% of its total risk based on a Tier 1 conservative dietary assessment), but it does have residential/nonoccupational uses. Fosthiazate is a new chemical submitted for registration (food use only) and, therefore, has not yet been surveyed in PDP. Nine OPs listed in Table 1 (acephate, bensulide, dichlorvos, disulfoton, fenamiphos, malathion, naled, tetrachlorvinphos, and trichlorfon) have residential/nonoccupational uses.

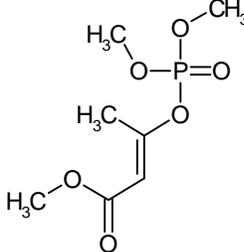
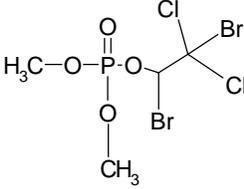
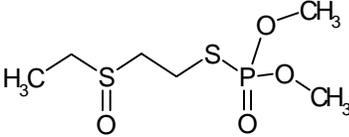
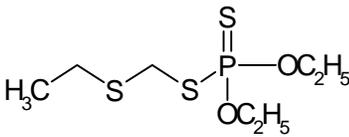
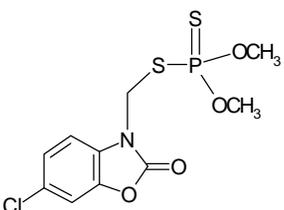
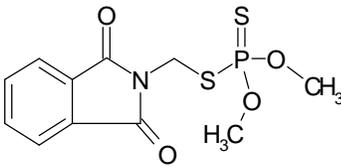
After this analysis was completed, four additional organophosphorus pesticides (ethoprop, fenthion, dicrotophos, and chlorpyrifos-methyl) were identified for consideration. These four OP pesticides are currently being evaluated for toxic potency and may be incorporated in the exposure portion of preliminary cumulative risk assessment. The analysis of their relative potency will follow the approach described in this document.

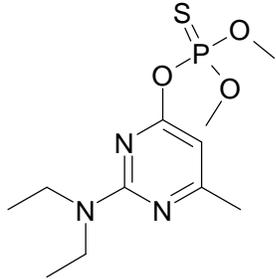
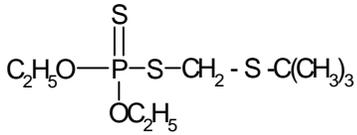
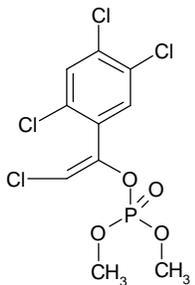
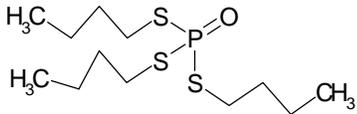
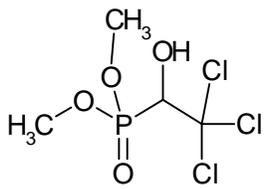
As indicated in the June 2000 Draft Cumulative Guidance Document (USEPA, 2000a), EPA is not going to quantify risk from chemicals with minimal hazard or exposure potential or from minor exposure pathways. Instead, the focus should be on pesticides and exposure scenarios that have potential to contribute to risk and may require mitigation actions. Although the 25 pesticides listed in Table 1 are likely to be included in the preliminary cumulative risk assessment, a route of exposure, a pathway of exposure, a specific use pattern, or an entire chemical may not be included in the quantification of cumulative risk following a detailed integration of the hazard/dose-response and exposure assessments. For example, for chlorpyrifos, previous Agency assessments indicate low risk potential for the wide area mosquito treatments and also treatments to golf courses. The chlorpyrifos use on residential lawns is restricted to applications by professional lawn care operators for the treatment of individual ant mounds. This use pattern also has been judged to have very low potential for exposure/risk. Finally, any mitigation measures presently imposed on these organophosphorus pesticides will be accounted for in the preliminary cumulative risk assessment.

| Table 1. Structures of the Organophosphorus Pesticides Included in this Document. | | | | |
|--|-------------------|---|---|-----------------|
| PC Code | Trade Name | Molecular Structure | Chemical Name | CAS Reg. |
| 103301 | Acephate |  | O,S-Dimethyl acetylphosphoramidothioate | 30560-19-1 |
| 058001 | Azinphos-methyl |  | O,O-dimethyl-S-((4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl)phosphorodithioate | 86-50-0 |
| 009801 | Bensulide |  | O,O-diisopropyl phosphorodithioate S-ester with N-(2-mercaptoethyl)benzenesulfonamide (8Cl) | 741-58-2 |

| Table 1. Structures of the Organophosphorus Pesticides Included in this Document. | | | | |
|---|--------------|---|---|------------|
| PC Code | Trade Name | Molecular Structure | Chemical Name | CAS Reg. |
| 59101 | Chlorpyrifos |  | O,O-Diethyl O-(3,5-trichloro-2-pyridinyl) phosphorothioate | 2921-88-2 |
| 057801 | Diazinon |  | O,O-Diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate | 333-41-5 |
| 84001 | Dichlorvos |  | 2,2-Dichlorovinyl dimethyl phosphate | 62-73-7 |
| 035001 | Dimethoate |  | O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate | 60-51-5 |
| 32501 | Disulfoton |  | Phosphorodithioic acid, O,O-diethyl-S-[2-(ethylthio)ethyl] ester (9CI) | 298-04-4 |
| 100601 | Fenamiphos |  | O-Ethyl-O-(3-methyl-4-methyl-thiophenyl)iso propylphosphoramidate | 22224-92-6 |

| Table 1. Structures of the Organophosphorus Pesticides Included in this Document. | | | | |
|---|------------------|---|---|------------|
| PC Code | Trade Name | Molecular Structure | Chemical Name | CAS Reg. |
| 129022 | Fosthiazate |  <p>Not yet registered</p> | (RS)-S-sec-butyl-O-ethyl-(2-oxo-1,3-thiazolidin-3-yl)phosphonothioate | 98886-44-3 |
| 057701 | Malathion |  | O,O-Dimethyl phosphorodithioate of diethyl mercaptosuccinate | 121-75-5 |
| 101201 | Methamidophos |  | O,S-Dimethyl phosphoramidothioate | 10265-92-6 |
| 100301 | Methidathion |  | O,O-Dimethyl phosphorodithioate, S-ester with 4-(mercaptomethyl-2-methoxy-1,3,4-thiadiazolin-5-one) | 950-37-8 |
| 053501 | Methyl parathion |  | O,O-Dimethyl-O-4-nitrophenyl phosphorothioate | 298-00-0 |

| Table 1. Structures of the Organophosphorus Pesticides Included in this Document. | | | | |
|---|-------------------|---|---|-----------|
| PC Code | Trade Name | Molecular Structure | Chemical Name | CAS Reg. |
| 15801 | Mevinphos |  | 2-Butenoic acid, 3-((dimethoxyphosphoryl)oxy)-, methyl ester (9CI) | 7786-34-7 |
| 034401 | Naled |  | 1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate | 300-76-5 |
| 58702 | Oxydemeton-methyl |  | S-[2-(Ethylsulfinyl)ethyl] O,O-dimethyl phosphorothioate | 301-12-2 |
| 057201 | Phorate |  | O,O-Diethyl S-[(ethylthio)methyl] phosphorodithioate | 298-02-2 |
| 97701 | Phosalone |  | Phosphorodithioic acid, S-((6-chloro-2-oxo-3(2H)-benzoxazolyl)methyl) O,O-diethyl ester | 2310-17-0 |
| 059201 | Phosmet |  | O,O-Dimethyl phosphorodithioate S-ester with N-(mercaptomethyl) phthalimide | 732-11-6 |

| Table 1. Structures of the Organophosphorus Pesticides Included in this Document. | | | | |
|---|-------------------|---|--|------------|
| PC Code | Trade Name | Molecular Structure | Chemical Name | CAS Reg. |
| 108102 | Pirimiphos-methyl |  | O-(2-diethylamino-6-methylpyrimidinyl) O,O-dimethyl phosphorothioate | 29232-93-7 |
| 105001 | Terbufos |  | S-[(1,1-dimethylethylthio)methyl]O,O-diethyl phosphorordithioate | 13071-79-9 |
| 83701 | Tetrachlorvinphos |  | (Z)-2-Chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate | 961-11-5 |
| 074801 | Tribufos |  | S,S,S-Tributyl phosphorotrithioate | 78-48-8 |
| 57901 | Trichlorfon |  | Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate | 52-68-6 |

IV. CUMULATIVE HAZARD AND DOSE-RESPONSE ASSESSMENT METHODS

A. Overview

Before the cumulative risk of organophosphorus pesticides can be quantified, the relative toxic potency of each OP must be determined. The determination of relative toxic potency should be calculated using a uniform basis of comparison, by using to the extent possible a common response derived from the comparable measurement methodology, species, and sex for all the exposure routes of interest.

B. Endpoints and Toxicology Studies

1. Selection of Endpoints

As part of the hazard analysis, all relevant responses are evaluated to identify the most appropriate and sensitive endpoint pertaining to the common mechanism of toxicity and to determine which endpoint(s) provide(s) a uniform and common basis for determining the relative potency of the cumulative assessment group. Organophosphorus pesticides exert their neurotoxicity by binding to and phosphorylation of the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems (Miles *et al.*, 1998). There are laboratory animal data on OPs for cholinesterase activity in plasma, red blood cell (RBC) and brain, as well as behavioral or functional neurological effects in submitted guideline studies. Measures of acetylcholinesterase inhibition in the peripheral nervous system (PNS) are very limited for the OP pesticides. As a matter of science policy, blood cholinesterase data (plasma and RBC) are considered appropriate surrogate measures of potential effects on PNS acetylcholinesterase activity and of potential effects on the central nervous system (CNS) when brain cholinesterase data are lacking (USEPA, 2000d). Behavioral changes in animal studies usually occur at higher doses compared to doses needed to inhibit cholinesterase. Also, behavioral measures are limited in terms of the scope of effects assessed and the measurements employed. **Therefore, this analysis focuses on the common mechanism endpoints for plasma, RBC, and brain cholinesterase inhibition.**

When assessing cholinesterase activity, it is important to carefully consider methodological issues that may affect the accuracy and variability of the data. There are many methods available for measuring cholinesterase activity. These methods include colorimetric, electrometric, titrimetric, radiometric, fluorimetric, gasometric, and immunochemical assays. The colorimetric method, based on the Ellman reaction, is the most commonly used method for measuring brain, plasma and erythrocyte cholinesterase activity (Ellman et al., 1961; USEPA 1992; ASCP, 1994). For this preliminary assessment, if the Data Evaluation Record (DER) for a particular study indicated that the study was acceptable, it was assumed that the methodology was also acceptable.

2. Selection of Routes and Duration of Exposure for Potency Determination

Humans may be exposed to the OPs through diet, in and around residences, schools, commercial building, etc. Therefore, the potency of OPs needs to be determined for the oral, dermal, and inhalation routes of exposure. Cholinesterase inhibition can result for single or short-term exposures. The OP cumulative risk assessment will use a series of one-day exposures integrating multiple sources of OPs.

Various toxicokinetic and toxicodynamic factors influence an individual OP's time to peak effect of inhibition, persistence of action following acute exposure, and the duration of exposure required to reach steady state inhibition. Steady state inhibition is that point at which continued dosing at the same level will result in no further increase in inhibition. The use of cholinesterase data for single-dose or short duration studies to model the comparative potency is problematic because the extent of inhibition is rapidly changing immediately following dosing. Measures of cholinesterase taken during this time will be highly variable and uncertain. Cholinesterase inhibition will continue to increase until steady state is reached. When the measurements are taken at steady state, the differences in toxicokinetics among the OPs are less likely to impact the assessment. At this point in the dosing scheme, it is possible to develop a stable estimate of relative inhibitory capacity (i.e., relative potency) between compounds.

