SAP Minutes No. 2005-01

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

DIMETHOATE: ISSUES RELATED TO HAZARD AND DOSE-RESPONSE ASSESSMENT

NOVEMBER 30 AND DECEMBER 1, 2004
FIFRA Scientific Advisory Panel Meeting, held at the Holiday Inn - Rosslyn at Key Bridge, Arlington, Virginia

NOTICE

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The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Myrta R. Christian, SAP Designated Federal Official, via e-mail at christian.myrta@.epa.gov.

In preparing the meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented by the Agency within the structure of the charge.

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Myrta R. Christian, M.S.
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: January 25, 2005

Stephen M. Roberts, Ph.D.
FIFRA SAP, Session Chair
FIFRA Scientific Advisory Panel
Date: January 25, 2005

DIMETHOATE: ISSUES RELATED TO HAZARD AND DOSE RESPONSE ASSESSMENT

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to dimethoate: issues related to the hazard and dose-response assessment. Advance notice of the meeting was published in the *Federal Register* on September 30, 2004. The review was conducted in an open Panel meeting held in Arlington, Virginia, on November 30 and December 1, 2004. Dr. Stephen M. Roberts chaired the meeting. Mrs. Myrta R. Christian served as the Designated Federal Official.

The FIFRA SAP met to consider and review dimethoate: issues related to hazard and dose response assessment. As part of tolerance reassessment activities underway at EPA's Office of Pesticide Programs as mandated by the Food Quality Protection Act (1996), EPA is developing a Registration Eligibility Decision document for dimethoate, an organophosphate (OP) pesticide. The purpose of this SAP meeting was to solicit comment on aspects of the dimethoate hazard and dose-response assessment. In particular, the discussion was focused on the results from the developmental neurotoxicity and cross-fostering studies performed with dimethoate. The agenda for this SAP meeting involved an introduction, background, and detailed presentations of the issues related to the dimethoate hazard and dose-response assessment provided by Dr. Diana Locke (Health Effects Division, Office of Pesticide Programs), Ms. Cheryl Chaffey (Pest Management Regulatory Agency (PMRA), Canada), Dr. Kathleen Raffaele (Health Effects Division, Office of Pesticide Programs), and Mr. Philip Villanueva (Health Effects Division, Office of Pesticide Programs). Mr. Joseph J. Merenda, Jr. (Director, Office of Science Coordination and Policy) and Dr. Randolph Perfetti (Health Effects Division, Office of Pesticide Programs) offered opening remarks at the meeting.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented at the meeting, especially the response to the Agency's charge.

PUBLIC COMMENTERS

Oral statements were presented as follows:

On behalf of Cheminova, Inc:

Abby Li, Ph.D., Exponent
David Gaylor, Ph.D., Gaylor and Associate, LLC
Rick Reiss, Ph.D., Sciences International, Inc.
John DeSesso, Ph.D., Mitretek Systems
Carl Keen, Ph.D., University of California, Department of Nutrition

On behalf of the Natural Resources Defense Council:
Jennifer Sass, Ph.D., Natural Resources Defense Council

On behalf of CropLife America:
Barbara H. Neal. D.A.B.T., The Weinberg Group, Inc.

Written statements were provided by or on behalf of the following group:
People for the Ethical Treatment of Animals (PETA)

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

The FIFRA SAP deliberated on the interpretation of the cholinesterase activity and pup mortality data from the dimethoate developmental neurotoxicity (DNT) and related studies. The Agency requested guidance regarding the use of benchmark dose (BMD) in this risk assessment and on the conservative nature of using brain cholinesterase (ChE) inhibition data to protect against pup mortality following dimethoate exposures.

