FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

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DIMETHOATE: ISSUES RELATED TO HAZARD AND DOSE RESPONSE ASSESSMENT

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C O N T E N T S

P R O C E E D I N G S

DR. ROBERTS: Good morning and welcome to the continuation of our session on dimethoate, issues related to hazard and does response assessment. My name is Steve Roberts and I will be the Chair for today's session.

Before we begin taking on our next question there are a couple of things I think we need to do, one is to re-introduce the panel in case we have any new members of the audience, so I would like to take just a moment to do that now and go around the table and have each member of the panel indicate their name, affiliation, and area expertise, and as we did yesterday I will begin on my right with Ruby Reed.

DR. REED: Nu-May Ruby Reed from California Environmental Protection Agency. I am a risk assessor and I do risk assessment for pesticides.

DR. RIVIERE: Jim Riviere from North Carolina State University. I do pharmacokinetics, dermal absorption and chemical mixtures.

DR. FISCHER: Larry Fischer from Michigan State University, I am interested in environmental

toxicology and on the side the use of science and risk assessment.

DR. CORY-SLECHTA: I'm Deborah Cory-Slechta from the Environmental and Occupational Health Sciences Institute. I am interested in work in developmental neurotox and neurodegenerative neurotox with an emphasis in behavioral toxicology.

DR. FOSTER: I'm Paul Foster from the National Institute of Environmental Health Sciences. I'm a reproductive and developmental toxicologist and my major interest is in the development of the reproductive system.

DR. COLLINS: I am Tom Collins, I am with the Food and Drug Administration, I work for CFSAN, Center for Food Safety and Nutrition. I am a developmental toxicologist.

DR. FRANCIS: Bettina Francis, University of Illinois. I'm a developmental toxicologist with a strong interest in pesticide toxicology.

DR. BRIMIJOIN: Steven Brimijoin, professor at Mayo Clinic, department of molecular pharmacology. I'm interested in all aspects of (inaudible) biology

including neurotoxicology.

DR. LEIN: Pamela Lein, Organ Health and Sciences University. I'm interested in developmental neurotoxicology.

DR. PESSAH: I'm Isaac Pessah, University of California Davis. I'm a molecular and cellular toxicologist.

DR. MACDONALD: Peter MacDonald, professor of mathematics and statistics at McMaster University in Canad with a general expertise in applied statistics.

DR. POPE: Carey Pope, professor of toxicology at Oklahoma State University and neurotoxicologist.

DR. HARRY: Jean Harry, NIEHS. I'm head of the neurotoxicology group.

DR. ISOM: Gary Isom, Perdue University, neurotoxicologist research interest and mechanisms in neural degeneration.

DR. FREY: I'm Chris Frey at North Carolina State, I'm in the environmental engineering program with interests in exposure and modeling.

DR. HANDWERGER: I'm Stuart Handwerger in the

departments of pediatrics and cell biology at the University of Cincinnati. My area of interest is molecular and developmental endocrinology.

DR. CHAMBERS: I'm Jan Chambers with the College of Veterinarian Medicine at Mississippi State University. I am a pesticide toxicologist emphasizing neurotoxicology and metabolism.

DR. PORTIER: Ken Portier, statistician, University of Florida, College of Agriculture and Life Sciences with interest in statistical issues and risk assessment.

DR. HEERINGA: Steve Heeringa, the Institute for Social Research at the University of Michigan. I am a biostatistician and my area of specialty is in the design of research for population-based studies.

DR. ROBERTS: And I'm Steve Roberts, I have a joint appointment in the College of Veterinarian Medicine and the College of Medicine at the University of Florida. My interests are in toxicology and risk assessment methodology.

Our designated federal official again today is Myrta Christian, her job is to keep us in line and

make sure we're fact of compliant and she has a few announcements for us.

MS. CHRISTIAN: Thank you, Dr. Roberts, good morning. I just want to welcome everyone and also to remind everyone that all the documents related to this SAP meeting are available in the OPP docket, also they're available in our EPA website. And I just look for another day full of great participation and stimulating discussions, thank you.

DR. ROBERTS: Dr. Perfetti, on reflection from our discussions yesterday I was going to offer the agency if they wanted to do sort of a follow-up on the topic yesterday or if they're satisfied with the discussion we can move onto the next question, but I thought I would give you a chance to re-cap or do a go-back if you would like.