OPP has elected to use data reflecting steady state conditions to estimate relative potencies for the OPs in the interest of producing relative potency factors (RPFs) that are reproducible and reflect less uncertainty due to rapidly changing, time-sensitive measures of cholinesterase. Although the data selected do not directly reflect one of the time frame(s) of interest (approximately one day), they are preferred to short-term estimates for developing comparative potencies among OPs. On average, cholinesterase activity reaches steady state by approximately 30 days. **This analysis focused on studies of a duration of 21 days or greater in order to use cholinesterase data that has attained steady state.** Twenty-one days of exposure was selected instead of 30 days because of the duration of exposure of available guideline toxicity studies; specifically, most dermal toxicity studies are 21 or 28 days in duration.

3. Available Toxicity Database

As stated previously, relative potency should be based whenever possible on data from the same species and sex to provide a uniform measure of relative potency among the cumulative assessment group (USEPA, 2000a). Under FIFRA, toxicology studies in various species (e.g., dog, mouse, rat and rabbit) are submitted to OPP. For the OP's, toxicology studies in the rat provided the most extensive cholinesterase activity data for all routes and in the three compartments in both sexes. **Thus, the focus of this analysis was on cholinesterase activity data derived from male and (non-pregnant) female rats.** EPA used rabbit studies for four chemicals with residential/nonoccupational exposure potential because dermal toxicity data in rats were not available. The cholinesterase data considered in this analysis were extracted from the study types listed in Table 2.

Studies used in this analysis were identified by their source MRID number. Studies previously determined to be "unacceptable" for reasons which were relevant to the determination of potency based on cholinesterase measurements were not used in the hazard assessment. A comprehensive list of all the MRIDs considered in this assessment in addition to specific study information concerning species, strain, and dose levels are given in Appendix 2.

| Table 2. Test Guideline Studies Evaluated for Cholinesterase Activity. | |
|---|----------------------------|
| Study Type | Guideline Type |
| Oral | |
| 90-day oral toxicity study in rat | OPPTS 870.3100 OPP 82-1 |
| Chronic oral toxicity in rat | OPPTS 870.4100 OPP 83-1 |
| Carcinogenicity in rat | OPPTS 870.4200 OPP 83-2 |
| Combined chronic toxicity/carcinogenicity in rat | OPPTS 870.4300 OPP 83-5 |
| Subchronic neurotoxicity study in rat | OPPTS 870.6200 OPP 82-7 |
| Range finding oral toxicity study in rat | Not applicable |
| Other —Special studies | Not applicable |
| Dermal | |
| 21/28-Day dermal toxicity in rat or rabbit | OPPTS 870.3200 OPP 82-2 |
| 90-Day dermal toxicity in rat | OPPTS 870.3200 OPP 82-2 |
| Inhalation | |
| 90-Day inhalation toxicity in rat | OPPTS 870.3465 OPP 82-4 |
| 21/28-Day inhalation toxicity in rat | OPPTS 870.3465 OPP 82-4 |
| Inhalation carcinogenicity in rat | OPPTS 870.3320 OPP 83-5 |

C. Determination of Chemical Potency

1. Pathway of Exposure: Oral Route

A preliminary review determined that numerous oral studies with comparable methodologies were available and suitable for dose-response analysis. Oral relative potency values were needed for all 25 OP pesticides considered in this analysis because of potential dietary exposures from food and drinking water and hand to mouth exposures associated with residential/nonoccupational uses.

a. Collection of Cholinesterase Activity Data from Oral Studies

Study type, route, duration, number of animals per dose group, species/strain/sex and the measured effect for each dose group (mean cholinesterase activity and standard deviation) were compiled into an electronic spreadsheet. In studies where the brain was dissected into anatomical sections, cholinesterase activity data were analyzed separately for each section. To allow for comparable units of measure, cholinesterase activity measurements were converted, when possible, to international units (U/L blood measure; and U/G for brain measures). Time of measurement was expressed as number of days on study where: number of days = number weeks x 7 and number of days = number months x 30. When the standard error was reported, it was converted to standard deviation.

b. Dose-Response Modeling

The key objectives of the dose-response analysis were to identify a common function to fit the cholinesterase activity data for the OPs and to determine if the dose-response curves were consistent with dose-additivity.

i. Selection of Dose-Response Function

In their review of the September, 2000 pilot analysis, the SAP recommended that the Agency reevaluate the selection of the probit model for determining RPFs and specifically suggested Michaelis-Menton kinetics or the exponential model as alternative methods (FIFRA SAP, 2001a). Preliminary simulations using a subset of studies (one study per 24 chemicals for a total of 521

cholinesterase measurement datasets) were performed using both the rectangular hyperbola (i.e., Michaelis-Menton kinetics) and the exponential function (Equation 1). The exponential model was selected over the rectangular hyperbola based on two criteria. First, fewer doses were dropped when using the exponential function (80 doses for rectangular hyperbola vs. 33 doses for exponential function) to achieve adequate model fit ($p \geq 0.05$) of the test subset. Secondly, on average, the Pearson Chi Square goodness-of-fit p-values of the dose-response relationships generated with the exponential function were greater than those for the rectangular hyperbola indicating that the exponential function gave the better fit for the cholinesterase data.

ii. Dose-Response Function and Parameters Estimated

The exponential function used for modeling the effect of the OPs on cholinesterase activity was:

$$y = B + (A - B) \times e^{-m \times \text{dose}} \quad \text{Equation 1}$$

where y is cholinesterase activity, dose is the dose of OP, in mg/kg/day, m is the dose scale factor and is the measure of absolute potency, A is background cholinesterase activity, and B is the y-asymptote. Both y (cholinesterase activity) and dose were extracted from the oral toxicity studies. Equation 1 reflects the observation that cholinesterase activity decreases to a limiting value (B) as dose increases. **The absolute potency is quantified by the dose scale factor, m .** The model has three parameters to be estimated: m (dose scale factor/absolute potency), A (background), and B (y-asymptote).

c. **Determination of Absolute Potency**

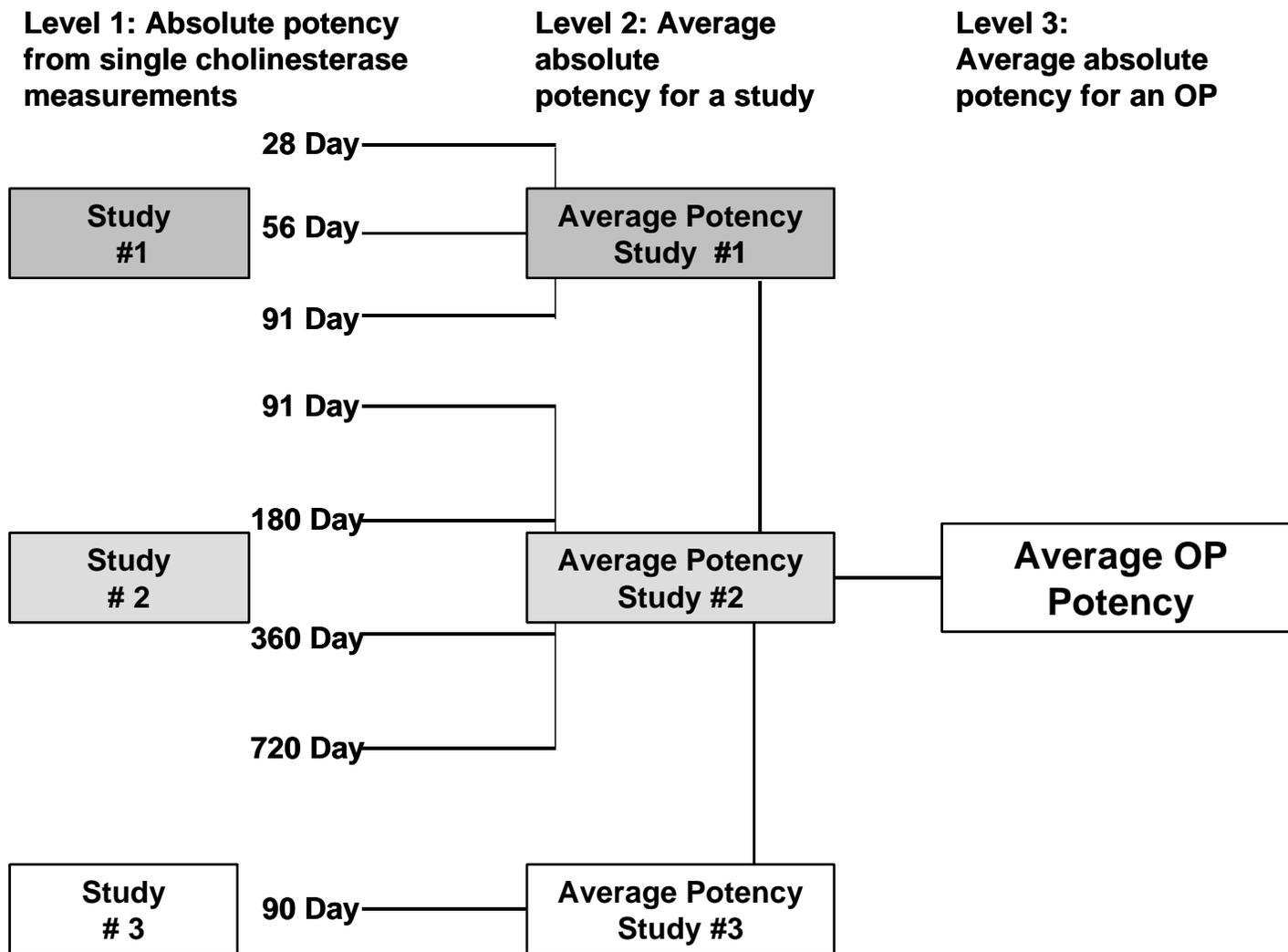
i. **Overview**

Separate potency values were first determined for all cholinesterase measurements taken at 21 days or more of exposure (Figure 1, Level 1).

Assuming the true potency varied among data sets within each study, a *study-specific* average potency was estimated. This reflected the average of potency estimates from all the single timepoint cholinesterase measurements from a particular study (Figure 1, Level 2). The purpose of this step was to ascertain potential study to study variation in pesticide potency.

Next, the *overall* average potency for an OP was estimated assuming that the true potencies for each study varied among themselves (Figure 1, Level 3). This reflected the average absolute potency value for a single chemical. Average absolute potency was determined for each chemical-sex-compartment combination (i.e., six absolute potency values were calculated for each chemical). **The average absolute potency was used in the calculation of RPFs.** Technical details of the statistical methods used are described in Appendix 1.

Figure 1. Schematic Diagram of Average Absolute Potency Calculations.



ii. **Determination of Potency for a Single Cholinesterase Measurement**

The procedure for fitting cholinesterase data sets (Figure 1, Level 1) to the exponential model is given below. This procedure is illustrated in Appendix 4 using a fictitious OP, Organophos.

- (1) For each cholinesterase measurement, data were first fit to the exponential function (Equation 1) using generalized least squares regression. Model parameters for A, B, and m were estimated. Confidence limits and standard errors for these estimates were also calculated.
- (2) If the p-value of the Pearson's Chi-Square statistic was ≥ 0.05 , model fitting was considered adequate. No doses were dropped (Figure 2).