- The use of BMD in this risk assessment was generally supported by the Panel. Concerns on the specific data analyzed using this BMD model and its results were discussed. The question of "sufficiently robust" data used to conduct the BMD analyses is difficult to answer unequivocally from an experimental point of view. The Panel was concerned that some data sets revealed very similar BMD_{10} and $BMDL_{10}$ values suggesting lack of power in this specific analysis. The estimate of BMD_{10} would be more reliable were there a number of doses that bracketed BMD_{10}. However, in the case of dimethoate specifically and cholinesterase inhibitors in general, it is difficult to make accurate measurements of ChE inhibition at doses below BMD_{10}. As dimethoate is a dimethoxy-compound, it would be expected to elicit relatively rapid cholinesterase inhibition and recovery. The time course of inhibition and recovery of brain cholinesterase inhibition in the fetus/pup would be expected to be markedly different than in the dam brain. Similarly, comparing effects after gavage versus dietary exposure would be expected to have different kinetic profiles which would affect ChE inhibition.

- The proposal to use brain ChE inhibition as the critical endpoint for risk assessment of dimethoate is inherently reasonable. However, the differences between the BMD and lower limit on the BMD (BMDL) for pup mortality and brain cholinesterase inhibition cannot be ascertained without a more thorough analysis of pup mortality data, especially focusing on post-natal day (PND) 1 and PND 1-4, including datasets with positive trend. According to the data presented and at the Agency predetermined benchmark response (BMR) (i.e., 10% for brain ChE inhibition and 5% for pup mortality), the BMD for brain ChE inhibition should be protective against pup mortality.

- The Panel deliberated on data presented with regard to the incidence of pup mortality in the DNT and cross fostering studies. Based upon an increased incidence of pup mortality in the DNT study, the potential disruption in dam behavior as a result of predicted ChE inhibition in the high dose group, a limited cross-fostering study was conducted by the registrant to determine the influence of post-natal factors on pup mortality in the high dose group only. These studies were not designed to address the issue of causality. There was a clear divergence of opinion among Panel members with regard to the utility of these studies to identify maternal factors versus direct exposure related effects on pup mortality. The Panel agreed that maternal stress is a critical factor in pup survival and development and that gestational effects of exposure can be influenced by lactational events. However, the limited design of the cross-fostering study, inclusion of natural control litters rather than cross-fostered control litters, lack of inclusion of all dose groups in the DNT, lack of systematic and quantitative measurements of maternal behavior, and the demise of pups within the first day of birth in the DNT served as the basis for the opinion that the cross-fostering study did not demonstrate that pup mortality was due to maternal factors. The contrary opinion considered the limited design of the cross-fostering study to be appropriate to address the question of whether the pup mortality was a function of gestational exposure or postnatal factors and that the inclusion of only the high dose group and natural control litters was not a fatal flaw to the study. This view was supported by the findings of clustering of pup mortalities in both the DNT and cross fostering studies in a small number of litters, the majority of which were born to dams exhibiting maternal neglect from crude observational data. In evaluating the available datasets, the Panel provided a number of recommendations to the Agency including, 1) evaluation of all data sets available that would include any measure of pup viability, mortality or body weight gain. 2) evaluation of other data sets to determine a possible overall correlation between pup viability and maternal ChE inhibition.

PANEL DELIBERATIONS AND RESPONSE TO QUESTIONS

The specific issues addressed by the Panel are keyed to the Agency's background documents, references, and the Agency’s charge questions.

Questions

Since the release of EPA’s 1999 preliminary risk assessment for dimethoate, new data related to developmental neurotoxicity and reproductive toxicity have become available. These new data have resulted in significant revisions to the hazard characterization and dose-response assessment for dimethoate. In July, 2004, EPA and PMRA jointly submitted a paper entitled “Dimethoate: Issues Related to the Hazard and

Dose-Response Assessment" to the FIFRA SAP for review. This meeting was postponed, however, because additional data pertinent to the assessment were brought the Agency's attention. Furthermore, benchmark dose (BMD) analyses have been conducted on the cholinesterase (ChE) activity and pup mortality data and are now presented in the paper dated November 2, 2004.