DR. PERFETTI: I don't think that's necessary, but I do want to welcome the panel for the session and yesterday was very, very informative and very valuable to us and we appreciate it very much.

The next question please.

DR. ROBERTS: Okay, terrific. Dr. Raffaele,

can you pose the second question then to the panel?

DR. RAFFAELE: 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 this evidence that supports or refutes this analysis.

DR. ROBERTS: In some discussion with some panel members I think there was somewhere maybe not entirely clear about what was being asked here and so if you wanted to expand on this or I might in fact give the panel the opportunity to sort of make sure that we understand the question before we respond.

DR. LEIN: I think I was the one who raised the issue yesterday. I'm not clear what you mean by a single exposure since none of the cross fostering studies consisted of a single exposure, could you elaborate on that?

DR. RAFFAELE: It has to do with the way we do risk assessment. We do separate risk assessments for acute and repeated exposures, so the question is whether or not there's information in these studies

which would allow us to determine whether the pup mortality was likely to occur after a single exposure or not and if it was then we would need to potentially use that end point in our acute risk assessments.

DR. ROBERTS: Okay, is that clear for everyone?

DR. BRIMIJOIN: It's clear but I still don't see any data about single exposures, so how can we answer such a question?

DR. RAFFAELE: Well in our analysis we discussed about the doses at which after -- for in the cross fostering study there was no single exposure but there was a repeated, a more limited exposure than what we have in the DNT study and if following repeated exposure at specific doses which was how we did our analysis, so if following repeated exposure at the lower dose people were fairly confident that the pup mortality was not observed then we might be able to say at least up to that dose it was unlikely since it didn't occur after repeated exposure that it would have occurred after a single exposure.

DR. BRIMIJOIN: Now the logic is clear, but

very Byzantine.

DR. ROBERTS: Is anyone else, would anyone else like clarification?

DR. FOSTER: I'm supposed to be leading of on this I thought -- I've made a interpretation of what I thought your question meant and I thought you were driving at the point that if any of this pup mortality had a gestational component to it that you could consider the toxicity due to any gestational component may be just through one single exposure as opposed to throughout that period.

DR. RAFFAELE: That's with the assumption that we generally make for developmental studies and the question is whether in this study given the data that we have we're able to make any judgment regarding the cross fostering study whether if we didn't see it at certain doses even following gestation exposure would we need to make that so, yes.

DR. ROBERTS: With that clarification do you want a moment to pause and reflect before you launch --

DR. FOSTER: It's the first thing I got right

all day. Okay, so I have a number of comments and just to reiterate there were no single exposures during this cross fostering study so there is no direct evidence that addresses this question, I think that's what you have to say up front.

And then my second point was if the agency is referring to this generally held belief that developmental effects occurring during gestation can be shown to occur as a result of a single exposure then increased pup mortality could be considered as an appropriate end point for the use in risk assessment for single dose exposures. I think yesterday the panel has already concluded that it hasn't -- there is no known cause or underlying mechanism that explains the instance of pup deaths seen in the DNT or I imagine that equally refers to the cross fostering study and increase neonatal demise of the pups is considered however to have both a pre and a post natal component to it, I think that's what we agreed on yesterday.

My personal believe is the cross fostering study has a number of design flaws and it was not

specifically designed to address this issue, so I think you're asking it to do things for which it was not specifically designed to do and that's not a fault of the registrant either. So for example there is no true cross fostering control in that study and the dose that would be directly comparable to the DNT study at three migs per kig we never had both the gestational and -- we didn't have a 3 and 3, basically, thank you, Tom, you said it a lot better than I did.

And so if we don't have that we can't really see, we don't really have a good feel for how the cross fostering study actually reiterated the DNT even though the exposure would have been -- because we have comparable exposures, but however you do have to say that in the 3 milligram per kilogram per day group that was just gestation exposed there did not seem to be a statistically significant increase in pup death.

I actually think you should treat that data with caution because this wasn't designed to do that and I think there are some problems with that study overall. I think you were better off addressing it in

a specific study and what I would suggest you do is a more standard teratology-type study where you have dose in from 6 to 20, gestation day 6 to 20, and then allow the pups to litter and just examine them very closely after birth to see what the gestational component is and then I think you would be at a much stronger position to be able to address the question that's posed, so I think they are my comments.

DR. ROBERTS: Dr. Collins, I'm going to go through the discussants first and then I'll get to you.