MRID1 360 D - MAIN

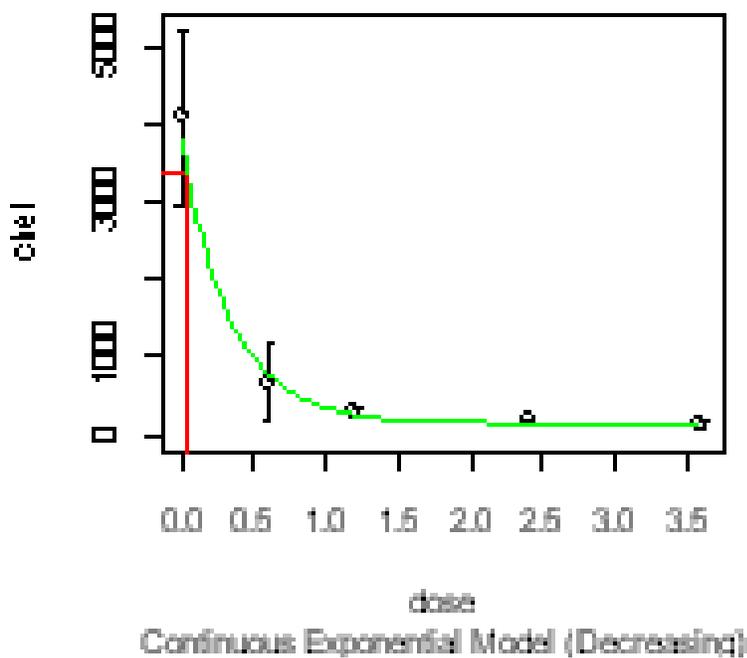


Figure 2. Dose-Response Analysis for a Single Cholinesterase Measurement (360 Day Measurement from RBC Cholinesterase from MRID no. 1) for the fictitious OP, Organophos.

- (3) If the p-value of the Pearson's Chi-Square statistic was < 0.05 or the model did not converge, the y-asymptote (i.e., the B-term in Equation 1) was set to 0. The dose-response model was fit again. At this point, if the p-value ≥ 0.05 , refinements were stopped. The bias of heterogeneous B values on the estimate of m (absolute potency) is discussed in detail in Appendix 1.

Rationale: Often, when activity declined exponentially over all the doses in the dataset, with no apparent leveling off, it was not possible to estimate B. This step in the procedure was designed to accommodate these data sets.

- (4) If the p-value < 0.05 , then high doses were sequentially dropped following by refitting the dose-response function (using $B = 0$) until 1) the p-value ≥ 0.05 or 2) only three dose groups remained (i.e., control and two pesticide treatment groups; Figure 3).

Rationale: The logic of the dose-dropping step(s) was to estimate the potency at the low dose end of the dose-response curve (USEPA, 1999c).

An annotated example of this fitting procedure is given in Appendix 4 for two cholinesterase measurements for a fictitious chemical, Organophos.

MRID2 90 D - MAIN

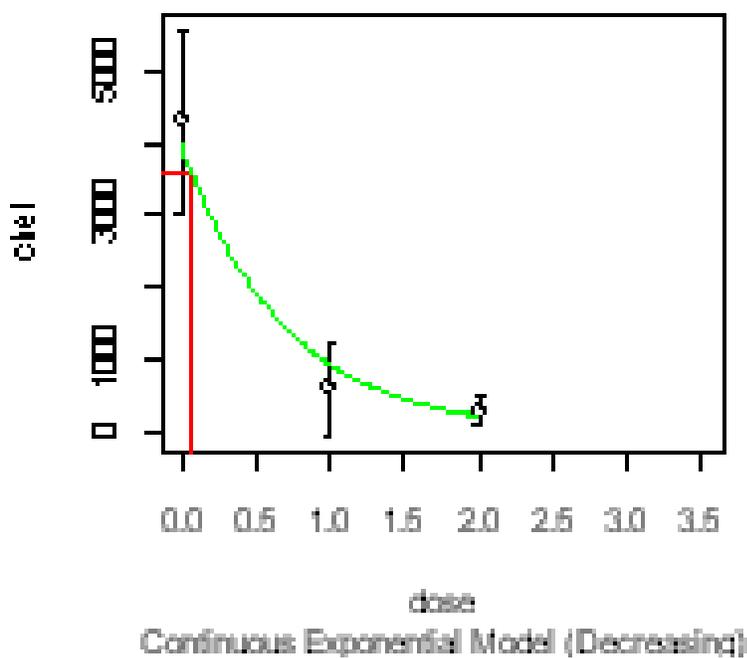


Figure 3. Dose-response Analysis for a Single Cholinesterase Measurement with One Dose Dropped and B =0 (90 Day Measurement from RBC Cholinesterase from MRID no. 2) for the Fictitious OP, Organophos.

iii. Calculation of Average Absolute Potency

The *study specific* average absolute potency (Figure 1, Level 2) and the *overall* average absolute potency (Figure 1, Level 3) for a given chemical-sex-compartment by estimating *mean* using maximum likelihood estimation was computed using the statistical model:

$$\ln(\text{obs. potency}) = \text{mean} + \text{Among} + \text{error} \quad \text{Equation 2.}$$

where: ***ln(obs. potency)*** is natural logarithm the observed potency;
mean is the combined mean potency for the particular stage (i.e., individual study or chemical-sex-compartment);
Among is a normally distributed random variable with mean 0 and unknown variance that reflects the individual (data set, study) deviation from the mean;
error is a normally distributed random variable with mean 0 and unknown variance that reflects the error of estimation of the mean + Among value.

In the first stage (Figure 1, Level 2), a *study-specific* average was calculated using Equation 2. The *Among* component allows the "true" potency to vary among data sets. The potency in each data set was estimated with *error* (estimated with the log potency in each data set). The *mean* value was estimated using maximum likelihood, assuming the *Among* component was normally distributed. Since this model was for *ln(potency)*, potency values were assumed to be log-normally distributed.

In the second stage (Figure 1, Level 3), the *overall* average was calculated using Equation 2. The *ln(observed potency)* values were the study specific means estimated in the first stage. The *errors* were the standard errors also estimated in the first stage. Further details of the methodology are described in Davidian and Giltinan (1995).

iv. Revisions and Refinements to Average Absolute Potency Values

Potential revisions and/or refinements to average absolute potency values could be made during the model fitting procedures. In particular, a choice to retain or remove high dose groups and/or choose to retain or remove single measurement potency values or entire studies was considered.

Following an initial review of the dose-response model fitting of all available data set, a series of test runs were performed to observe the sensitivity of the average absolute potency values. Revisions were made to average absolute potency values based on the following criteria:

1. Dose-response models with Pearson Chi-Square with $p \leq 0.01$ were removed.
2. Data sets where cholinesterase was significantly inhibited at only the high dose level (i.e., activity of low and mid dose was similar to control) were removed.
3. Absolute potency values which appeared to be visual outliers were removed.
4. Potency values with large confidence limits were removed ($\pm 10x$ of the potency value).

The revised average absolute potency values did not differ considerably from the average absolute potency values using all the available data; the majority of refined values were less than $\pm 2X$ the absolute potency using all data. Therefore, in order to prevent the introduction of subjectivity into the assessment and to generate potency estimates that were representative of a particular OP, only minimal revisions were made to the final average absolute potency values. Table 3 lists these revisions.

| Table 3. Data Sets with Refined/Revised Potency Estimates. | | |
|---|------------------------|---|
| Pesticide name | Study ID number | Endpoint(s) |
| Disulfoton | 42977401 | 91 day measurement for brain cholinesterase in female rats. For this measurement, the cholinesterase activity of the mid-dose level could not be read. The high-dose group was removed, only two dose (control and low dose) levels remained. |
| Mevinphos | 43088601 | All available timepoints from this study were removed due to concerns by the reviewers about the health of the animals in this study; all of the female control rats died prior to termination. DER was not finalized as of July 2, 2001. |
| Terbufos | 44842302 | The high dose group was removed in the 28, 56, and 91 day measurements for RBC cholinesterase in female rats due to large difference between predicted and observed for control (Appendix 2). |
| Tribufos | 42335101 | The high dose group was removed in the 343 day measurement for plasma cholinesterase in female rats. The high-dose group was removed, only two dose (control and low dose) levels remained. |

d. Identification of Steady State

Cholinesterase measurements taken prior to 21 days on study were not included in this analysis. This decision was based on the time generally required for OPs to reach steady state. The available data sets for each chemical-sex-compartment included a range of exposure durations from 21 days to > 700 days. Steady state for each OP was determined qualitatively, both visually with graphs of absolute potency versus time and with a simple regression of absolute potency against exposure duration. In the regression analysis, absolute potency was weighted by the reciprocal of the squared estimated standard errors. The data for

the shortest remaining exposure duration in the data set was repeatedly removed until a data set was derived in which the slope of potency versus time was not significant (i.e., the p-value ≥ 0.05). This process was stopped when only three distinct durations remained. The results of this regression are given in Appendix 2 for each chemical-compartment-sex combination. Summary graphs of potency versus duration of exposure contain a solid vertical line; this vertical line represents the duration required for the slope of the regression to exceed a p-value of 0.05 (See Appendix 2).

This method was not meant to be a definitive determination of steady state but rather a qualitative method to observe the change in potency with time.

e. OPCumRisk

The dose-response analysis was performed using a computer program, called OPCumRisk. This program was used to calculate and revise average absolute potency values, to calculate relative potency factors (RPFs), and to estimate points of departure (PoD) for the index chemical. OPCumRisk was developed at ORD's NHEERL specifically for use in the OP dose-response assessment.

OPCumRisk is written in R (Ihaka and Gentleman, 1996), a freely distributable implementation of the S programming language available for download on the internet at <http://www.R-project.org>. The output from OPCumRisk is in html format and can therefore be viewed in widely used internet browsers such as Netscape (4.7 or better) or Microsoft Explorer (5.0 or better) without the need for installation of OPCumRisk. OPCumRisk is described in detail in Appendix 1.

2. Pathway of Exposure: Dermal Route

Due to the limited number of dermal studies with quality dose-response data, it was determined that the database of dermal toxicology studies was not amenable to dose-response modeling. Chemical potency was determined using no-observed-adverse-effect-levels (NOAELs) for this route of exposure.

a. Collection of Cholinesterase Activity Data From Dermal Studies for OPs with Residential/nonoccupational Exposure Potential

Cholinesterase activity data were collected from dermal toxicity studies for eight chemicals with residential/nonoccupational exposure. One chemical, dichlorvos, had no dermal exposure study. The requirement for a dermal toxicity study with dichlorvos was waived because the volatility of the chemical renders it technically difficult to conduct such a study. It has not yet been determined whether or how residential/nonoccupational dermal exposure potential will be assessed for dichlorvos in the preliminary cumulative risk assessment of OPs.

As stated above, comparisons of chemicals tested by the same route of exposure and in the same sex and species were preferred. However, only four chemicals were tested by the dermal route in rats. Only rabbit studies were available for the other four other OP's. Thus, it was not possible to compare cholinesterase activity data from dermal studies in only one species. Of the chemicals with potential dermal exposure, only three chemicals (acephate, disulfoton, and naled) had more than one dermal toxicology study which could be used for assessing relative potency. Five chemicals had one suitable dermal exposure study.

b. Establish NOAELs for Dermal Studies

Relative potencies of the chemicals with residential/non-occupational uses were determined by comparing NOAELs observed in the dermal toxicity studies. As described in detail later, for the organophosphorus pesticides it was determined that the RPFs derived from data on inhibition of cholinesterase activity in male rat RBC should be used to estimate potential cumulative risk. Therefore, the NOAELs for cholinesterase measured in RBC were used. Both male and female NOAELs were established for purposes of comparison. The NOAEL was defined as the lowest dose where a maximum 10-15% RBC cholinesterase inhibition (compared to control) occurred.

