SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

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Reported by: Frances M. Freeman

C O N T E N T S

DR. ROBERTS: Good morning. And welcome to the June 26th meeting of the FIFRA Scientific Advisory Panel.

The objective of our meeting is to advise the agency on some specific issues related to risk to children from exposure to OP pesticides in the context of the OP pesticide cumulative risk assessment.

I would like to begin the meeting today by introducing our designated federal official, Mr. Paul Lewis, and turning the microphone over to him for any announcements or instructions to the panel that he might have.

MR. LEWIS: Thank you, Dr. Roberts.

I am Paul Lewis. And I will be serving as the designated federal official for today's meeting and tomorrow's meeting with the FIFRA Scientific Advisory Panel.

And I would like to welcome panel members and the public to this important meeting of the FIFRA SAP addressing determination of the appropriate FQPA safety factors in the

organophosphorous pesticide cumulative risk assessment addressing susceptibility and sensitivity to the common mechanism, acetylcholinesterase inhibition.

I would like to, again, thank the panel members for agreeing to serve and for their time and effort in preparing for the meeting, taking into account their busy schedules and time commitments preparing for this meeting.

As you notice, on the agenda for this two-meeting, we have several challenging science issues being presented. And the agenda for both days is the full agenda, and meeting times and presentation are approximate, thus, may not keep to the exact times as noted due to panel discussions, panel clarification and public comments.

With that in mind, we want to ensure that adequate time for presentations by the agency, public comments to be presented and panel deliberations are allowed.

For presenters, panel members and public

commenters, we request that you please identify yourselves and speak into the microphones provided, since the meeting is being recorded.

And for panel members, we have distributed copies of overheads being presented today for your interest and for making any additional notes.

For members of the public, and we will be having a public comment period during the afternoon of today's meeting, I request that members of the public limit their remarks to five minutes, unless prior arrangements have been made.

And if you have not preregistered, please either notify myself or my colleagues with the SAP staff to the side of me here to arrange a time for making a public comment this afternoon.

For this SAP meeting, we have established a public docket. All background materials, questions posed to the panel by the agency and other documents related to this SAP meeting are available in the docket.

The docket address and contact

information is available on the top of the agenda outside this room, on the greeting table outside this room. And overheads will be available in a few days.

In addition, the major agency background materials, the agenda, list of panel members are available on the EPA web site.

I want to just touch upon the composition of the FIFRA Scientific Advisory Panel. There are seven permanent members of the SAP, and panel membership represents several scientific disciplines, including, but not limited to, toxicology, pathology, environmental biology and related sciences.

In addition, the expertise of the panel is augmented through a science review board. These science review board members serve as ad hoc members, temporary members of the FIFRA SAP providing additional scientific expertise to assist in reviews conducted by the panel.

My role as the designated federal official for the next two days is serving as

liaison between the agency and the panel. I am responsible for ensuring provisions of the federal advisory committee act are met.

A critical responsibility for my role as a designated federal official is to work with appropriate agency officials to ensure all appropriate ethics regulations are satisfied.

In that capacity, panel members are briefed with provisions of the federal conflict of interest laws.

Each participant has filed a standard government ethics report commonly known as a financial disclosure report.

I, along with our deputy ethics officer for the office of prevention of pesticides and toxic substances and in consultation with the office of general counsel have reviewed the report to ensure all ethics requirements are met.

At the conclusion of this meeting, the SAP will prepare a report as a response to questions posed by the agency, background materials, presentations and public comments. The

report serves as meeting minutes for this meeting.

The agency has requested the panel report and minutes be available as soon as possible. And with that in mind, we anticipate having the report available in approximately two to three weeks that will be posted on our SAP web site. In addition, to be available in our public docket.

I want to again thank all the panel members for agreeing to serve for today. I'm looking forward to very interesting and challenging meeting.

Dr. Roberts?

DR. ROBERTS: Thank you, Paul.

The SAP staff has assembled an outstanding panel of experts for this meeting. And I would like to introduce them now beginning on my immediate right with Dr. Brimijoin and proceeding counterclockwise around the table.

I would like each of the members of the panel to state their name, their affiliation and their expertise related to the subject today.

Dr. Brimijoin?