DR. FRANCIS: Well again, I also have the point that there are really no data on single exposure within the range of the doses used but within the range of the doses used in the present study again, yes, the three milligram per kilogram level did not seem to show definite effects, so apparently it wouldn't do that under single exposure either, but I also feel uncomfortable using this and think that a study that involves single doses would be preferable, and other than that I agree with Dr. Foster.

DR. FISCHER: I agree with Dr. Foster. I

don't see any reason why it couldn't be a single exposure to produce some effect theoretically particularly with short acting compounds and interest in a peak of the response as opposed to an accumulation of factors, a single dose might do it, but again I'm not going to repeat it, but I will -- there's just no way we can tell with no data.

DR. LEIN: Given the logic that you just explained to us I guess that I would agree with the panel's discussion thus far that the three mig per kig exposure data would indicate that there are no effects following gestational exposure to that level and therefore perhaps it would be safe to assume that a single exposure at that dose level would not have an effect.

I disagree with the previous panel members and I believe the cross fostering study despite some of the study limitations does indicate that gestational exposure does not have an effect on pup mortality, I don't think we can -- other aspects of developmental neurotox because other end points have not been very well analyzed, but with respect to pup

mortality I think the data despite its limitations does indicate that gestational exposure is not responsible, it's primary lactational exposure that causes pup mortality.

DR. PESSAH: I agree with everything that's been said including Dr. Lein's uncertainty about gestational exposure questioning of influence of gestational exposure. I actually think that if there is a component that occurs during gestation it's perhaps silent and sets up for the later problem.

What really troubles me is that no attempt in any of these studies was made to address this very rapid toxicity that occurs soon after birth within one to four days or actually within hours but within one to four days in the DNT study. I think that needs to be looked at quite a bit because there they may be some factor or factors transmitted from the mother other than behavioral factors that contribute to toxicity that may have been overlooked.

DR. ROBERTS: Let me open it to other panel members for comment.

DR. COLLINS: You know I agree with what Dr.

Foster has said, just for the record though getting away from the cross fostering study, we did have one study in the comparative cholinesterase where they did do acute dosing, unfortunately the animals they chose, the adult an animals obviously weren't pregnant and they look at post natal date of eleven animals and of course they were killed or sacrificed shortly thereafter, but there was some acute dosing, but there is no study that answers the question, so I am in agreement with Dr. Foster.

DR. HARRY: Can I ask for a point of clarification from one of the presenters earlier just to make sure I'm right on this?

DR. ROBERTS: Sure.

DR. HARRY: Is Dr. Desesso (ph) here? When you did the retrospective analysis and you were looking at all of the litters that were impregnated to use for the cross fostering study and you looked at the pup mortality that happened within those excess litters how far out after birth did you take that, was that just birth or --

DR. DESESSO: John Desesso, that was just day

one, that was just day one, it wasn't the day four, it was the day one so although it's not six hours it went a little bit further than that, but it's close.

DR. HARRY: So on that point I think you do have little pieces of data that may help the Agency, one is if you look at the day one time point and I guess you could look back in the DNT and see which day did those animals die between the one and four if it was primarily very early, then you have a pool of data you could go back and say and in that one there wasn't that much of an effect happening at day one, so it might offer you a source to look at whether there was something happening gestationally.

The other one is you started direct dosing in the DNT at eleven days of age and at least at that dose you know that the first -- there's an assumption that during the lactational period of when you are relying on the milk delivery that the animal is probably getting very little, there's no data on that in these animals but that's an assumption, then you would have a one-shot acute dose on day eleven that you would directly be giving the animals and you had

no mortality from that one delivered dose, so you've got little pieces that may help you support your decision to whether an acute exposure can do that or not.

DR. CHAMBERS: I agree with Dr. Lein's comments earlier that the data don't really indicated that there is a gestational component to the pup mortality, the data suggests that there is something maternal going on, some internal influence in that early few days that are influencing the pups, so I agree with that comment.

DR. LEIN: Just to follow-up on Dr. Chamber's comment, I agree that the data's consistent with the maternal effect however I think it cannot be ruled out that there's lactational exposures causing direct toxicity in the pup since it is becoming well established in the open literature that organophosphate pesticides can cause developmental neurotoxic effects at concentrations that do not inhibit cholinesterase.