3. Pathway of Exposure: Inhalation Route

Similar to the dermal toxicity database, the number of available inhalation toxicity studies with quality dose-response data was limited. Chemical potency was, therefore, determined using NOAELs for RBC cholinesterase activity for the inhalation route of exposure.

a. Collection of Cholinesterase Activity Data from Inhalation Studies for OPs with Residential/nonoccupational Exposure Potential

There are nine chemicals with residential/nonoccupational exposure in which cholinesterase activity measured from inhalation studies were needed.

Inhalation studies were available for seven of the nine. Two inhalation exposure studies were available for acephate whereas only one suitable study was available for the other six OPs. No inhalation toxicity study was available for two chemicals, bensulide and tetrachlorvinphos. It has not yet been determined whether or how residential/nonoccupational inhalation exposure potential will be assessed for bensulide and tetrachlorvinphos in the Preliminary Cumulative Risk Assessment of OPs.

Although all the inhalation studies were performed with the same species (rat), four different strains of rats were used. Furthermore, the exposure conditions varied among the chemicals tested. There were four whole-body exposure studies, and three nose only exposure studies.

Inhalation studies selected for analyses of cholinesterase activity were of subchronic duration (21 to 90 days), with the exception of dichlorvos, which had a chronic inhalation study.

b. Establish NOAELs for Inhalation Studies

As described for the NOAELs for the dermal studies, relative potency was calculated from NOAELs for RBC cholinesterase activity determined from inhalation toxicity studies. The NOAEL was defined as the lowest dose where a maximum 10-15% RBC cholinesterase inhibition (compared to control) occurred.

4. Selection of the Index Chemical and Calculation of Points of Departure (PoDs)

a. Criteria for Selection

The cumulative risk assessment guidance document (USEPA, 2000a) states that the index chemical should be selected based on the availability of high quality dose-response data for the common mechanism endpoint and that it acts toxicologically similar to other members of the common mechanism group. For the cholinesterase inhibiting OP pesticides, the ideal index chemical should exhibit high quality dose-response data in plasma, RBC, and brain for both sexes of a single species for all exposure routes of interest. Such high quality data allows calculation of points of departure (PoD) for oral, dermal, and inhalation exposures with confidence. A PoD is a point estimate on the index chemical's dose-response curve that is used to extrapolate risk to the exposure levels anticipated in the human population. Thus, any error or uncertainty in an index chemical's PoD value will be carried forward in the cumulative risk estimates. Although not required by the RPF method, EPA preferred to use one index chemical for all of the exposure routes of interest in order to express cumulative risk for all pathways using the same index chemical exposure equivalents.

b. Studies Reviewed

In order to do a comprehensive search for the index chemical, initially all 25 chemicals were considered candidates. All available oral, dermal, and inhalation study types listed in Table 2 were reviewed.

c. Points of Departure (PoD)

The oral, dermal, and inhalation PoDs for the index chemical were based on benchmark dose modeling of its RBC cholinesterase data. The benchmark dose where cholinesterase activity is reduced 10% compared to background activity (BMD_{10}) was selected as the effect level for the PoD as a response level close to the background cholinesterase. This level was generally at or near the limit of sensitivity for discerning a statistically significant decrease in cholinesterase activity across the blood and brain compartments in toxicity studies. The lower 95% confidence

interval on these BMD₁₀ (BMDL) was also calculated. The average BMD₁₀ for each route of exposure was calculated by combining estimates of BMD₁₀ across all the dose-response data sets available for the selected sex and compartment for the index chemical using the same statistical method that was used for combining absolute potency (Section IV.C.1.b.4). The combined BMD₁₀ estimate was computed by assuming a nested hierarchical statistical model for the individual estimates: estimates from individual data sets were nested within study, which were nested within compartment x sex. The lower confidence limit was based on the estimated standard error of the combined estimate. This approach combined all the information available for estimating the BMD₁₀, and incorporated uncertainty in the confidence limit (resulting from experiment to experiment and study-to-study variability). The technical details of the computation are discussed in Appendix 1.

5. Calculation of Relative Potency Factors (RPFs)

a. Oral Route

Oral RPFs were calculated from average absolute potency by the following equation:

$$\text{Oral RPF}_{\text{Chemical X}} = m_{\text{Chemical X}} / m_{\text{Index Chemical}}$$

Equation 3

where $m_{\text{Index Chemical}}$ is the overall absolute potency of the index chemical
and $m_{\text{Chemical X}}$ is the overall absolute potency of Chemical X.

The standard error for the $\ln(\text{relative potency}_{\text{Chemical X}})$ and confidence intervals for RPFs were also calculated (see Appendix 1).

b. Dermal Route

NOAELs for RBC cholinesterase activity measured in dermal studies were determined in order to calculate RPFs. Dermal RPFs were calculated using Equation 4.

$$\text{Dermal RPF}_{\text{Chemical X}} = \text{NOAEL}_{\text{Chemical Index}} / \text{NOAEL}_{\text{Chemical X}}$$

Equation 4

c. Inhalation Route

NOAELs for RBC cholinesterase activity measured in inhalation studies were determined in order to calculate RPFs. Inhalation RPFs were calculated using Equation 5.

$$\text{Inhalation RPF}_{\text{Chemical X}} = \text{NOAEL}_{\text{Chemical Index}} / \text{NOAEL}_{\text{Chemical X}}$$

Equation 5

V. RESULTS

A. Dose-Response Modeling: Oral Route of Exposure

1. Overview

A total of 1496 data sets from 69 studies were collected. The data sets represent cholinesterase activity measured in plasma (516 data sets), RBC (524 data sets), and brain (213 data sets from whole brain and 243 from separate anatomical sections should total 456) from male and female rats orally exposed to each of the 25 OPs from 21 to > 700 days. On average there were 59 ± 33 data sets per chemical ranging from 24 for tetrachlorvinphos and naled to 106 for acephate. At least one data set was available for the 25 chemicals in each compartment in both sexes.

Appendix 2 and 3 present the specific results of dose-response modeling for each chemical-sex-compartment combination. Appendix 2 includes exponential model parameters, goodness-of-fit information, potency values, dose-response graphs, and summary plots of potency versus duration of exposure. Only whole brain results are reported in Appendix 2 because the actual anatomical sections assayed for cholinesterase varied from study to study; absolute potency values estimated from cholinesterase measured in specific brain anatomical sections are not given. Appendix 3 gives the results of distributional analyses of dose-response parameters for each chemical-sex-compartment combination in addition to summary and comparative plots of potency estimates from the different compartments and sexes.

The following section summarizes the results of the dose-response modeling. Specifically, the adequacy of the exponential function to fit the data sets, the potency determinations, and the qualitative steady state observations are described.

2. Exponential Model Fit

a. Adequacy of Model Fit

Of the 1496 total data sets collected, 1312 were analyzed with OPCumRisk (See Table 4). The goodness-of-fit was adequate according to the Pearson Chi Square statistic ($p \geq 0.05$) for the majority of the data sets (84%) used in this analysis. Each of the 25 OP's had at least one data set that exhibited inadequate fit. Most of the failures of fit were due to individual dose groups either slightly above or slightly below the fitted curve (but large enough relative to the reported standard errors of the means that the goodness of fit statistics were affected). These observations indicated that there may be an additional source of variance acting among dose groups within a study. These observations of inadequate fit may reflect the likelihood that although the exponential model was sufficient for determination of potency, it may not *exactly* be the correct model for these data.

| Table 4. Datasets Fit to Exponential Function. | |
|---|---------------------------|
| OPCumRisk Decision Logic | Number of datasets |
| Total number of datasets | 1496 |
| Data sets with fewer than 3 dose groups to begin | 156 |
| Data sets in MRID 43088601 from mevinphos data, all dropped | 28 |
| Data sets available for modeling | 1312 |
| Number that did not converge to exponential function | 6 (0.5%) |
| No dose groups dropped, total | 1044 (80%) |
| No dose groups dropped, B > 0 | 535 (41%) |
| No dose groups dropped, B = 0 | 509 (39%) |
| One dose group dropped, B = 0 | 190 (14%) |
| Two dose groups dropped, B = 0 | 70 (5%) |
| Three dose groups dropped, B = 0 | 2 (0.2%) |

It is important to note that the potency estimates in most of the data sets with inadequate goodness-of-fit were comparable to potency estimates for data sets with adequate fit. As part of the sensitivity analysis, all data sets with goodness-of-fit with $p \leq 0.01$ were removed; average absolute potencies did not change significantly. A notable exception was plasma cholinesterase measured in female rats exposed to diazinon in a two-year study (MRID 41942002). In this study, model goodness-of-fit was not adequate (p -values < 0.05) for all five timepoints. The average study potency for this study was 8.64 whereas the study potency for the other oral studies for plasma cholinesterase measured in female rats exposed to diazinon ranged from 0.74 to 3.82.

The algorithm used to estimate parameters did not converge to a solution for six data sets (Table 5). In other words, out of 1312 data sets available for modeling, there were only six data sets in which parameter estimates including absolute potency could not be calculated. Cholinesterase activity in these data sets actually increased with dose; the exponential function was unable to fit these data. Lack of convergence to the exponential function by these six data sets does not impact the average absolute potency values for these chemical-sex-compartment combinations. Several data sets with adequate goodness-of-fit remained for calculation of average absolute potency for each of the chemical-sex-compartment combinations.

| Table 5. Model Fitting: Data Sets That Did Not Converge to Exponential Function* | | |
|--|-----------------|--|
| Name of OP | Study ID number | Endpoints |
| Chlorpyrifos | 42172802 | 546 day measurement for RBC cholinesterase in male rats |
| Malathion | 43269501 | 91 day measurement for brain cholinesterase (hippocampus) in male rats |
| Methyl-parathion | 43490501 | 98 day measurement for brain cholinesterase (brainstem) in male rats |
| Pirimiphos-methyl | 129343 | 21 and 28 day measurements for RBC cholinesterase in male rats |
| Terbufos | 44842302 | 28 day measurement for RBC cholinesterase in female rats. |

