

FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

NOVEMBER 30 - DECEMBER 1, 2004

DIMETHOATE: ISSUES RELATED TO HAZARD AND DOSE
RESPONSE ASSESSMENT

WEDNESDAY, DECEMBER 1, 2004
VOLUME II OF II

Located at: Holiday Inn Rosslyn at Key Bridge
1900 North Fort Myer Drive
Arlington, VA 22209

Reported by: Monica Knight Weiss, Stenographer

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1 P R O C E E D I N G S

2 DR. ROBERTS: Good morning and welcome to the
3 continuation of our session on dimethoate, issues
4 related to hazard and does response assessment. My
5 name is Steve Roberts and I will be the Chair for
6 today's session.
7 Before we begin taking on our next question there are
8 a couple of things I think we need to do, one is to
9 re-introduce the panel in case we have any new members
10 of the audience, so I would like to take just a moment
11 to do that now and go around the table and have each
12 member of the panel indicate their name, affiliation,
13 and area expertise, and as we did yesterday I will
14 begin on my right with Ruby Reed.

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

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

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

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

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

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

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

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

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

1 including neurotoxicology.

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

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

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

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

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

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

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

22 DR. HANDWERGER: I'm Stuart Handwerger in the

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

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

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

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

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

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

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

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

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

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

21 The next question please.

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

1 can you pose the second question then to the panel?

2 DR. RAFFAELE: The results of the cross
3 fostering study suggest that the pup mortality
4 observed at lower doses in the main DNT study may not
5 be attributable to a single dimethoate exposure.
6 Please comment on this evidence that supports or
7 refutes this analysis.

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

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

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

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

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

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

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

22 DR. BRIMIJOIN: Now the logic is clear, but

1 very Byzantine.

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

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

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

19 DR. ROBERTS: With that clarification do you
20 want a moment to pause and reflect before you launch
21 --

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

14 DR. ROBERTS: Sure.

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

22 DR. DESESSO: John Desesso, that was just day

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3 DR. FOSTER: That's right.

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

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

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

16 DR. LEIN: In which document.

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

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

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

5 DR. FOSTER: I'm sorry?

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

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

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

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

18 DR. LEIN: Yes.

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

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

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

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

10 DR. CHAMBERS: There was a comment earlier of
11 your experience, I will tell you of our experience of
12 looking at an animal that is exposed to a fairly high
13 level of an organophosphate anticholinesterase they
14 exhibit very strange behavior with a dimethoate
15 compound they exhibit that behavior very transiently
16 (ph) or tremors and very unusual behavior it still is
17 perfectly logical in my mind that those dams were
18 exposed to levels of the compound that were given high
19 cholinesterase inhibition, it was not monitored
20 because of the quick recovery of the phosphorylate
21 cholinesterase and a transient (ph) disruptive behavior
22 that would have impacted the ability of the pups to

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

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

10 DR. REED: Yes, I do want to reiterate what I
11 said yesterday. I think I would feel much more
12 comfortable if analysis could be done on the first day
13 of death and I haven't seen that data. And my reasons
14 for these sort of uneasy feelings we vouted (ph) is
15 because I see that in table 9 page 24 of November the
16 1st a document from Kim Inova (ph) showing that there
17 is some indication of that pre cross fostering death
18 data although separately compared to the control they
19 are not statistically significant, it does indicate a
20 positive train of increase and that should be looked
21 at and any data related to that should be looked at.
22 I would say the first day is a good time frame, I

1 would appreciate that analysis.

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

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

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

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

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

3 Let's go onto the next one then.

4 DR. RAFFAELE: This one is a bit longer, but
5 after considering the results of the BMD analyses for
6 brain cholinesterase inhibition and for pup mortality
7 it is preposed that brain cholinesterase inhibition be
8 used as endpoint for the dimethoate risk assessment
9 for all durations of exposure, for example, acute and
10 chronic. This would also be protective for the pup
11 mortality endpoint, because available data indicate
12 that brain cholinesterase inhibition occurs at doses
13 similar to or lower than those causing increases in
14 pup mortality. A number of factors were considered in
15 developing this proposal, brain inhibition
16 cholinesterase occurs at doses similar to or lower
17 than those causing cholinesterase inhibition in other
18 compartments, BMD analyses results indicate a very
19 robust dose-response curve for brain cholinesterase
20 inhibition, with similar BMD 10 values from studies
21 with varying modes of administration (dietary or
22 gavage) and durations (short term for DNT studies and

1 longer term for the reproduction studies).

2 BMD analyses results similar dose-response
3 curve at all ages, with no difference in BMD 10 values
4 for different age groups following similar exposure
5 durations.

6 Comparison of BMR dose levels for brain
7 cholinesterase inhibition and pup mortality following
8 repeated dosing indicates that cholinesterase
9 inhibition occurs at doses similar to those associated
10 with increases in pup mortality.

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

17 Comparison of the NOAEL for increased pup
18 mortality from limited dosing with the BMD 10 for the
19 brain cholinesterase inhibition following a single
20 dose indicates that brain cholinesterase inhibition
21 occurs at doses below those causing a clear increase
22 in pup mortality.

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

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

8 DR. RAFFAELE: Yes.

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

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

1 appropriate.

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

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

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

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

1 times used to evaluate cholinesterase inhibition.

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

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

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

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

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

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

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

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

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

17 DR. ROBERTS: Thank you, Dr. Pope.

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

1 the study started.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

22 Just a minor comment about the third bullet

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

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

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

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

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

1 opinions I have heard expressed agreed with that.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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