DR. POPE: Let me ring in in support of Dr. Lein and Dr. Chambers. My review of the table in the

mortality and that whether they're in the cross fostering study looks clear to me that there's no major effect with the prenatally dosed only animals and this is at a dose of even 6 milligrams per kilogram, unless I'm missing something it looks clear to me that this is a post natal effect of some sort.

DR. ROBERTS: Anyone else like to weigh in on this one? It looks I guess consistent with yesterday, it looks like we do have a divided opinion on the panel regarding the evidence for gestational component.

Let me put Dr. Foster on the spot since he's the lead discussant to sort of summarize what you have heard in our discussion in terms of what you think the panel's response is?

DR. FOSTER: I was going to say confused but I'm sure that's really not the case. I think we have a measure of agreement on there is no direct evidence for single dose exposure that you can put your hand on your heart and say it's related to the pup mortality, on the other hand I think what we have is a difference of opinion on whether there's an gestational component

involved in that pup mortality. I think there is evidence probability to support both cases, you can't rule it out and you can't definitively rule in either, so I think you're in the situation there that makes it even a weaker case to talk about whether or not you can use this for single dose exposures in your risk assessment, you don't really have a concurrence of views on whether a developmental mediated effect has indeed occurred.

DR. FISCHER: Dr. Foster, what's the evidence for a gestational component?

DR. FOSTER: I think when I start to look at this is that when I see having done a lot of reproduction studies is that when I see changes in pup death of the older of one or two per litter over control that causes me concern, the other thing is that if you think about when you're most likely to see effects in the offspring from a gestational exposure in a littering study it tends to be almost immediately after birth when that occurs, and that's exactly what we saw in this study, that the animals were dying in the first few hours after birth.

DR. FISCHER: But this isn't evidence this is a speculation that you're making from experience.

DR. FOSTER: That's right.

DR. ROBERTS: I think Dr. Lein wanted to also make a point and then Dr. Reed.

DR. LEIN: How do you explain in the fact that the animals that were exposed gestationally to three migs per kig and six migs per kig did not exhibit increased mortality when cross fostered to control dames?

DR. FOSTER: Well if you look at it they did, it would depend how you decide which animals you're going exclude or not. And in fact on one of the tables, I have it here in front of me, I think it was table 5.

DR. LEIN: In which document.

DR. FOSTER: This was in the expert document you know there were in the early period, post natal day one to four there were three in the control animals, there were three animals from three litters, and in the ones where it is exposed at six and then cross fostered onto a control there was twelve animals

that were dead from five litters, well that seems to be four times the amount to me.

DR. LEIN: I guess I was going with the assumption that that wasn't aberrant dame.

DR. FOSTER: I'm sorry?

DR. LEIN: That was the group that had the aberrant dame?

DR. FOSTER: No because the aberrant dame was actually of control.

DR. LEIN: She was a control, but she was the one exhibiting the abnormal behavior.

DR. FOSTER: But you still got the 12 from here, if it was treated just according to the table I have in front of me it was gestationally exposed at six migs per kilogram and then cross fostered onto a control. You think it was the aberrant dame that was the control, it was cross fostered off?

DR. LEIN: Yes.

DR. FOSTER: Well it still is from five different litters, I mean it depends -- I'm not so sure I consider an aberrant dame one that delivers six, seventeen pups anyway.

DR. RAFFAELE: I think the table that you actually want to look at is table 4 because that's the one that includes the very early deaths that then were excluded.

DR. FOSTER: And then that's so if you go to table 4 it's ten pups from ten litters in the control and in the six and zero it was twenty-four from nine litters, I consider that more than double.

DR. LEIN: It doesn't change my opinion.

DR. CHAMBERS: There was a comment earlier of your experience, I will tell you of our experience of looking at an animal that is exposed to a fairly high level of an organophosphate anticholinesterase they exhibit very strange behavior with a dimethoate compound they exhibit that behavior very trangently (ph) or tremors and very unusual behavior it still is perfectly logical in my mind that those dames were exposed to levels of the compound that were given high cholinesterase inhibition, it was not monitored because of the quick recovery of the phosphorylate cholinesterase and a tranging (ph) disruptive behavior that would have impacted the ability of the pups to

survive there, that's logical, again it's speculation but it's no more speculation than some of the other comments.

DR. ROBERTS: Dr. Reed let me just say we're sort of revisiting this, I think ultimately the panel is going to have differences of opinion, we can sort of articulate our viewpoints, but I suspect that after doing that it's still going to be a divided panel on this topic.