Interpretation of the cholinesterase activity and pup mortality results from the dimethoate developmental neurotoxicity (DNT) study and related studies.

A few years ago, EPA developed a BMD approach for modeling the ChE inhibition caused by the OP pesticides for purposes of conducting a cumulative risk assessment (EPA, 2002). This model has been applied for dimethoate data from several studies, and the results are presented in the current paper. (It should be noted that the Agency is not requesting comment on this model per se since it received extensive comment from the FIFRA SAP in 2001 and 2002.) The calculated BMD_{10} for brain ChE inhibition following repeated dosing ranged from 0.2-1.0 mg/kg/day and the $BMDL_{10}$ ranged from 0.2-0.7 mg/kg/day. The calculated brain ChE BMDs are very consistent across age groups, between males and females, and across different studies.

In addition, the pup mortality data from the rat DNT study was also modeled using BMD analysis, with models from the EPA Benchmark Dose Software (www.epa.gov/ncea/bmds.htm). The calculated BMD_5 is 0.5 mg/kg/day and the $BMDL_5$ is 0.3 mg/kg/day.

The EPA and PMRA would like to ask the Panel several questions relating to the interpretation of the pup mortality data, including the use of ChE activity data versus pup mortality data as the appropriate endpoint for use in the risk assessment on dimethoate.

Question 1.1

Please comment on the information available for dimethoate which characterizes the underlying cause(s) of the pup mortality in the dimethoate DNT study and the degree to which this information can be used to determine the impact of maternal neglect/maternal toxicity on pup mortality. [Section II B and Sections II C 2, 3, 5b-d]

Response

The Panel focused a large part of its deliberations on the interpretation of pup mortality data in both the DNT and cross-fostering study. However, these studies were not designed to address the issues of causality. The Panel is in consensus that the database is insufficient to characterize the underlying cause of pup mortality. There is a divergence of opinion among Panel members as to whether the available information can be used to discriminate the contribution of maternal neglect from other post-natal factors on pup mortality.

Several observations were made regarding the design, outcome, and data analysis of the cross-fostering study, particularly pertaining to pup death. A number of Panel members believe that the cross fostering study was sufficient to assess the questions posed. Others felt there was a lack of clarity due to the absence of a cross-fostered control group in the study. Therefore the actual mortality rate for pups as related to cross-fostering per se is unknown. Moreover, measures of maternal behavior said to indicate neglect as another potential basis for the mortality were not done in a rigorous manner or defined sufficiently well to make the findings credible. Observations of the cross-fostering study were apparently obtained by individuals not blinded to treatment, and indications of observer reliability or inter-observer reliability are not available. Thus, the impact of maternal neglect is undefined. It was also noted that the large litter size in this specific cross-fostering study may have added a background stress factor that accentuates any post-natal maternal effects on pup death.

Supplemental information provided by the Registrant on the day of the meeting addressed a number of critical questions with regard to the data set. The larger data set from all litters initiated to provide appropriately timed subsets for use in cross-fostering exposures showed no differences among groups with regard to pup mortality within 24-hours of birth and was helpful in interpreting the overall data.

The possible cause of pup death was extensively discussed regarding the increased pup mortality noted in the DNT, range-finding DNT, and cross-fostering studies. One possible cause of pup mortality may be that maternal toxicity caused the dam to become restless or scatter her pups, or somehow prevent the pups from adequately being nursed, and thus resulting in their death. However, maternal parameters such as food and fluid consumption, as well as change in gestation weight showed minimal changes during gestation and lactation periods. On the other hand, while exposed dams did not display these general signs of toxicity, other biological effects could have occurred to alter normal maternal behaviors (e.g., hormonal).

