I. Preliminary OP Cumulative Risk Assessment

B. Hazard/RPF

1. Introduction

Over the last two years, the Office of Pesticide Programs (OPP) has presented proposed guidance, tools and methodologies for conducting cumulative risk assessments to the FIFRA Scientific Advisory Panel (SAP). Specifically, the hazard and dose-response have been presented to the FIFRA SAP in September 1999 (FIFRA SAP, 2000a), September 2000 (FIFRA SAP, 2001a), and September 2001 (FIFRA SAP, 2001c). The present document will be reviewed by the SAP in February 2002. Following the previous SAP reviews, the constructive comments and recommendations of the SAP have been incorporated into revisions and refinements of the hazard and dose-response assessment for the organophosphorus pesticides (OPs). In its review of a pilot dose-response analysis of 24 OPs in September 2000 (FIFRA SAP, 2001b), the SAP suggested that OPP derive potencies from several relatively consistent studies rather than a single study. The SAP also suggested that the Agency consider Michaelis-Menton kinetics or an exponential function as a dose-response model. In collaboration with Office of Research and Development's (ORD) National Health and Environmental Effects Research Laboratory (NHEERL), OPP released a Preliminary Dose-Response Assessment for 25 OPs on July 31, 2001 (USEPA 2001b), which was reviewed by the SAP in September, 2001 (FIFRA SAP 2001c). The July 2001 analysis incorporated both of these key recommendations.

Overall, the SAP was very supportive of the approach used in the July 2001 dose-response analysis, calling the approach both 'skillful' and 'creative.' Some additional analyses and revisions were recommended (FIFRA SAP 2001c). Several recommendations were identified in the SAP report from the September 2001 meeting. Each of these has been addressed, and the OPP responses are described in detail in Appendix III.B.3. The key recommendations incorporated into this document include: (1) reevaluation of the procedure for estimating the horizontal-asymptote (i.e., the 'B' term); (2) consideration of repeated measures of cholinesterase in potency estimates; (3) a formal analysis of residuals; (4) revision of the statistical procedures for weighting the cholinesterase data and calculating confidence intervals; and (5) determination of the appropriate measure for relative potency.

2. Cumulative Hazard and Dose-Response Assessment Methods

a. Overview

Before the cumulative risk of exposure to OPs 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 (USEPA 2001a, 2000a).

b. Summary of Preliminary Hazard and Dose-Response Assessment from July 2001

i. Selection of Endpoints

As part of the hazard analysis, all relevant responses were evaluated to identify the most appropriate 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 phosphorylating of the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems (Mileson *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, the July 2001 version of the Preliminary Cumulative Risk Assessment for OPs focused on the common mechanism endpoints for plasma, RBC, and brain cholinesterase inhibition.**

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

Humans may be exposed to the OPs through food, drinking water, in and around residences, schools, commercial buildings, etc. Therefore, the potency of OPs needs to be determined for the oral, dermal, and inhalation routes of exposure.

OPP has elected to estimate relative potencies and points of departure (POD) using measurements where cholinesterase inhibition in the laboratory animal is not changing with time. OPP defines this point where continued dosing at the same level results in no further increase in enzyme inhibition as steady state. The use of cholinesterase data for single-dose or short duration studies to model the comparative potency is problematic because the extent of inhibition changes rapidly immediately following dosing. Measures of cholinesterase taken during this time will be highly variable and, therefore, relative potency estimates based on such measures would be uncertain. Use of steady state data for relative potency determination generates relative potency factors (RPFs) that are reproducible and reflect less variability due to rapidly changing, time-sensitive measures of cholinesterase. On average, cholinesterase activity reaches steady state by approximately 3-4 weeks. **The hazard and dose-response assessment focused on studies of a duration of 21 days or greater in order to use cholinesterase data that have attained steady state.**

iii. 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 OPs, 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 pesticides with residential/nonoccupational exposure potential when dermal toxicity data in rats were not available. The cholinesterase data considered in this analysis were extracted from the subchronic and chronic studies for the oral, dermal, and inhalation routes of exposure. Some range finding and special studies were also included. Studies submitted to OPP are reviewed for their quality of cholinesterase measurements and consistency of their experimental protocol with the OPPTS Guidelines (http://www.epa.gov/opptsfrs/home/guidelin.htm).

A comprehensive list of all the studies considered in the present analysis is given in Appendix III.B.2. *The electronic dataset used in the July 2001 is the same one used in the current analysis. The cholinesterase data are available to the public at* http://www.epa.gov/pesticides/cumulative/*.*

iv. Collection of Cholinesterase Activity Data from Oral Studies

Study type, duration of exposure, number of animals per dose group, species/strain/sex, compartment, and the measured effect for each dose group (mean cholinesterase activity, activity units, and standard deviation) were compiled into an electronic spreadsheet. In feeding studies, average compound intake (mg/kg/day) over the entire study was used. At least one oral toxicity study was available for all the OPs. In present analysis, comparative effect levels (CELs) have been used (see below).

vi. Collection of Cholinesterase Activity Data From Dermal and Inhalation Studies

Because of the limited availability of dermal and inhalation toxicity studies, dose-response modeling of dermal and inhalation toxicity studies was not performed in the July 2001 or the present analysis. In the July analysis, relative potency of OPs with nonoccupational/residential exposure potential was determined using no-observed-adverse-effect-levels (NOAELs). Dermal and inhalation exposure studies were not available for all OPs with nonoccupational/residential exposure potential.

vi. Dose-Response Analysis used for the Oral Route

In the review of the July 2001 hazard and dose-response assessment, the SAP was generally supportive of the approach calling it both “skillful” and “creative” (FIFRA SAP 2001c). The panel indicated that no alternative to the exponential model would be more appropriate at the present time. The methods and results of the July 2001 analysis are briefly summarized here. The exponential equation used for modeling the effect of the OPs on cholinesterase activity was:

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

where ***y*** is cholinesterase activity,
dose is the dose of OP, in mg/kg/day,
m is the slope scale factor. The slope scale factor was used in the July, 2001 assessment as the measure of absolute potency,
A is the estimated background cholinesterase activity,
and ***B*** is the horizontal-asymptote or the limiting value of maximum cholinesterase inhibition.

Both *y* (cholinesterase activity) and *dose* were extracted from the oral toxicity studies. Equation I.B-1 has three parameters to be estimated: *m* (absolute potency used in the July 2001 analysis), *A* (background), and *B* (horizontal asymptote). The fitting procedure for Equation I.B-1 used an iterative decision process. First, all parameters in Equation I.B-1 were estimated using all available doses. If adequate model fit ($p < 0.05$) was not achieved, B was set to zero and the model refit. If adequate fit was still not achieved, high doses were dropped one at a time followed by refitting. This process was followed until adequate fit ($p > 0.05$) was achieved or only three dose groups remained. Over 1300 datasets from cholinesterase measurements in plasma, RBC, and brain from male and female rats exposed to 25 different OPs were analyzed using this procedure. (A dataset was defined as the cholinesterase activity measurements from all dose levels plus the controls from a single chemical-sex-time point-compartment combination.) Overall, the exponential model performed well in fitting the cholinesterase data.

Average absolute potency was determined in the July 2001 dose-response analysis using a nested hierarchal statistical model. Using the iterative fitting procedure described above, separate potency values were first determined for each cholinesterase measurement separately. A study-specific average potency was then estimated. This reflected the average of potency estimates from all the single timepoint cholinesterase measurements from a particular study. The overall average potency for an OP was estimated. This reflected the average absolute potency value for a single chemical. Average absolute potency was determined for male and female rats for the brain, RBC, and plasma cholinesterase data. This procedure is described in detail in the July 2001 document (USEPA 2001b).

vii. Identification of Steady State

In the July 2001 dose-response assessment, qualitative observation of steady state was made by noting the change in potency with duration of exposure both graphically and using a simple regression (USEPA, 2001b). The slope-scale factor (m) for each cholinesterase measurement was plotted against time. Separate graphs for each OP, sex and compartment combination are given in Appendix 2 of the July 2001 document. A vertical line in these graphs indicates the time where potency was no longer increasing with time. The results of the regression procedure were not meant to be a definitive method for determining steady state but rather as a qualitative guide. Overall, potency values were generally consistent across time for the majority of chemical-sex-compartment combinations for all of the 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.** *No further analysis of the time course data was performed in the revised dose-response assessment.*

vii. Selection of the Index Chemical (Methamidophos)

The cumulative risk assessment guidance document (USEPA, 2001a) 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. High quality dose-response data allowed the 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. 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.

In the July 2001 dose-response assessment, methamidophos was utilized as the index chemical for the OPs. The selection criteria and the potential candidates for the index chemical were discussed in the July, 2001 document (USEPA 2001b). **Methamidophos remains the index chemical for the Preliminary Cumulative Risk Assessment for OPs because this chemical has a high quality database for the common mechanism endpoint of inhibition of acetylcholinesterase for the oral, dermal, and inhalation routes of exposure**.

ix. Points of Departure (POD)

The oral, dermal, and inhalation PODs for the index chemical are based on the benchmark dose where cholinesterase activity is reduced 10% compared to background activity (BMD_{10}). The BMD_{10} was selected as the effect level for the POD because this level is generally at or near the limit of sensitivity for discerning a statistically significant decrease in cholinesterase activity across the blood and brain compartments and is a response level close to the background cholinesterase. The revised PODs used in the December 2001 Preliminary Cumulative Risk Assessment for OPs are given in Table I.B-6.

c. Summary of Refinements and Revisions to the Preliminary Hazard and Dose-Response Assessment

Based on the recommendations of the SAP from September 2001 described above and in Appendix III.B.3, the Preliminary Hazard and Dose-Response Assessment for OPs has been revised. The following text highlights these revisions.

i. Selection of Relative Potency Factors for the Female Brain Cholinesterase Data Set

A key component of cumulative hazard assessment is to select an endpoint pertinent to the common mechanism of toxicity that can be used to quantify cumulative risk. In the July 2001 dose-response assessment, OPP prepared a dose-response analysis for 25 OPs in which a large body of toxicity data on a common mechanism endpoint for these OPs – the ability to inhibit cholinesterase in plasma, RBC, and brain – was analyzed. To determine which compartment would provide a strong basis for determination of relative potency, OPP reviewed data in each compartment. In the July 2001analysis, RPFs based on the male RBC database were proposed. It was stated in that document that the RBC RPFs proved to be a reliable and sensitive endpoint considered protective of both the peripheral and central nervous systems for the majority of the chemicals. The major advantage of the RBC database was its large size compared to the whole brain ChE database; this large database allowed the examination of time course information and observation of a steady state response.

After considering the comments from the September 2001 SAP meeting in addition to the comments from the public and stakeholder groups, at this time OPP has decided to use female brain ChE data for quantifying cumulative risk for OPs. OPP has decided to estimate cumulative risk based on RPFs and PODs from the female brain ChE database for several reasons. Principally, compared to relative potency estimates based on RBC, estimates of relative potency based on brain ChE have tighter confidence intervals and therefore will confer less uncertainty on cumulative risk estimates. Also, these data represent a direct measure of the common mechanism of toxicity as opposed to using surrogate measures. The toxic potencies and PODs for brain cholinesterase inhibition for these OPs are generally similar to the RBC data for the oral, inhalation, and dermal exposures. The SAP recommended the Agency address the issue of repeated measures in its revised analysis. This issue concerning repeated cholinesterase activity measures only pertains to the plasma and RBC ChE data because blood can be collected several times from a single animal, whereas brain ChE can only be collected once. Finally, in the present analysis, although male and female rats were equally sensitive for 25 OPs, female rats were more sensitive to four OPs. Therefore, OPP has chosen to based its RPFs on female brain measurements.

Using the revised methodology described below, potency estimates have been recalculated only from the brain ChE database. The plasma and RBC databases were thoroughly examined in the July 2001 analysis. Re-analysis of the plasma and RBC databases using the revised methodology is unlikely to significantly change potency estimates from these compartments (See Comparison of Relative Potency Factors from July and December Analyses).

ii. Exponential Equations Used

In the current analysis, two variations of the exponential function were used. The first equation (Equation I.B-2) is equivalent to the function used in the July assessment and was described above (Equation I.B-1) except for the parameter representing the horizontal asymptote. In the July 2001 dose-response assessment, the horizontal-asymptote was represented by the variable 'B' and had units of cholinesterase activity. In the current analysis, the horizontal-asymptote is represented by P_B. P_B expresses the horizontal-asymptote as a fraction of background cholinesterase activity and is equivalent to the ratio of B/A (parameters used in Equation I.B-1). P_B does not have any units. For technical reasons, *tB* was actually estimated (*tB* is the natural logarithm of $(P_B/(1-P_B))$. As appropriate, descriptions of modeling methods in addition to the results below refer to both the horizontal-asymptote as *tB* or P_B.

The exponential functions in Equations I.B-1 and I.B-2 decrease in a linear fashion in the low dose region. Considerable discussion at the Technical Briefing (August 2001) and the SAP meeting of September 5-6, 2001 centered around the potential for a flat region in the low dose portion of the dose-response curve. This potential of a low-dose flat region was explored in the current analysis. The second equation (Equation I.B-3) is a modified version of the exponential function in Equation I.B-2. Equation I.B-3 contains two variables, *S* (shape) and *D* (displacement), which describe a low-dose flat region of the dose-response curve. Figure I.B-1 shows the relationship between the basic and expanded models and also how the shape and displacement variables impact the dose-response curve.

For ease of discussion, Equation I.B-2 will be called the '*basic*' model and Equation I.B-3 will be called the '*expanded*' model.

iii. Joint Analysis of OP Cholinesterase Data

In the July 2001 dose-response analysis, potency of each cholinesterase dataset was determined *separately* followed by calculation of mean *within study* potency and then *mean chemical* potency. For example for brain ChE measured in female rats exposed to methamidophos, five datasets from three oral toxicity studies are available. Each of these five were first analyzed separately (Figure 1.B.1). Next, the mean potency from the three studies was calculated. The overall mean potency for brain ChE measured in female rats exposed to methamidophos was calculated in the last step.

In the present joint analysis, all the datasets for each chemical are modeled *together* all at once. In the example described above for the methamidophos brain ChE data from female rats, all five datasets were analyzed at once. This approach allows information about the shape of the dose-response curve to be "shared" among individual data sets. The dataset of cholinesterase information used in the July analysis has also been used in the present analysis (Appendix III.B.2).

iv. Estimates of the Horizontal Asymptote

In the July 2001, analysis an iterative approach was used to fit the cholinesterase data to the exponential model. In the first step of this iterative process a non-zero horizontal asymptote (the B-term) was fit. If adequate fit was not achieved, the horizontal asymptote was set to zero and the data refit. The SAP recommended the Agency reevaluate the decision tree of the fitting procedure in order to generate more consistent values of the horizontal asymptote.

In response, OPP developed a joint analysis for each chemical and sex combination was developed. Because only *one* dose-response model for each chemical and sex combination was developed, only *one* value of the horizontal-asymptote was generated per chemical.

v. Incorporation of Fixed and Random Effects

To perform the joint analysis of all the datasets for each chemical, several aspects of the data need to be accommodated . First, measurements of cholinesterase activities can have different units (mainly U/G, U/L, and ΔpH), which need to be accommodated in the same analysis. Model parameters may also differ between males and females. Finally, it is likely that model parameters vary randomly among studies and among datasets within a study.

