Cumulative Hazard Assessment: Issues for the FIFRA Scientific Advisory Panel

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Table of Contents

List of Tables

List of Figures

I.   Introduction

As discussed in the Background document prepared for the February 2005 meeting of the FIFRA Scientific Advisory Panel (SAP), the Office of Pesticide Programs (OPP) established the N-methyl carbamate pesticides as a common mechanism group (USEPA, 2001) and thus established the need to develop a cumulative risk assessment for this group of pesticides. The common mechanism determination for the chemicals in this group was based on the shared structural characteristics and similarity and their shared ability to inhibit acetylcholinesterase (AChE) by carbamylation of the serine hydroxyl group located in the active site of the enzyme. Following maximal inhibition of cholinesterase, recovery typically occurs rapidly (minutes to hours). This rapid recovery of AChE activity is an important toxicological characteristic of the N-methyl carbamates which needs to be considered in the cumulative risk assessment. The purpose of this document is to describe EPA's on-going efforts to incorporate recovery of AChE activity into its cumulative hazard and risk assessment for the N-methyl carbamate pesticides and to get feedback from the FIFRA SAP regarding these efforts. This document represents the collaborative efforts of scientists from OPP and EPA's National Health and Environmental Effects Research Laboratory (NHEERL). Three major areas are covered here: 1) laboratory experiments conducted in rats to evaluate time to recovery and dose-response relationships using two measurement techniques; 2) brief descriptions of additional and/or on-going laboratory experiments involving measurement of behavioral endpoints and testing mixtures of N-methyl carbamates; and 3) empirical modeling efforts to estimate benchmark dose estimates and to incorporate timing of exposure along with pharmacokinetics (PK) of AChE inhibition and recovery into risk estimates.

II.   Laboratory Measurement of AChE Inhibition

A.   Background

In toxicology studies submitted to EPA for pesticide registration, measurements of AChE inhibition are typically performed using some variation of the Ellman spectrophotometric method (Ellman et al., 1961). This method usually involves extensive sample dilution, prolonged incubation, and temperatures around 37ºC; all of which promote reversal of the enzyme inhibition. If precautions are not taken to prevent recovery using this method, then reported AChE activities can underestimate actual AChE inhibition (Winteringham and Fowler, 1966; Williams and Casterline, 1969; Nostrandt et al, 1993; Hunter et al., 1997) and could thus impact relative potency estimates important for cumulative risk assessment. Furthermore, the reversibility encountered during the assay varies for each N-methyl carbamate and laboratory conditions for this assay vary across laboratories. Thus, in the event that relative potency estimates are indeed affected, a standard correction factor cannot be used. A radiometric method as that reported by Johnson and Russell (1975) provides a more appropriate method for measuring AChE inhibition, because the factors which promote reversibility are minimized. For example, dilution is minimized (1:30 vs. more than 1:1000 dilution for the Ellman method), incubation time may be more rapid for the radiometric method (one to three minutes

compared to 10 minutes or greater), and the radiometric method may be conducted at lower temperatures. Lowering the tissue dilution, time, and temperature of the assay all limit the amount of spontaneous decarbamalyation of the inhibited enzyme (Hunter et al., 1997).

Laboratory scientists from EPA's NHEERL have systematically evaluated AChE inhibition following acute exposures of adult rats to seven N-methyl carbamates using both Ellman and radiometric techniques. The purpose of these studies was to characterize the degree to which available data submitted by the pesticide registrants may or may not underestimate potency and to characterize the potential impact on benchmark dose and relative potency estimates. As the studies submitted for purposes of pesticide registration make up the majority of the available studies for the N-methyl carbamates, the EPA studies were NOT designed to replace existing toxicology databases submitted for purposes of registration. They were, instead, designed to supplement existing information and provide characterization important for the cumulative risk assessment.

B.   Brief Description of the Methods

Detailed description of the methods used in the AChE experiments is provided in Appendix 1. The preliminary N-methyl carbamate cumulative risk assessment is expected to be released in the summer of 2005. At that time, all of the data generated from these studies will be provided to the public.

Adult, male Long Evans rats were given acute, oral dosages of either carbaryl, carbofuran, methiocarb, propoxur, formetanate, oxamyl or methomyl. Red blood cell (RBC) and brain cholinesterase were measured using both the Ellman method and the radiometric method. Two studies were performed with each compound: (1) a time course study and (2) a dose response study. In the time course study, rats were given one dose level of the chosen N-methyl carbamate and tissues were taken at various times after dosing to identify the time of peak effect, the approximate recovery time and also at 24 hours after dosing to determine if AChE activity was normal at that time. In the dose response study, rats were given 5 different dosages levels of one N-methyl carbamate (plus control) and tissues taken at the same time for analyses.