DR. BRIMIJOIN: Thank you. It says here William Brimijoin. I'm actually William Steven Brimijoin.

I'm chair of pharmacology at Mayo Clinic. And I have had a longstanding interest in the biology of cholinesterases and in their potential roles in development of the brain and nervous system.

DR. HATTIS: I'm Dale Hattis. Clark University. I'm a risk analysis modeler. I'm originally a geneticist. I have done a fair amount of pharmacokinetic analyses. And I specialize in studies of human interindividual variability, in particular, variability comparing children and adults.

DR. POPE: I'm Carey Pope. I'm from Oklahoma State University. My area is neurotoxicology of pesticides. And over about the last 10 years, we have been studying age-related differences in sensitivity organophosphorous compounds.

DR. SULTATOS: I'm Les Sultatos. I'm from the New Jersey Medical School. And I'm a pesticide toxicologist with interest in the toxicokinetic disposition of pesticides and the kinetics of the interaction of pesticides with acetylcholinesterase.

DR. ELDEFRAWI: I'm Amira Eldefrawi. I'm professor in the department of pharmacology and experimental therapeutics, University of Maryland School of Medicine.

My research interest span toxicology as well as pharmacology and mainly with a focus on neurotoxicology.

DR. REED: I'm Nu-May Ruby Reed from California Environmental Protection Agency. I'm a staff toxicologist with department of pesticide regulation. Do I pesticide risk assessment.

DR. MCCLAIN: My name is Michael McClain. I'm a toxicologist. I have spent most of my career in the pharmaceutical industry doing pharmaceutical development.

I have worked for Hoffman LaRoche for 28

years. The last three years, I have been working as a consultant in toxicology doing mostly pharmaceutical development and some work in the area of dietary supplements.

The name of my company is McClain Associates. Most of my work, as I said, is associated with pharmaceutical development.

DR. LAMBERT: I'm George Lambert from the environmental occupational health science institute at Rutgers in the university of medicine and dentists in New Jersey.

I'm the director of the childhood center for neurotoxicology and exposure assessment.

And I'm a pediatrician and a newborn medicine specialist.

DR. MATSUMURA: I'm Fumio Matsumura from the University of California at Davis. I serve as the director of the environmental health sciences there.

My area of expertise is for the pesticide toxicology. And I'm interested in organophosphate for a long, long time.

I'm looking forward to this session.

DR. NEEDLEMAN: I'm Herbert Needleman. I'm professor of psychiatry and pediatrics at the University of Pittsburgh.

My interest has been in the effects of toxicants on children's brain and development.

DR. PORTIER: I'm Chris Portier from the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina.

There, I direct the environmental toxicology program and manage the national toxicology program. And I'm chief of the laboratory of computational biology and risk analysis.

DR. ROBERTS: And my name is Steve Roberts. I'm a toxicologist and professor at the University of Florida with joint appointment in the college of medicine and college of veterinary medicine.

I also serve as director of the center for environmental and human there.

I'm a toxicologist with a research interest in mechanisms of toxicity, primarily involving the immune system and liver, as well as risk assessment and toxicokinetics.

And it is my pleasure to serve as the chair for this session.

I'm pleased that we have with us Ms. Sherell Sterling who is acting director of the office of science coordination and policy.

Good morning and welcome.

MS. STERLING: Good morning. I too would like to welcome the panel and thank you.

Many of the faces that I see around the table are quite familiar. And I think that's indicative of the journey that we're taking together in developing this cumulative risk assessment process.

And I think that this working together through many Science Advisory Panels looking at many of the issues, the foundational issues for this risk assessment process, it is indicative of the real value that the agency is putting on sound

science.

The agency has come such a long distance in a very short time. And I think that's much to the credit of the scientists within the agency. But it is equally important to have had you with us, the Science Advisory Panel, to ensure that we were going in the right direction on this long journey and keeping us going, kind of us a compass in this journey.

I would like to say thank you once again, and we look forward to hearing how you help to steer us today and tomorrow.

Thank you.

DR. ROBERTS: Thank you, Ms. Sterling, for those remarks and for joining us this morning.

We also have with us Ms. Marcia Mulkey who is the director of office of pesticide programs.