An additional potential cause for pup death could be due to stress on the dam that would affect her ability to nurture her young. There was no consistent correlation between maternal ChE inhibition and pup death to support the notion that pup mortality is due to the effect of dimethoate on ChE inhibition. On the other hand, it is reasonable to consider that the dams may have exhibited transient aberrant behavior shortly after each gavage dose of dimethoate. Dimethoate's active metabolite omethoate is a dimethyl phosphate. ChE phosphorylated by dimethyl phosphates has a relatively fast reactivation time, with a half-life of about 2-3 hours. While dimethoate is an O,O,S-compound, if it follows the same reactivation rates as the O,O,O-compounds, it is expected that the dams may have had higher ChE inhibition shortly after dosing at the time of monitoring. In other words, it is likely that the procedures followed in sample handling and ChE assay would have allowed some reactivation of ChE activity. The presumed higher ChE inhibition may have resulted in transient aberrant behavior that was detrimental to pups.

Another possible cause of pup mortality could be due to an unidentified metabolite of dimethoate. Some Panel members agreed that the data from the cross fostering study has not ruled out a contribution from pre-natal exposure, in addition to that resulting from post-natal events, as contributors to pup death in the early post-natal period.

Several suggestions were also made regarding data analysis that may provide further information on the contribution of pre- and post-natal factors to pup death. Opinions of Panel members varied in terms of the justification for excluding dams/litters in the analysis of the cross-fostering data based on observations of maternal behavior. Focusing on the data from Group 1A, 1B, and 1C, it was suggested that pup death data should be more carefully analyzed, especially concentrating on the data of earlier pup mortality (PND 1-4), rather than focusing only on the overall data up to PND 11. Considerations were given to two variations of data exclusion: pup death prior to cross-fostering, and the death of 8 pups from the dam (Dam #19) that exhibited "aggressive" behavior (later re-designated as "abnormal" behavior) said to begin prior to cross-fostering. By focusing on the PND 1-4 death, two observations were made: 1) There was an apparent increase in PND 1-4 pup death in Group 1A and 1B when stillbirths and pre-fostering deaths were included in the analysis (Table 4 in DeSesso et al., "*Dimethoate: Key issues for the assessment of potential human health risks*"; November 20, 2004). The pup death per litter index still appeared to remain elevated when data from Dam #19 was excluded. 2) The pre-fostering data showed an apparent trend of increase in death with pups that received 3 and 6 mg/kg/day pre-natally, although pair-wise comparison to the controls showed no statistical significance (Table 9 in "*Response from Cheminova on EPA's data evaluation record for the Dimethoate cross-fostering study*"; November 1, 2004). The Agency is encouraged to closely analyze these and other similar data on early post-natal pup death from all available studies (e.g., DNT, reproductive toxicity studies, dose range study for DNT), and conduct BMD dose-response analysis not just on datasets showing statistically significant effects by pair wise comparison but on those with positive dose-related trends as well. In this regard, one Panel member suggested that the severity of endpoint could warrant BMD analysis at multiple BMR levels, including a level lower than the Agency pre-determined 5%. Similarly, the neurobehavioral observations in the pups could be a part of the overall effects from pre-natal exposure and should be conducted and analyzed more rigorously. This would require systematic collection by individuals blinded to treatment conditions of behaviors which have been operationally defined and with appropriate statistical analysis if they are repeated measures.

There was some attempt to explore data consistency across all available studies. It was noted that there is a major difference between ordinary systemic toxicity and developmental toxicity. The fact that direct dosing of pups between PND 11-20 did not cause pup mortality does not speak to the possibility of developmental toxicity occurring during a specific developmental window, even at lower doses. Specifically, the possibility of direct developmental toxicity during PND 1-4 is not excluded. It was difficult to draw comparisons between the gavage-dosed DNT with the dietary

reproduction studies without some comparison of dietary versus gavage kinetics and dose to the target. The dietary studies indicated very limited apparent effects on pup mortality even in the presence of high inhibition of ChE. Thus, as noted previously, these two events – pup death and cholinesterase inhibition – may not be causally related. Pup deaths may also be caused by some unknown metabolites or stress. Limitations in the study design that compromise the usefulness of this study included the need of a cross-fostered control group, and a lack of quantitative rigor in reporting behavioral observations.