In the July 2001 analysis, discreet data sets were analyzed separately; thus the issue about mixing units and differences between sexes did not arise. The issue of random effects was addressed in the July 2001 analysis after the individual sets were modeled by combining estimates across studies and datasets. In the present analysis, a mixed effects model was used. Mixed effects models in statistics are models for data in which some parameters vary among subsets of the data. For example, in this analysis, The background level (*lA*), scale factor (*lm*), and horizontal asymptote (*tB*) for the basic and expanded nonlinear models were presumed to vary among studies and among datasets nested within studies, whenever there was more than one study or dataset. The estimation problem for mixed effects models is to estimate both the fixed effects (parameters that do not vary), and the distribution parameters (for example, means and standard deviations) for the random parameters. In addition to estimating population means and variances for the parameters that were expected to vary randomly among studies and among datasets within studies, by using a mixed effects model, separate values could be estimated for males and females.

vi. Weighting: Modeling the Relationship of Variance-to-Mean

In the July 2001 analysis, it was assumed that the variance was proportional to the square of the mean (that is, that coefficients of variation were constant across doses). Analysis of the residuals suggested that a lower power for the relationship between variance and mean might be more appropriate. In the current analysis, variance was assumed to be proportional to the mean.

vii. Use of BMD_{10} for Relative Potency Determination

In the July 2001 analysis, the slope-scaling factor (m) was used as the measure of potency. In the present analysis, the BMD_{10} has been used as the measure of potency. The BMD_{10} was selected for two reasons. First, the model fit was significantly improved using the expanded model for 8 of the 29 OPs. Potency of the remaining 21 OPs was fit with the basic model. Because the shapes of the dose-response curves produced by the basic and expanded models differ, the slope-scaling factor is not an appropriate measure of potency. Second, the value of the slope-scaling factor is dependent on the value of the horizontal asymptote. As shown below, for the 29 OPs, the values for the horizontal asymptote (P_B) are not similar to each other; thus, the slope-scaling factor is not an appropriate measure for determining relative potency.

viii. Use of Comparative Effect Levels (CELs) for Dermal and Inhalation Relative Potency Determination

The database for residential chemicals was not suitable for dose-response modeling. Cholinesterase determinations in these studies were typically made at only one timepoint, and several of the studies had no cholinesterase inhibition at the high dose. For these reasons, relative potencies in the previous assessment were evaluated by comparing no-observed-adverse-effect-levels (NOAELs). The NOAEL is not preferred for comparing potency because its value does not represent a uniform measure of response. In order to compare the dermal and inhalation ChE studies on a common basis, relative potencies by the dermal and inhalation routes were compared using comparative-effect-levels (CEL). The CEL was defined as the dose causing a maximum of 15% brain cholinesterase inhibition.

d. Determination of Chemical Potency

i. Pathway of Exposure: Oral Route

Oral relative potency values were needed for all 29 OP pesticides considered in this analysis because of potential oral exposures from food and drinking water and hand to mouth exposures associated with residential/nonoccupational uses.

The key objectives of the dose-response analysis were to estimate parameters for the exponential model for calculation of relative potencies and PODs, as well as to explore the low-dose behavior of the dose-response curves.

1) Joint Analysis of OP Cholinesterase Data

While the July 2001 analysis fit models to discreet data sets, and then combined estimates of potency for each OP across data sets, the goal of the present analysis was to model all the data for a given chemical together all at once. This approach allows information about the shape of the dose-response curve to be "shared" among individual data sets. One of the recommendations of the SAP was to develop more consistent estimates of the horizontal asymptote than were developed in the July 2001 analysis. This issue is alleviated because there is a single estimate of the horizontal asymptote for each sex and chemical. Furthermore, this estimate is always estimated to be consistent with the data, in contrast to the previous approach in which the value defaulted to 0.0 when it could not be estimated. Also, because the overall dose-response curve is generally based on more dose levels in the joint analysis, it proved possible to look in more detail at the shape of the low-dose end of the dose-response curve.

Equations Used: Two equations were used in this analysis. The first is equivalent to the equation used in the analysis of discreet data sets. The shape of this curve is the same as the function used in the July 2001 analysis (Equation I.B-1). The only difference between Equation I.B-1 and I.B-2 is that instead of expressing the horizontal asymptote in activity units, in this equation it is expressed as a fraction of background activity. Equation I.B-2 is shown here:

Equation I.B-2

$$y = f(Dose; A, P_B, m) = A\left[P_B + (1 - P_B)\mathrm{e}^{-m \times Dose} \right]$$

where ***y*** is cholinesterase activity,
Dose is the dose level of the OP, in mg/kg/day,
A is the background (similar to control) AChE activity,
m is the slope-scale factor,
and $\boldsymbol{P_B}$ is the horizontal asymptote (i.e., limiting value of minimum cholinesterase activity), expressed as a fraction of the background activity.

The parameter P_B in this model is equivalent to the ratio *B/A* in the equation described in Equation I.B-1. This change simplified the interpretation of the parameters and improved convergence of the models. For technical reasons, the parameters actually estimated were *lA* = ln*(A)*, *lm* = ln*(m)*, and *tB* = ln$(P_B/(1-P_B))$. Figure I.B-2 shows an example of the equation in Equation I.B-2. In the present document, Equation I.B-2 is referred to as the 'basic' equation.

The second equation resulted from combining Equation I.B-2 with an equation which describes the relationship between administered dose and internal dose:

Equation I.B-3

$$idose = g(Dose; S, D) = 0.5\left[(Dose - S - D) + \sqrt{(Dose - S - D)^2 + 4 \times Dose \times S} \right]$$

where ***idose*** is the scaled internal dose,
Dose is the administered dose level (mg/kg/day),
S controls the low-dose shape of the curve, and
D controls the ultimate horizontal displacement of the curve relative to the identity line (i.e., the line with *idose* = *Dose*).

The value *idose* replaces *Dose* in the previous equation. As shown in Figure I.B-2, the low-dose end of the dose-response curve has a shallower slope (more flat), which increases to the slope of the basic equation (Equation I.B-2) as dose increases. As *S* grows large, or *D* approaches 0, the relationship between *idose* and *Dose* approaches the line *idose = Dose*. *In other words, as S increases or D decreases, the shape of the expanded equation approaches the shape of the basic equation*. Again, for technical reasons the estimated parameters were transformations of the parameters shown here: *lS* = ln*(S)*, and *lD* = ln*(D)*. The derivation of this equation and some of its properties are described in more detail in Appendix III.B.1. In the present document, the equation resulting from combining Equation I.B-2 with Equation I.B-3 is referred to as the 'expanded' equation.

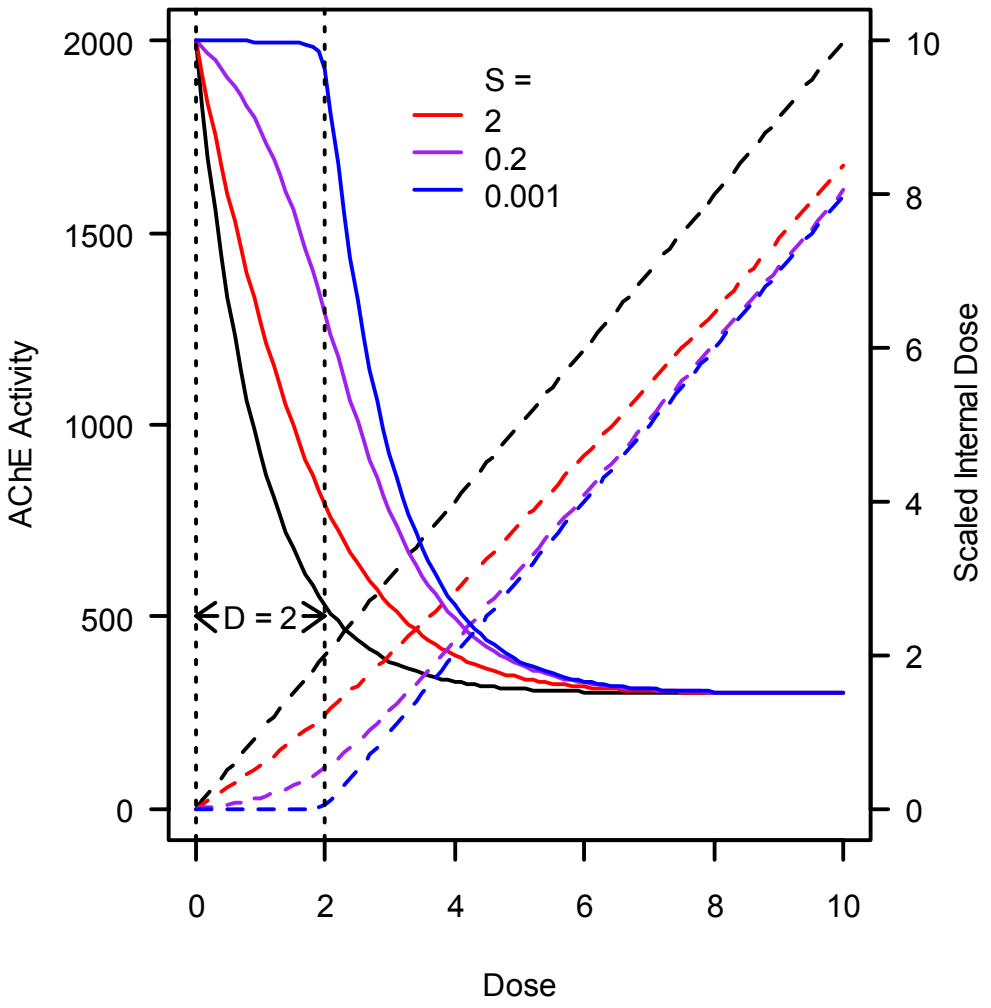


Figure 1.B-1. Basic and expanded equations. The black solid curve is the basic equation of Equation I.B-2 with A = 2000, P_B = 0.15, and m = 1 The dashed curves represent the relationship between internal dose and administered dose expressed in the expanded equation (Equation I.B-3), with D = 2 and varying values of S. The colored solid curves show the results of combining the two equations

Estimating Parameters: As stated above, several aspects of the data that did not impact the analyses of discreet datasets separately (in July analysis) need accommodating in the joint analysis of all the datasets for each chemical. First of all, cholinesterase activities in different studies have different units (mainly U/G, U/L, and ΔpH) which need to be accommodated in the same analysis. Measurements of cholinesterase activity may also vary randomly between males and females. Finally, it is likely that model parameters vary randomly among studies and also among datasets within a study. All of these features were addressed with nonlinear mixed effects (*nlme*) models using the method of Lindstrom and Bates (1990) as implemented in the *nlme* package of *R* (statistics computer program). In general terms, this procedure fits a nonlinear model which assumes some parameters vary randomly about a population mean among subsets of the data. In this analysis, *lA*, *lm*, and, if estimated, *tB* are allowed to vary among studies and among datasets within studies.

Since *nlme* uses an iterative algorithm to estimate model parameters, initial values for the fixed parameters (that is, in the basic equation *lA* for all unit x sex combinations, *lm* and *tB* for each sex; in the expanded equation, in addition, *lS* and *lD*) are required, and are iteratively updated until successive changes are sufficiently small. The result of the estimation process is an estimate of the population mean of the parameters and the standard deviations of all the parameters assumed to vary randomly, as well as approximate standard errors of all the parameters.

The implementation in *nlme* allows the fixed parameters in the model to be expressed as linear combinations of other variables (e.g., an indicator variable that indicates sex). In addition, this method also allows the error variance to be modeled as a power function of the mean (i.e., the error variance is proportional to the predicted mean raised to a power). In the July 2001 analysis, the variance was taken to be proportional to the square of the mean. The analysis of residuals from the July 2001 analysis suggested that a lower power would probably be more appropriate. In this analysis, the variance was modeled as proportional to the mean. A more detailed discussion of the statistical methodology is contained in Appendix III.B.1.

Separate values of *lA* (log of background cholinesterase activity) were estimated for each sex-units combination. Values of the fixed effects for *tB* (transformed horizontal asymptote) and *lm* (log slope-scale factor) were allowed to differ between sexes. In addition, separate random effects were estimated for variation of all three parameters among studies and also among datasets within study. The random effects for *lA*, *tB*, and *lm* were assumed to be uncorrelated because there was insufficient data to estimate correlations among them. When estimating parameters of the model that included the low-dose modification, both *lS* and *lD* were modeled as fixed parameters for each chemical.

The cholinesterase data were modeled in the following steps:

① **Selecting acceptable values for horizontal asymptote (*tB*) in the basic equation**

It was particularly important for the initial estimates of *tB* to be well-chosen for the iterative algorithm of *nlme* to converge successfully. To select reasonable values for *tB*, likelihood values were calculated for each point on the grid defined by fixing male and female values of P_B to eleven evenly spaced points from 0.01 to 0.99. An 11 × 11 grid equals 121 total optimizations. Often, not all 121 models converged; usually this occurred when parameter values were distant from the likelihood peak. For those models which converged, the log-likelihood of the result was recorded as a function of the corresponding values of P_{BF} and P_{BM}, the female and male specific values of P_B. The resulting values form a mound- or hill-like surface, called a profile likelihood. Larger (higher) likelihood values are better supported by the data than are smaller (lower) values. Interpolation on this grid was used to visually identify the approximate maximum. Parameters were then selected that corresponded to this maximum, and transformed to values of *tB* that could be used in the model.

Two example profile likelihood surfaces are shown in Figure I.B-2. Figure I.B-2A is a plot of the profile likelihood surface to estimate P_B for acephate (an OP for which P_B could be estimated). The log-likelihood is represented as color, with dark red the lowest value and bright yellow the highest. "+" marks indicate points on the grid where likelihood estimates were available. There is a clear narrow 'peak' around (0.3,0.3) for acephate, where the log likelihood is about 906. One step away, the log likelihood is about 830. The white areas around the edges of Figure I.B-2a indicate models which did not converge.

Figure I.B-2b is the profile likelihood surface for bensulide. Here the profile likelihood surface has a broad flat place below about 0.5 in both dimensions. It was not possible to estimate *tB* jointly with other model parameters. The peak value on the grid was at (0.01,0.01), where the log likelihood is 274. The transition between the lightest yellow and the next darker shade occurs where the log likelihood is about 270.

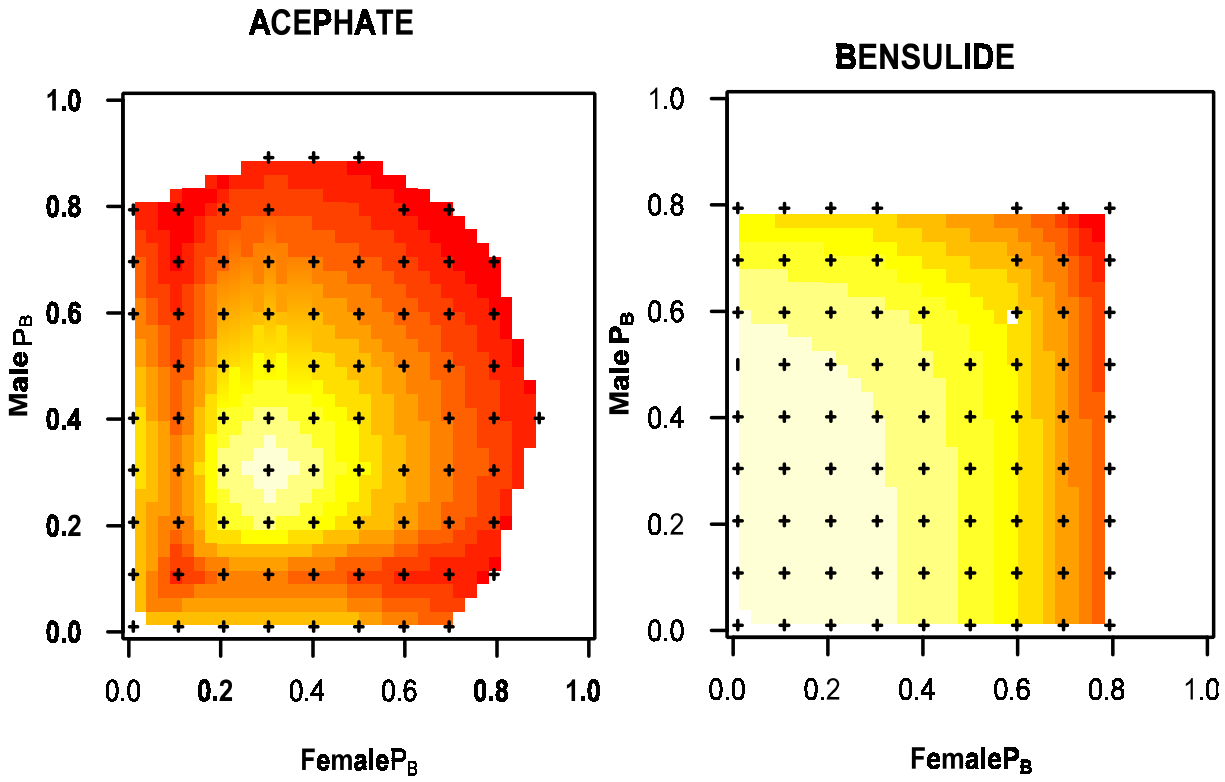


Figure I.B-2a. Plot of the profile likelihood surface to estimate P_B for acephate (an OP for which P_B could be estimated)

Figure I.B-2b. Plot of the profile likelihood surface to estimate P_B for bensulide (an OP for which P_B could not be estimated)

② Fitting the basic equation

The basic equation was first fit to the data for each OP with *nime*, using as initial values for *tB* the values for *tB* selected in the previous step. The basic model converged (all parameters were successfully estimated) for acephate, chlorpyrifos, chlorpyrifos-methyl, dicrotophos, dimethoate, ethoprop, fenthion, fosthiazate, methamidophos, methidathion, mevinphos, naled, and ODM.