Good to see you again.

MS. MULKEY: Thank you. It is good to be here.

DR. ROBERTS: Did you have any comments

for the panel?

MS. MULKEY: I have some brief, mainly in the nature of a hearty welcome, as well as a thank you for your service.

I was sitting here thinking that technically you all are known as special government employees. I think that that technical designation has as much to do with the ethics compliance as anything else, but actually, the terminology fits very well in at least two important ways.

One is that you are truly special, that while we are very proud of our capacity within government to produce science, we are very mindful that the kind of work that goes on in this country in the academic institutions and other governments and in the private sector offers such a rich trove beyond that which our government can produce.

And so you are special in what you add to government's capacity.

And my favorite synonym for government employee is public servant. And as you sit today,

you sit as public servants. You sit here doing something, not just for your government, but for the people at large.

And for that, we are grateful at EPA, but I believe that your fellow citizens are and should be grateful too.

So I hope that you look forward to this opportunity to share with us the joys of public service over this two days.

We are today after a brief sort of update about the various risk assessment models that are being considered or available to consider with regard to the overall cumulative risk assessment for organophosphates going to go into a very what we hope will be a very rich dialogue around all of the science issues that inform and work into the FQPA safety factor determination.

That is to say, the application of the provision of the Food Quality Protection Act that requires an additional safety margin to protect children unless on the basis of reliable evidence the administrator can determine that some

difference safety margin is sufficient for that purpose.

So we have been working with this provision of the statute since the statute passed in 1996. We have engaged with you and others around a lot of the issues. We have produced a science policy paper on the application of the factor in the context of individual chemicals.

And while there are many challenges and there is a great deal of science, there are many elements of the science that go into that thinking in the context of each individual chemical, now we are faced with having to think through this issue in the context of a group of chemicals operating by a common mechanism.

So it is the science that relates to that, not fundamentally different, but in some important ways different from the way we think about it with regard to the individual chemical.

So it is the context of thinking about it with respect to this class of chemicals that we brought forward our science product together with

a set of questions that we hope will frame a dialogue between us about the science that goes into this issue.

So we look forward to that.

DR. ROBERTS: Thank you, Ms. Mulkey.

She wasn't listed on the program, but I see that she is sitting at the table. So let me introduce Margaret Stasikowski who is the director of the health effects division for office of pesticide programs.

I don't know if you had any remarks you wanted to make, but at least I was going to introduce you.

MS. STASIKOWSKI: Thank you. And I look forward to hearing the debate and the advice that you will provide us over the next two days. Thank you.

DR. ROBERTS: And sitting to your left is Dr. Randy Perfetti who is the associate director of the health effects division.

Good morning and welcome.

DR. PERFETTI: Thank you, Dr. Roberts.

I would simply like to thank this panel for this long journey we have been on.

And for this, at least hopefully for the OP chemicals and the cumulative risk assessment, perhaps this will be the last step in this journey. And we can move on to some other stuff so that you will not get bored. Again, thank you very much.

DR. ROBERTS: We're never bored.

I'm not sure this is -- anyway, I won't comment on the other part.

The first presentation scheduled this morning is, in fact, an update on the comparison of outputs of different models that are used in the cumulative risk assessment.

And Mr. Bart Suhre from the office of pesticide programs is here to give that presentation for us.

Welcome, Mr. Suhre.

MR. SUHRE: Thank you. Good morning. My name is Bart Suhre, for the record.

It is my pleasure today to update the

SAP on the status of EPA's involvement in efforts to develop software for conducting a cumulative risk assessment.

EPA's office of pesticide program continues to encourage several efforts along these lines. This slide shows three of those modeling efforts. The DEEM/Calendex, that's a model that was actually used for the assessment that has been posted on the web just recently. And then there is the CARES model and the LifeLine model.

Again, the purpose of today's briefing is to update the SAP on the current modeling efforts applicable to performing a cumulative risk assessment for the organophosphorous pesticides.

The models discussed today have all received a detailed review by the SAP prior to this presentation.

As always, we find it necessary to state our official position on models. Regardless of how many times we state this position, we get the question directed to us, what model is EPA going to use.