During the Agency presentation phase of the SAP meeting, a question was raised regarding whether mortality is a common endpoint for organophosphate pesticides. While it was speculated that although fetal or pup death has been observed with other OPs, it may not be a sensitive endpoint. This is an important area that could provide useful context for the evaluation of pup death associated with dimethoate, and the Agency is encouraged to pursue a comparative analysis of data from other OPs regarding this specific endpoint.

Question 1.2

> The results of the cross fostering study suggest that the pup mortality observed at lower doses in the main DNT study may not be attributable to a single dimethoate exposure. Please comment on the evidence that supports or refutes this analysis. [Section II B 2 and II C 5 d]

Response

The Panel noted that there were no single exposures during the cross-fostering study to directly address this issue. If the Agency is referring to the commonly held belief that many developmental effects, including increased mortality, have been shown to occur as a result of single exposures during development, then increased pup mortality could be considered as an appropriate end point to use in risk assessment for single dose exposures.

The Panel has already concluded (Question 1.1) that there is serious uncertainty about the cause (or underlying mechanism) that explains the incidence of pup death in the DNT (or cross-fostering study). The increased neonatal demise of the pups could, however, have both a pre-natal and post-natal component, although the Panel did not reach consensus on the role of gestational exposure in the observed pup death due to limitations in study design.

The cross-fostering study has a number of design flaws and was not specifically designed to address the Agency's question. For example, no true cross-fostered control group was used and the dose comparable to the DNT study did not have a non-cross fostered group exposed gestationally and lactationally for direct comparisons to the DNT study. However, the 3 mg/kg/day group exposed gestationally and then cross-fostered

onto control dams did not exhibit any statistically significant increase in pup death. These data should be treated with caution. Current data indicates that a causative factor responsible for pup mortality is unlikely to be transmitted through the mother's milk, but these data are neither conclusive nor exhaustive. Dimethoate administered to the pups later in life (PND 5-11) did not result in pup mortality, underscoring a window of susceptibility to the developmental toxicity of dimethoate. A number of Panel members felt that the cross fostering study was sufficient to address the specific questions raised by EPA. Panel members noted that a more complete analysis of all pup deaths on PND 1 would aid interpretation of gestational versus post-natal components. Exactly what specific factor(s) from exposed dams is/are responsible for transmitting pup mortality is the key issue, and is not adequately addressed in these studies.

There were two contrary views expressed by the Panel on the involvement of gestational exposure in neonatal pup mortality. The evidence to support a gestational component was provided by the increase in the number of pup deaths in those animals gestationally exposed at 6 mg/kg/day and cross-fostered onto control dams. This was higher than that noted in the controls when the analyses included similarly treated animals that also had still births. Moreover, the demise of the pups very rapidly after birth (within hours) is characteristic of agents producing an in utero developmental effect.

The contrary view of no gestational exposure component in the observed pup death is supported by the findings of: 1) clustering of pup mortalities in both the DNT and cross fostering studies in a small number of litters, the majority of which were born to dams exhibiting maternal neglect from crude observational data; and 2) statistical analyses of cross-fostering data after excluding 2 aberrant dams and the still births, which indicated that mortality in pups exposed to dimethoate during gestation, but not during lactation did not differ significantly from that of controls, but that mortality in pups exposed to dimethoate during lactation but not gestation was significantly increased relative to controls. Moreover, the larger data set from all litters initiated to provide appropriately timed subsets for use in cross-fostering exposures showed no differences between groups with regard to pup mortality within 24-hour of birth. Information provided by the Registrant also indicated that it is biologically possible for maternal neglect to result in pup mortality within the first day after birth since there is evidence that pups can die from improper thermoregulation during this time period. However, measures of maternal behavior said to indicate neglect as another potential basis for the mortality were not done in a rigorous manner, or defined sufficiently well to make the findings credible. Observations of the cross-fostering study were obtained by individuals not blinded to treatment and indications of observer reliability, or inter-observer reliability is not available. Thus, the impact of maternal neglect is undefined. It must be noted that in the deliberation of Question 1.1, some Panel members agreed that the cross-fostering study design was sufficient to conclude that mortality seen at the high dose of 6 mg/kg/day was influenced by the exposure of the dam during the early lactational period whereas a low or no increase in pup mortality was observed with exposure during gestation with cross fostering provided by control animals.