C.   Summary of Results

In general, all the N-methyl carbamates were very quick acting with time of peak effect within the first hour after dosing. Moreover, all animals had recovered to control levels by 24 hours after dosing. For the dose response assessment, there were wide variations in the potency of the various N-methyl carbamates, but all compounds showed a smooth dose response curve. There were variations in the relationship between RBC and brain AChE inhibition, but in general, the brain and RBC showed similar levels of AChE inhibition. A comparison of the two methods for analyses showed that the Ellman method was much more variable than the radiometric method. In EPA's studies, the Ellman method tended to

underestimate the level of AChE inhibition; this underestimation was most prominent at high doses levels. At lower doses relevant for risk assessment purposes, particularly at or around 10% inhibition, in general, there is good visual similarity between the two measurement methods. Figures 1 and 2 provide example graphs of the time course and dose-response studies. Plots of the time course and dose-response studies are provided for each of the seven *N*-methyl carbamates in Appendix 2. Figure 2 also provides dose-response data from a registration study submitted to EPA for Chemical G. Plots of registration data for the remaining chemicals tested by EPA are provided in Appendix 2. A comparison of the study conditions in EPA studies and registration studies is provided in Table 1. Among the registration studies, acute rat studies which most closely matched the conditions used in EPA's studies were selected—e.g., unless noted data presented are for male rats only; gavage administration; time points at/or near 40 minutes post-dosing. Statistical analysis comparing these studies has not yet been performed. For all seven pesticides, there is remarkable similarity between EPA studies using the radiometric technique and registration studies, particularly at doses at or near 10% inhibition. This similarity is notable given the differences in protocol (e.g., rat strain, gavage administration vehicle) and the difference in purpose for these studies (determination of no-observed-adverse-effect level for the registration studies; definition of the dose-response curve for EPA studies).

D.   Discussion

As mentioned previously, one purpose of EPA's time course and dose-response studies was to systematically evaluate radiometric and spectrophotometric methods for measuring AChE inhibition. Prior to the completion of these studies, there was a concern that studies submitted to EPA for pesticide registration may underestimate relative potency. Based on the results noted above and those presented in Appendix 2, in this preliminary analysis, it appears that the AChE data provided in the registration studies are sufficient quality for evaluating relative potency.

EPA does not typically receive detailed protocols or standard operating procedures for laboratory measurements of AChE. Thus, EPA does not know the exact conditions used in various laboratories performing registration studies. However, not only does the choice of assay method influence the amount of inhibition seen in tissues from animals treated with *N*-methyl carbamate pesticides, but exactly how the assay is conducted also can influence the results. It is possible to conduct an Ellman-based assay on brain and erythrocytes and obtained data comparable to the radiometric assay on the same tissue (Nostrandt *et al.*, 1993). In this paper a comparison was made between the conventional Ellman assay, a modified Ellman assay and the radiometric assay. It was found that the modified Ellman assay gave answers comparable to the radiometric assay if some special precautions were taken: (1) minimize the dilution of the tissue prior to the assay; (2) keep the homogenate on ice prior to the assay; (3) minimize the duration of the assay (5 minutes or less) (4) keep the assay temperature as low as possible (23-26°C). EPA did not take similar

precautions in the Ellman assays; thus particularly at high doses, the EPA's Ellman results underestimate inhibition measured in the radiometric assays. Uncertainties remain regarding the exact protocols and conditions used by the laboratories conducting registration studies. However, based on the remarkable similarity between the results of EPA's radiometric studies and the registration studies, at this time, EPA believes that the majority of studies submitted for pesticide registration will provide reliable estimates of relative potency. EPA will soon begin empirical dose-response modeling the toxicity data available for the *N*-methyl carbamates. As EPA moves forward with the cumulative hazard assessment and estimation of relative potency, EPA will continue to critically evaluate all the available AChE data.

Table 1. Comparison of acute rat studies available for seven _N_-methyl carbamate pesticides.