*Eq. 1 $y = B + (A - B) \times e^{-m \times \text{dose}}$

b. Dropping of High Doses

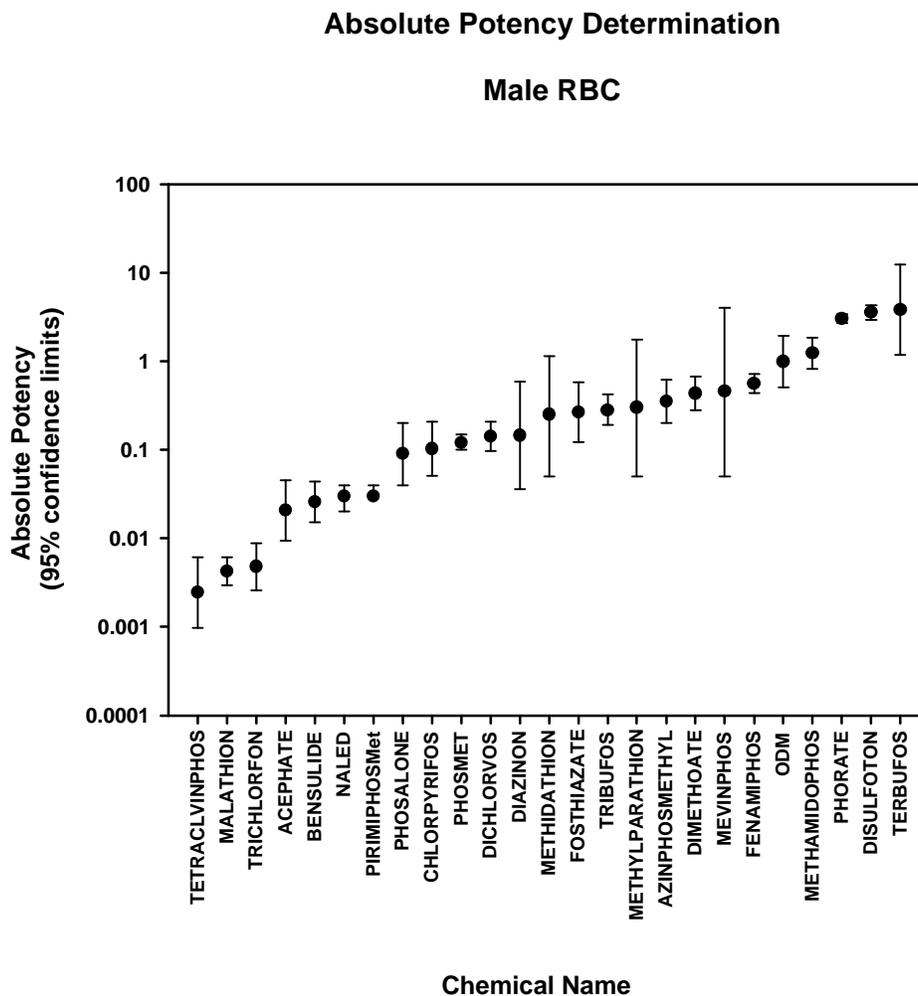
As described above, an iterative process was used to fit each single cholinesterase data set to the exponential function. The first step included the estimation of B, A, and m (Equation 1) using all available data. If adequate goodness-of-fit (p-value ≥ 0.05) was achieved on the first iteration, then the process stopped. If adequate fit was not achieved, B was set to zero. The next step(s) of the curve fitting procedure included the sequential dropping of high doses. The process continued until 1) the p-value was ≥ 0.05 (i.e., adequate goodness-of-fit) or 2) only three dose groups remained (i.e., control and two pesticide treatment groups).

The absolute potency of 80% of available single cholinesterase data sets was calculated with all available dose levels. Absolute potency for 14% of the data sets was estimated with one high dose removed while two or more high doses were removed from < 6% of data sets (Table 4). One dose was dropped from at least one data set for all the OPs, except bensulide. No doses were dropped in any of the absolute potency calculations for bensulide. Diazinon (39/72) and fosthiazate (24/70) comprise 24% of the 262 data sets where at least one dose was dropped (Appendix 2).

3. Average Absolute Potency Values

Six average absolute potency estimates were calculated for each OP. Plots of average absolute with confidence intervals are given in Appendix 3 for the six sex-compartment combinations. Figure 4 gives an example of these graphs.

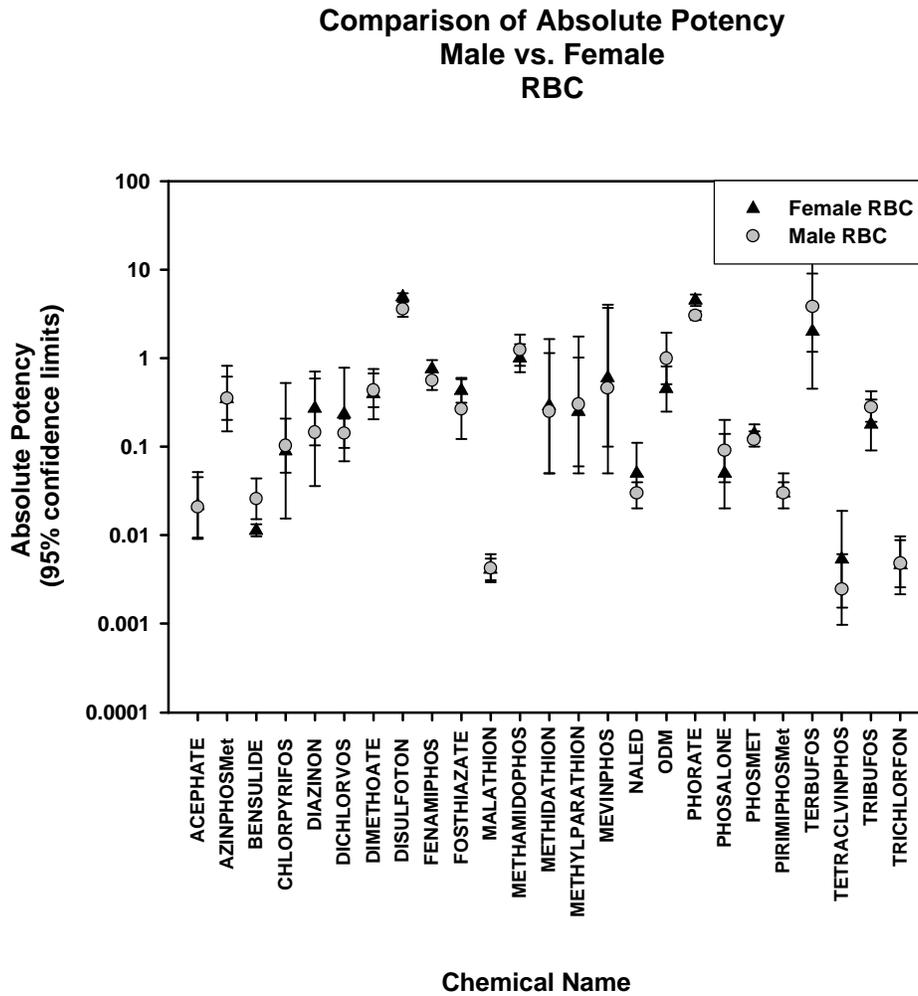
Figure 4. Average Absolute Potency of OPs for RBC Cholinesterase Measured in Male Rats (Error Bars Are 95% Confidence Limits).



a. Comparison of Sexes

Overall, there was good concordance (Figure 5) between average absolute potency values calculated from cholinesterase activity measured in brain, RBC, and plasma between male and female rats for each chemical (Appendix 3). A notable exception was the average absolute potency value for tetrachlorvinphos measured in brain; the male average absolute potency value was >15X larger than the female value.

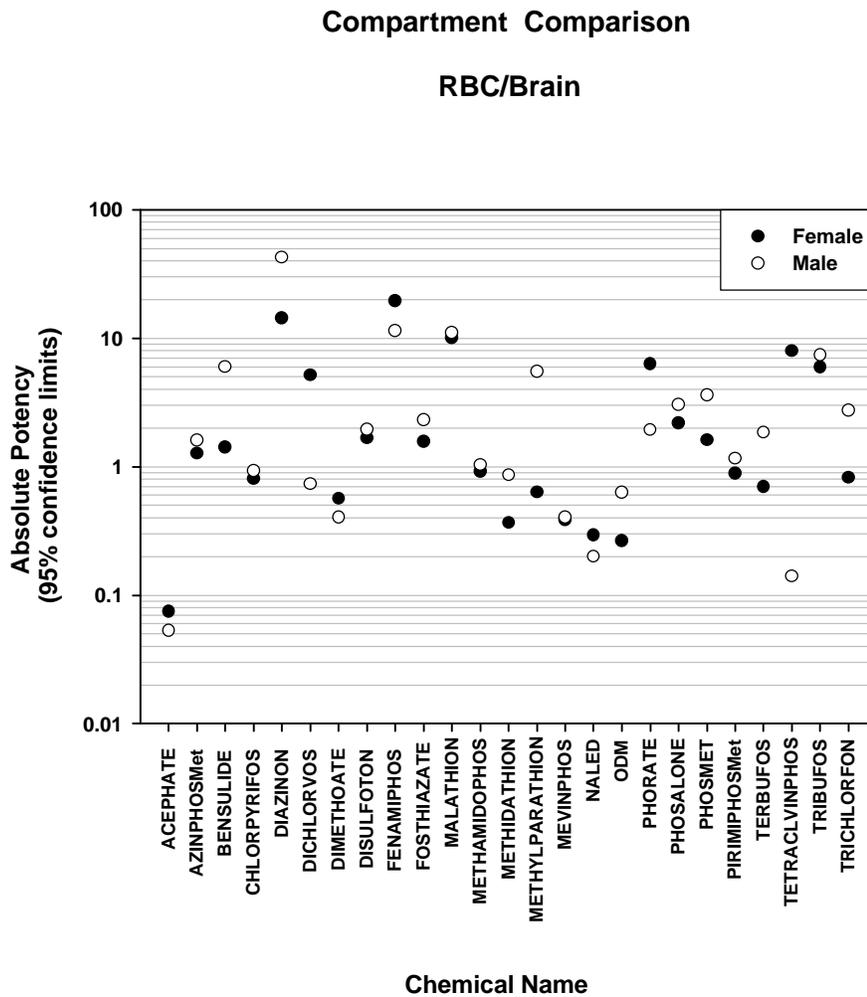
Figure 5. Comparison of Average Absolute Potency of OPs for RBC Cholinesterase Measured in Male and Female Rats (Error Bars Are 95% Confidence Limits).



b. Comparison of Compartments

For most OP's, average absolute potencies were similar between the plasma, RBC, and whole brain measurements (Figure 6 and Appendix 3). Average absolute potencies of diazinon and fenamiphos were 20-100X larger in the RBC and plasma cholinesterase measurements than the whole brain indicating that the plasma and RBC measures were more sensitive to diazinon and fenamiphos exposure than cholinesterase activity measured in whole brain.

