III. Appendices

B. Hazard/RPF

1. Technical Aspects of Dose-Response Analysis

Background

EPA released a Preliminary Dose-Response Assessment for OPs on July 31, 2001 (USEPA 2001b) followed by a revised dose-response assessment on December 3, 2001. Both of these analyzes were reviewed by the FIFRA SAP in September 2001 and February 2002, respectively (FIFRA SAP 2001b, 2002). The current approach was supported by the SAP (FIFRA 2002). At the February 5-8, 2002 meeting of the SAP, EPA discussed some programming errors found after the December 3, 2001 release of the Preliminary Cumulative Risk Assessment. These errors have been corrected; the contents of III.B.4 (R programming code) reflect the corrections.

Dose-Response Modeling

The goal of the statistical methods was to estimate the dose that would be expected to result in a 10% reduction in brain AChE activity, the BMD_{10}. The data for this study were in the form of dose-response studies which measured the effect of different dose rates of OP pesticides on cholinesterase activities in brain, red blood cells, and plasma. The mean and standard deviation of cholinesterase activity, and number of animals examined were available for several dosages in each data set. Females and males were analyzed separately in each study. For each chemical there were several groups of studies labeled by separate MRIDs. Within each major study, one or more studies were conducted, each with measurements taken for several durations of exposure.

It is useful to describe the approach to modeling the dose-response data in three parts:

- the shape of the dose-response curve to be used;
- how multiple data sets were modeled at the same time;
- the statistical methods used to estimate values for the model parameters.

In this analysis, the dose-response function had to accommodate three important features of the data. First, since the data came from multiple studies, perhaps carried out in different laboratories and at different times, and even sometimes reporting cholinesterase activity in different units, activity at a given dosage was expressed as a fraction of control activity. Second, it was observed that, as dose increased, cholinesterase activity in quite a few data sets approached a lower non-zero asymptote. This asymptote varied among chemicals and possibly sexes. Finally, for many of the chemicals it was apparent that there is a “shoulder” on the dose response curve, such that the dose-response curve was shallower at lower doses than at higher.

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These features of the dose-response were incorporated in the dose-response model in two phases. First, a model was developed relating dose to cholinesterase activity which allowed for a horizontal asympote, and expressed activity at a given dose level as a fraction of background, or control, activity. In this document, this first model is called the "basic" model. Next, a submodel relating internal dose to administered dose, was combined with the first model to make a new model with that could have a shoulder in the low-dose region. The next subsections discuss these two models in more detail.

Basic Dose-Response Model

The basic model is described by the equation:

$$y = A\left[P_B + (1 - P_B)\,\mathrm{e}^{\frac{\log\left(\frac{1-P_B-BMR}{1-P_B}\right)}{BMD}\times Dose} \right] \qquad \textbf{Eqn. 1}$$

Here, A is the level of cholinesterase activity in the absence of exposure to organophosphate, P_B is the fraction of cholinesterase activity remaining at a very high dose of organophosphate, *BMR* is the level of inhibition at which to estimate the benchmark dose (in this study, *BMR* is always 0.10), *BMD* is the benchmark dose, and *Dose* is the dose of organophosphate pesticide, generally in units of mg/kg/day. This model is essentially the same as was described in FIFRA SAP (2001b, 2002), only reparametrized so that *BMD* appears as an explicit parameter, thus simplifying the calculations. Note that the model is undefined if $P_B + BMR \geq 1$.

Expanded Dose-Response Model

A submodel relating internal dose to administered dose was combined with the basic model to make the expanded model which allows for a shoulder in the low-dose region.

1. Biologically Inspired Model: Accounting for Potential First-Pass Metabolism

At this time, the appropriate kinetic data needed for the development of a physiologically based pharmacokinetc model (PBPK model) for all OPs are not available. EPA has developed a *biologically inspired* model based on metabolic pathways for first-pass metabolism which are *theorized* to influence the shape of the dose-response curve.