Chemical	Endpoints	Strain/Sex	N per dose group	Time of AChE Measurement	Gavage Vehicle and Volume
EPA Studies	Brain RBC	Long Evans	5	40 minutes	corn oil (A, F, G, H) water (C, D, E) all were given at 1 mL/kg
Registration Studies					
Chemical A	Brain RBC	Sprague-Dawley	3	30 minutes	carboxymethylcellulose; polyoxythylene sorbitan mono_oleate (Tween 80) 10 ml/kg
Chemical C	Brain RBC	Crl:CD	10	30-60 minutes	deionized water 10 mL/kg
Chemical D	Brain Whole blood	Crl:CD[SD]IGS BR (Sprague Dawley, CD)	5	30 to 60 minutes	deionized water 5 mL/kg
Chemical E	Brain RBC	Crl:CD BR	10	30 minutes	deionized water 10 mL/kg
Chemical F	RBC	Crl:CD®(SD)IGS BR	9	30 minutes	corn oil 2 mL/kg
Chemical G	Brain RBC	Wistar	6	45 minutes	Polyethylene glycol 5 ml/kg
Chemical H	RBC	Sprague Dawley (females only tested)	5	30 minutes	Carbowax 5 ml/kg

Figure 1. Example time course data collected for *N*-methyl carbamates

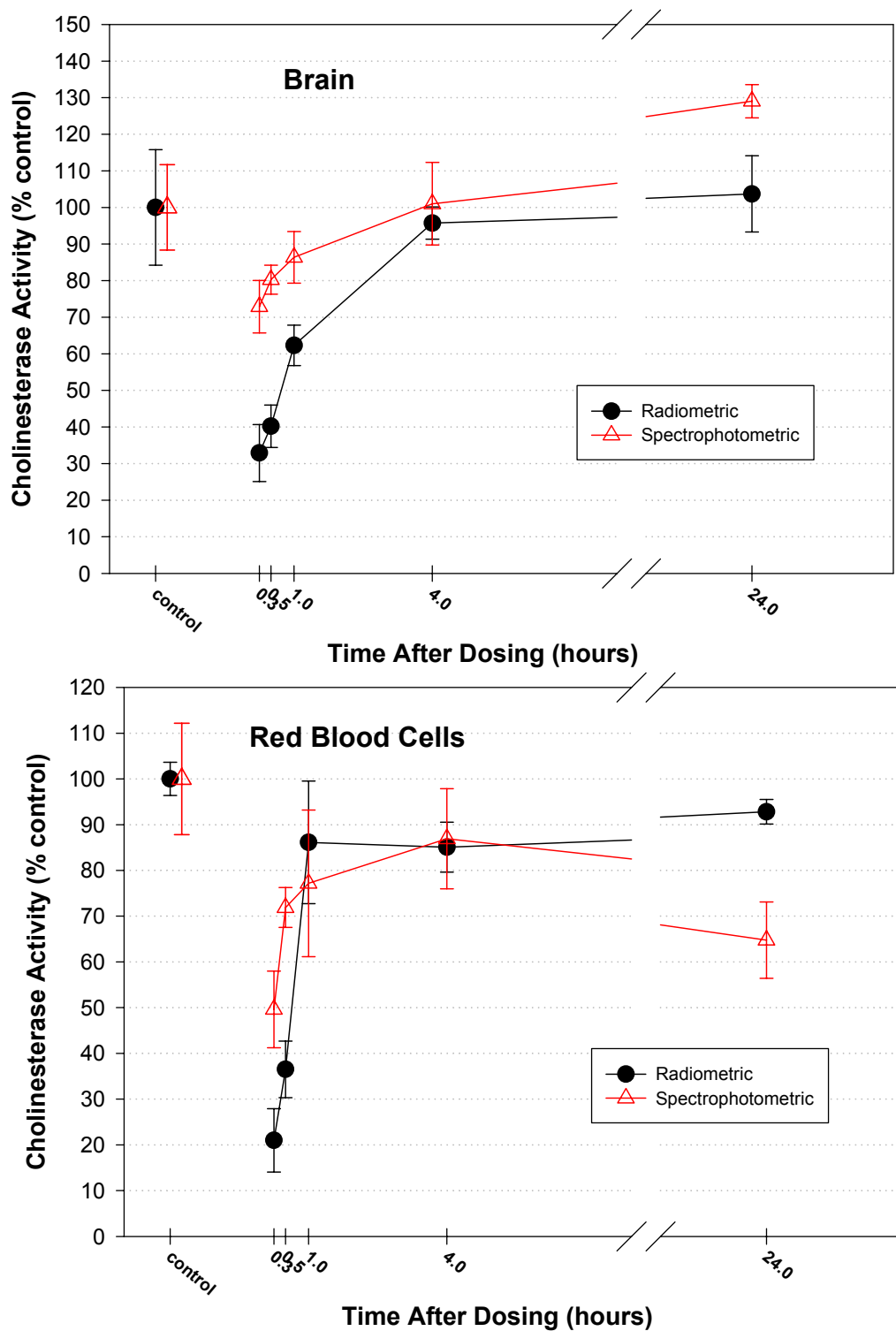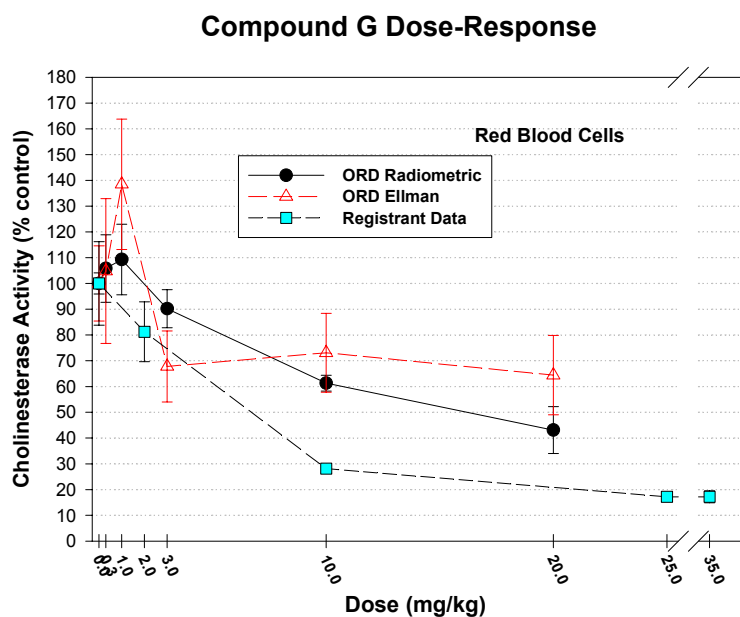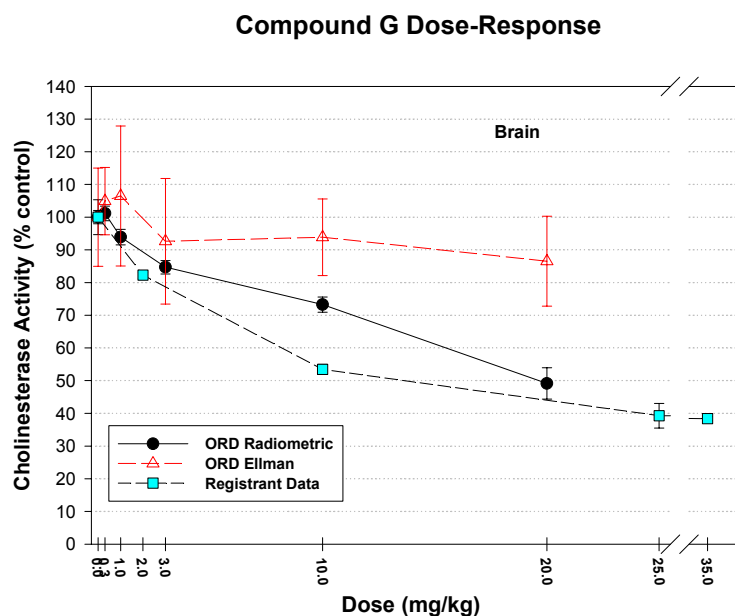## Time Course of Brain and RBC Cholinesterase in Compound G Treated Rats (20 mg/kg in corn oil) orally