If that model failed to converge, then *tB* was fixed to those same values, and a simpler model, conditional on the fixed values of *tB* and lacking random effects for that parameter, was fit. In this latter situation, where the surface appeared relatively flat, as in Figure I.B-2B, the choice of *tB* was out of necessity rather arbitrary. However, the very flatness of the surface is assurance that a range of choices of *tB* should result in nearly equally good fits. *Since potency is based on the interpolated BMD_{10}, and not on any one model parameter, as long as the model describes the data adequately, this should have little practical impact on the estimates of potency*.

③ **Selecting acceptable values for *IS* and starting values for *ID* in the expanded equation**

The expanded equation adds two new parameters, *IS* (shape) and *ID* (distance), to those of the basic equation. The initial values for parameters that appear in both models used to fit the expanded model were those estimated using the basic model. If it was not possible to jointly estimate *tB* with other parameters in the basic model, *tB* was fixed at the same value it had in the basic model when the expanded model was fit.

Just as for the value of *tB* for males and females in the basic model, the log-likelihood of the expanded model was calculated over a grid of values for *IS* and *ID* (both selected to correspond to *S* and *D* varying over the range [0.001,0.4]. Values that coincided with the maximum of the profile likelihood were selected as starting values for jointly estimating all the parameters.

④ **Fitting the expanded equation**

It was substantially more difficult to estimate parameters in the expanded equation than in the basic equation. This difficulty led to a series of discrete decision points for proceeding with the estimation of the parameters in the expanded equation:

a) *It was not possible to compute the likelihood for enough points on the IS × ID grid to estimate the profile likelihood.* The expanded model was not estimated for these chemicals because it was presumed that if models could not be fit with any fixed value of *IS* and *ID*, it would certainly not be possible to estimate those parameters. For these chemicals (acephate, chlorpyrifos, chlorpyrifos-methyl, ethoprop, fenthion, ODM), potency was determined using the basic model.

b) *There was no clear maximum on the profile likelihood surface.* These chemicals, these chemicals were not modeled further. For these chemicals (dicrotophos, fosthiazate, naled, trichlorfon), potency was determined using the basic model.

c) *The peak of the profile likelihood surface occurred at parameters that resulted in identical predictions to the basic model (idose = Dose)*. For these chemicals (diazinon, dichlorvos, fenamiphos, methamidophos, pirimiphos-methyl, tetrachlorvinphos), potency was determined using the basic model.

d) *The remaining chemicals were modeled with the expanded model.* It was not possible to get convergence for any chemical while estimating *both IS* and *ID*. Consequently, *IS* was fixed to a value consistent with the maximum of the profile likelihood surface, and only *ID* was estimated. Estimation in this case used the same basic modeling approach as the basic model: *nlme* with the same random effects and sex-specific effects. The expanded model was used to estimate potency for azinphos-methyl, bensulide, disulfoton, malathion, methyl-parathion, phorate, phosmet, and terbufos . For dimethoate, methidathion, mevinphos, phosalone, and tribufos, the expanded model did not converge, and the basic model was used to determine potency.

Testing for differences among estimated *tB*s: A Chi-square test of whether the estimated values of *tB* (i.e., transformed horizontal asymptote) varied significantly among chemicals was performed. This test was based on the assumption that estimates are approximately normally distributed. Thus, if all the estimates are of the same parameter, the quantity $\sum_{i}^{k} \frac{\left(tB_i - \overline{tB} \right)}{se_{tBi}^2}$ should be approximately chi-square distributed, with $k - 1$ degrees of freedom, if the estimates are independent. To guarantee independence, this test was conducted separately for males and females.

Testing for the significance of the expanded model: When the expanded model could be fit to the cholinesterase data, the significance of the improvement in fit afforded by thelow-dose modification was assessed by a standard log-likelihood ratio test.

Estimating Benchmark Dose: The benchmark dose resulting in a 10% reduction in cholinesterase activity compared to background (BMD_{10}) was calculated for the basic equation using Equation I.B-4.

$$BMD = \frac{\log[1 - BMR \times (1 + e^{tB})]}{e^{lm}}$$

Equation I.B-4

In addition, it was possible to rewrite the equation described in Equation I.B-2 by solving Equation I.B-4 for *lm* in terms of *BMD*, *BMR*, and *tB*, and replacing *m* in Equation I.B-2 with the exponential of the resulting expression. When this model was fit to the data set, a direct estimate of BMD and its standard error resulted (as for the model that includes *m*, actually it was log(BMD) that was estimated; appropriate for computing relative potencies and corresponding confidence intervals). This was the approach used for computing BMDs from the basic equation, as it is a slightly more direct approach. These calculations were then checked with a direct application of Equation I.B-4 to the parameter estimates of the basic model using *lm*.

When the expanded equation was used, the BMD was calculated in two stages. First the previous BMD value was calculated and was taken to be an "internal" BMD_{10} (*iBMD*). Equation I.B-2 was then solved for dose in terms of internal dose, which gives the following expression:

$$BMD = \frac{iBMD^2 + iBMD \times \left(e^{lS} + e^{lD}\right)}{iBMD + e^{lS}}$$

Equation I.B-5

When the internal BMD_{10} is substituted for *iBMD* in Equation I.B-5, the result is the BMD in terms of administered dose.

2) Calculation of Relative Potency Factors

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

$$Oral\ RPF_{\text{Chemical X}} = BMD_{10\ \text{Index Chemical}} / BMD_{10\ \text{Chemical X}}$$

Equation I.B-6

where $BMD_{10\ \text{Chemical X}}$ is the BMD_{10} for Chemical X

and $BMD_{10\ \text{Index Chemical}}$ is the BMD_{10} of the index chemical.

3) Software Used in Potency Determination

In the July 2001 dose-response analysis, a computer program, OPCumRisk, was used to determine relative potency estimates and PODs for the index chemical. OPCumRisk was developed at ORD's NHEERL specifically for use in the July 2001 OP dose-response assessment and is available at http://www.epa.gov/scipoly/sap/index.htm. 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. Minor revisions recommended by the SAP have been incorporated into the OPCumRisk program (See Appendix III.B.3).

The statistical methodology used in the present document has **not** been incorporated into the OPCumRisk program. The programming code in R-language used to develop the relative potency factors and the PODs for the index chemical in the current analysis has been included in Appendix III.B.4.

ii. 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 CELs for this route of exposure. These CELs are experimental dose levels which elicit a similar toxicological response to the selected endpoint. CELs are used as common response for comparison purposes in cases where a benchmark dose estimate can not be attained.

Cholinesterase activity data were collected from dermal toxicity studies for nine chemicals with residential/nonoccupational exposure and the index chemical (methamidophos). Four OPs were tested by the dermal route in rats. Only rabbit studies were available for the other five OPs. 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. 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.

1) Establish CELs for Dermal Studies

Relative potencies of the chemicals with residential/non-occupational uses were determined by using CELs derived from data on inhibition of cholinesterase activity in female rat brain. The CEL was defined as the lowest dose where a maximum 15% brain cholinesterase inhibition (compared to control) occurred. For comparison, the respective CELs for RBC cholinesterase in female and male rats are also given.

b. Calculation of Relative Potency Factors

CELs for brain cholinesterase activity measured in dermal studies were determined in order to calculate RPFs. Dermal RPFs were calculated using Equation I.B-7.

$$Dermal\ RPF_{\text{Chemical X}} = CEL_{\text{Index Chemical}} / CEL_{\text{Chemical X}}$$

Equation I.B-7

iii. 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 CELs for brain cholinesterase activity for the inhalation route of exposure.

Cholinesterase activity data were collected from inhalation toxicity studies for seven chemicals with residential/nonoccupational exposure and the index chemical (methamidophos). Two inhalation exposure studies were available for acephate whereas only one suitable study was available for the other OPs. Although all of 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, one head-nose, and three nose only exposure studies.

No inhalation toxicity study was available for three chemicals, bensulide, fenthion, and tetrachlorvinphos.

1) Establish CELs for Inhalation Studies

Relative potency was calculated from CELs for brain cholinesterase activity determined from inhalation toxicity studies. The CEL was defined as the lowest dose where a maximal response [brain cholinesterase inhibition] of 15% (compared to control) occurred. For comparison, the respective CELs for RBC cholinesterase in female and male rats are also given.

2) Calculation of Relative Potency Factors

CELs for brain cholinesterase activity measured in inhalation studies were determined in order to calculated RPFs. Inhalation RPFs were calculated using Equation I.B-8.

$$Inhalation\ RPF_{\text{Chemical X}} = CEL_{\text{Index Chemical}} / CEL_{\text{Chemical X}}$$

Equation I.B-8

3. Results

a. Dose-Response Modeling: Oral Route of Exposure

A joint analysis using the basic (low dose linear) and/or the expanded (low dose flat region) equations of brain cholinesterase data for each chemical was performed. The potency of eight chemicals (azinphos-methyl, bensulide, disulfoton, malathion, methyl-parathion, phorate, phosmet, and terbufos) were modeled with the expanded. The potency of the remaining 21 were modeled with the basic. The expanded model fit was significantly improved; i.e., the p-value of the likelihood test for the expanded model was ≤ 0.0002 for all eight chemicals. Plots of dose-response data, residuals, and profile likelihoods for all 29 OPs are given in Appendix III.B.2. One annotated example each of the basic and expanded models are given in Figures I.B-3 and I.B-4. BMD_{10}s and RPFs for all 29 OPs are also given below. Analysis of the residual plots indicates that the models generally capture the trend of the mean of the data, and that the weighting function (variance $\propto$mean^2) used in this reanalysis is generally superior to that used in the original analysis.

i. Results of Model Fitting: Basic vs. Expanded Equation

Table I.B-1 below shows the outcome of the model fitting procedure including the decision points for fitting the shape (S) and distance (D) parameters for each chemical. The fit for eight OPs (azinphos-methyl, bensulide, disulfoton, malathion, methyl-parathion, phorate, phosmet, and terbufos) was significantly improved with the expanded model.

The joint analysis using the basic model is shown in Figure I.B-3.a [male (red) and female (blue)]. The solid line represents the dose-response curve for the mean model parameters, and the dotted lines are curves for the study-specific model parameters. Figure I.B-3b shows the scaled residuals for the basic model. The residual pattern shown here indicates that the model adequately describes the data: the residuals are symmetrically distributed around zero, so the model captures the shape of the dose-response curve; their spread is about the same over the range, so the weighting (using the variance proportional to the mean) is adequate. The indicated expected fraction of inhibition ranges from 0 to just under 0.8, with a number of observations around 0.10, so the data span the region of inhibition that is used to calculate potency. Figure I.B-3c is the profile likelihood plot for the estimation of P_B.

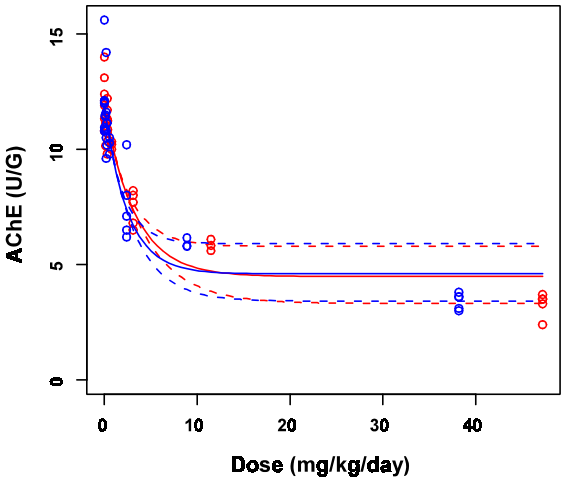
Table I.B-1. Outline of results of model fitting procedure for the basic and expanded models

Chemical	All Parameters fit by Basic Equation	Pb fixed based on Profile Likelihood	Fit by Basic Equation Only	Enough Fits to form Profile for *IS* and *ID*	Apparent peak in Profile for *IS* and *ID*	Fit with Expanded Equation	P-value of Likelihood test for Expanded Model
Acephate	Yes	No	Yes	No	--	--	
Azinphos-methyl	No	Yes	No	Yes	Yes	Yes	<0.0001
Bensulide	No	Yes	No	Yes	Yes	Yes	0.0002
Chlorpyrifos	Yes	No	Yes	No	--	--	
Chlorpyrifos-methyl	Yes	No	Yes	No	--	--	
Diazinon	No	Yes	Yes	Yes	Yes (large S,Small D)	--	
Dichlorvos	No	Yes	Yes	Yes	Yes (large S,Small D)	--	
Dicrotophos	Yes	No	Yes	Yes	No (broad flat large S, Small D	--	
Dimethoate	Yes	No	Yes	Yes	Yes	No	
Disulfoton	No	Yes	No	Yes	Yes	Yes	<0.0001
Ethoprop	Yes	No	Yes	No	--	--	
Fenamiphos	No	Yes	Yes	Yes	Yes (large S, Small D)	--	
Fenthion	Yes	No	Yes	No	--	--	
Fosthiazate	Yes	No	Yes	Yes	No	--	
Malathion	No	Yes	No	Yes	Yes	Yes	<0.0001
Methamidophos	Yes	No	Yes	Yes	Yes (large S, Small D)	--	
Methidathion	Yes	No	Yes	Yes	Yes	No	
Methyl-parathion	No	Yes	No	Yes	Yes	Yes	<0.0001
Mevinphos	Yes	No	Yes	Yes	Yes	No	
Naled	Yes	No	Yes	Yes	No	--	
Oxydemeton-methyl	Yes	No	Yes	No	--	--	
Phorate	No	Yes	No	Yes	Yes	Yes	<0.0001

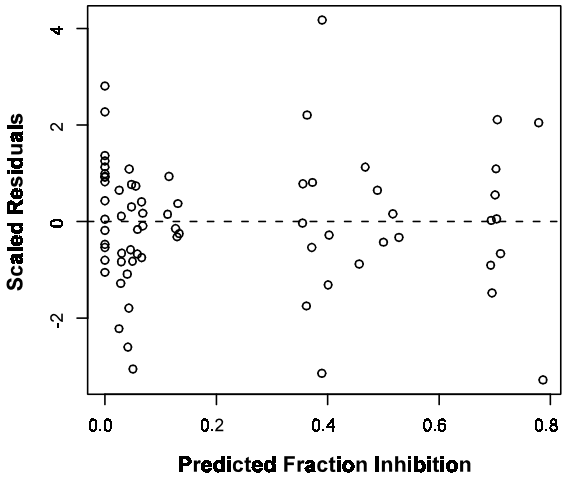
Chemical	All Parameters fit by Basic Equation	Pb fixed based on Profile Likelihood	Fit by Basic Equation Only	Enough Fits to form Profile for *IS* and *ID*	Apparent peak in Profile for *IS* and *ID*	Fit with Expanded Equation	P-value of Likelihood test for Expanded Model
Phosalone	No	Yes	Yes	Yes	Yes	No	
Phosmet	No	Yes	No	Yes	Yes	Yes	<0.0001
Pirmiphos-methyl	No	Yes	Yes	Yes	Yes (large S, Small D)	--	
Terbufos	No	Yes	No	Yes	Yes	Yes	<0.0001
Tetrachlorvinphos	No	Yes	Yes	Yes	Yes (large S, Small D)	--	
Tribufos	No	Yes	Yes	Yes	Yes	No	
Trichlorfon	No	Yes	Yes	No	--	--	

Figure I.B-3. Dose-response curve, plot of the scaled residuals versus predicted inhibition, and the profile likelihood plot for acephate using the basic model.

I.B-3.a. Dose-response curve for basic model



I.B-3.b. Scaled Residuals versus Predicted Inhibition



I.B-3.c. Profile Likelihood for P_B

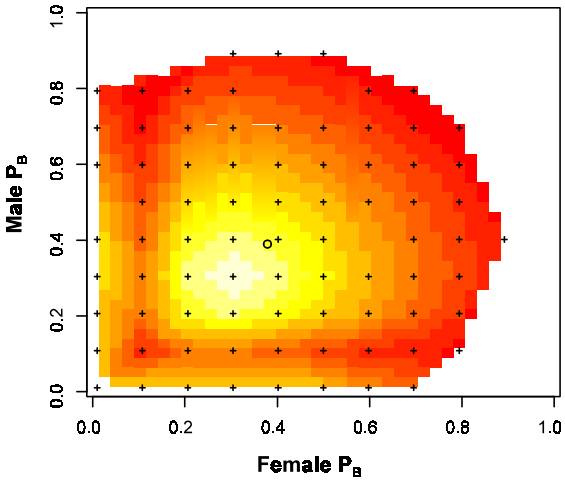


Figure I.B-4a and I.B-4e give the dose-response curves of the joint analysis for both male (red) and female (blue) rats using the basic and expanded models, respectively. The solid line represents the dose-response curve for the overall mean parameters and the dotted lines represent the curves based on study-specific parameters. Figure I.B-4b and I.B-4 f show the scaled residuals for the basic and expanded models, respectively. The residual patterns shown here indicate that the basic model seems to overestimate the degree of inhibition for low levels of inhibition (less that about 8% inhibition) and underestimate it at slightly higher levels of inhibition. Above approximately 25% inhibition, the model captures the mean level fairly well. The expanded model seems to capture the mean a bit better, since the residuals are more symmetrically distributed about 0. Figure I.B-4c is the profile likehood plot for the estimation of P_B. The area of bright yellow is fairly broad and flat. Figure I.B-4d is the profile likehood plot for the estimation of *D and S*. The slope of the surface is relatively more steep in the direction of increasing *S*, so small values of S are clearly to be preferred. Fixing *S* to a small value (e^{-15}) and estimating *D* resulted in the estimate marked by the small circle.

ii. Parameter Estimates

The parameters estimated from the basic and expanded models are given in Table I.B-2 below. A simple chi-square test of the estimated *tB*s, indicates that the P_Bs clearly differ among chemicals (for females, X^2 = 385.6 w/ 12 df; for males, X^2 = 201.1 w/12 df; both P-values << 10^{-6})

Figure I.B-5 is a plot of P_B (horizontal-asymptote) for each chemical. The OPs in this plot are sorted into two groups: those which are direct acting and those which require activation. There appears to be no correlation between P_B and requirement for activation.

iii. Benchmark Dose Calculations

The BMD_{10}s for brain cholinesterase measured in male and female rats using the joint analysis procedures are shown in Figures I.B-6 and I.B-7 and listed in Table I.B-3. Among the OPs, BMD_{10}s range widely over approximately five orders of magnitude.