When many chemicals are administered orally, much of the absorbed chemical is carried to the liver by the portal circulation, where they may be metabolized. In the presence of saturable metabolism the dose-response curve would be expected to have a shallower slope at lower doses, and the slope would gradually increase as metabolism became saturated and more of the active chemical enters the general circulation. Although a detailed treatment of this process for each chemical is beyond the scope of this project, this basic idea was used to derive a two-parameter function of dose that relates administered dose to internal dose. The resulting function was combined with the basic exponential model giving a model that has a

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low dose shoulder while retaining the dose-response shape of the basic model for larger doses.

Consider the simple two-compartment pharmacokinetic model illustrated in Figure III.B.1-1.

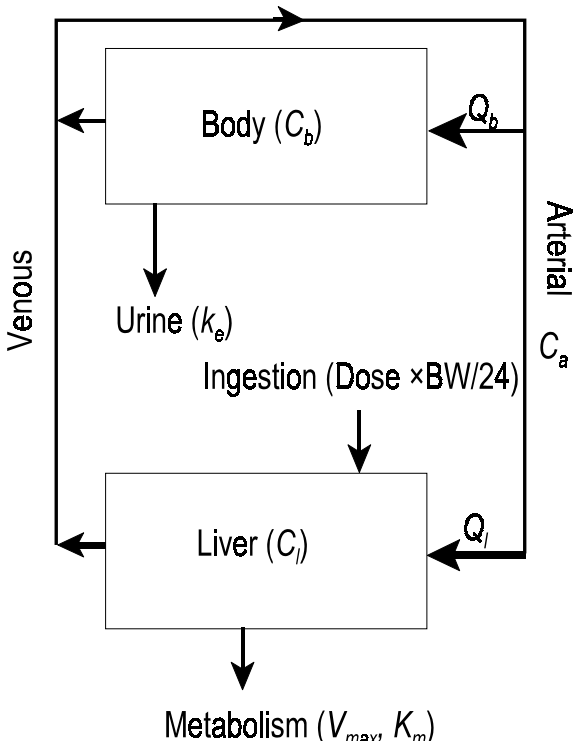

Figure III.B.1-1: Diagram for two-compartment PBPK model for the extension to the basic model

In this simple model, all the ingested chemical is taken directly to the liver, where it is metabolized. The residual unmetabolized chemical is then distributed to the rest of the body through the circulation. Intake of chemical is continuous. In this case, two differential equations and one algebraic equation describe the concentration in the liver and the rest of the body:

$$V_b \frac{dC_b}{dt} = Q_b \times (C_a - C_b) - k_e C_b$$

$$V_l \frac{dC_l}{dt} = Q_l \times (C_a - C_l) + \frac{Dose \times BW}{24} - \frac{V_{\max} C_l}{K_m + C_l}$$

$$C_a = \frac{Q_b C_b + Q_l C_l}{Q_b + Q_l}$$

Here, C_x is the concentration in compartment *x*, where *x* is *a* for arterial blood, *b* for the body other than liver, and *l* for liver. The volume of and blood flow to

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compartment *x* are V_x and Q_x, where *x* is either *b* or *l*. V_{max} and K_m describe saturable metabolism of the chemical in the liver. The constant k_e is a first-order clearance term. Dose is expressed in milligrams per kilogram per day (hence the constant "24" to convert to hours), and body weight is expressed in kilograms. Thus, volumes in this parametrization are expressed in liters and concentrations in milligrams per liter.

At steady state, the derivatives are both 0: clearance just balances the dose rate. It can be shown (by solving the system of equations with derivatives set to zero) that the concentration in the body (C_b) at steady state is:

$$C_b = 0.5 * \frac{BW}{24 \times k_e} \left\{ \left(Dose - \frac{24 Q_l Q_b K_m k_e}{BW\,(Q_l k_e + Q_b k_e + Q_l Q_b)} - \frac{24 V_{\max}}{BW} \right) + \sqrt{\left(Dose - \frac{24 Q_l Q_b K_m k_e}{BW\,(Q_l k_e + Q_b k_e + Q_l Q_b)} - \frac{24 V_{\max}}{BW} \right)^2 + 4 Dose \frac{24 Q_l Q_b K_m k_e}{BW\,(Q_l k_e + Q_b k_e + Q_l Q_b)}} \right\} \quad \textbf{Eqn (2)}$$

Here, the odd constants 0.5 and 4 arise because the solution involves finding the roots of a quadratic polynomial, and 24 arises because dose rates are usually expressed in terms of "per day", while other coefficients in the model are "per hour".