Figure 2. Example dose-response data using the radiometric and Ellman techniques collected for the *N*-methyl carbamate

Compound G Dose-Response

Compound G Dose-Response

III. Additional and/or On-going Experiments

A. Behavioral Evaluations

Motor activity data were collected in the dose-response studies for each *N*-methyl carbamate pesticide. Tests of motor activity have been widely used in toxicology and pharmacology for decades. Activity is an apical measure of overall neurological function, and can be sensitive to many types of neurotoxicants, including cholinesterase-inhibiting pesticides (Moser, 1995; 1999). The figure-8 chambers used in these studies provide evaluation of both horizontally-directed activity via photodiode/detectors spaced throughout the chamber, and vertically-directed activity via a row of detectors located approximately 15 cm above the floor of the chamber. Motor activity data were collected in the same animals for which cholinesterase inhibition was determined. Thus, the magnitude of change for the behavioral vs. biochemical endpoints can be directly compared. This analysis is still on-going and will NOT be presented to the SAP in February 2005. However, these data will be presented at the March 2005 meeting of the Society of Toxicology.

In addition to motor activity assessments, clinical observations were conducted on the rats right before they were placed in the activity chambers. Rats were ranked based on their observable signs of toxicity (signs such as tremor, lacrimation, etc.) typically seen with *N*-methyl carbamates, and termed a "tox score". This allowed a comparison between activity data and clinical signs in terms of sensitivity and potential value added with the activity data. The tox score data will also be presented at the Society of Toxicology meeting.

Note to reader: Some studies submitted for pesticide registration have also measured motor activity. EPA has not yet compiled these data but may in the coming months.