Figure I.B-4. Dose-response curve, plot of the scaled residuals versus predicted inhibition, and the profile likelihood plots for P_B, D, and S for bensulide using the basic and expanded models

Fig. I.B-4a Dose-Response curve for basic model

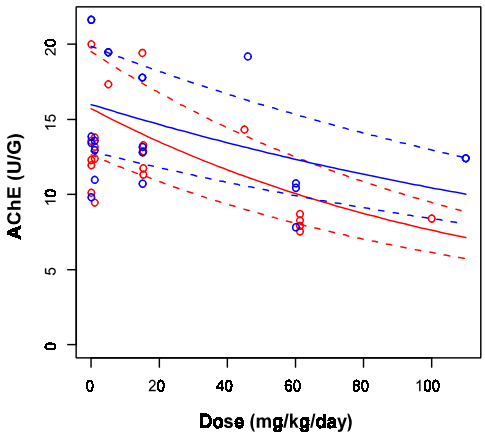


Fig. I.B-4b Scaled Residuals versus Predicted Inhibition (Basic)

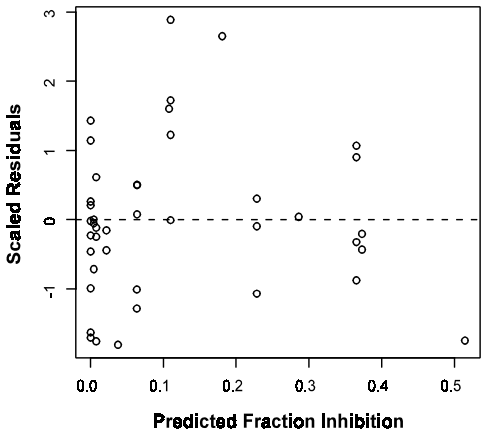


Fig. I.B-4c Profile Likelihood for P_B

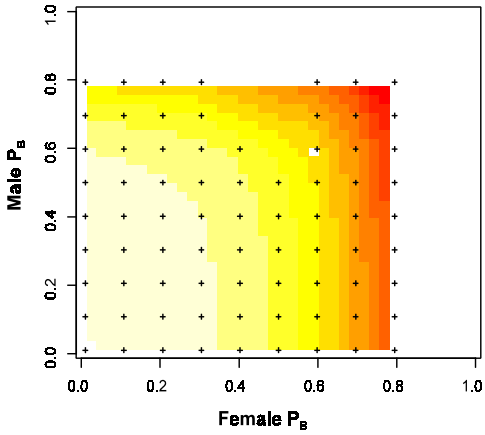


Fig. I.B-4d Profile Likelihood for D and S

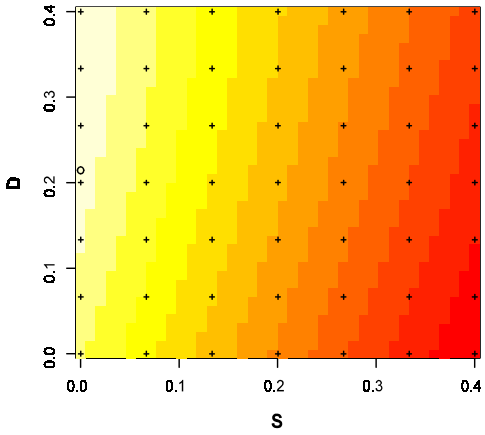


Fig. I.B4-e Dose-response curve for expanded model

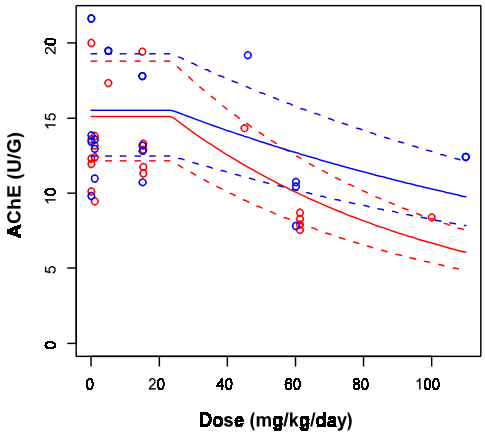
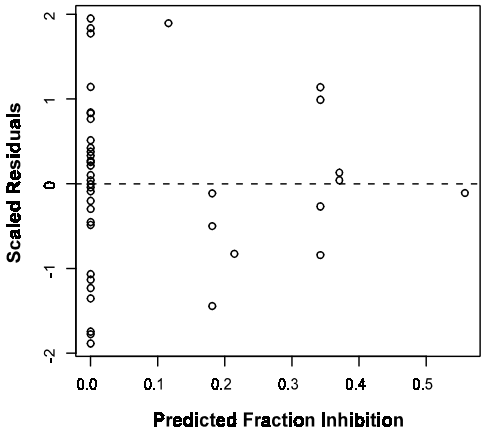


Fig. I.B-4f Scaled Residuals versus Predicted Inhibition (Expanded)



iii. Comparison of Sexes

Both male and female brain data were analyzed with the revised dose-response approach. As shown in Figure I.B-8, for 24 out of the 29 OPs, females and males were equally sensitive for cholinesterase inhibition. However, for the remaining five OPs (diazinon, dichlorvos, pirimiphos-methyl, tetrachlorvinphos, and trichlorfon), females were ~2- to 7-fold more sensitive compared to male rats. These results provide support for OPPs decision to base RPFs and PODs on the female brain cholinesterase data.

b. CELs Determined for Dermal Endpoints for OPs with Residential/Nonoccupational Exposure

Table I.B-4 lists CELs and the next higher dose levels for brain ChE inhibition from dermal exposure studies of OPs with residential/occupational exposure plus the index chemical, along with the level of ChE inhibition (compared to control values). The CELs for RBC cholinesterase inhibition are also given for comparison.

c. CELs Determined for Inhalation Endpoints for OPs with Residential/Nonoccupational Exposure

Table I.B-5 lists CELs for brain cholinesterase inhibition determined for inhalation toxicity studies for OPs with residential/nonoccupational exposure plus the index chemical, along with the level of cholinesterase inhibition (compared to control values). The CELs for RBC cholinesterase inhibition are also given for comparison.

Table I.B-2a. Exponential model parameters for female brain cholinesterase data

	log (*A*) [a]	Standard error of log (*A*)	Units of log (*A*)	l*m* [b]	Standard error of l*m*	*tB* [c]	Standard error of *tB*	*lS* [d, f]	*lD* [e, f]	Standard error of *lD*
Acephate	2.47	0.03	U/G	-1.21	0.18	-0.49	0.34	--	--	--
Azinphosmethyl	1.51	0.85	U/G	-0.72	0.15	-2.10	--	-15.00	-2.49	0.07
Bensulide	2.72	0.17	U/G	-4.36	0.16	-2.13	--	-15.00	-1.54	0.27
Chlorpyrifos	2.09	0.26	U/G	-1.81	0.23	-1.31	0.32	--	--	--
Chlorpyrifos-methyl	2.39	0.03	U/G	-3.65	0.20	0.21	0.39	--	--	--
Diazinon	7.96	0.05	U/L	-2.83	0.61	-0.18	--	--	--	--
Dichlorvos	7.20	0.06	U/L	-2.94	0.25	-2.13	--	--	--	--
Dicrotophos	2.25	0.05	U/G	1.04	0.10	-2.07	0.07	--	--	--
Dimethoate	-0.68	0.01	U/G	-0.37	0.09	-0.55	0.06	--	--	--
Disulfoton	2.68	0.03	U/G	1.35	0.19	-1.34	--	-7.35	-3.69	0.16
Ethoprop	1.97	0.04	U/G	-2.40	0.35	-0.87	0.08	--	--	--
	5.30	0.01	U/G							
	-0.40	0.12	U/G							
	-0.47	0.22	ΔpH							
Fenamiphos	2.59	0.08	U/G	-2.45	0.32	-0.40	--	--	--	--
Fenthion	2.58	0.04	U/G	-0.60	0.11	-1.54	0.16	--	--	--
Fosthiazate	2.40	0.03	U/G	-1.44	0.29	-2.19	0.12	--	--	--
Malathion	2.33	0.02	U/G	-6.91	0.23	-2.13	--	-7.35	-1.43	0.11
Methamidophos	2.68	0.05	U/G	0.46	0.11	-1.51	0.18	--	--	--
Methidathion	8.00	0.05	U/L	-0.37	0.11	-0.80	0.13	--	--	--
Methylparathion	2.32	0.18	U/G	-0.82	0.41	-2.15	--	-7.35	-1.78	0.19
Mevinphos	2.51	0.01	U/G	0.96	0.20	-0.76	0.21	--	--	--
Naled	2.18	0.14	U/G	-1.91	0.11	-1.00	0.05	--	--	--
Oxydemetonmethyl	1.97	0.44	U/G	0.34	0.03	-1.38	0.19	--	--	--
Phorate	2.91	0.03	U/mL	2.05	0.16	-2.20	--	-15.00	-1.27	0.04
Phosalone	1.92	0.09	U/G	-3.35	0.13	-2.10	--	--	--	--
Phosmet	1.79	0.92	U/G	-2.74	0.48	-2.10	--	-6.53	-2.45	0.10
Pirimiphosmethyl	3.37	0.04	ΔpH	-2.76	0.14	-0.40	--	--	--	--
Terbufos	1.56	0.34	U/G	2.97	0.69	-2.13	--	-6.53	-1.33	0.19
	0.36	0.48	ΔpH							
	2.86	0.48	U/mL							
Tetrachlorvinphos	2.49	0.07	U/G	-6.63	0.22	-1.35	--	--	--	--
	-0.26	0.14	ΔpH							
Tribufos	2.60	0.01	U/G	-2.73	0.08	-2.15	--	--	--	--
Trichlorfon	2.69	0.02	U/G	-3.49	0.40	-0.40	--	--	--	--

a. *lA* = ln*(A) and A* is the background (similar to control) AChE activity.
b. *tB* = ln$(P_B/(1-P_B))$ where P_B is the horizontal asymptote, expressed as a fraction of the background activity.
c. lm = ln (*m*) where *m* is the slope-scale factor.
d. *lS* = ln*(S)* where *S* controls the low-dose shape of the curve.
e. *lD* = ln*(D)* where *D* controls the ultimate horizontal displacement of the curve relative to the identity line (i.e., the line with *idose* = *Dose*).
f. Parameters for S and D only available for those chemicals with the expanded model

Table I.B-2b. Exponential model parameters for male brain cholinesterase data

	log (*A*) [a]	Standard error of log (*A*)	Units of log (*A*)	ln*m* [b]	Standard error of ln*m*	*tB* [c]	Standard error of *tB*	*IS* [d, f]	*ID* [e, f]	Standard error of *ID*
Acephate	2.47	0.03	U/G	-0.93	0.18	-0.45	0.34	--	--	--
Azinphosmethyl	1.65	0.85	U/G	-1.38	0.16	-2.08	--	-15.00	-2.49	0.07
Bensulide	2.74	0.16	U/G	-5.08	0.14	-2.10	--	-15.00	-1.54	0.27
Chlorpyrifos	2.06	0.26	U/G	-1.79	0.25	-1.05	0.29	--	--	--
Chlorpyrifos-methyl	2.35	0.03	U/G	-3.48	0.20	0.15	0.39	--	--	--
Diazinon	7.96	0.05	U/L	-3.80	0.62	-0.17	--	--	--	--
Dichlorvos	7.04	0.07	U/L	-3.55	0.41	-2.10	--	--	--	--
Dicrotophos	2.25	0.05	U/G	1.23	0.10	-2.03	0.08	--	--	--
Dimethoate	-0.80	0.01	U/G	-0.68	0.15	-0.63	0.13	--	--	--
Disulfoton	2.69	0.03	U/G	1.17	0.19	-1.33	--	-7.35	-3.69	0.16
Ethoprop	1.85	0.04	U/G	-2.29	0.35	-1.09	0.11	--	--	--
	5.37	0.01	U/G							
	-0.42	0.13	U/G							
	-0.44	0.22	ΔpH							
Fenamiphos	2.63	0.07	U/G	-2.23	0.33	-0.38	--	--	--	--
Fenthion	2.55	0.04	U/G	-0.34	0.11	-1.40	0.16	--	--	--
Fosthiazate	2.35	0.03	U/G	-1.72	0.29	-1.79	0.13	--	--	--
Malathion	2.32	0.02	U/G	-7.52	0.25	-2.10	--	-7.35	-1.43	0.11
Methamidophos	2.69	0.05	U/G	0.58	0.11	-1.54	0.18	--	--	--
Methidathion	7.96	0.05	U/L	-0.14	0.13	-0.53	0.14	--	--	--
Methylparathion	2.32	0.18	U/G	-0.47	0.42	-2.10	--	-7.35	-1.78	0.19
Mevinphos	2.49	0.02	U/G	0.51	0.31	-0.81	0.38	--	--	--
Naled	2.15	0.14	U/G	-1.94	0.11	-1.07	0.05	--	--	--
Oxydemeton-methyl	1.93	0.44	U/G	0.59	0.03	-1.30	0.19	--	--	--
Phorate	2.90	0.03	U/mL	0.65	0.16	-2.20	--	-15.00	-1.27	0.04
Phosalone	1.90	0.09	U/G	-3.59	0.14	-2.08	--	--	--	--
Phosmet	1.78	0.92	U/G	-3.05	0.48	-2.08	--	-6.53	-2.45	0.10
Pirimiphosmethyl	3.39	0.04	ΔpH	-3.39	0.17	-0.37	--	--	--	--
Terbufos	1.55	0.34	U/G	1.96	0.66	-2.05	--	-6.53	-1.33	0.19
	0.34	0.48	ΔpH							
	2.87	0.48	U/mL							
Tetrachlorvinphos	--	--	U/G	-8.05	7.42	-1.34	--	--	--	--
	-0.22	0.14	ΔpH							
Tribufos	2.59	0.01	U/G	-2.88	0.09	-2.08	--	--	--	--
Trichlorfon	2.67	0.02	U/G	-5.32	0.36	-0.39	--	--	--	--

a. $lA = \ln(A)$ *and A* is the background (similar to control) AChE activity.
b. $tB = \ln(P_B/(1-P_B))$ where P_B is the horizontal asymptote, expressed as a fraction of the background activity.
c. lm = ln (*m*) where *m* is the slope-scale factor.
d. $lS = \ln(S)$ where *S* controls the low-dose shape of the curve.
e. $lD = \ln(D)$ where *D* controls the ultimate horizontal displacement of the curve relative to the identity line (i.e., the line with *idose* = *Dose*).
f. Parameters for S and D only available for those chemicals with the expanded model

Figure I.B-5. Plot of horizontal asymptotes with 95% confidence limits for 29 OPs (red = females; blue = males)