Figure 6. Ratio of Average Absolute Potency of OPs for RBC/Brain Cholinesterase Measured in Male and Female Rats.



c. Effects of Various Rat Strains on Average Absolute Potency Values

At least one oral feeding study was available for all of the OPs in this assessment. Among the 69 oral rat studies, three different strains were used: Sprague-Dawley, Wistar, and Fischer. Although the same strain was not used for all of the chemicals, average absolute potency values were based on a single rat strain for 16 pesticides. For example, all three methamidophos studies used in this analysis were tested in the Fischer rat, whereas absolute potency of acephate was determined with three studies tested with the Sprague Dawley rat. Average absolute potencies for azinphos-methyl, chlorpyrifos, dichlorvos, dimethoate, fenamiphos, malathion, ODM, pirimiphos methyl, and tetrachlorvinphos were calculated from studies in at least two different strains. Among these 9 pesticides, no significant differences in absolute potency attributable to strain differences were noted.

d. Graphical Observations: Visual Outliers

A plot of the average absolute potency values versus duration of exposure was generated for each chemical (Appendix 2). As part of the process for refining average absolute potency values, visual outliers were removed. These were defined as average potency outside of the 95% confidence limits. The removal of visual outliers did not significantly change the average potency values. Average absolute potency values were: 1) based on several single values for most chemical-sex-combinations and 2) were calculated by weighting their variance (outliers tended to have large variances).

e. Potency Values with Large Confidence Limits

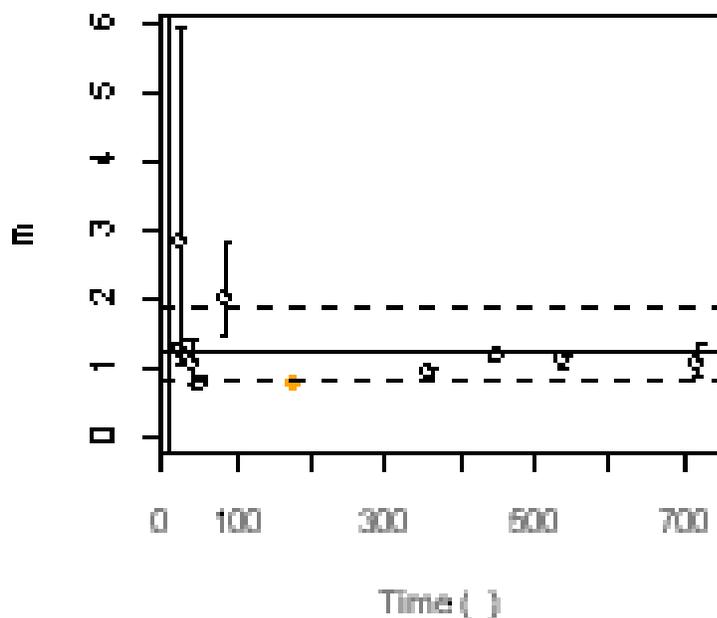
As shown in the plots of potency versus duration of exposure (Appendix 2), single measurement potency estimates with large confidence limits were observed. During the potency refinement process, these single potency values with large confidence limits were removed; their removal did not impact the average absolute potency values. The average absolute potency was calculated by weighting the variance. As variance increased, 95% confidence limits also increased, and, therefore, the influence of those values on the average absolute potency decreased.

4. Time Course Analysis

As stated previously, another key aspect of this analysis was to base relative potency determinations on cholinesterase measurements that had attained steady state.

Qualitative observation of steady state was made by visual evaluation of the dose-response graphs and by using a simple regression of potency at single timepoints versus duration of exposure. This regression procedure was not meant to be a definitive tool but rather as a helpful guide. Plots of potency at single timepoints versus duration of exposure are given for each pesticide in Appendix 2. An example of these plots is given below for RBC cholinesterase measured in male rats following methamidophos exposure (Figure 7).

Figure 7. Plot of Absolute Potency Estimates Versus Duration of Exposure for RBC Cholinesterase Measured in Male Rats Exposed to Methamidophos.



Overall, potency values were generally consistent across time for the majority of chemical-sex-compartment combinations for all of the 25 OPs. Most chemicals appeared to reach steady state by 21 or 28 days of exposure in both sexes and all three compartments. **The available data indicate that it is reasonable to assume that the relative potency determinations were made using steady state responses.**

B. NOAELs Determined for Dermal Endpoints for OPs with Residential/nonoccupational Exposure

Table 6 lists NOAELs and lowest-observable-adverse-effect-levels (LOAELs) determined for dermal toxicity studies of eight pesticides with residential/occupational exposure. The respective level of RBC cholinesterase inhibition (compared to control values) is also given. NOAELs were selected from male and female laboratory animals of two species (rat and rabbit).

| Table 6. NOAELs and LOAELs for RBC Cholinesterase Activity from Dermal Toxicity Studies (% Cholinesterase Inhibition Compared to Control Value). | | | | | |
|---|---|-------------------------------|-------------------------------|---------------------------------|---------------------------------|
| Chemical | Species | Male NOAEL (mg/kg/day) | Male LOAEL (mg/kg/day) | Female NOAEL (mg/kg/day) | Female LOAEL (mg/kg/day) |
| Acephate | rat | 300 * 9% | >300 * 9% | 300* 13% | >300 * 13% |
| Bensulide | rat | 500 * 4% | >500 * 4% | 500 * 2% | >500 * 2% |
| Dichlorvos | Dermal exposure study waived due to volatility of compound. | | | | |
| Disulfoton | rabbit | 0.8 4% | 1.0 18% | 0.8 16% | 1.0 21% |
| Fenamiphos | rabbit | 2.5 0 | 10 32% | 2.5 0 | 10 35% |
| Malathion | rabbit | 50 0% | 300 17% | 50 8% | 300 26% |
| Naled | rat | 10 0% | 20 21% | 10 7% | 20 25% |
| Tetrachlorvinphos | rat | 1000* 0% | >1000* 0% | 1000 * 0% | >1000* 0% |
| Trichlorfon | rabbit | 100 6% | 300 25% | 100 0% | 300 19% |

* Highest dose tested.

C. NOAELs Determined for Inhalation Exposure for OPs with Residential/nonoccupational Exposure

Table 7 lists NOAELs and LOAELs determined for inhalation toxicity studies for seven chemicals with residential/nonoccupational exposure. In addition, the level of RBC cholinesterase inhibition (compared to control values) is given.

| Table 7. NOAELs and LOAELs for RBC Cholinesterase Activity from Inhalation Toxicity Studies (% Cholinesterase Inhibition Compared to Control Value). | | | | | |
|---|---|--------------------------|--------------------------|----------------------------|----------------------------|
| Chemical | Method | Male NOAEL (mg/L) | Male LOAEL (mg/L) | Female NOAEL (mg/L) | Female LOAEL (mg/L) |
| Acephate | nose only | 0.0056* 13% | >0.0056* 13% | 0.0056* 9% | >0.0056* 9% |
| Bensulide | No inhalation toxicity study was available for bensulide | | | | |
| Dichlorvos | whole body | 0.00005 0% | 0.0005 28% | 0.00005 12% | 0.0005 31% |
| Disulfoton | nose only | 0.00016 0-2% | 0.0014 22-28% | 0.00016 5-11% | 0.0014 26-34% |
| Fenamiphos | nose only | 0.0035* 8% | >0.0035* 8% | 0.00025 5% | >0.0035 17% |
| Malathion | whole body | 0.1 9% | 0.45 22% | 0.1 11% | 0.45 27% |
| Naled | whole body | 0.0013 0-11% | 0.0058 70-81% | 0.00023 2% | 0.0013 17-25% |
| Tetrachlorvinphos | No inhalation toxicity study was available for tetrachlorvinphos. | | | | |
| Trichlorfon | whole body | 0.035 0% | 0.104 24% | 0.013 0% | 0.035 20% |

*Highest dose tested.

D. Selection of Index Chemical

1. Candidates for the Index Chemical

The qualities of an ideal index chemical were previously discussed. These qualities included: 1) high quality dose-response brain, plasma, and RBC cholinesterase data measured in male and female laboratory animals of a single species 2) availability of data for all of the exposure routes of interest so that only one chemical could be used as the index.

Dermal toxicity studies were available for 20/25 OPs. Rat studies were available for 9 OPs. Rabbit studies were available for 11 chemicals. Inhalation studies were available for only 13/25 OPs. Acephate, methamidophos, naled, and terbufos were the only pesticides with both dermal and inhalation studies performed in the rat. Of these four chemicals, the dermal terbufos studies (MRID nos. 44690501 and 44520501) were performed with a formulation instead of the preferred technical material and could not be used.

One of the largest oral databases was available for acephate (106 data sets). Although there was good concordance between male and female absolute potencies, average values calculated from brain cholinesterase measures were approximately ten-fold larger (i.e., more potent) than either blood measure (Table 8).

Naled had one of the smallest oral databases (22 data sets). Although average absolute potency values for naled were similar between male and female rats the average absolute potency values for RBC were three to five-fold smaller (i.e., less potent) compared to plasma and brain (Table 8).

A moderate size oral database (56 data sets) was available for methamidophos. As shown in Table 8, unlike acephate and naled, average absolute potency values for methamidophos were *comparable for all three cholinesterase measures in both sexes*. The 95% confidence limits on these average values were also relatively small, thus increasing confidence in the methamidophos potencies. As described in detail below, the available oral, dermal, and inhalation studies had dose-response data conducive for benchmark dose calculation.

Therefore, based on the availability of high-quality RBC, plasma, and brain cholinesterase data in male and female rats in oral, dermal, and inhalation toxicity studies, *methamidophos* was selected as the index chemical for the Preliminary OP Cumulative Risk Assessment.

| Table 8. Average Absolute Potency for Acephate, Methamidophos, and Naled Oral Studies. | | | |
|--|---------------------------|-------------------------|----------------------|
| | RBC | Plasma | Brain |
| Acephate | | | |
| Male | 0.021 (0.0094- 0.046) | 0.045 (0.0092- 0.22) | 0.39 (0.27- 0.56) |
| Female | 0.022 (0.0091 - 0.052) | 0.14 (0.092- 0.23) | 0.29 (0.26- 0.32) |
| Methamidophos | | | |
| Male | 1.23 (0.82-1.83) | 0.87 (0.69-1.10) | 1.19 (0.95 -1.5) |
| Female | 1.00 (0.701-1.44) | 1.24 (0.94-1.64) | 1.09 (0.90-1.33) |
| Naled | | | |
| Male | 0.033 (0.024-0.045) | 0.15 (0.026-0.81) | 0.15 (0.11-0.21) |
| Female | 0.055 (0.026-0.11) | 0.16 (0.087-0.28) | 0.17 (0.11-0.26) |

2. Description of Methamidophos Database

a. Oral Studies

Of the four methamidophos oral studies available (MRID nos. 148452, 41867201, 14155 and 43197901), three were analyzed. The study report for MRID no. 14155 lacked standard deviation calculations which were not calculated by reviewers and consequently, this study was not used. MRID nos. 148452, 41867201, and 43197901 each had different length and purpose (two year combined chronic-carcinogenicity study, special cholinesterase study, and subchronic neurotoxicity study). Whole brain cholinesterase activity was measured three, two, and one time respectively whereas plasma and RBC cholinesterase activity were measured five, three, and two times, respectively. All three studies utilized the Fischer rat. As shown in Table 8, within compartment-sex endpoint, individual potency estimates did not vary significantly across time or study. Steady state was apparently achieved in all three compartments and both sexes.

The combined results of the three studies characterize the whole dose-response range from low doses with little cholinesterase inhibition to high doses with considerable cholinesterase inhibition. The dosing regime of the special cholinesterase study characterized the low dose range. This special study included only low doses (i.e., the *highest* dose tested in the special study was 0.24-0.28 mg/kg/day whereas the *lowest* dose tested in the combined chronic-carcinogenicity study was 0.2 mg/kg/day). Even at extremely low doses, a dose-dependent reduction in cholinesterase activity was observed at all tested doses in RBC, plasma, and brain cholinesterase of female rats and in RBC and brain cholinesterase activity of male rats (Appendix 2). Both the combined chronic-carcinogenicity and subchronic neurotoxicity studies characterize the dose-response curve at the mid and high dose range; both included at least one high dose where cholinesterase activity was < 20% of background in both sexes and in the blood and brain measures (Appendix 2).

b. Dermal Studies

Two dermal toxicity studies were available for methamidophos, one in rat and one in rabbit. The NOAEL value used for RPF determination was selected from the rat dermal exposure study of 21 days duration (Table 9, MRID no. 44525301). This chemical exhibited a good dose-response relationship for assessing cholinesterase activity with RBC, brain, and plasma (Appendix 2; Pearson Chi-Square p-value = 0.99). Similar NOAEL values were observed for RBC, brain and plasma cholinesterase activity in both sexes in this study. Blood and brain cholinesterase activity was determined only once, at the end of the study.