B. Mixture experiments

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 2002b). While there are a few interaction studies of *N*-methyl carbamate and OP pesticides in the literature (e.g., Gupta and Dettbarn, 1993; Takahashi et al., 1987), no studies conducted using mixtures of more than two *N*-methyl carbamates and which use lower dose levels (i.e., that do not produce lethality or profound toxicity) have been identified. NHEERL scientists will conduct mixture studies of seven *N*-methyl carbamates. The overall purpose of these studies will be to determine if the interaction of these seven pesticides in a mixture produces a dose-additive interaction following acute exposure. Brain and RBC AChE and motor activity will be used as indicators of neurochemical and behavioral impact of the pesticides. Since the data will be collected in the same animals, the magnitude of effect on the biochemical and behavioral endpoints can be directly compared for a better understanding of the biological

effect of specific levels of inhibition. The single chemical dose-response data will be used to construct the expected model of dose-additivity for the mixture.

For the *N*-methyl carbamate mixtures, two approaches will be used in the mixture experiments. A fixed-ratio ray design will be used to model the mixture data (Gennings et al., 2004). The ray design approach allows for testing along a range of mixture doses wherein the proportion of pesticides within the mixture is the same. That proportion can be based on many factors, but perhaps the most defensible mixture is based on projected environmental exposures. A simpler approach using proportions based on the relative potency of the pesticides will also be used.

IV.   Empirical Modeling: Benchmark Dose Estimations and Simple Pharmacokinetic Modeling Approach

A.   Overview

The following text provides a summary of how the EPA will calculate benchmark dose estimates for use in relative potency calculations. The hazard assessment portion of the *N*-methyl carbamate cumulative risk assessment will rely on estimates of benchmark doses for 10% AChE inhibition as well as estimates of parameters to characterize the time course of AChE inhibition after an acute exposure, both in brain and in RBC.

The following text also provides an overview of a simple PK approach which could be used to evaluate the risk to *N*-methyl carbamates following oral exposures (i.e., food and/or drinking water). This simple PK approach considers chemical specific information regarding potency, time to recovery, and timing and magnitude of exposure. A simple example using the PK approach is provided below.

As described in detail in the "Background Document," the current capabilities of the exposure assessment models prevent the implementation of this approach in the cumulative risk assessment. Specifically, this approach requires that model output include chemical specific exposures which are separated in time. This issue was discussed in the white paper developed by the LifeLine group and presented to the FIFRA SAP in December 2004. It is unknown at this time whether this simple PK approach will be used by EPA in its cumulative risk assessment for the *N*-methyl carbamates.

B.   Benchmark Dose & Empirical Dose-Time Response Modeling

EPA is extracting RBC and brain AChE data in male and female animals (mean, standard deviation, sample size) from toxicology studies submitted for pesticide registration. Similar to previous practice with the OP cumulative risk assessment, all of the data used by EPA to develop benchmark dose estimates will be provided to the public when the preliminary *N*-methyl carbamate cumulative risk assessment is released.

1.   AChE Inhibition Data

EPA is extracting data from both single- and multiple-dose studies, particularly rat studies as rats are the most commonly used species in toxicology studies with *N*-methyl carbamates. Single dose, acute studies will likely provide the basis for benchmark dose estimate as these studies are generally designed to evaluate AChE inhibition at or near the maximal inhibition level. Time course and AChE recovery studies will also be analyzed.

As recovery of AChE inhibition is rapid for this group of pesticides, the cumulative exposure and risk assessment will focus on daily, acute exposures to the *N*-methyl carbamates. There is however potential that recovery rates could change following multiple or chronic exposures. In order characterize this potential, EPA is also evaluating results of multi-dosing studies. Specifically EPA plans analyze AChE inhibition over various durations of exposure to evaluate changes in benchmark dose with time.

Data sets for analysis come from several different kinds of studies:

❑   Oral studies quantifying the relationship between maximum inhibition and a single administered dose

❑   Oral studies quantifying the relationship between maximum inhibition, but with multiple administered doses, spaced at one per day

❑   Oral studies quantifying the *in vivo* recovery time course, usually at several doses, and beginning at or around the time of maximum inhibition (which had typically been determined in preliminary studies)

❑   Combinations of 1 or 2 with 3

❑   For those pesticides with residential exposure, any inhalation and dermal studies

The analysis described here will be for two endpoints: AChE activity in whole brain (or a surrogate, like "half brain", "left brain", "right brain") and AChE activity in RBC. RBC data can provide an additional complication in statistical analysis as the same animal may be sampled at multiple of time points. This means the statistical analysis for this endpoint in these studies needs to take repeated measures into account. EPA is currently evaluating the degree to which repeated measures may impact estimates by evaluating the individual animal from a subset of studies.