The rapidity of the mortality upon cross fostering is troubling as it might indicate an effect of the parent compound or metabolite acting on a critical target essential for the survival of the pups between PND 1-4. Thus 'maternal behavior' may not be the direct cause of early PN pup mortality. In the absence of data on the effects of directly dosing pups from PND1 through PND4, it is not possible to make any definitive conclusions on the relative roles of maternal neglect vs. direct toxic effects in the pups. One Panel member pointed out that the data do not rule out the possibility that lactational exposure may also cause toxic effects directly in pups. This is particularly a concern in light of growing evidence in the open literature that some OPs exert DNT at doses that do NOT inhibit cholinesterase (Campbell *et al.* 1997; Garcia *et al.* 2001; Jett *et al.* 2001; Johnson *et al.* 1998; Roy *et al.* 2004; Schuh *et al.* 2002; Slotkin 2004).

Question 1.3

After considering the results of the BMD analyses for brain ChE inhibition and for pup mortality, it is proposed that brain ChE inhibition be used as the endpoint for the dimethoate risk assessment for all durations of exposure (e.g. acute, chronic). This would also be protective for the pup mortality endpoint, because available data indicate that brain ChE inhibition occurs at doses similar to or lower than those causing increases in pup mortality. A number of factors were considered in developing this proposal:

Brain ChE inhibition occurs at doses similar to or lower than those causing ChE inhibition in other compartments;

BMD analyses results indicate a very robust dose-response curve for brain ChE inhibition, with similar BMD_{10} values from studies with varying modes of administration (dietary or gavage) and durations (short term for DNT studies and longer term for reproduction studies);

BMD analyses results indicate similar dose-response curves at all ages, with no difference in BMD_{10} values for different age groups following similar exposure durations;

Comparison of BMR dose levels for brain ChE inhibition and pup mortality following repeated dosing indicates that ChE inhibition occurs at doses similar to those associated with increases in pup mortality;

Evaluation of pup mortality data from the cross-fostering study reveals clear increases in mortality only at the highest dose following short-term exposure, indicating that increased mortality at lower doses occurs only with repeated dosing;

Comparison of the No-Observed-Adverse-Effect Level (NOAEL) for

increased pup mortality from limited dosing with the BMD_{10} for brain ChE inhibition following a single dose indicates that brain ChE inhibition occurs at doses below those causing a clear increase in pup mortality.

Please comment on the evidence that supports or refutes this proposal (Sections IIB 4, and II C).

Response

The Agency requested guidance regarding the conservative nature of using brain ChE inhibition data to protect against pup mortality following dimethoate exposures. Benchmark dose analyses of both pup mortality and brain cholinesterase inhibition following dimethoate exposure have been conducted by both EPA and PMRA, and also by the registrant. The report states "In cases where data are sufficiently robust to support an analysis, BMD modeling is preferred over the use of NOAELs and Lowest-Observed-Adverse-Effect Levels (LOAELs)…". From an experimental point of view, the question of "sufficiently robust" data used to conduct these analyses is difficult to answer unequivocally. While the report indicates that the general model of determining benchmark doses has already been reviewed by SAP, some comments on this method appear appropriate. First, review of data used to estimate BMD_{10} for cholinesterase inhibition (e.g. Table 1) suggests that data containing effect levels of 2-12 percent are considered "sufficiently robust" for benchmark analyses, though this conclusion could be debated.