In the rabbit study (MRID no. 00147935), six rabbits/sex/dose were tested. Each dose group was split into two sets: three rabbits with abraded skin and three rabbits with unabraded skin. It is notable that the NOAEL of 0.5 mg/kg/day for cholinesterase activity in plasma, RBC, and brain in the unabraded rabbit group was similar to the NOAEL of 0.75 mg/kg/day identified in the rat study.

| Table 9. NOAELs and LOAELS for RBC Cholinesterase Activity from Rat Dermal Toxicity Study with Index Chemical (% Cholinesterase Inhibition Compared to Control Value). | | | | | |
|---|----------------|-------------------------------|-------------------------------|---------------------------------|---------------------------------|
| Chemical | Species | Male NOAEL (mg/kg/day) | Male LOAEL (mg/kg/day) | Female NOAEL (mg/kg/day) | Female LOAEL (mg/kg/day) |
| Methamidophos | rat | 0.75 6% | 11.2 55% | 0.75 1% | 11.2 46% |

c. Inhalation Study

A 90-day, head/nose, inhalation toxicology study (MRID no. 41402401) was available for methamidophos (Table 10). Five measurements for plasma and RBC cholinesterase activity were taken in this study. Brain cholinesterase activity was determined at study termination. There was a good dose-response relationship with respect to the cholinesterase activity observed in both blood compartments (plasma, RBC) monitored throughout the study and in the brain at study termination (Appendix 2; Pearson Chi-Square p-value > 0.05 for 4 of 5 timepoints). The NOAEL for cholinesterase inhibition was the similar for all three compartments in both sexes.

| Table 10. NOAELs and LOAELs for RBC Cholinesterase Activity from Inhalation Toxicity Study with Index Chemical (% Cholinesterase Inhibition Compared to Control Value). | | | | | |
|--|---------------|--------------------------|--------------------------|----------------------------|----------------------------|
| Chemical | Method | Male NOAEL (mg/L) | Male LOAEL (mg/L) | Female NOAEL (mg/L) | Female LOAEL (mg/L) |
| Methamidophos | head/nose | 0.001 0-8% | 0.005 2-25% | 0.001 0-11% | 0.005 8-28% |

3. Points of Departure for the Index Chemical (Methamidophos)

Table 11 lists the PoDs for the oral, dermal, and inhalation routes calculated for methamidophos. EPA plans to use the endpoints in the Preliminary OP Cumulative Risk Assessment.

RBC cholinesterase was only measured once in the methamidophos rat dermal toxicity study. Therefore, only one absolute potency value was available for calculation of the dermal PoD (dPoD). The dermal BMD₁₀ calculated from this measurement is expected to serve as the dPoD.

Because multiple measures of RBC cholinesterase activity were available for both oral and inhalation toxicity studies, average BMD₁₀s were calculated for these two routes. The BMD₁₀s and BMDL for individual timepoints of RBC cholinesterase measured in male rats exposed to methamidophos are listed in Appendix 2.

The BMD₁₀s given in Table 11 are for male rats only. The analogous values for RBC cholinesterase activity in female (0.12 mg/kg/day, 1.71 mg/kg/day, and 0.01 mg/L for oral, dermal, and inhalation, respectively) were similar to the male values. The male and female plasma and brain BMD₁₀s were also similar to the male RBC BMD₁₀ for the oral route (0.15 and 0.10 mg/kg/day for male plasma and brain, respectively; 0.09 and 0.11 mg/kg/day for female plasma and brain, respectively). As noted in Table 8, the confidence intervals for the average absolute potency values were fairly tight. Similarly, Table 11 shows that the values of the BMD₁₀s and BMDL were roughly in the same order of magnitude. These observations increase the confidence not only in the selection of methamidophos as the index chemical but also the utilization of the central estimate of the male data (BMD₁₀) for cumulative risk extrapolation rather than its lower limit (BMDL).

BMD₁₀ and BMDL values were similar to but slightly larger than NOAELs established for the oral (chronic NOAEL used for RfD derivation), dermal, and inhalation routes.

Table 11. Points of Departure for Index Chemical (Methamidophos) by Route of Exposure for RBC Cholinesterase Activity Measured in Male Rats.

| Route of Administration | BMD ₁₀ | BMDL | NOAELs |
|-------------------------|-------------------|----------------|-----------------|
| Oral ^a | 0.09 mg/kg/day | 0.07 mg/kg/day | 0.03 mg/kg/day* |
| Dermal ^b | 1.21 mg/kg/day | 0.91 mg/kg/day | 0.75 mg/kg/day |
| Inhalation ^c | 0.0046 mg/L | 0.0031 mg/L | 0.001 mg/L |

^a MRID nos. 41867201, 43197901, 00148452

^b MRID no. 44525301

^c MRID no. 41402401

*NOAEL used for chronic RfD derivation in the single chemical assessment.

E. Relative Potency Factors (RPFs)

Table 12 provides the RPFs for the oral, dermal, and inhalation routes of exposure which are expected to be used in the preliminary OP cumulative risk assessment. Figure 8 shows the oral RPFs with 95% confidence limits. Due to narrow confidence limits on methamidophos, the confidence limits on RPFs (Figure 8) were quite similar to confidence limits on absolute potencies (Figure 4).

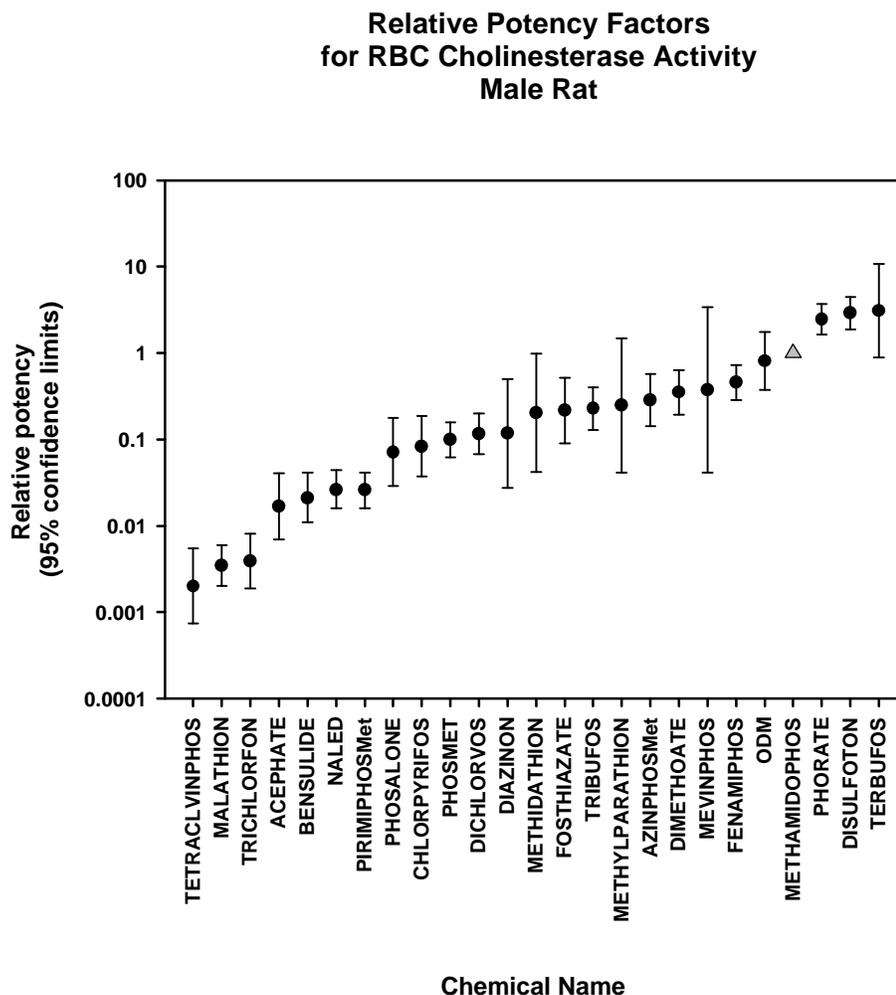
These values were calculated with Equations 3, 4, and 5 for oral, dermal, and inhalation routes, respectively, and using methamidophos as the index chemical. Oral average absolute potency values for all of the chemicals are listed in Appendix 2. Dermal and inhalation NOAELs are given in Tables 6 and 7 for the OPs with residential/nonoccupational exposure and Tables 9 and 10 for the index chemical.

| Table 12. Relative Potency Factors for the Oral, Dermal, and Inhalation Routes of Exposure. | | | |
|--|-------------|---------------|-------------------|
| Chemical | Oral | Dermal | Inhalation |
| RPFs based on RBC cholinesterase activity measured from male rats. | | | |
| Acephate | 0.02 | 0.002 | 0.18 |
| Azinphos-methyl | 0.29 | | |
| Bensulide | 0.02 | 0.001 | NA* |
| Chlorpyrifos | 0.08 | | |
| Diazinon | 0.12 | | |
| Dichlorvos | 0.12 | NA* | 20 |
| Dimethoate | 0.35 | | |
| Disulfoton | 2.89 | 0.94 | 6.25 |
| Fenamiphos | 0.46 | 0.30 | 0.29 |
| Fosthiazate | 0.22 | | |
| Malathion | 0.003 | 0.02 | 0.01 |
| <i>Methamidophos</i> | <i>1.00</i> | <i>1.00</i> | <i>1.00</i> |
| Methidathion | 0.20 | | |
| Methyl-parathion | 0.25 | | |
| Mevinphos | 0.37 | | |
| Naled | 0.03 | 0.075 | 0.78 |
| Oxydemeton-methyl | 0.81 | | |
| Phorate | 2.46 | | |
| Phosalone | 0.07 | | |

| Chemical | Oral | Dermal | Inhalation |
|-------------------|-------|--------|------------|
| Phosmet | 0.10 | | |
| Pirimiphos-methyl | 0.03 | | |
| Terbufos | 3.08 | | |
| Tetrachlorvinphos | 0.002 | 0.0008 | NA* |
| Tribufos | 0.23 | | |
| Trichlorfon | 0.004 | 0.007 | 0.028 |

*NA, not available. It has not yet been determined if or how residential/occupational exposure to dichlorvos via the dermal pathway or to bensulide or tetrachlorvinphos via the inhalation pathway will be assessed in the Preliminary Cumulative Assessment of OPs.

Figure 8. Relative Potency Factors for Oral Route of OPs for RBC Cholinesterase Measured in Male Rats (Error Bars Are 95% Confidence Limits).



Even though dermal and inhalation RPFs were based on NOAELs whereas the oral RPFs were based on potency values estimated from dose-response modeling, the relative rank order of the potency for the residential chemicals with route specific data was comparable to the order of the oral potencies (Table 12).

Table 12. Relative Rank Score of RPFs for Residential Chemicals.

| Chemical Name | Oral Rank Score | Dermal Rank Score^a | Inhalation Rank Score^b |
|----------------------|------------------------|--------------------------------------|--|
| Acephate | 22 | 7 | 6 |
| Bensulide | 21 | 8 | NA |
| Dichlorvos | 14 | NA | NA |
| Disulfoton | 2 | 2 | 2 |
| Fenamiphos | 6 | 3 | 5 |
| Malathion | 24 | 5 | 8 |
| Methamidophos | 4 | 1 | 3 |
| Naled | 20 | 4 | 4 |
| Tetrachlorvinphos | 25 | 9 | NA |
| Trichlorfon | 23 | 6 | 7 |

^aDermal toxicity studies were not available for dichlorvos

^bInhalation toxicity studies were not available for bensulide and tetrachlorvinphos

VI. DISCUSSION

A. Determination of Potency

Relative potency has been determined for 25 OPs with potential oral exposure through food or water and for nine of these pesticides with residential/nonoccupational exposure potential. This analysis included a comprehensive review of oral, dermal, inhalation rat toxicity studies (and four dermal toxicity studies in rabbit) which include cholinesterase activity measured in plasma, RBC, and brain. Relative potency of oral toxicity studies was determined using a novel approach. The database of dermal and inhalation toxicity studies was not as extensive as the oral database. NOAELs were used to determine relative potency of the chemicals with residential/nonoccupational exposure potential. Although NOAELs are not the preferred method for determination of relative potency, it is notable that the rank order of the dermal and inhalation RPFs was similar to that of the oral RPFs.