2. Dose-Time Response Model

Several features of the dose-time response for the *N*-methyl carbamates need to be captured in an empirical model:

❑ AChE activity declines rapidly with increasing dose, perhaps after a "shoulder" at the low-dose end of the dose-response curve;

❑ For many, perhaps most or all, AChE inhibitors, there is a minimum level below which AChE activity will not drop, regardless of dose;

❑ AChE activity drops rapidly after dosing to a minimum level which depends upon dose, then returns to the background level over a period of minutes to hours, at a rate that may also depend upon dose;

❑ Most of the time course studies do not provide adequate early time points to accurately estimate the time of maximum effect, instead starting at around a previously estimated time of maximum effect.

The model proposed here (and discussed in more detail below and in Appendix 3) is the result of multiplying a dose-response model for inhibition that is closely related to the model that was successful at characterizing OP dose-response curves (USEPA, 2002a) and a time-course model for inhibition. Transformations of parameters are used to enforce constraints, since the proposed statistical software for estimating model parameters does not incorporate bounded estimation (for example, to require that half-life estimates remain positive).

The model for inhibition, before parameters are transformed to enforce constraints, is

$$g(d) = g(d; R, P, D_R, \gamma) = (1-P)\left(1 - e^{\log\left(\frac{1-R-P}{1-P}\right)\left(\frac{d}{D_R}\right)^{\gamma}}\right)$$

(Eq. 1)

where:

❑ d is administered dose, and is part of the data set;
❑ P is the minimum fraction of background AChE activity, and is constrained to fall between 0 and 1;
❑ R is the inhibition fraction associated with the desired benchmark dose (that is, the benchmark dose is the dose expected to yield 100×R% inhibition at the time of maximum effect), and is set to 0.10 in this analysis;
❑ D_R is the benchmark dose, constrained to be greater than 0.0;
❑ γ is a shape parameter to allow a shoulder at the low-dose end of the dose-response curve, and is constrained to be greater than 0.0.

The time course model is the difference of two exponential functions, scaled so that the maximum is always 1:

$$h(t) = h(t; T_A, T_R) = C_0\left(e^{-\frac{\ln(2)t}{T_R}} - e^{-\frac{\ln(2)t}{T_A}}\right)$$

(Eq. 2)

where:

❑ T_A is the half-life of the process that results in an increase in inhibition, and
❑ T_R is the half-life of the process that results in a decrease in inhibition (recovery or reactivation).

The maximum of *h(t)* occurs at:

$$T^* = \frac{T_R T_A \left(\ln\left(T_R\right) - \ln\left(T_A\right) \right)}{\ln\left(2\right)\left(T_R - T_A\right)}$$

(Eq. 3)

so $C_0 = 1 \left/ \left(e^{-\frac{\ln(2)T^*}{T_R}} - e^{-\frac{\ln(2)T^*}{T_A}} \right) \right.$. With this scaling, h(t) is symmetric in the
two parameters (that is, *h(t; a, b)* = *h(t; b, a)*), which complicates
statistical estimation unless a constraint is added to keep $T_R > T_A$. Also,
many data sets require that *T** be specified (not estimated from the data),
because the designs were inadequate for estimating *T**. For these
reasons, it is convenient to reparameterize the model in terms of *T** and α
= T_R / T_A and make sure α is constrained to be greater than 1.0 (see
Appendix 3 for details).

Multiplying *g(d)* and *h(t)* together gives a function for AChE inhibition as a
function of dose and time. Thus,

f(t, d) = A×(1 − g(d)×h(t))

(Eq. 4)

is a model for AChE activity as a function of dose and time, where *A* gives
the background (that is, control) level of AChE activity.

3.   Statistical Methodology

Information about the parameters of interest for each chemical and
endpoint is distributed among multiple datasets. The model described
above will be fit to all these datasets simultaneously, using mixed effects
models to estimate the variance of key parameters (such as benchmark
dose and α) among data sets, and otherwise fitting separate values for
different data sets or, sometimes, doses (for example, preliminary
analyses suggest that α will need to depend on dose). Generally, *T** will
be fixed to the value specified by the original investigators, except in the
rare cases where the design allows it to be estimated directly from the
data. Data from both sexes will be analyzed at the same time (to improve
variance estimation); whether parameters differ in value between the
sexes will be tested.

All statistical analysis will use the statistical software environment R (version 2.01 or later; R Development Core Team, 2004) and its nonlinear mixed effects modeling function *nlme()* (Pinheiro, et al, 2004) Statistical testing will be based on likelihood ratio tests as well as comparison of AIC values.