DR. REED: Yes, I do want to reiterate what I said yesterday. I think I would feel much more comfortable if analysis could be done on the first day of death and I haven't seen that data. And my reasons for these sort of uneasy feelings we vouted (ph) is because I see that in table 9 page 24 of November the 1st a document from Kim Inova (ph) showing that there is some indication of that pre cross fostering death data although separately compared to the control they are not statistically significant, it does indicate a positive train of increase and that should be looked at and any data related to that should be looked at. I would say the first day is a good time frame, I

would appreciate that analysis.

DR. ROBERTS: Getting back to the question about the inference about single exposure, can there seem to be maybe not necessarily differences of opinion, I don't know that I heard a dichotomous response, but it seemed be graded levels of confidence that inferences could be made about what might happen in terms of a single exposure although as Dr. Foster said unfortunately there are no direct data that as the Agency acknowledged in posing this question that to with which to address that.

Is there anything anyone else on the panel would like to add in terms of a response or clarify?

Dr. Raffaele and Dr. Locke, is the panel's response I guess on this going to be as clear it is going to be given that there are differences of opinion or would you like some clarification or follow-up question related to this?

DR. RAFFAELE: I think that we have the panel's input on this and it doesn't look like it's going to get any clearer to us so that's helpful to us.

DR. ROBERTS: Maybe it's clearer but it's not a consensus that's for sure.

Let's go onto the next one then.

DR. RAFFAELE: This one is a bit longer, but after considering the results of the BMD analyses for brain cholinesterase inhibition and for pup mortality it is preposed that brain cholinesterase inhibition be used as endpoint for the dimethoate risk assessment for all durations of exposure, for example, acute and chronic. This would also be protective for the pup mortality endpoint, because available data indicate that brain cholinesterase 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 inhibition cholinesterase occurs at doses similar to or lower than those causing cholinesterase inhibition in other compartments, BMD analyses results indicate a very robust dose-response curve for brain cholinesterase 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 the reproduction studies).

BMD analyses results similar dose-response curve 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 cholinesterase inhibition and pup mortality following repeated dosing indicates that cholinesterase 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 NOAEL for increased pup mortality from limited dosing with the BMD 10 for the brain cholinesterase inhibition following a single dose indicates that brain cholinesterase inhibition occurs at doses below those causing a clear increase in pup mortality.

Please comment on the evidence that supports or refutes this proposal.

DR. ROBERTS: Let me ask a clarification question, I just want to be clear that the question here it relates to whether the brain cholinesterase would be protective of the pup mortality endpoints specifically, not necessarily all endpoints?

DR. RAFFAELE: Yes.

DR. POPE: That makes it a little easier. Well let me start by talking a little bit off about the bench mark dose and the modeling. The Agency and others have conducted these bench mark dose estimates and in the report it states, in the EPA report it states that in cases where data are sufficiently robust is supported analysis, bench mark dose modeling is preferred over the use of NOAEL's and LOAEL's.

And from an experimental point of view the question of whether some of these data are sufficiently robust I think is uncertain. While the report indicates that the general model of determining bench mark doses has been previously reviewed by SAP, I think some comments on this method here are

appropriate.

First, the review of the data used to estimate benchmark dose 10 for cholinesterase inhibition in table 1 of the EPA's report suggest the data containing effect levels of 2 to 12 percent are considered sufficiently robust for these bench mark dose analyses. One of things I'm not is a statistician and I can imagine that there are statistical methods that you can use to determine effective dose 10 levels with data such as this, however from an experimental point of view it seems very questionable.

Calculation of a 10 percent effect dose using other dosages with effect levels 2 to 12 percent seems difficult, furthermore in some cases the bench mark dose low is the same value as the bench mark dose. In table one dames repeatedly treated with dimethoate had a bench mark dose of .3 and a bench mark dose low of .3. In table 2 dames treated with 3 or 6 milligrams per kilogram dimethoate showed 75 or 88 percent of inhibition of brain activity given a bench mark dose of .2 and a bench mark dose low of .2.

I don't see how this is possible to have no variation from the bench mark dose to the 95 percent confidence interval lower bound. While a statistical model based on dose response relationships of the cholinesterase other anticholinesterases may provide a framework for conducting these analyses. It's hard for me at least to imagine how the exact number of the bench mark dose and bench mark dose low estimates can be generated from any model.