Historically, OPP has selected single data sets or data points (such as reference doses [RfD] or NOAELs) for use in single chemical risk assessment. The present approach of combining estimates of potency for the oral studies (average absolute potency values) and combining estimates of benchmark dose for the oral, dermal, and inhalation routes (average BMD₁₀s for the index chemical) has several advantages. These estimates incorporate the variability among data sets into the estimate of uncertainty (standard errors or confidence limits). In addition, combining potency estimates for data sets within and across studies maximizes the use of the available. As opposed to using single data sets or data points, it was possible to evaluate the change in potency over time and to assess steady state. Overall, potency estimates for the OPs were consistent across time. Most chemicals appeared to reach steady state by 21 or 28 days of exposure in both sexes and all three compartments thereby increasing confidence in the average absolute potency measures used to calculate the RPFs.

B. Assumption of Dose Additivity

The cumulative risk assessment for the OPs is based on the assumption of dose additivity. Dose additivity is the Agency's assumption when evaluating the joint risk of chemicals that are toxicologically similar and act at the same target site (USEPA, 2001). The SAP (FIFRA SAP, 2001a) indicated that substantial reliance would have to be placed on what is known about the mechanism of toxicity because it is very difficult to prove dose additivity at human exposure levels. They further pointed out that studies likely to be available on individual chemicals were usually not designed to address the issue of dose additivity.

The organophosphorus pesticides all act on the same target site— namely, the inhibition of acetylcholinesterase by phosphorylation in nerve tissue, which elicits a variety of cholinergic effects. Dose addition is regarded as a reasonable and appropriate approach for estimating the cumulative risk associated with joint exposure to the OP common mechanism group. In reality, these common mechanism chemicals may not behave ideally (i.e., the exact same pharmacokinetics and pharmacodynamics). Biotransformation of OPs is extremely complex and involves several metabolic systems in different organs (e.g., reactions involving cytochrome P450 isoenzymes, hydrolysis by esterases, and transferase reactions; see Nigg and Knaak, 2000). The differential activation and/or deactivation of OP pesticides has not been well documented in the literature, nor have the human metabolic pathways (Milesen et al., 1998). At this time, these pesticides can not be separated into subgroups based on pharmacokinetic or pharmacodynamic characteristics. Thus, current information on OP metabolism does not provide a sufficient basis to depart from dose additivity.

The application of dose additivity requires the assumption of no interactions other than additive among the chemicals at low doses. OPP conducted a review of the literature on chemical interactions among organophosphorus chemicals. This review identified a limited number of investigations of the toxicity of combinations of organophosphorus substances, not necessarily pesticides, that are known to inhibit cholinesterase enzymes (For example see Dubois, 1961 and 1969; Frawley et al., 1957 and 1963; Calabrese, 1991; Cohen, 1984; Eto, 1974; Su et al., 1971; Casida et al., 1963; Keplinger and Deichman, 1967; Rosenberg and Coon, 1958; El-Sebee, et al., 1978; Seume and O'Brien, 1971). Most of the studies reviewed were high dose studies that investigated the acute lethality (LD50) of combinations, mostly binary, and not the cumulative effects of low exposure levels from multiple OPs. A number of these studies were conducted using intraperitoneal (i.p.) administration which confounds interpretations of effects that may be expected by the oral, dermal, or inhalation routes.

Overall, the studies reported in the literature do not provide a basis for concluding that interactions between organophosphorus pesticides will result in significant departure from dose addition at low doses. Most studies used high dose levels or inappropriate routes of administration. Nevertheless, this literature provides data showing that different types of interactions can occur between OPs and that the magnitude of the interaction appears to depend on the specific combination of OPs investigated, the dose-levels administered, and also the sequence of exposure (Singh, 1986; Pope and Padilla, 1990). In particular, the data available are not sufficient to establish the nature of interactive effects on cholinesterase activity that may be expected among OPs at low exposure levels.

The mathematical definition of dose addition requires a constant proportionality among the effectiveness of the chemicals (USEPA 2001; Hertzberg et al., 1999). Thus, an important objective in the dose response assessment is to evaluate the OPs considered in this analysis to determine whether they exhibit similarly shaped dose-response curves by examination of each dose-response curve and to evaluate whether these dose-response relations are consistent with the assumption of dose additivity. In general, the same model function adequately described the cholinesterase inhibition data sets for the OPs evaluated in this analysis. Analysis of the parameter estimates (particularly the B term) and the achievement of a similar maximal response among the dose-response curves, and the potential impact on the assumption dose-additivity are discussed in Appendix 1. In general, although the value of estimated maximal response was not the same for all the datasets, the results of the analysis did not contradict the assumption of dose-additivity, especially at low exposure levels appropriate for extrapolation of the cumulative risk to

humans. Thus, there was not a sufficient basis to depart from dose additivity. The default assumption of dose additivity was then used in the calculation of RPFs of the organophosphorus pesticides.

C. Selection of the Index Chemical

The SAP commented in their recommendations from the September 2000 meeting on the importance of modeling RPFs after a chemical that has the best and most complete data for the common endpoint(s) in order to minimize the uncertainties in the exposure assessment and risk estimation. Based on similar criteria, **methamidophos was selected as the index chemical for the Preliminary OP Cumulative Risk Assessment.** The oral database contains studies that characterize the entire dose-response range from very low doses to high doses. Additionally, within the oral route of exposure, potency values for methamidophos were consistent between adult male and female rats and among the three compartments (i.e., plasma, RBC, and brain measures). Dermal toxicity studies in two species with comparable NOAELs were available in addition to an inhalation toxicity study where multiple blood cholinesterase measurements were taken. Finally, adequate dose-response data were available for calculation of points of departure for extrapolating risk associated with oral, dermal and inhalation exposure.

The SAP also commented that it is preferable for the index chemical to act “toxicologically as purely as possible by the common mechanism defining the group.” Although “toxicological purity” would be extremely difficult to prove, there is strong evidence to support the use of methamidophos as the index chemical for the Preliminary OP Cumulative Risk Assessment. Methamidophos is a direct acting anticholinesterase OP that appears to selectively inhibit cholinesterase, the target enzyme. The binding of OPs to carboxylesterases, A-esterases, neurotoxic esterase, and carboxyamidase can influence the capability of OPs to inhibit AChE by lowering the amount of pesticide available to AChE. Binding of methamidophos to these nontarget enzymes does not seem to influence its metabolism or does so only at dose-levels above which cholinesterase inhibition is induced (Khasawinah et al., 1978; Bertolazzi et al., 1991; Mahajna et al., 1997). Additionally, other mechanisms may influence an OP overall cholinergic toxicity as a result of interactions with nicotinic and muscarinic receptors (for review see, e.g., Mileson et al., 1998). There is evidence that methamidophos does not alter acetylcholine release by binding to muscarinic receptors, nor does it bind to and desensitize nicotinic receptors (Camara et al., 1997). There also appears to be no difference in the inhibition of whole blood and brain cholinesterase between adult and weanling rats following treatment with methamidophos by oral gavage (Moser, 1999).

D. Relative Potency Factors for the Preliminary Cumulative Risk Assessment

An important part of the cumulative hazard and dose-response assessment was to identify an endpoint pertinent to the common mechanism of toxicity which will be protective of both the peripheral and central nervous system that has the high quality and extensive data to establish the relative potency of the chemical group. For the organophosphorus pesticides it was determined that the relative potency factors derived from data on **inhibition of cholinesterase activity in male rat RBC should be used to estimate potential cumulative risk**. This determination was based on an evaluation of all cholinesterase activity data in three biological compartments (plasma, RBC, and brain) in male and female rats. This evaluation included consideration of the quality of data and number of studies on each endpoint; dose-dependency of responses; time course; and similarities or differences of responses observed among compartments and between the sexes.

Acetylcholinesterase inhibition in the nervous system is viewed as a key event in the mechanism of toxicity of these compounds and an important critical effect to consider in the cumulative assessment. Direct measures of acetylcholinesterase inhibition in the neuronal tissues (i.e., central and peripheral nervous system) would be the preferred choice for characterizing potential cumulative risk for this class of pesticides. Because of likely variation in pharmacodynamic properties of different chemicals and different measures of cholinesterase inhibition in both the central and peripheral nervous systems, it was important to perform a thorough evaluation of potential hazard. Direct measurements of cholinesterase activity in peripheral nervous system tissues were rarely available. The brain cholinesterase activity data had limitations compared to the blood data mainly because brain cholinesterase activity was generally only determined at terminal sacrifice, and thus time course information was rarely available.

When direct measures of neuronal cholinesterase activity are not available or limited, as a matter of prudent science policy protective of human health, OPP will treat cholinesterase inhibition in the blood as a surrogate measure for the peripheral nervous system in animals and for both the peripheral and central nervous systems in humans. The OPP's Science Policy on "The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides" (USEPA, 2000d) states that *"RBC measures of acetylcholinesterase (AChE) are generally preferred over plasma measures of cholinesterase activity because data on red blood cells may provide a better representation of the inhibition of the neural target enzyme, acetylcholinesterase. OPP may use plasma cholinesterase inhibition data under*

certain circumstances, such as if red blood cell data are insufficient, of poor quality, or unavailable; if there is a lack of dose-dependency for the red blood cell acetylcholinesterase inhibition; or, if the dose responses for inhibition of plasma cholinesterase more closely approximate those for AChE inhibition in the nervous system than do the dose responses for RBC acetylcholinesterase inhibition.”

In general, the number of available studies and the quality of dose-response data for plasma and RBC inhibition were essentially the same. Furthermore, when the potency factors for RBC and brain were compared for all 25 OPs, the RBC relative potency proved to be a sensitive predictor of the brain relative potency for the majority of the chemicals. Therefore, RBC cholinesterase inhibition is considered to be an appropriate endpoint for estimating cumulative risk.

There was good concordance between average absolute potency values calculated from cholinesterase activity measured in RBC for male and female rats for the 25 OPs evaluated. Furthermore, PoDs in female and male rats for RBC cholinesterase inhibition for methamidophos, the index chemical, were of similar, high quality for the oral, dermal, and inhalation routes of exposure. Therefore, the male rat data for RBC cholinesterase activity was selected for the preliminary cumulative risk assessment.

VII. CONCLUSION

As part of the tolerance reassessment process of the organophosphates, a Preliminary Cumulative Risk Assessment of the organophosphate pesticides is ongoing. The present document represents the hazard and dose-response portion of this preliminary cumulative assessment. Relative potencies for 25 OPs for the oral route of exposure plus nine chemicals with residential/nonoccupational exposure have been determined in addition to endpoints needed for the extrapolation of cumulative risk to humans. The exponential function performed well in the determination of potency for cholinesterase inhibition for the oral studies. The use of NOAELs for potency determination for the dermal and inhalation routes, although not the preferred method, was adequate and generated reasonable estimates.

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