The fact that the data are aggregated presents two distinct problems for the statistical analysis. First of all, the aggregation of the data may mask features of the data distributions, such as extreme observations, that may bias or otherwise compromise the interpretation of the statistical analysis. However, in the analysis of OP AChE data (USEPA, 2002a), in which the individual data were acquired and analyzed for a sample of data sets, it was found that while there was a tendency for the distributions of AChE activities to be somewhat long-tailed, this had a relatively small effect on parameter estimates and their estimated standard errors.   Since the design and chemical analysis of the *N*-methyl carbamate data is similar to that of the OP data, it is expected that analyses based on aggregated data should be adequate, in the absence of repeated measures (for example, all brain data). Secondly, aggregation seriously complicates the analysis in the presence of repeated measures. As mentioned above, a more detailed analysis will be conducted to determine the extent to which repeated measures are a problem in these data. If necessary, analyses maybe based on individual subject data for studies with repeated measures.

C.   Using Recovery Half Life And Dose-Response To Assess Consequences Of Human Exposure To *N*-Methyl Carbamates

The physiologically-based pharmacokinetic (PBPK) and pharmacodynamic (PD) data and models for carbaryl suggest that the parent compound is cleared relatively quickly after small exposures to carbaryl, and that metabolites are relatively unimportant factors in AChE inhibition, leaving the reactivation of enzyme as the main determinant of the recovery phase of AChE inhibition

This suggests that the following simple model to account for dose-response and recovery for enzyme inhibition would offer a reasonable approximation to reality (the following section provides a more formal treatment):

❑   After exposure to an *N*-methyl carbamate, the total fraction of newly inhibited enzyme increases rapidly to a maximum that depends on dose, then declines exponentially as the inhibited enzyme molecules are decarbamylated. The amount of inhibited enzyme is the product of the fraction inhibited and the amount of active enzyme available at the time of exposure.

❑ If a subsequent exposure (to the same or a different *N*-methyl carbamate) occurs before complete recovery from the previous exposure, again a fraction (which depends upon dose and time after exposure) of the remaining active enzyme is inhibited.

❑ If there are new exposures to multiple *N*-methyl carbamates at the same time, the total new fraction of inhibition is just the sum of the fractions of inhibition that would be expected from the individual doses.

❑ The amount of inhibited enzyme at any time is calculated by adding up all the enzyme still inhibited after all the previous exposures.

The following text provides an example of this simple PK approach. This example is not based on actual data or exposure estimates; this example is provided for illustration purposes only. The following table (Table 2) gives the daily exposure of a 70 kg individual to three imaginary *N*-methyl carbamates (X, Y, and Z):

Table 2. Daily exposures in illustrative example

	Amount (mg)		
Time	X	Y	Z
0:00	0	0	0
8:00	1	.5	0.02
11:00	0	.1	0
12:00	2	.2	.1

Table 3 provides parameters for chemical potency (the benchmark dose for 10% inhibition, BMD_{10}), the time of peak effect (T^*) and the "recovery half-life" (T_R) for these three chemicals. These parameters were estimated using the statistical methodology outlined in the previous section.

Table 3. Parameters in illustrative example

Chemical	BMD_{10} (mg/kg)	T^* (hours)	T_R (hours)	P
X	300	0.25	0.75	0.3
Y	150	0.25	1.5	0.3
Z	100	0.25	1.25	0.3