I think now shifting more to the question, as I mentioned yesterday one uncertainty in the evaluation of these data regards the unclear nature of brain cholinesterase inhibition in the dames and the pups. As dimethoate is a dimethyl compound as Dr. Chambers pointed out yesterday would be expected to elicit relatively rapid cholinesterase inhibition and recovery from inhibition, furthermore the time course of inhibition and recovery of brain cholinesterase inhibition in the fetus and pup would be expected to be markedly different in the dame, however no time course stated were provided in the review that would allow you to interpret the appropriateness of the

times used to evaluate cholinesterase inhibition.

In EPA's presentation yesterday in issue 1B they stated that increased mortality occurred at doses causing various levels of inhibition, in some studies considerable brain cholinesterase inhibition was seen without pup mortality, and finally low level brain cholinesterase inhibition was noted in pups at doses with increased mortality in the main DNT study.

First it has to be realized that all of these results are collected from a number of studies and unfortunately all studies are not the same. And just considering pup mortality and the full DNT compared to the companion cholinesterase study which I would like to have clarified whether this is a true companion study or if it was just another study done to get cholinesterase data, it is hard to interpret that the cholinesterase data could have been markedly different between those two studies if tissues had indeed been analyzed from the full DNT study.

Second, it would make some sense that any sign or indicator of toxicity might be different from studies using gavage or dietary exposures hidden with

peaks of inhibition possible with gavage dosing that are not possible with dietary exposures one might expect more toxicity and possibly differential development of tolerance with gavage dosing relative to the brain cholinesterase inhibition.

Finally, the degrees of brain cholinesterase inhibition and the companion cholinesterase study noted at doses causing pup mortality in the full DNT study because they don't appear to have listed similar effects on pup survival, don't provide a very good degree of certainty that correlations between cholinesterase inhibition and pup mortality in these two studies should be made.

Now, having said that the proposal to use brain cholinesterase inhibition as a critical effect I think is inherently reasonable. Typically with cholinesterase inhibiting pesticides there's somewhat of a gap between cholinesterase inhibition and any elicited toxicity in particular in mortality. In the EPA bench mark dose analyses there was restriction of data for analysis for pup mortality, in other words they focused only on the full DNT study whereas when

they did the bench mark doses for the cholinesterase data they included it all in a meta (ph) analysis.

In some presentations we saw yesterday when you collected all the pup mortality data the difference in bench mark dose between mortality and brain cholinesterase inhibition was actually increased, the dose response relationship for brain cholinesterase inhibition appeared to be relatively similar across age groups and dosing strategies.

The bench mark dose for brain cholinesterase inhibition should be protective against pup mortality, however this potential for missing the peak brain cholinesterase inhibition by assaying at an inappropriate time after exposure and its influence on the relative sensitivity between the dame and the pup is still an uncertainty.

DR. ROBERTS: Thank you, Dr. Pope.

DR. BRIMIJOIN: I agree with every Carey has just said there. You know if this were a research study we would fault it for not going after the key unknowns. It's given us a lot of data, but it's left us with almost as such uncertainty as we had before

the study started.

On the other hand, so I think we -- the key variables that Dr. Pope has pointed out are two, and one is the one that Dr. Chambers initially mentioned namely that the measured levels of brain cholinesterase inhibition are probably wrong probably because of the potential for spontaneous reactivation from the nature of this compound, and the second one being that we don't know -- we could probably also guess that there is at least a substantial chance that the levels of inhibition achieved in the pups are even more seriously wrong because of the potential for rapid resynthesis of the enzyme that complicates things.

Now these two factors have different effects. The first one would actually be protective, that is to say if there was some variable or even consistent underestimation of cholinesterase inhibition because of reactivation then we could say that at the doses that cause true brain inhibition are in fact even lower than the doses that -- they're even farther below the doses causing mortality then in the data we

have now, so we would have to say that brain ChEi would be protective for then other endpoint mortality especially if it were measured correctly, but probably if we used the existing values it would also be protective.

As to what might be going on in the pups I think it is really key that you know how -- what is the optimal time, what is the peak time and we don't know it. The significance of that information gap is lessened a little bit by the fact that the pups in general, the actual direct dosing studies show that the pups are -- inhibition of the pups are markedly lower at a given does than the adults, so it could be that if we had the correct measurements inhibition of the pup brain might be closer to or even identical with the -- so there's some let's say room for error in that estimate without upsetting the EPA's plan to use brain ChEi in the adults as the regulatory endpoint, so I wish we had more complete information but I think Carey's really summarized the situation well.