Equation (2) suggests using the function:

$$idose = 0.5 * \left\{ (Dose - S - D) + \sqrt{(Dose - S - D)^2 + 4 \times Dose \times S} \right\} \quad \textbf{Eqn (3)}$$

to describe the relationship between administered dose (*Dose*) and a scaled internal dose, where

$$S = \frac{24 Q_l Q_b K_m k_e}{BW\,(Q_l k_e + Q_b k_e + Q_l Q_b)},$$

and

$D = \frac{24 V_{\max}}{BW}$. In this parameterization of the model, V_{max}, k_e, and total blood flow (= Q_b + Q_l) should be proportional to body weight, so both *S* and *D* are independent of body weight. This is a function of two parameters (*S* and *D*), and approaches the function *idose* = *Dose* − *D* for larger doses; the slope with respect to dose when *Dose* is close to 0 is *S/(S + D)*. *D* quantifies the displacement of the relationship between *Dose* and *idose* from the identity relationship, and *S* controls the shape of the relationship at low doses. In the limit as $D \to 0$ or $S \to \infty$, Equation (7) converges to *idose* = *Dose*.

In fact, it is reasonable to use Equation (3) to approximate the relationship between internal dose and administered dose in the chronic dosing setting, even in the absence of a detailed pharmacokinetic justification. The general properties of the equation capture the expected effects of first-pass metabolic clearance of an active compound: a shallow shoulder of the curve at lower doses, with a slope that

increases to a limit as the dose increases. As long as *S* and *D* are non-negative, varying these two parameters should result in a good approximation to virtually any low-dose deviation due to metabolic clearance, at least at the resolution available in bioassay dose-response data.

2. Equation for the Expanded Model.

The expanded model is just the basic model (Eqn. 1), in which *Dose* is replaced by an expression relating administered dose to internal dose. Note that, in this use of the model, the parameter *BMD* is the *internal dose* that corresponds to a *BMR* level of inhibition. Calculating the benchmark dose that corresponds to that internal dose requires setting Eqn. 3 equal to *BMD*, and solving for *Dose*.

Incorporating Differences among Datasets in the Modeling and Modeling Variability

The data for each chemical were modeled independently of all other chemicals. However, the data for any one chemical were to some extent from heterogeneous studies, grouped hierarchically. At the highest level of the hierarchy, the data could come from multiple major studies, indicated by different MRID numbers (A MRID no. is an identification code for a particular study; MRID is used in this discussion to describe the major studies). At that level, it could be expected that analytic methods could differ most distinctly, and different major studies might use different units to express cholinesterase activity. Within a major study were individual dose-response studies, often the result of multiple intermediate observations in a sub-chronic or chronic study. Although these were part of the same study, since the data collection was separated by relatively wide time intervals, there is still a reasonable expectation that details of method might vary among such data sets. Finally, within each individual dose-response study were data for both males and females. In order to combine all the data for a given chemical with a single model, all this variability needed to be incorporated in the model. This was done with a combination of allowing fixed effects to take different values in different dose-response data sets and sexes, treating a parameter as if it varied randomly across data sets, and treating some parameters as fixed for any given chemical. The following describes how each parameter was treated in the modeling.