Figure 3. Plot of simulation
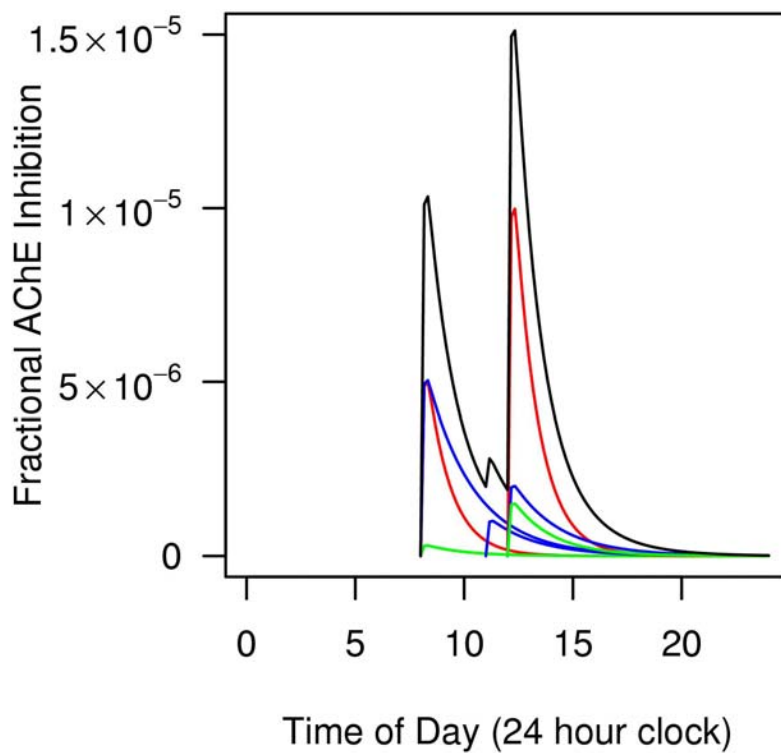
As shown in Figure 3, the simulation starts at midnight, and there are three exposure episodes, at 8:00, 11:00, and 12:00 noon (for example, these might be the result of dietary exposures at breakfast and lunch, and drinking water in late morning). There is exposure to three different chemicals at the 8:00 AM event, and, since there is no preexisting AChE inhibition, the total inhibition contributed by this exposure is the sum of the inhibitions due to each chemical separately. At 11:00 AM, there is a new exposure to chemical Y. That exposure acts to inhibit some of the remaining uninhibited AChE, and the total inhibition is the sum of the remaining inhibition from the 8:00 AM exposures and the new exposure at 11:00. Similarly, the exposures to all three chemicals at 12:00 noon contribute new inhibition. All inhibition decays exponentially after the final exposure. Appendix 4 provides the computer code (R code) used to generate Figure 3.

Several assumptions are needed for this simple model for the effects of exposure to be reasonable:

❑   The inhibitor is cleared quickly from the target tissue, so that recovery time mostly depends upon the rate of decarbamylation of AChE. This seems to be the case at least for one common *N*-methyl carbamate, carbaryl.

❑   Inhibitors do not compete for AChE or clearance pathways. While probably not strictly true, at low concentrations this is a reasonable approximation.

❑ Inhibitors do not modify the affinity of AChE for other inhibitors (e.g., by binding to a site on the AChE molecule that has allosteric effects). Again, even if there is such a site, there should be a minimal effect at low concentrations.

❑ It is appropriate to ignore resynthesis of new enzyme molecules on the time frames of interest.

❑ The model for human effects can be calibrated by scaling parameters of models fit to rodent data.

D.   Implementation of the Simple PK Approach

1.   Distributions of exposure

A cumulative risk assessment for the *N*-methyl carbamates based on this simple PK model would be based on the distribution of a functional (for example, the daily maximum inhibition, daily area under the curve of inhibition, fraction of a day during which AChE inhibition exceeds some critical level) of the inhibition time course. The distribution would be generated by simulating dietary, drinking water, and residential exposure scenarios. As mentioned above, at present time, exposure assessment models do not output information in this form potentially convoluting these with distributions based on the uncertainty and variability of the dose-time response parameters.

2.   Uncertainty and Variability

There are two general approaches for incorporating population variability and account for parameter uncertainty in health effects risk assessments—use of uncertainty and extrapolation factors and probabilistic analysis. The more common approach is to use factors to scale points of departure (such as benchmark doses) to account for uncertainties in interspecies extrapolation and variability in sensitivity among humans. To use such an approach to the risk assessment model described here would involve scaling critical parameters of the model: at least D_R, by uncertainty factors such as interspecies and intraspecies extrapolation or the FQPA 10x factor for the sensitivity of infants and children.

The other approach, used commonly in exposure assessments, now, but still only rarely in health effects or dose-response assessments, is a probabilistic analysis. Such an analysis needs to account for the same sources of variability and uncertainty as the uncertainty factor approach, but differs from it by using random variables to characterize the variability and uncertainty. The risk assessment could then be based on Monte Carlo simulation of the resulting stochastic model.

V.   Summary

Characterization of relative potency and time to recovery are important aspects of cumulative hazard assessment for the *N*-methyl carbamates. This document has provided a summary of laboratory experiments conducted by EPA's NHEERL to evaluate the time-course and dose-response relationship for seven *N*-methyl carbamates using two techniques. Prior to the conduct of these experiments, there was a concern that the registration studies, which typically use some variation of a modified Ellman technique, could underestimate toxic potency. However, based on preliminary analysis, there is remarkable similarity between the registration studies and EPA's radiometric studies. This document has also provided conceptual and technical aspects of empirical approaches proposed by EPA to calculate benchmark dose estimates and to consider the timing of exposure and time to recovery in risk estimates.

EPA is soliciting comment from the FIFRA SAP on each of these key issues: comparison of results between EPA and registration studies; empirical modeling to estimate benchmark dose estimates; and empirical modeling to consider timing of exposure and time to recovery.

VI.   References

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