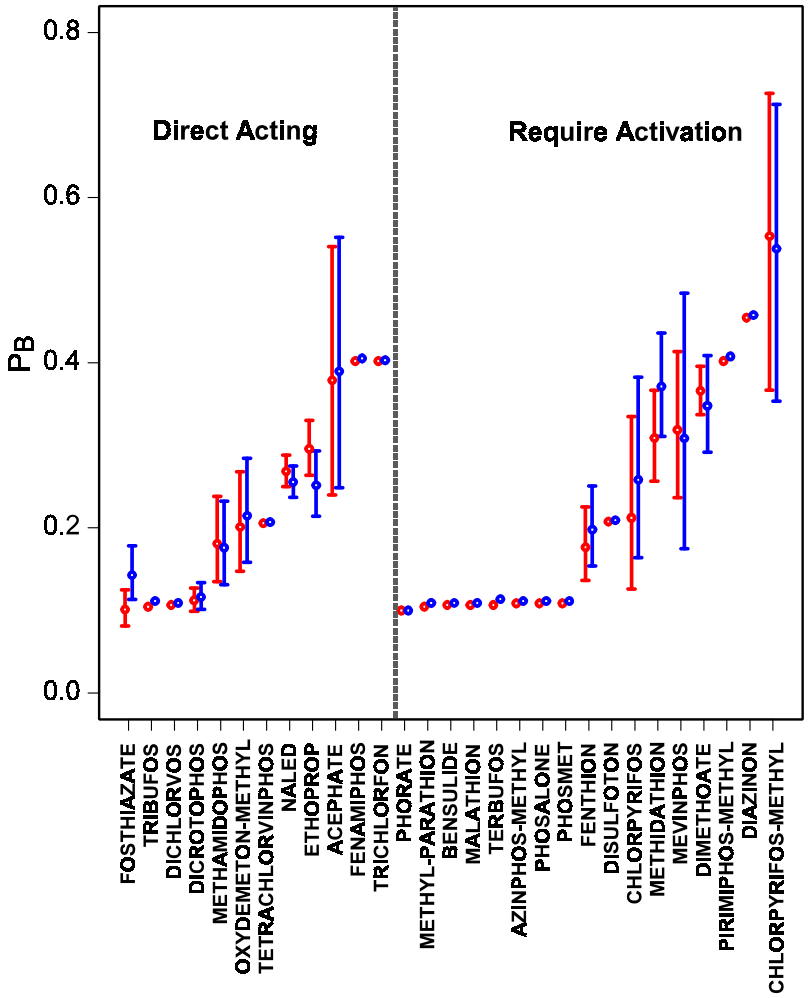


Figure I.B-6. BMD_{10}s (mg/kg/day) for female brain ChE activity for 29 OPs

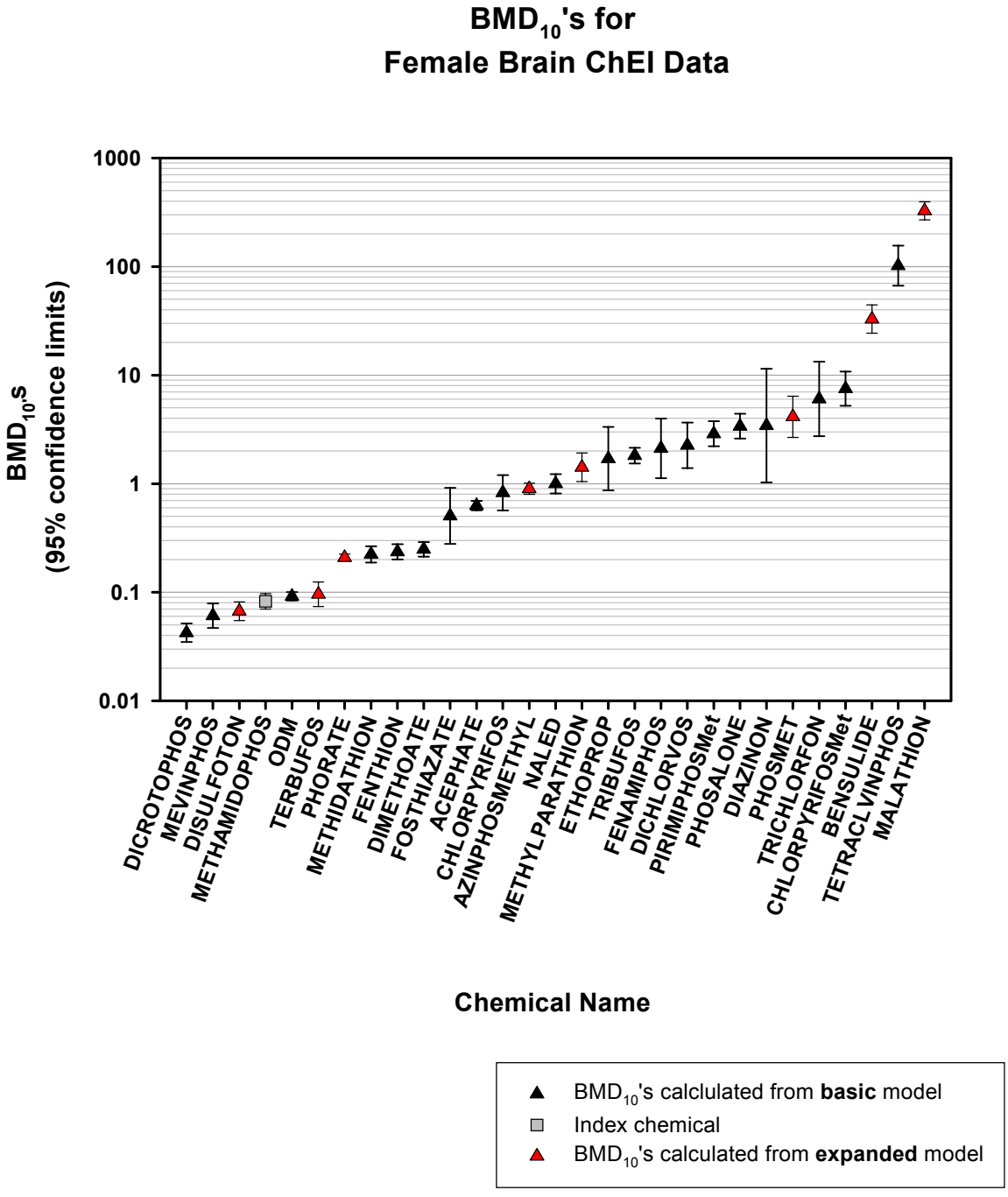


Figure I.B-7. BMD_{10}s (mg/kg/day) for male brain ChE activity for 29 OPs

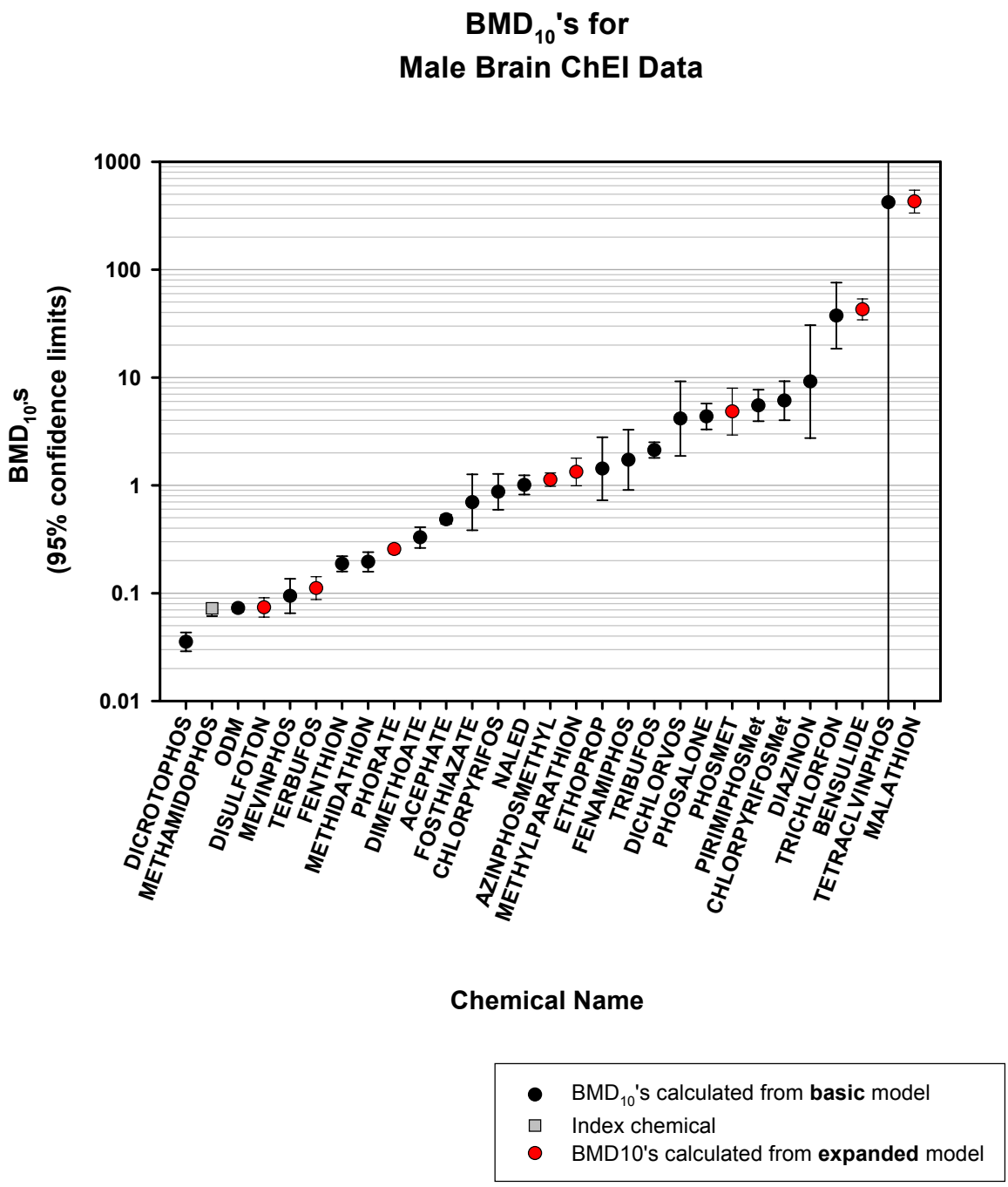


Table I.B-3. Oral BMD_{10}s and BMDLs (mg/kg/day) estimated for brain ChE activity

Chemical name	Female		Male	
	BMD_{10}	BMDL	BMD_{10}	BMDL
Acephate	0.63	0.57	0.48	0.44
Azinphos-methyl	0.90	0.80	1.13	0.98
Bensulide	32.85	24.32	42.70	34.18
Chlorpyrifos	0.83	0.57	0.87	0.59
Chlorpyrifos-methyl	7.51	5.23	6.09	4.02
Diazinon	3.43	1.03	9.16	2.74
Dichlorvos	2.25	1.39	4.15	1.87
Dicrotophos	0.04	0.03	0.04	0.03
Dimethoate	0.25	0.21	0.33	0.26
Disulfoton	0.07	0.05	0.07	0.06
Ethoprop	1.70	0.87	1.42	0.73
Fenamiphos	2.11	1.12	1.72	0.90
Fenthion	0.24	0.20	0.19	0.16
Fosthiazate	0.50	0.28	0.70	0.38
Malathion	326.37	269.66	427.30	335.23
Methamidophos	0.08	0.07	0.07	0.06
Methidathion	0.22	0.19	0.20	0.16
Methyl-parathion	1.41	1.05	1.33	0.99
Mevinphos	0.06	0.05	0.09	0.07
Naled	1.00	0.81	1.01	0.82
ODM	0.09	0.08	0.07	0.07
Phorate	0.21	0.19	0.26	0.23
Phosalone	3.38	2.60	4.34	3.29
Phosmet	4.13	2.67	4.83	2.93
Pirimiphos-methyl	2.88	2.21	5.50	3.93
Terbufos	0.10	0.07	0.11	0.09
Tetrachlorvinphos	101.92	66.64	420.97	0.0002
Tribufos	1.81	1.54	2.12	1.79
Trichlorfon	6.03	2.74	37.35	18.44

Figure I.B-8. Comparison of relative potencies for brain ChE activity measured in female and male rats

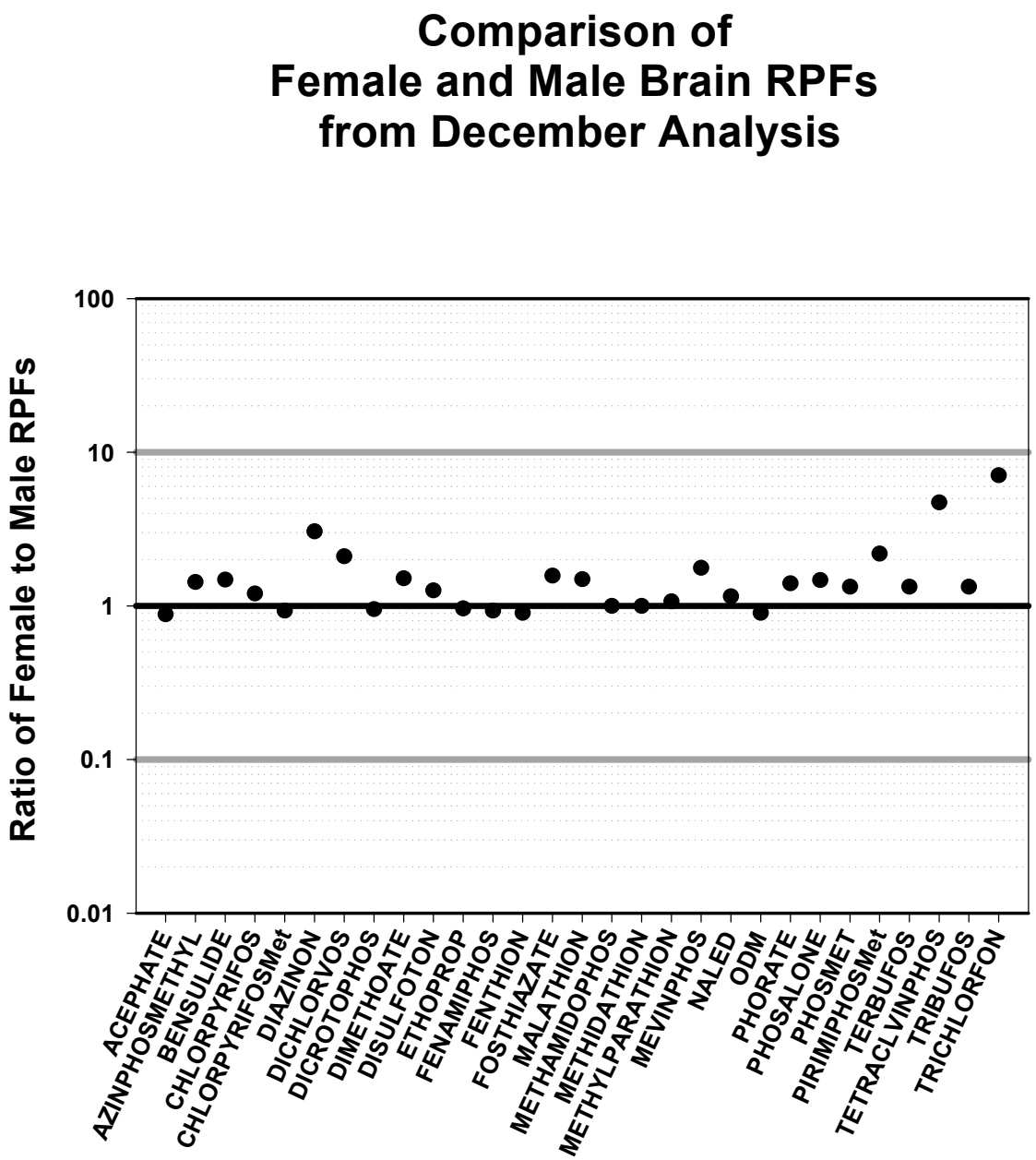


Table I.B-4. CELs for brain and RBC cholinesterase activity from dermal exposure studies (% cholinesterase inhibition compared to control value)

Chemical	Species	Male RBC CEL mg/kg/day	Male RBC Next Higher Dose mg/kg/day	Female RBC CEL mg/kg/day	Female RBC Next Higher Dose mg/kg/day	Male Brain CEL mg/kg/day	Male Brain Next Higher Dose mg/kg/day	Female Brain CEL mg/kg/day	Female Brain Next Higher Dose mg/kg/day
Acephate	rat	300 9%	>300 * 9%	300 13%	>300 * 13%	300 9%	>300* 9%	**300** **14%**	>300* 14%
Bensulide	rat	500 4%	>500 * 4%	500 2%	>500 * 2%	500[a] 0-9%	>500*[a] 0-9%	**500[a]** **2-10%**	>500*[a] 2-10%
Dichlorvos	Dermal exposure study waived due to volatility of compound.								
Disulfoton	rabbit	0.8 4%	1.0 18%	0.8 16%	1.0 21%	1.6 7%	3 55%	**1.6** **8%**	3 27%
Fenamiphos	rabbit	2.5 0%	10 32%	2.5 0%	10 38%	10 * 0%	>10 * 0%	**0.5** **0%**	2.5 18%
Fenthion	rabbit	100 0%	150 56%	100 0%	150 17%	100 13%	150 65%	**50** **13%**	100 24%
Malathion	rabbit	50 0%	300 17%	50 8%	300 26%	300[a] 2%	1000[a] 65%	**50[a]** **0%**	300[a] 19%
Methamidophos	rat	0.75 6%	11.2 55%	0.75 1%	11.2 46%	0.75 0%	11.2 41%	**0.75** **5%**	11.2 38%
Naled	rat	10 0%	20 21%	10 7%	20 25%	10 0%	20 60%	**10** **0%**	20 60%
Tetrachlorvinphos	rat	1000 0%	>1000* 0%	1000 0%	>1000* 0%	1000 0%	>1000 * 0%	**1000** **0%**	>1000 * 0%
Trichlorfon	rabbit	100 6%	300 25%	100 0%	300 19%	1000 0%	>1000 * 0%	**100** **4%**	300 18%

* Highest dose tested.