The official position is that EPA will evaluate and use all modeling tools that use criterion laid out by existing policy documents and we will continue to encourage development of any of these models.

A little background is shown on this slide. In October of 2001, EPA, at the direction of the SAP, conducted a modeling workshop. That was sponsored by the office of pesticide programs and the office of research and development.

Today's presentation reflects a continuation of this process of comparing models.

The focus of that workshop in October of '01 was limited to residential exposure pathways, since that was a less well-defined pathway.

Today we will concentrate and focus on the dietary pathway.

The October 2001 workshop reflected our initial effort to consider similarities and differences the among these models.

The models included in that October workshop were Lifeline, CARES Calendex and SHEDS.

Today we will be looking at three of those four models. We will be talking about Calendex, CARES and Lifeline.

The October workshop provided those in attendance the opportunity to have first-hand -- to see the first-hand impact that model design has on model outputs.

Designs of model -- examples of model designs that impact the output were things like the algorithm used to estimate the exposure, assumptions used as inputs or hard wired into the models, the methodology used for formatting input data and for sampling that data and the techniques used to generate reports, what was saved by the model and how the models presented that material.

At this time I would like to give you just a real quick summary of what is going to be said for the rest of this presentation. And these will be single slide discussion items.

First, I would like to explain the relationship of the three modeling development groups to EPA and the SAP, describe very briefly

the three models, since they have been reviewed in detail by the SAP, to describe how each model simulates a cohort of one to two-year olds, to describe the enhancements that the models have undergone since they were presented to the SAP, present a slide on some dietary results comparing Calendex and Lifeline results and to briefly talk about the next steps with respect to these models.

We'll start with DEEM/Calendex. This, again, is the model that was used to generate the results that have been posted by EPA. It is a model that was developed by N O V I G E N Sciences, which has just very recently been merged into Exponent.

EPA has a license to use this model. And this model was reviewed by the SAP. The DEEM component of it was reviewed in March of 2000, and the Calendex component was reviewed in September of 2000.

A one slide description of DEEM, it is a probabilistic models that combines dietary

consumption records with residues in or on food with the consumption records in order to produce an exposure distribution.

DEEM is in fact essentially the dietary components of the Calendex model.

Calendex incorporates a time element. And it also aggregates multiple pathways of exposures.

The time element of Calendex is a 365-day, single year, hypothetical year. It aggregates the multiple pathways with multiple routes and chemicals. Buried in that bullet is the implication that it is a cumulative risk assessment tool.

It provides the temporal, spatial and demographic specificity. And it uses -- it draws its cohort from the continuing survey of food intake by individuals, the CSFII records.

This slide attempts to kind of give you an idea of the exposure events that are occurring in the California model.

There are approximately 2000 children

age one to two in the CSFII survey. There are two records per individuals. The assessment that the agency ran included 10 iterations. And of course there is 365 days in a year.

Therefore, for any single day of exposure, there are 40,000 potential exposures -- 40,000 exposure events. If one multiplies that by the 365 days in a year, there are 14,600,000 exposure events.

So when we talk about the 99.9 percentile of a distribution, if we were talking about a single day, we are looking at those 40,000 events.

Enhancements of this model, since the SAP had a chance to do a full model review, a new version of the survey, of the consumption survey has been implemented and incorporated into the model.

Translation files associated with the new consumption surveys were implemented in a CEC enhancements, which is really a critical exposure contribution.

This is a method that the model uses to do some sensitivity analysis once the run is completed.

On to the second model very briefly. It was developed and sponsored by Crop Life America. EPA provided -- EPA and USDA provided technical support to that development team. And CARES was very recently reviewed by the SAP April 30, May 1st of 2002.

CARES is like the Calendex. It's a calendar-based tool. It is based on a hypothetical year, 365 days.

Again, it estimates the multiple pathways, routes and chemicals. Same thing with the previous slide. It provides temporal, spatial and demographic specificity.

What is a little different with this model is that the exposure cohorts are drawn from a census reference population of 100,000.

Again, to look at the simulated cohort for one to two-year olds, out of that 100,000 individuals in their reference population, there

are 3,367 one to two-year olds.

Each of these individuals is mapped to 365 days. Therefore, if one were looking at a single day and they were looking at a percentile of exposures, they would be pooling 3,367 individuals.