DR. CHAMBERS: I agree with Dr. Pope and Dr.

Brimijoin both, I think they both bring out very important points.

I think one thing that probably hasn't been mentioned again with the biochemistry of cholinesterase inhibitors is that the dimethoate phosphates, the phosphorylated, the enzyme phosphorylated by dimethoatyl phosphates not only reactivates more quickly it also ages more quickly and once it ages then that cholinesterase inhibition will be stabled to the analysis, and I think part of the reason why you're getting a lot of consistency amongst the various studies is that you're probably looking at the amount that is aged, so that probably comes into play there in the Data sets.

But both of the previous speakers agreed that this probability is a protective, the BMD on the cholinesterase inhibition probably is protective of pup mortality because it probably is underestimating the amount of cholinesterase inhibition and given that it was obtained in the way that it was coming out the way that it did it would be protective of the pup mortality endpoint.

DR. MACDONALD: I don't have a lot to add to what's already been said, but I wanted to make some comments on fitting models for the bench mark dose data.

The examples that I have seen definitely are rather short of data points and I don't think we should be expecting a model fitting to make up for deficiencies in the data, in particular I think we should always be trying to get more points near the bench mark dose, that extreme high doses will tend to only confuse the issue, also if you don't have enough points the goodness of fit test isn't going to be powerful enough.

And finally just reiterate some remarks we ha made yesterday about the interpretation of the goodness of fit tests. Usually in statistical hypothesis testing you pejoratively hope that you will be rejecting the hypothesis and you're looking for all your P values to be small, but in goodness of fit testing it's the other way around, you want to accept your hypothesis but remember that you can't prove a hypothesis is true, so the evidence that the

hypothesis is true here is that your P values will follow a normal distribution -- not a normal -- a uniform distribution so you will get the right proportion of small ones and the right proportion of large ones, and then that over a lot of model fittings the evidence that you may have the correct model, but I think the most important point here is getting enough data around the bench mark dose before doing the model fitting.

DR. REED: I don't have a whole lot to add either, but I do endorse the approach of bench mark dose, I think it is a good approach to look at large sets of data in terms of comparison.

My comments is manly on sort of the uncertainties. I would encourage the Agency to get more coverage on the uncertainties, for example as I mentioned earlier more data analysis on post natal day one pup death. Also that regarding pup death I felt that because the adversity at the endpoint I think it is advisable to run or to present BMD and BMDL for a different BMR response level, for example one percent.

Just a minor comment about the third bullet

presented in the question to state that the analysis results indicate that similar dose response curve at all ages, you are truly not referring to the dose response curve because I think the curves are different but it's at the BMD and BMDL of 10 percent response is similar, and so that's different than saying the curves are similar because if you look at the coefficients for example for M they are all over the place and so you might want to consider stating this as a similar BMDL at 10 percent response or 5 percent response for pup death instead of the same curve shape.

DR. ROBERTS: Let me ask other panel members if they have opinions they would like to offer on this question?

DR. POPE: Well, I would kind of like to re-address the issue that Dr. MacDonald brought up with the bench mark dose and the idea of selecting more doses around the bench mark dose in order to model the effect at these levels. It doesn't -- what time is it, 9:30 -- it doesn't really address this question, however I think that in some responses

cholinesterase inhibition is not very well suited for this extrapolation at low levels and that's because I can -- again, forgive me, I'm not a statistician, I don't understand these models -- however when you start having responses that you can here's some response, it's at a 5 percent level, here's a response at 10 percent level so you can measure something going up, but with cholinesterase inhibition you are measuring something going down, so you've got a lot of enzyme activity and you're inhibiting it some, and so the idea of measuring this small difference at let's say 5 percent inhibition is very difficult to do experimentally, and I think that is a problem for bench mark dose analysis if you're looking at very low levels of effect.

DR. ROBERTS: Other comments or points? I think what I have heard is a general endorsement of the bench mark dose approach, some technical concerns about bench mark dose calculations in this case, but a consensus that the fundamental question that you asked is whether or not brain cholinesterase would be protective of the pup mortality endpoint, all the

opinions I have heard expressed agreed with that.