Table I.B-5. CELs for brain and RBC cholinesterase activity from inhalation toxicity studies (% cholinesterase inhibition compared to control value)

Chemical	Method	RBC				Brain			
		Male CEL (mg/kg/day)	Male Next higher dose (mg/kg/day)	Female CEL (mg/kg/day)	Female Next higher dose (mg/kg/day)	Male CEL (mg/kg/day)	Male Next higher dose (mg/kg/day)	Female CEL mg/kg/day	Female Next higher dose (mg/kg/day)
Acephate	nose only	1.419 13%	>1.419* 13%	1.492 9%	>1.492* 9%	1.419 14%	1.419* 14%	**1.492** **13%**	1.492* 13%
Bensulide	No inhalation toxicity study was available for bensulide								
Dichlorvos	whole body	0.044 0%	0.436 28%	0.046 12%	0.458 31%	0.436 10%	0.436 10%	**0.458** **11%**	0.458 11%
Disulfoton	nose only	0.044 0-2%	0.384 22-28%	0.047 5-11%	0.410 26-34%	0.044 4%	0.384 24%	**0.047** **5%**	0.410 28%
Fenamiphos	nose only	0.928 8%	>0.928* 8%	0.070 5%	0.984 17%	0.928 0%	>0.928* 0%	**0.984** **0%**	>0.984* 0%
Fenthion	No inhalation toxicity study was available for fenthion								
Malathion	whole body	25.56 9%	115 22%	26.88 11%	121 27%	115 3%	514 17%	**121** **8%**	540 41%
Methamidophos	head/ nose	0.292 0-8%	1.432 2-25%	0.31 0 0-11%	1.520 8-28%	0.292 8%	1.432 29%	**0.310** **11%**	1.520 25%
Naled	whole body	0.354 0-11%	1.594 70-81%	0.067 2%	0.378 17-25%	0.354 0%	1.594 38%	**0.378** **4%**	1.702 46%
Tetrachlorvinphos	No inhalation toxicity study was available for tetrachlorvinphos.								
Trichlorfon	whole body	9.388 0%	27.44 24%	3.574 0%	9.96 20%	9.388 0%	27.44 21%	**3.574** **0%**	9.96 27%

*Highest dose tested.

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

Table I.B-6 lists the PODs and NOAELs for the oral, dermal, and inhalation routes for methamidophos. The PODs for all three routes were calculated with dose-response modeling using the basic model of Equation I.B-2. OPP has used these endpoints in the Preliminary OP Cumulative Risk Assessment.

Brain cholinesterase was only measured once (at study termination) in the methamidophos 21-day dermal and 90-day inhalation studies. Therefore only one data set was available for calculation of the PODs for these routes.

Within route of exposure, the BMD_{10}s for brain cholinesterase shown in Table I.B-6 were similar for males and females. The values of the BMDLs were close to the BMD_{10}s. This observation increases the confidence not only in the selection of methamidophos as the index chemical but also the utilization of the central estimate of the female data (BMD_{10}) for cumulative risk extrapolation rather than its lower limit (BMDL). It is notable that the BMD_{10} 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 I.B-6. Points of departure for index chemical (methamidophos) by route of exposure for brain cholinesterase activity measured in female and male rats

Route of Administration	Sex	BMD_{10} (mg/kg/day)	BMDL (mg/kg/day)	NOAELs (mg/kg/day)
Oral[a]	F	**0.08** [d]	0.07	0.03*
	M	0.07	0.06	
Dermal[b]	F	**2.12** [d]	1.77	0.75
	M	1.88	1.41	
Inhalation[c]	F	**0.39** [d]	0.21	0.31
	M	0.30	0.20	0.29

[a] MRID nos. 41867201, 43197901, 00148452

[b] MRID no. 44525301

[c] MRID no. 41402401

[d] PODs for Preliminary Cumulative Risk Assessment of OPs.

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

e. Relative Potency Factors (RPFs)

Table I.B-7 provides the RPFs for the oral, dermal, and inhalation routes of exposure based on brain cholinesterase in female rats which were used in the Preliminary Cumulative Risk Assessment for OPs. Figure I.B-9 shows the oral RPFs with 95% confidence limits. Due to the narrow confidence limits on the BMD_{10} for methamidophos, the appearance of Figure I.B-9 is similar to Figure I.B-6.

These values were calculated with Equations I.B-6, I.B-7, and I.B-8 for oral, dermal, and inhalation routes, respectively, and using methamidophos as the index chemical. BMD_{10}s for all of the chemicals are listed in Table I.B-3. Dermal and inhalation CELs are given in Tables I.B-4 and I.B-5. It should be noted that the oral RPFs for phostebupirim and profenofos were assigned a value of 25. Model-derived RPFs were not determined for these two OPs because they were not found to be significant contributors to risk from water in their individual REDs. It should be further noted that a RPF value of 25 is an overestimation of these two OP's toxic potencies Although a model-derived oral RPF was determined for fosthiazate, this is a new OP that is not yet registered. Fosthiazate has no appropriate monitoring data to support characterization of exposure from food, and therefore, was not included in the quantification of cumulative risk.

4. Comparison of relative potency factors from July and December analyses

a. RPFs Derived From Different Methodology: Analysis of Discreet Data Sets vs. Joint Analysis

Based on the September 2001 SAP recommendations, OPP has revised the dose-response analysis conducted in July. The revised statistical analyses of RPFs and PODs are available for the brain data but not the RBC oral data. A comparison of the BMD_{10}s from the July 2001 analysis for female brain cholinesterase inhibition with the current revised female brain RPFs indicates that the revised statistical methods, while providing a scientifically refined use of statistical methodology, had very little impact on the July BMD_{10} values as the September 2001 SAP anticipated.

As shown in Figure I.B-10, 20 of the 29 OPs had similar RPFs for female brain when the July and revised values were compared. The potency of trichlorfon is greater in the present analysis compared to the July 2001 methods. The eight remaining OPs were modeled using the expanded model with incorporated variables to describe a low dose flat region. OPP believes the potency of these eight OPs was overestimated in the July analysis.

Figure I.B-9. Oral relative potency factors for the inhibition of female brain cholinesterase activity of OPs (error bars are 95% confidence limits)

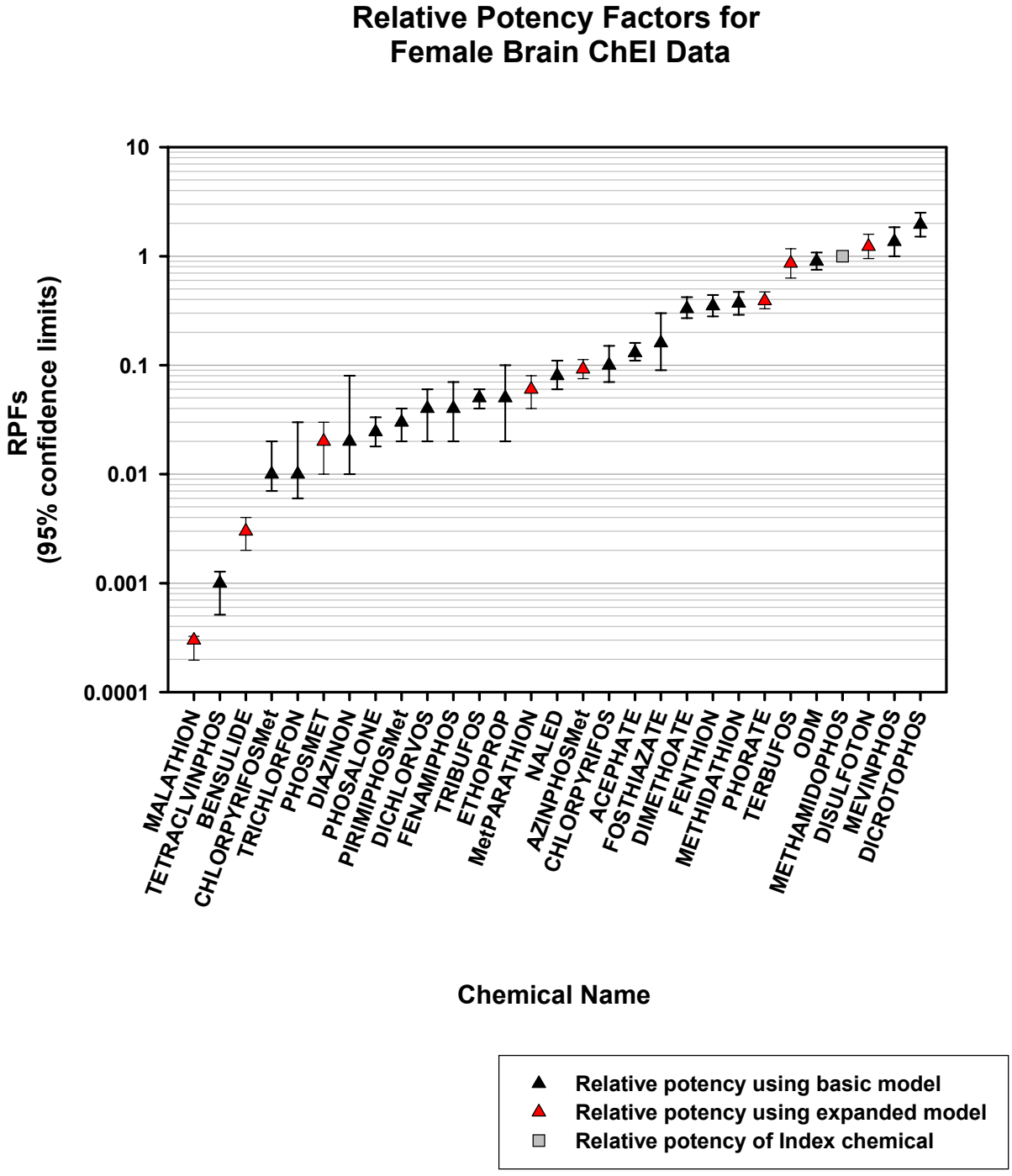
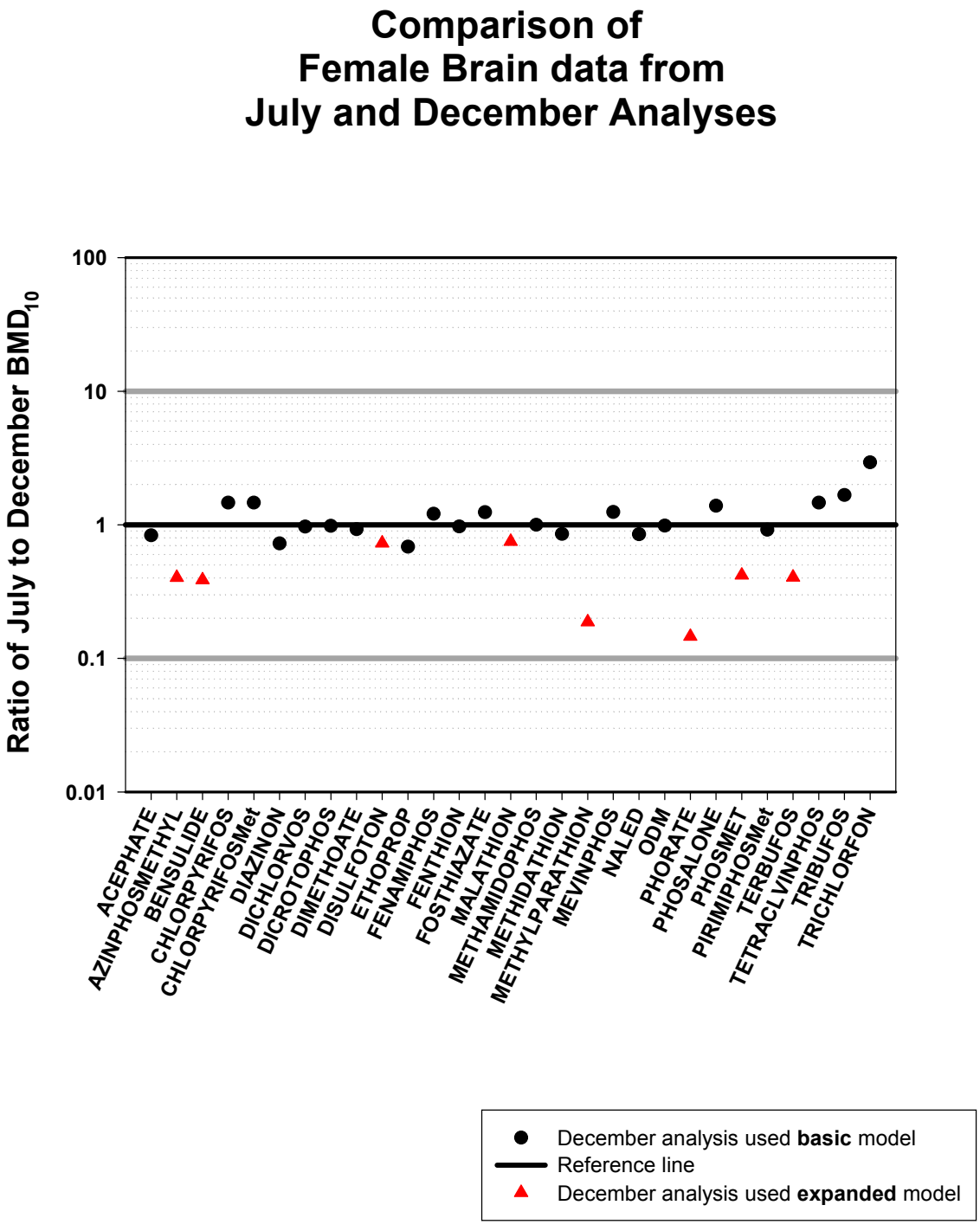


Table I.B-7. Relative potency factors for the oral, dermal, and inhalation routes of exposure

Chemical	Oral	Dermal	Inhalation
RPFs based on brain cholinesterase activity measured from female rats.			
Acephate	0.13	0.0025	0.208
Azinphos-methyl	0.092		
Bensulide	0.003	0.0015	
Chlorpyrifos	0.10		
Chlorpyrifos-methyl	0.012		
Diazinon	0.024		
Dichlorvos	0.037		0.677
Dicrotophos	1.95		
Dimethoate	0.33		
Disulfoton	1.23	0.47	6.596
Ethoprop	0.049		
Fenamiphos	0.039	1.5	0.315
Fenthion	0.35	0.015	
Fosthiazate	0.16		
Malathion	0.0003	0.015	0.003
Methamidophos	1.00	1.00	1.000
Methidathion	0.37		
Methyl-parathion	0.058		
Mevinphos	1.36		
Naled	0.083	0.075	0.820
ODM	0.90		
Phorate	0.39		
Phosalone	0.024		
Phosmet	0.020		
Pirimiphos-methyl	0.029		
Terbufos	0.84		
Tetrachlorvinphos	0.0008	0.00075	
Tribufos	0.045		
Trichlorfon	0.014	0.0075	0.087

Figure I.B-10. Comparison of BMD_{10}s using the present analysis and the July 2001 analysis for female brain cholinesterase inhibition



b. RPFs Proposed for Extrapolation of Cumulative Risk: Male RBC Database vs. Female Brain Database

i. Brain Acetylcholinesterase Inhibition Dose-Response Data show Tighter Confidence Intervals Compared to RBC ChEI

As stated in the July 2001 analysis, the brain cholinesterase activity data had limitations compared to the blood data mainly because brain cholinesterase activity was generally determined at terminal sacrifice, and thus time course information was rarely available. On the other hand, the relative potency estimates based on the brain data generally have tighter confidence limits compared to the RBC potency estimates (see Figures I.B-11-14). Mathematically, statistical values derived from larger datasets tend to have smaller confidence intervals than values derived from smaller datasets. It is noteworthy that the confidence intervals calculated for the brain-based RPFs from July 2001 and the present analysis are tighter than the confidence intervals calculated for RBC-based RPFs even though two to three times more RBC ChE data are available compared to brain ChE data. *Relative potencies and PODs based on the brain ChEI have tighter confidence intervals and less uncertainty than those developed using RBC ChEI data and, therefore, confer less uncertainty onto cumulative risk estimates*.

ii. Brain Cholinesterase is Concordant with RBC Cholinesterase

As described above, the brain RPFs from the July 2001 analysis are similar to the current brain RPFs for most of the OPs. Based on this observation, it was assumed that the methodology used in the present analysis would not significantly change the RPF values derived from RBC ChEI data in July 2001. Therefore, it is reasonable to compare the RBC RPF values from the July 2001 analysis with the brain RPFs from the present analysis to evaluate the relative sensitivity of these two proposed sets of RPFs (Figure I.B-15 and Table I.B-8)

This figure shows that 12 of the 29 OPs had very similar RPFs for RBC and brain RPFs (less than 2-fold). Another 9 OPs had only very slight differences (~ 2-fold to 3-fold). As shown by overlapping error bars for 18 of 21, these slight differences most likely represent experimental variability and error; and, thus are not likely due to differences in sensitivity between the RBC and brain for cholinesterase inhibition. Therefore, 21 of the 29 OPs have comparable potencies for RBC and brain ChE.