The use of BMD in this process was generally supported by the Panel. The estimate of BMD_{10} will be more reliable when there are a number of doses close to BMD_{10}, below and above, but in the case of dimethoate specifically and cholinesterase inhibitors in general, it is difficult to make accurate measures of ChE inhibition at doses below BMD_{10}. Adding doses much higher than BMD_{10} may give a fit that looks good, but will not substantially improve the estimate of BMD_{10}.

P-values for goodness-of-fit Chi Square tests for the BMD models are presented and generally indicate significant model fits. However, these p-values must be interpreted with caution. The null hypothesis for goodness-of-fit tests is that the model explains a large fraction of response variability. Unlike experimental hypotheses tests where one hopes to reject the null hypothesis, in goodness-of-fit tests one hopes to not reject the null hypothesis and thus conclude adequate model fit. In situations where there are few data points and large experimental error, the goodness-of-fit test is not very powerful, hence the probability of not rejecting the null hypothesis when the alternative is true is quite high. The conclusion of an adequate model fit does not necessarily imply that the model form used is the best and the low power of these test increases the uncertainty in BMDL10 estimates that are derived from the model.

Furthermore, in some cases the $BMDL_{10}$ is the same value as the BMD_{10}. In Table 1, dams repeatedly treated with dimethoate had a BMD_{10} of 0.3 mg/kg/day and a

$BMDL_{10}$ of 0.3 mg/kg/day. In Table 2, dams treated with 3 or 6 mg/kg/day dimethoate showed 75 or 88% inhibition of brain cholinesterase activity, giving a BMD_{10} of 0.2 mg/kg/day. The $BMDL_{10}$ from this same data set provided the same number, i.e., 0.2 mg/kg/day. Other data sets revealed very similar BMD_{10} and $BMDL_{10}$ values using relatively minimal data sets for dose-related analysis. While a statistical model based on dose-response relationships of anticholinesterases may provide some framework for conducting these analyses, it is hard to imagine how any model could generate exactly the same numbers for BMD_{10} and $BMDL_{10}$.

One uncertainty in the evaluation of the data presented regards the unclear nature of brain ChE inhibition in both dams and pups. As dimethoate is a dimethoxy-compound, it would be expected to elicit relatively rapid cholinesterase inhibition and recovery. Furthermore, the time course of inhibition and recovery of brain cholinesterase inhibition in the fetus/pup would be expected to be markedly different than in the dam brain. However, no time course data were provided in the review that would allow the Panel to judge the appropriateness of times used to evaluate cholinesterase inhibition.

Issue 1b in the presentation by the Agency states that "increased mortality occurred at doses causing various levels of inhibition", "in some studies, considerable brain ChE inhibition was seen without … pup mortality", and "low level of brain ChE inhibition (was noted) in pups at doses with increased mortality in the main DNT study". In considering this information, it must first be realized that the results were collected from several different studies with obviously different outcomes. Just considering pup mortality in the full DNT and companion ChE studies, it is not difficult to imagine that the effects on cholinesterase could have been markedly different between those two studies. That is, we might have seen different levels of inhibition if tissue ChE had been assayed in the full DNT study. Second, it is entirely possible that any sign or indicator of toxicity might be different from studies using gavage or dietary exposures. With the more extensive peaks of inhibition that can occur with gavage dosing relative to dietary exposures, one might expect more toxicity and possibly altered development of tolerance relative to the degree of brain ChE inhibition. Finally, there is a problem in using the full DNT study and the companion ChE study to determine the relationship between the degree of ChE inhibition, on one hand, and pup death, on the other. Since pup mortality at comparable doses of dimethoate was much lower in the ChE study than the DNT study, the true correlation between these variables remains unclear.