If there's anyone on the panel that disagrees with my assessment please let me know. Let me ask then the Agency is our response reasonably clear or are there any follow-up questions that's related to this that you would like to ask?

DR. RAFFAELE: It seemed very clear to us and we thank all the panel members for their input.

DR. ROBERTS: Okay, that concludes the questions that were formally posed to the panel. I told the panel members that we would allow them the opportunity the if there was a comment perhaps that they felt needed to be made related to these issues but was not covered in the question we would have the opportunity to make that comment at the end of the session, so let me create that opportunity now, let me ask the panel members if there are any related issues for which you feel some comment is necessary.

DR. HARRY: Well I think Dr. Brimijoin said this excellently when he said that we were given a lot of data but it's left us with almost as many uncertainties as we had when we started. And that's

raising an issue as we sort of sat here in the last couple of days where we've had a number of questions that have come up and a lot of those have dealt with the experimental design, the conduction, the handling and analysis of data and the presentation of data that comes across to you in a DNT study, not a question necessarily on the protocol, but maybe there are some things that it could be helped in how to interpret or better present the data or suggestions of how to analyze it as Dr. Cory-Slechta mentioned, but they're going to be raised in any DNT study and not just this one.

At our previous meeting too many years ago now, and we will check back to find exactly the document on that, issues were raised regarding the DNT protocol and at that time if I remember correctly a retrospective analysis was given upon the limited number of chemicals that had been evaluated, and the questions were how well was the DNT going to be able to predict useful for the Agency to predict for protecting children's health. And at that time it was then the efforts to go out and try to obtain more data

to be able to evaluate this and it's usefulness.

I think given the questions that have come up around the table regarding that, that it might now be a time to revisit you know what you have and in a constructive-type way that we might be able to come back in as a panel and offer suggestions to say what are other ways to look at this data, what are better ways to help you guys get more information out of the data, just with the example on the motor activity for one, that the data sets may be there, just if you look at them a different way they may offer you a lot more data or other suggestions that could be made.

DR. PERFETTI: Dr. Harry you're absolutely right. We have received now 51 DNT studies and these include chemicals that are not cholinesterase inhibitors, chemicals that other than the OP's, we have reviewed most of them, the rest of them are in various stages of review and we are already putting together a retrospective study because you're absolutely right, we firmly, firmly believe that a retrospective study is needed to tell us what we learned from the various endpoints that you look at in

a DNT and what you don't learn and whether we need to adjust in the presentation of the data.

The retrospective study as I said we're putting together, it's a mammoth project, 51 DNT's and even as we go along another one comes in, another one comes in, and the more studies we have to look at the better it is, and this is of course a study that has many, many facets, so it's a mammoth undertaking.

We are starting on it, in fact we're working on it even as we speak, so we hope -- in the future we will bring a retrospective study on the DNT to this panel.

DR. ROBERTS: Dr. Harry did you want to follow up?

DR. HARRY: What I would like to follow up on is to make the suggestion that you might find some help around the panel as you're progressing through this, so it might be that at a mid-stage of what you're doing the panel might be more helpful to you then all of the effort to get to a final stage of a group and then go back and re-look at things, so just not being a standing member I will open that up for

them to commit that they will do, but I think it would be a benefit to do it in stages.

DR. PERFETTI: We will certainly try to do that.

DR. ROBERTS: Anyone else, any other points? Okay then let me make I guess the same offer to the Agency if having gone through these questions if there are other questions that have come up that you would like to pose to the panel we're here.

DR. PERFETTI: This has been tremendously helpful, believe me it really has, we thank the panel very much.

DR. ROBERTS: Well then if there is no other business on this topic let me thank the panel members for the time and effort spent in preparation, your advice and consideration have been excellent.

Let me also thank the Agency for assembling the materials and presenting it in a way that's really helped us understand the complex data set and understand the issues that were involved. Let me also thank the public commenters for their input, obviously they spent a lot of time sorting through these data

and made some excellent points and their input was very helpful to the panel. And finally let me thank the SAP staff because they're the ones that do all the work behind the scenes to get us all here and get us all the material and make the meetings possible.

If there is no other business to conduct on this, this session on dimethoate is now closed.

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I, Monica Knight Weiss, Stenotype Reporter, do hereby certify that the foregoing proceedings were reported by me in stenotypy, transcribed under my direction and are a verbatim record of the proceedings had.

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MONICA KNIGHT WEISS