- The parameters for the submodel relating administered dose to internal dose (*S* and *D* in Eqn 3) were given a single value for a given chemical, though they could differ between chemicals.
- The parameter governing the horizontal asymptote, P_B was allowed to differ between sexes, but otherwise to be the same value for all datasets for a given chemical.
- The background parameter, *A*, was estimated as a fixed value for each individual data set for each sex.
- The parameter *BMD* was treated as a random effect. Specifically,

  $$\ln(BMD) = \mu_{lBMD} + E_{MRID} + E_{Data\ Set},$$

  where μ_{lBMD} is the log of the geometric mean of the distribution of BMD among data sets, E_{MRID} and $E_{Data\ Set}$ are normally distributed random variables with mean 0 and different standard deviations that reflect variation of ln(*BMD*) among MRIDs and

among datasets within MRID, respectively. The parameter μ_{lBMD} was allowed to vary between males and females, but for each sex was constant over all data sets for a chemical. Some chemicals were represented by only one MRID, and some were represented by MRIDs with only a single data set in them. The above formula was reduced in the logical way for such chemicals. In particular, when only a single data set was available for a chemical, all the random effect terms would drop away, leaving only the log geometric mean for each sex.

- The variation among individual observations from animals of the same sex within a data set was assumed to be normal, with mean determined by the above model, and variance proportional to the mean cholinesterase activity level. An earlier version of this analysis (FIFRA SAP, 2001b) had treated the variance to be proportional to the square of the mean, and was based on analyzing the relationship between mean and variance across studies and chemicals. The current model is due to reexamining the relationship, focusing on the relationship within studies. The constant of proportionality was allowed to differ among MRIDs for a chemical, to allow for differences in units.

Estimating Parameters

It proved to be impossible to jointly estimate all the parameters for either the basic or the expanded model simultaneously. Therefore, parameters in these models were estimated using a combination of either *nlme*, a method for nonlinear mixed effects models (when there were multiple MRIDs and/or data sets for a chemical; almost all the chemicals) or *gnls*, generalized least squares, and profile likelihood. The functions *nlme* and *gnls* are from the package *nlme* for the statistical package *R* (Ihaka and Gentleman, 1996).

The *R* package *nlme* estimates parameters for nonlinear mixed effects models using the approach described in Lindstrom and Bates (1990). Davidian and Giltinan (1995, pp 164 – 174) give a good description of this model, where they refer to it as being based on "conditional first-order linearization". This approach involves approximating the nonlinear function using a Taylor expansion before carrying out maximum likelihood estimation. The implementation in *nlme* allows the fixed and random effects to be expressed as linear models of other independent variables. In this analysis, for example, *lBMD* was allowed to differ between sexes by modeling *lBMD ~ sex - 1*, where *sex* is a categorical variable in the data set that takes the values *"F"* or *"M"*. The term "*- 1* " indicates that an intercept term should not be fit for this model, so there would be an estimate of *lBMD* for each sex.

The function *gnls* in the *R* package *nlme* has a similar user interface as does the function *nlme*, but is appropriate when there are no random effects terms other than the error variance. Generalized least squares as a method is well described in Chapter 2 of Davidian and Giltinan (1995).

Parameters for the basic model were estimated first, and served as the basis for estimating parameters for the expanded model.

To fit the basic model, the values of P_{BF} and P_{BM} were set to each value on a grid of appropriate values, and the remaining parameters (background parameter for each individual data set, mean of ln(BMD) for males and females, standard deviations of ln(BMD) among MRIDs and among datasets within MRID, and parameters for the error variance) were estimated by the method appropriate to the dataset (that is, either *gnls* or *nlme*; see the discussion of these two methods, below). When all the models for that particular grid of P_B values were fit, a new grid was constructed by using the values of P_B on either side of the grid point with the largest loglikelihood as the new extremes, and repeating the process. When no BMD estimate on grid points surrounding the point with the largest loglikelihood differed from the BMD at the maximum by more than 5%, the process of iterative refining the grid stopped.

A similar method was used to estimate *S* and *D* in the expanded model. First, the values of P_{BF} and P_{BM} were fixed to their best estimates for the basic model, and were not further modified. In the expanded model, the grid being explored and refined was of values for *S* and *D*, but otherwise the process was the same as for the basic model. In the expanded model, the criterion for convergence was no difference between the maximum on the grid and neighboring points of greater than 10%.

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