If all of those individuals were pooled together for the year, it would be 1,228,000.

CARES hasn't been around that long and the enhancements aren't that great. Although the SAP and EPA was having a little trouble with the robustness of the model that was provided, that has been worked on. And version 1.1, test 2, is much more robust. And there are some additional entry and reporting features added to the model.

The third and final model that we'll talk about today is the LifeLine model that was developed by Hampshire Research and the Lifeline group.

EPA and HRI had a co-operative agreement that resulted in the development of version 1.0 and 1.1.

EPA has recently contracted with the Lifeline group to develop a version 2.0 and to perform an OP cumulative risk assessment with that model.

The Lifeline version 1.0 was reviewed by the SAP in March of 2001.

Lifeline is a little different from the other two that we described, in that the time line associated with Lifeline is not a hypothetical year, but a hypothetical lifetime.

So it runs from birth to death, indicated as zero to 85 years in this slide. It's probabalistic by nature. It estimates again the multiple pathways, routes. So it's an aggregate cumulative model. Provides the standard temporal, spatial and demographic specificity.

The cohort for Lifeline is drawn from natality records, from birth records, Census birth records, and it starts with drawing from 3.8 million records, birth records.

For Lifeline 1, again, always start at birth. So we really don't have a cohort of one to

two-year olds. You have to start from zero.

So for this particular example we took zero to three years olds as the time frame. And EPA simulated 100,000 individuals for three years, 365 days a year.

Some of the numbers associated with that on any single day or season of the year there would be 10,000 exposure events from which to pull a percentile.

If one were to total up all the exposure events in that three years, it would end up to be 10.9 million.

This diagram is necessary in order to understand a little bit about what Lifeline saves. The previous slide tells you the actual calculations as the model is running. But the model does not save every day that it assesses.

It is set up to run in seasons. And every season, every 90-day period an assessment is made on every day.

At the end of that 90-day period, however, only three numbers are saved. The

numbers that are saved are designated in this slide. The yellow line at top represents the maximum value that was calculated for that season.

The purple line in the center there represents the average for that season, that 90-day period. And the orange square represents a random draw from that particular season.

These are the actual values that are saved and are available to generate reports at the end of the run.

Recent enhancements. Like Calendex, Lifeline has been revised to incorporate the new consumption survey, 9496. And also as shown on a previous slide, we have asked the Lifeline group, we have contracted with the Lifeline group to develop version 2.0 specifically to conduct an OP cumulative risk assessment.

Here are some of the enhancements that go along with that version 2.0 in conducting the risk assessment. One was to track risks using data on route specific toxicity to determine a total -- the MOE, one over MOE, one over MOE

approach.

The original model version 1.1 was using a systemic dose in order to calculate the total MOE.

We added the ability to track multiple chemicals dermal and inhalation absorption factors. The Lifeline group also added the ability to select that random day. And the previous model only had two choices at the end of the season, the average and the maximum.

So the modeling team has added that random day draw. That allows us to compare the two models more closely.

There has been some various modifications to models. One was on tracking ornamentals.

I told you I'd try to be brief. A single slide on the OP cumulative risk assessment. We have spent days and days talking about this.

It has a dietary component, a residential. Dietary component includes drinking water. And there are thousands and thousands of

pages on the web at the site listed there.

The first thing I would like to do in the next two slides is to characterize the results in words and then show some numbers.

So EPA's completed cumulative risk assessments for organophosphorous pesticides using Calendex and Lifeline. These were done by EPA.

MOEs for the dietary pathways are essentially identical for these two models. The MOEs for the residential pathways tend to diverge somewhat. However, both models clearly show that the indoor inhalation is a primary route of exposure on the residential pathway.

EPA at this time is going to present the dietary numbers only. We have not really fully investigated or interpreted the residential MOEs generated by Lifeline.

And we would like to have some time to look at those and understand them before we start showing those numbers.

We have a much better understanding of what is going on with the dietary pathway. It's a

much simpler algorithm. So we will present those today.

The CARES results. There is an organization, Sound Science Policy Alliance is what they call themselves. They have completed a cumulative risk analysis for organophosphates using the CARES model and they have provided those results to EPA.