Figure I.B-11. Relative potency factors from the July 2001 analysis for the inhibition of female brain cholinesterase activity of OPs (error bars are 95% confidence limits)

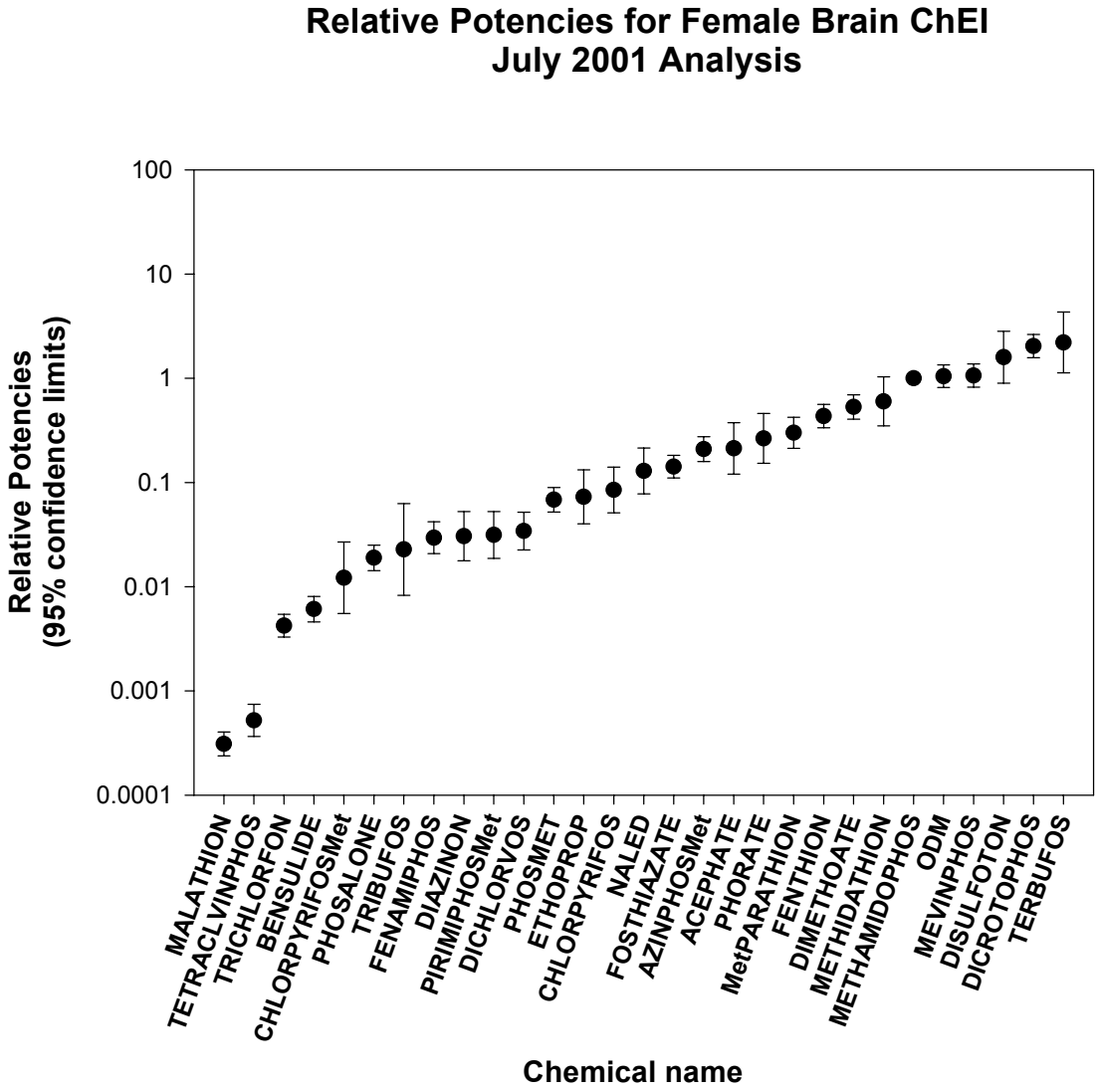


Figure I.B-12. Relative potency factors from the July 2001 analysis for the inhibition of female RBC cholinesterase activity of OPs (error bars are 95% confidence limits)

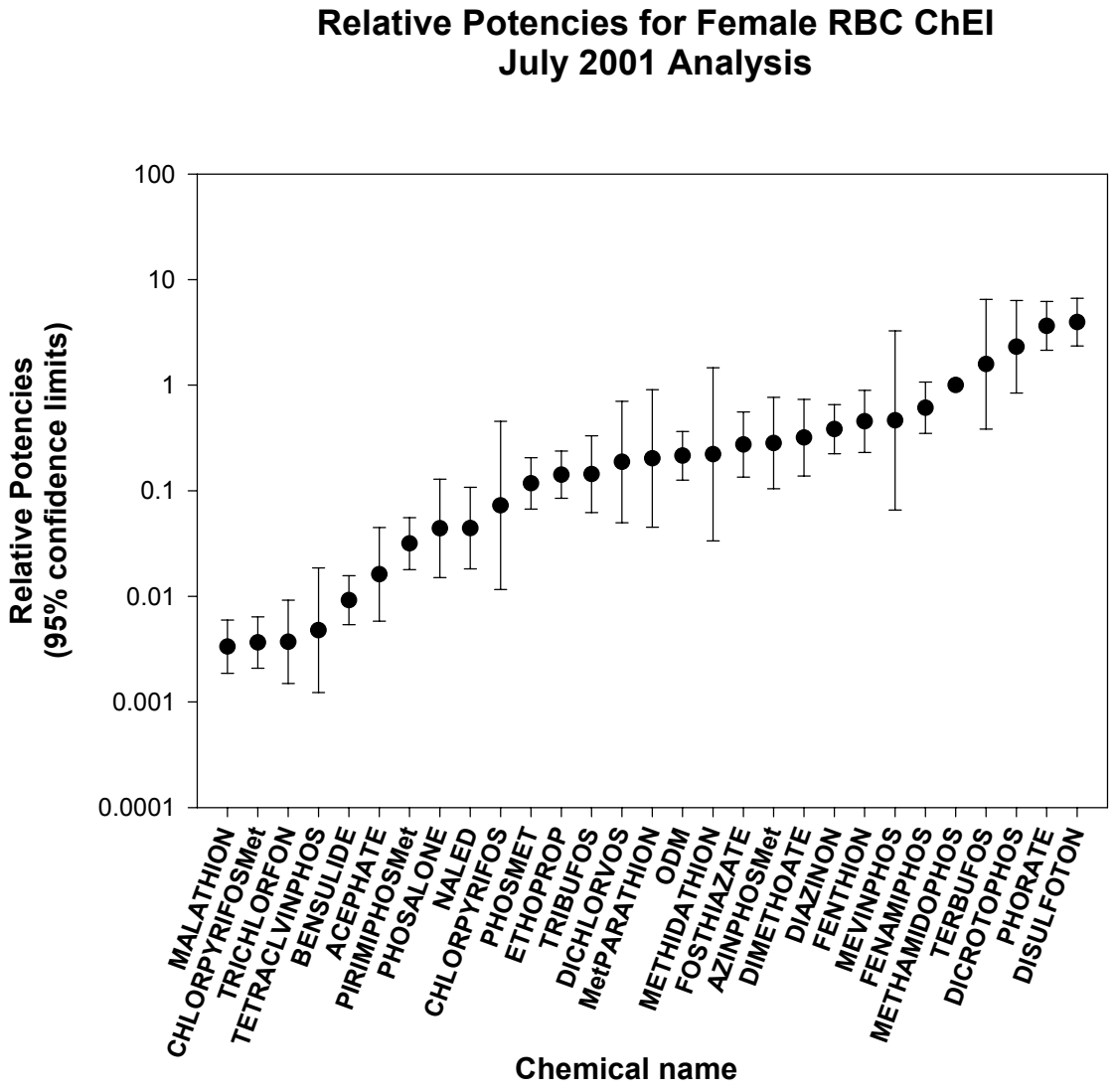


Figure I.B-13. Relative potency factors from the July 2001 analysis for the inhibition of male brain cholinesterase activity of OPs (error bars are 95% confidence limits)

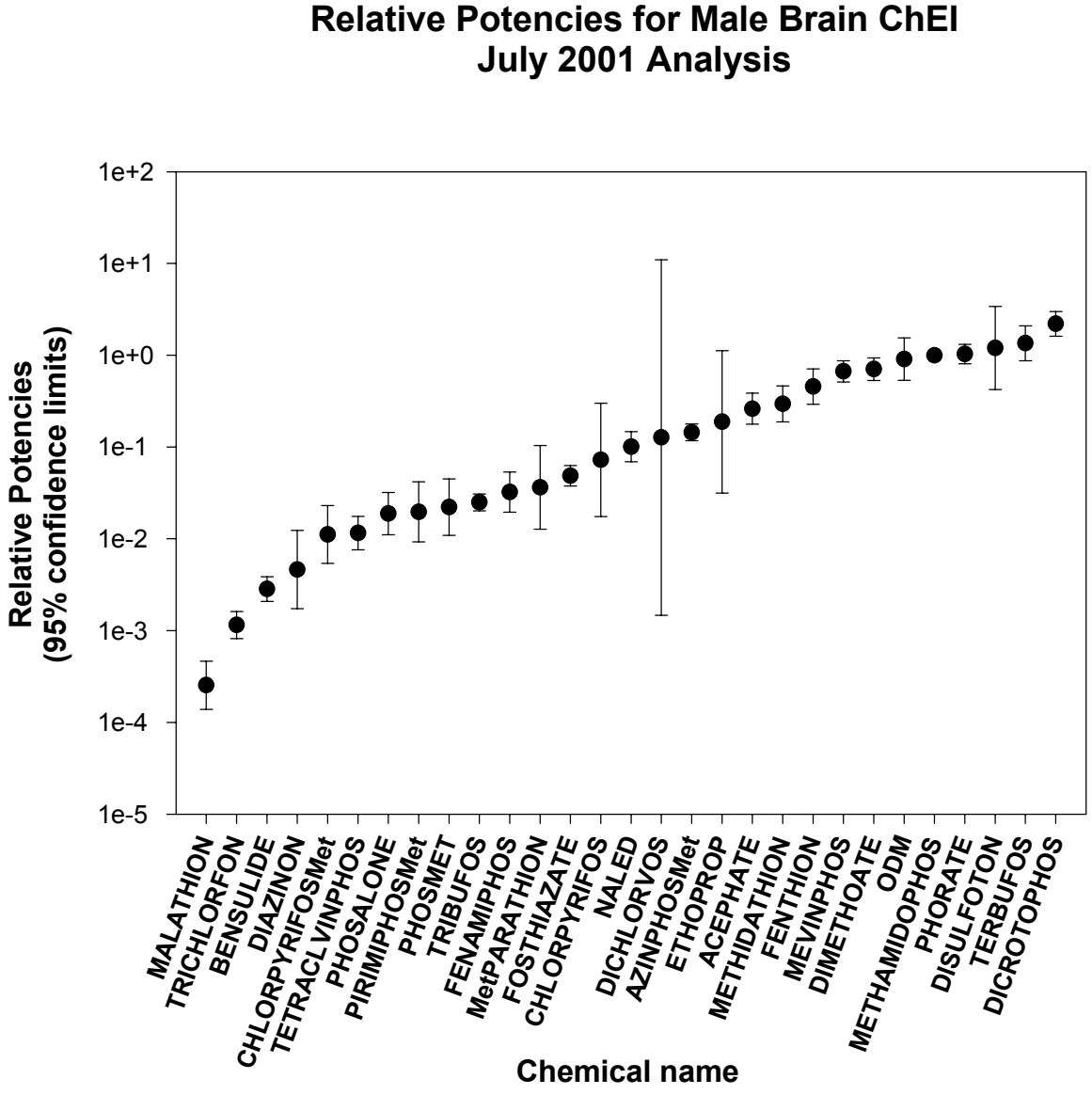
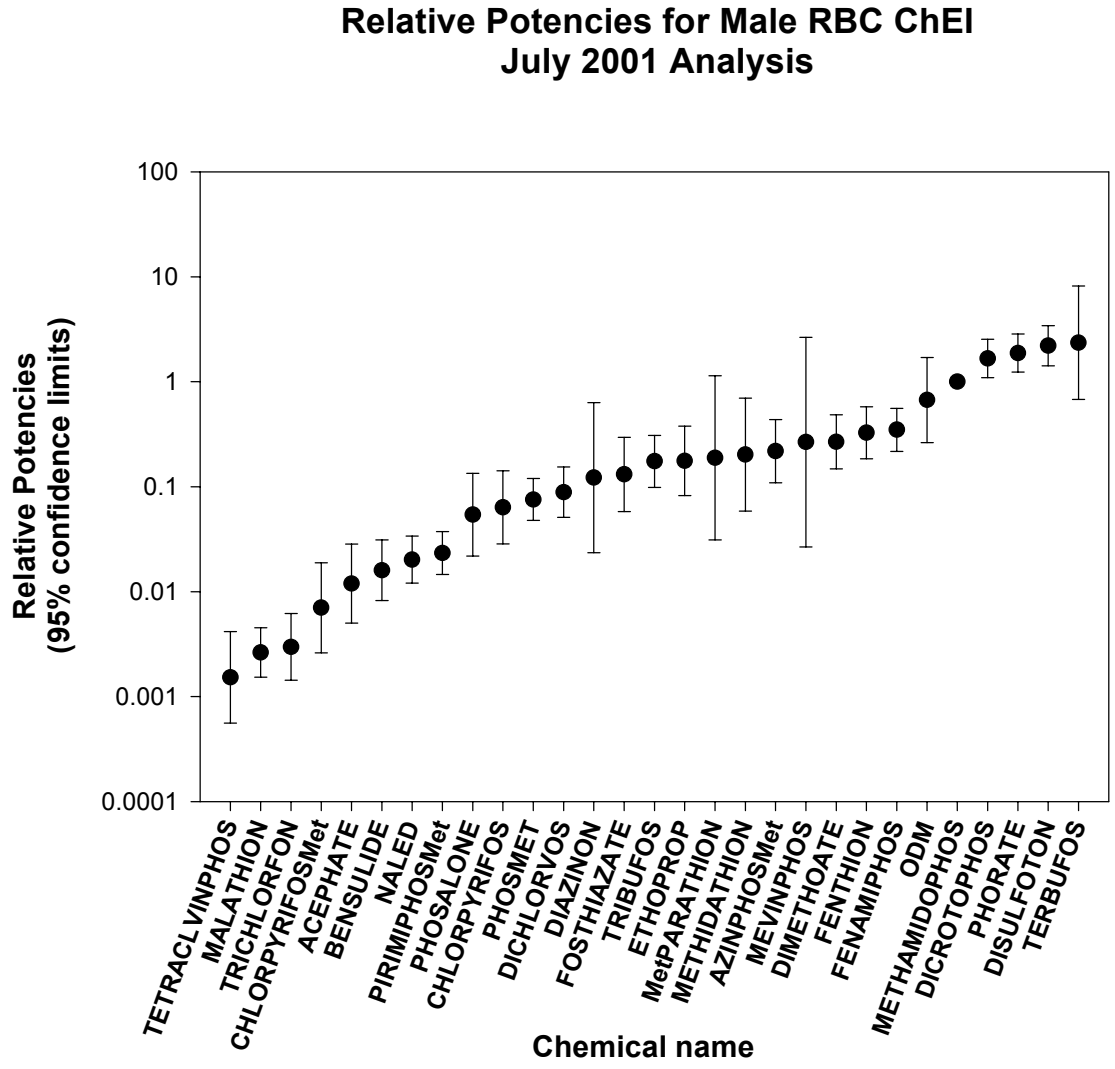


Figure I.B-14. Relative potency factors from the July 2001 analysis for the inhibition of male RBC cholinesterase activity of OPs (error bars are 95% confidence limits)



There are eight pesticides (acephate, diazinon, fenamiphos, malathion, mevinphos, methidathion, naled, and tribufos) with potential four to ten fold differences between RBC and brain RPFs. For these OPs where one compartment appeared to be more sensitive than another, it is important to characterize the difference.