Having said all this, the proposal to use brain ChE inhibition as the critical endpoint for risk assessment of dimethoate is inherently reasonable. Typically with ChE-inhibiting pesticides, there is a dose "gap" between ChE inhibition and any toxicity, in particular mortality. In the EPA BMD analyses, a restricted data set was used to assess pup mortality, but all ChE data were analyzed. However, the differences between the BMD and BMDL for pup mortality and brain cholinesterase inhibition cannot be ascertained without a more thorough analysis of pup mortality data, especially focusing on PND 1 and PND 1-4, including datasets with positive trend, as recommended under question 1.1.

On review, it appears correct to say that the data indicate that brain ChE is inhibited at doses equal to or lower than those causing inhibition of plasma or RBC ChE. Another issue regarding ChE inhibition is whether inhibition in pups was inappropriately measured because of rapid resynthesis of acetylcholinesterase in young animals and the failure to determine an optimal time for analysis after bolus dimethoate administration. This issue was also addressed in the context of Question 1.1. The dose-response relationship for brain ChE inhibition appears relatively similar across age groups and dosing strategies, but as noted before, these results may be biased by potential lack of concordance in peak times of enzyme inhibition between fetus/pups and the dam. The consistency among the ChE inhibition curves among studies may well be the result of the rapid reactivation of the dimethyl-phosphorylated ChE together with the rapid "aging" of the dimethyl-phosphorylated ChE. The rapid aging could lead to a stably-inhibited ChE with similar levels of inhibition from study to study. But, because of the potential for partial reactivation before aging, the level of ChE inhibition detected could be less than the real level at the time of peak effect.

The essence of this question is whether brain ChE inhibition can be used as the endpoint for dimethoate risk assessment for all durations of exposure and whether this use would be conservative and protective of the endpoint of pup mortality. It can be argued that the answer is affirmative. According to the data presented and at the Agency predetermined BMR (i.e., 10% for brain ChE inhibition and 5% for pup mortality), the BMD for brain ChE inhibition should be protective against pup mortality. If the present data do underestimate the degree of ChE inhibition, then it is even more likely that enzyme inhibition occurs at lower doses than those associated with pup mortality. If pup ChE inhibition is still more seriously underestimated than adult brain, a margin of safety is suggested by the fact that in direct dosing, dimethoate caused much less inhibition in pups than in adults. However, the brain ChE inhibition dose-response curves may not be similar for all ages. In fact, the diverse model estimates of the m parameter would indicate that the dose-response curves for all brain ChEI datasets are not similar. For this reason, and in view of the potential error introduced by assaying at an inappropriate time after exposure, the calculation of an exact value for relative sensitivity of pups and adults remains an unresolved issue.

Additional General Comments from the Panel

A number of questions were raised during the course of the Panel discussion regarding experimental design, conduct of study, variability of endpoints, data presentation and statistical analysis related to developmental neurotoxicology studies as components of risk evaluations. These are issues that would normally be raised with in any scientifically valid DNT study. The inclusion of behavioral assays in DNT studies has the potential to provide needed information on developmental neurotoxicity that

cannot easily be obtained by other means. It is important that such assays be carefully designed with clear objectives, carefully defined and implemented measurement protocols with state-of-the-art and generally accepted statistical methods that are appropriate for the types of data collected (e.g., survival analysis and incorporation of random effects and repeated measures components). This will ensure that these studies provide the greatest level of useful and rigorous information for assessment of developmental toxicity. With respect to behavioral assays that rely on observational approaches, it is imperative to operationally define the measured behavior, confirm inter-rater reliability and monitor implementation for consistency over time.

At the (1999) SAP meeting, in response to questions raised by the SAP regarding the DNT protocol, a retrospective analysis of existing study data was presented by the Agency. Since that time, additional DNT studies have been conducted and submitted to the Agency. Given the questions raised by the current Panel, it is recommended that the Agency present a review of this data to the SAP for advice with regard to such issues.

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