The RPFs derived from male RBC ChEI data are more sensitive compared to the RPFs from female brain ChEI for diazinon, malathion, fenamiphos, and tribufos. In the present analysis, malathion data were modeled using the expanded model and were overestimated in the July 2001 analysis. Malathion was the least potent OP included in the analysis, and the results indicate that the RPF was approximately 300-fold less potent for RBC ChEI and approximately 3,000-fold less potent for brain ChEI compared to the index compound. Using either the brain or RBC RPF for malathion would have little if any impact on the total cumulative risk estimates.

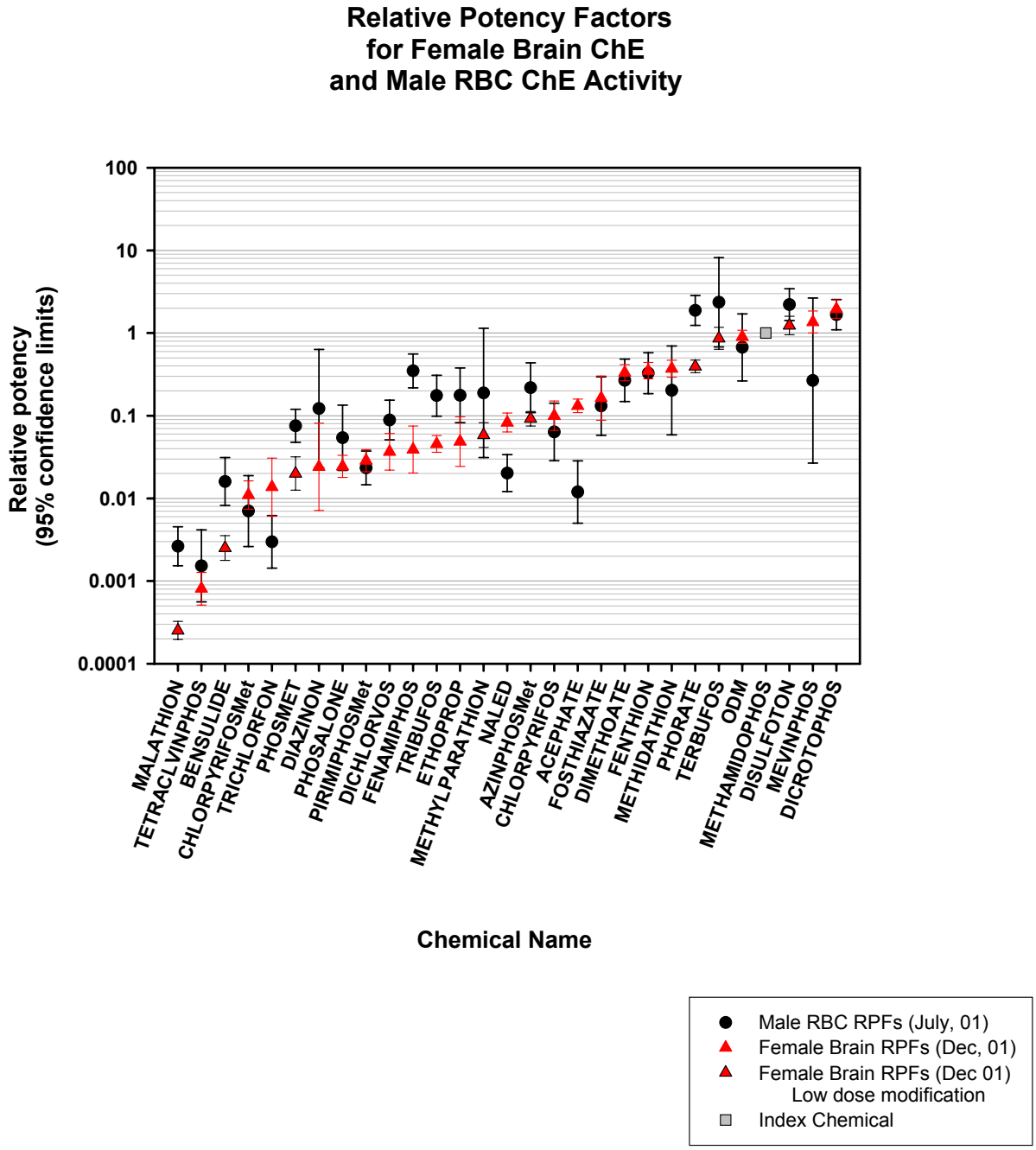
Tribufos and diazinon cannot be easily discounted because of a minimal hazard potential for cholinesterase inhibition. However, tribufos does not have residential uses and is only used as a defoliate on cotton seed. Given that individuals are exposed only to highly refined and processed and blended cotton seed oil, its exposure potential is very low. The residential uses of diazinon are being phased out as well as many of its agricultural uses. The potency of fenamiphos was only three-fold less than the index compound, based on RBC ChEI but was ~35-fold less potent based on brain ChEI. The only residential/nonoccupational exposure to fenamiphos is on golf courses and fenamiphos is only applied to golf courses by professional applicators. Although fenamiphos is used on a number of agricultural commodities, it has few detections in PDP. Thus dietary exposure to fenamiphos residues is not expected to be high. Because of limited exposure potential, using either the brain or RBC RPF for diazinon, fenamiphos, and tribufos would have little impact on the total cumulative risk estimates.

Alternatively, the RPFs derived from female brain ChEI data are more sensitive compared to the RPF from male RBC ChEI for mevinphos, methidathion, acephate, and naled. Mevinphos is among the most potent OPs for both RBC and brain potency estimates. Dietary exposure to mevinphos is very low because the only existing tolerance is an import tolerance on bananas. Methidathion does not have many detects in PDP. Dietary risk to acephate and naled could be underestimated for these two OPs because both pesticides are approved for agricultural uses on numerous commodities.

Table I.B-8. RPFs for the OPs considered in the Preliminary Cumulative Risk Assessment

Chemical	July RBC (m) Male	December Brain (BMD) Female
Acephate	0.01	0.13
Azinphos-methyl	0.22	0.092
Bensulide	0.02	0.003
Chlorpyrfos-methyl	0.007	0.012
Chlorpyrifos	0.06	0.10
Diazinon	0.12	0.024
Dichlorvos	0.09	0.037
Dicrotophos	1.67	1.95
Dimethoate	0.27	0.33
Disulfoton	2.21	1.23
Ethoprop	0.18	0.049
Fenamiphos	0.35	0.039
Fenthion	0.33	0.35
Fosthiazate	0.13	0.16
Malathion	0.003	0.0003
Methamidophos	1.0	1.0
Methidathion	0.20	0.37
Methyl-parathion	0.19	0.058
Mevinphos	0.27	1.36
Naled	0.02	0.083
Oxydemeton-methyl	0.67	0.90
Phorate	1.88	0.39
Phosalone	0.05	0.024
Phosmet	0.08	0.020
Pirimiphos-methyl	0.02	0.029
Terbufos	2.36	0.84
Tetrachlorvinphos	0.002	0.0008
Tribufos	0.17	0.045
Trichlorfon	0.003	0.01

Figure I.B-15. Comparison of RPFs for female brain cholinesterase inhibition from the present analysis and RPFs for male RBC cholinesterase inhibition from the July 2001 analysis



iii. Inhalation and Dermal RPFs

As shown in Table I.B-9, the RPFs for the dermal and inhalation routes are similar for the RBC and brain compartments for most of the OPs with nonoccupational/residential exposure potential.

For the dermal route, based on RPFs, disulfoton was two-fold less potent based on the brain ChEI RPF. Both fenamiphos and fenthion appeared more potent (5-fold and 2-fold, respectively) based on the brain ChE RPF

For the inhalation route, brain and RBC RPFs were equally sensitive for all the OPs with nonoccupational/ residential exposure potential except for dichlorvos, malathion, and trichlorfon. Trichlorfon is more potent with the brain RPF than the RBC RPF. As remarked for the oral RPFs, malathion has low potency compared to the OPs and using either the brain or RBC RPF for malathion would have little if any impact to the total cumulative risk estimates. Conversely, dichlorvos was among the more potent OPs with nonoccupational/residential exposure via the inhalation route and has high exposure potential.

Table 1.B-9. Comparison of relative potency factors for dermal and inhalation routes of exposure based on RBC and brain data

CHEMICAL	RPF based on Male RBC ChE Activity [July 2001]		RPF based on Female brain ChE Activity [December 2001]	
	Dermal	Inhalation	Dermal	Inhalation
Acephate	0.002	0.18	0.0025	0.208
Bensulide	0.001	N/A	0.0015	N/A
Dichlorvos	N/A	6.64	N/A	0.677
Disulfoton	0.94	6.25	0.47	6.596
Fenamiphos	0.30	0.29	1.5	0.315
Fenthion	0.0075	N/A	0.015	N/A
Malathion	0.02	0.01	0.015	0.003
Methamidophos	1.00	1.00	1.00	1.00
Naled	0.075	0.78	0.075	0.820
Tetrachlorvinphos	0.0008	N/A	0.00075	N/A
Trichlorfon	0.007	0.028	0.0075	0.087

N/A not available

iv. Points of Departure for Brain Cholinesterase Compared to RBC Cholinesterase

As shown in Table I.B-10, the PODs calculated for methamidophos were similar for the brain and RBC compartments. The only exception is BMD_{10} for brain and RBC ChE measured in female rats from the inhalation study (0.39 vs. 2.24 mg/kg/day).

Table I.B-10. Points of departure for index chemical (methamidophos) by route of exposure for brain and RBC cholinesterase activity measured in female and male rats

Route of Administration	Sex	Brain		RBC	
		BMD_{10}	BMDL	BMD_{10}	BMDL
Oral	F	**0.08**	0.07	0.09	0.07
	M	0.07	0.06	0.07	0.05
Dermal	F	**2.12**	1.77	1.71	1.27
	M	1.88	1.41	1.21	0.91
Inhalation	F	**0.39**	0.21	2.24	1.74
	M	0.30	0.20	0.87	0.62

5. Discussion

a. Determination of Relative Potency

With the passage of the FQPA in 1996, EPA was faced with numerous challenges such as the reassessment of 66% of all tolerances by 2002 and notably the development of methodology for doing cumulative risk assessment. As part of the methodology development, EPA has participated in the public process with technical briefings and reviews by outside experts who make up the SAP. The SAP has offered constructive and thoughtful guidance in the development of the hazard and dose-response component of cumulative risk assessment. With each review, EPA has taken the recommendations into consideration and has made appropriate revisions or refinements. The combined results of the July 2001 analysis and the current analysis represent an innovative and novel approach to hazard and dose-response assessment, and by taking advantage of the large database of oral toxicity studies available to OPP, offer a comprehensive review of the common mechanism endpoint (i.e., cholinesterase inhibition) from available toxicity studies. By incorporating dose-response information from multiple studies into one estimate of potency for the oral route, potency estimates are representative of the overall toxicity of each pesticide.

The OPs have been analyzed using two distinct yet complementary dose-response analyses. In the July analysis, potency was estimated for each dataset and each toxicity study for three different biological compartments which allowed OPP to investigate both study-to-study variability and compartment variability. The July analysis also allowed qualitative observation of time course data and steady state response. The present joint analysis concentrated on a single compartment, brain. This joint analysis generated single estimates of the horizontal asymptote and allowed the exploration of low dose issues. The strong similarity in the potency estimates from the two different methods and using two different measures of potency (i.e., slope-scaling factor and BMD_{10}) increase the confidence in the determinations and importantly decreases uncertainty in the overall cumulative risk assessment. The data for the inhalation and dermal routes were less extensive. Potency estimates using CELs from the dermal and inhalation studies are not as robust as those calculated for the oral route but are adequate for use in the cumulative assessment. It is also notable that the relative order of estimated potencies for all three routes of exposure are consistent with current knowledge about their toxicology. The selection of methamidophos as the index chemical was supported by the SAP. Methamidophos had the highest quality database for the common mechanism endpoint in three routes of exposure and three biological compartments. The PODs calculated with methamidophos have narrow confidence limits which reduces overall uncertainty in the cumulative risk assessments. In this assessment, administered dose was used to estimate RPFs and PODs. At this time there are inadequate pharmacokinetic data for these OPs to incorporate information about dose at the target site or species to species extrapolation.

b. 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 2001a). The SAP (FIFRA SAP, 2001c) 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 available on individual chemicals were usually not designed to address the issue of dose additivity.

The mathematical definition of dose addition requires a constant proportionality among the effectiveness of the chemicals (USEPA 2001c; Hertzberg et al.,1999). Thus, an important objective in the dose response assessment is to evaluate whether dose-response relationships are consistent with the assumption of dose additivity. There is some uncertainty surrounding the assumption. Two different versions of the exponential model have been used in this assessment. Eight OPs were fit using a model with a flat low dose region while the remaining 21 were fit using a model which is linear in the low dose region. In addition, the OPs did not exhibit a common horizontal asymptotes (P_B); rather the P_Bs vary among chemicals. Both of these factors indicate that the dose-response curves are not parallel.

Dose additivity assumes that the common mechanism chemicals behave in a similar fashion (i.e., same pharmacokinetics and pharmacodynamics). 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 (Mileson 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 at low levels of exposure anticipated to be encountered environmentally.

The application of dose additivity requires the assumption of no interactions other than additive among the chemicals at low doses. There are 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, 1960; Singh, 1986; Mahajna et al., 1997; Serat and Bailey, 1974; Richardson, et al., 2001; Karanth et al., in press; Abu-Qare , et al., 2001a; Abu-Qare et al., 2001b). Most of the studies reviewed were high dose studies that investigated the acute lethality (LD_{50}) 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 OPs will result in significant departure from dose addition at low doses. 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 OPs 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. At this time, there is not sufficient basis to depart from dose additivity.

Although a biological or pharmacokinetic modeling approach would be preferred to determine the cumulative risk for these OPs, the input parameters for such an approach are not available. Thus, the pharmacokinetic (PK) characteristics of the OPs could not be incorporated in the dose-response assessment which would allow for a more refined estimate of the combined risk to humans. Therefore, OPP has applied simple dose addition and used an empirical curve fitting model (i.e., the exponential model) to determine RPFs and PODs.

c. Future Directions in Cumulative Dose-Response Assessment: Physiologically Based Pharmacokinetic (PBPK) Modeling

Physiologically based pharmacokinetic [PBPK] models, which describe the time course disposition of chemicals and their metabolites, are well suited to help assess cumulative risk. PBPK models are excellent tools to quantify the cumulative toxicity that can result from multiple exposures (multiple exposures and multiple pathways) and from exposure to multiple chemicals with a common mechanism or mode of action. These models typically are systems of first order differential equations describing the mass balances and disposition of the chemicals and their metabolites in the body. While these models are excellent tools, numerous input parameters are necessary for each chemical. Organ specific thermodynamic parameters (such as tissue to blood equilibrium partition coefficients) are required for each pesticide entering the body and for each of its metabolites. Additionally, values for all of the metabolic rates governing all the biotransformation steps for each pesticide would be necessary. The complex processes for the common mechanism effect would be necessary. Using the OPs as an example, compound specific

inputs such as binding constants and values for the rates of enzyme degradation, aging, and resynthesis would be needed.

The U.S. EPA [Office of Research and Development's (ORD) National Exposure Research Laboratory (NERL)] has formulated such a model that has been used to simultaneously model the disposition of three OPs and their metabolites (Blancato, et al., in review). Another PBPK model has been developed to describe the complex pharmacodynamics of acetylcholinesterase inhibition following OP exposure, based almost entirely on *in vitro* information (Gearhart, et al., 1994).

At present, these types of data/information on the majority of the OPs are not available to EPA. PBPK modeling techniques offer good promise despite the current limitations regarding the necessary input information. Continued development and testing of the models is necessary and should be pursued. Pharmacokinetic studies (*in vivo* and *in vitro* experiments to determine key values for PK parameters and the time course disposition of the compounds in the body) need to be done with many compounds to determine the key parameters of use in PBPK modeling. It is anticipated that data and methods will continue to improve and evolve as more experience is gained in this area.