In words, the MOEs for the dietary pathways produced by the initial run were similar to those produced by the other two models within 30 percent.

Subsequent runs have been conducted. Those actually are coming closer to the Calendex and Lifeline results.

The MOEs for residential, again, tend to diverge with these three models, but, once again, clearly show that the inhalation pathway for this OP cumulative is the primary route of exposure.

The Sound Science Policy Alliance has requested of EPA to make this particular statement. They are in the process of completing

their OP cumulative risk assessment modeling efforts and interpreting the results and they have requested EPA not to present the numbers today.

However, we do have a few numbers. This is a comparison of MOEs for the dietary pathway. Children, 1 to 2, Calendex, these are the results that are posted on the web, and comparable results for the LifeLine model.

As can be seen, the one day numbers are essentially the same. The MOEs do increase as the percentile of exposure decreases, as we would expect.

The 7 and 21 days, though, there tends to be some divergence there between the two models, not much, but some. The Lifeline MOEs are in fact higher in each case. Not significantly, but they are higher.

The only other thing I would point out is that as you move from a single day of exposure to -- the 7 to 21 days are actually 7 and 21 day averages. But as you move from the single day exposure to the averages, the MOEs do increase.

Next steps. EPA is still actively involved with the contract with Lifeline. It will end in August of 2002. There is still a lot of work to do. The interpretation of results, understanding results, are these occurring because of modeling errors, entry errors? Is a true difference in just the way the model treats the information. So that's ongoing.

The Sound Science Policy Alliance will be completing their OP on cumulative risk assessment. We are talking with them and assisting them in that effort.

And we will be involved with interpreting these results and considering them in making decisions down the road.

That concludes my presentation, my update of what is going on with these various models. I'm open for clarifying questions, and then Vicki will take over from there.

DR. ROBERTS: Thank you, Mr. Suhre.

The panel appreciates the efforts of the agency to keep us advised and informed as the

models are developed and implemented for cumulative risk assessment.

Let me just ask the panel members if they have any questions for Mr. Suhre.

I see none. Thank you very much.

MR. SUHRE: Thank you.

DR. ROBERTS: Let us proceed, then, with the next item on the agenda, which is a presentation from Dr. Vicki Dellarco.

It's an introduction and an overview of the approach to evaluating susceptibility and sensitivity of children in the cumulative risk assessment. Welcome, Dr. Dellarco.

DR. DELLARCO: Thank you.

Before I begin, I would like to introduce my colleagues at the table with me. To my left I have Dr. Stephanie Padilla. She is a branch chief within our neurotoxicology division at the National Health Environmental Effects Research Laboratory down at Research Triangle Park.

To her left, we have Dr. Carl Baetcke,

who is a senior scientist in our Health Effects Division, Pesticides.

We have a couple of presentations for you this morning. We want to walk you through our analysis. And what we're going to do is give you a little background. And then I'm going to explain some of the cholinesterase.

I want to point out some of the data we have in hand to inform our analysis on uncertainty and safety factors. And then Dr. Padilla will follow me and talk about the possible causes behind increased sensitivity observed in our animal studies.

And then I'll comment at the end and kind of put it altogether bringing together and the hazard and exposure conclusions and kind of explaining the rationale behind our decision for the FQPA safety factor.

Before I start, I just want to acknowledge the other scientists who helped us with this assessment. There were several scientists from our office of research and

development as well as additional people in our own program.

With respect to background, just a little bit of history, very briefly. And I want to talk about the scope and purpose of this SAP review and explain to you the overall approach that we took in this analysis.

First, what is a cumulative assessment. I think we're all very familiar with this. It is multi chemicals, multi sources of exposures. Therefore, this analysis is made in that context.

As it was said this morning, it has been a long journey. We have been to this panel numerous times to seek your advice and your input. I think this is the 25th session. And I can think back to the first time we came to you in '99 refer guidance where we had three OPs in just the food pathway to just this past February where we presented our full preliminary assessment of 30 OPs in all sources of exposure.

We have revised that assessment after considering public comments and the SAP comments.

And that was released in early June.

But at the February review, one of the comments that we got from you and also from the public is that we needed to address the potential risk to children. We had discussed the exposure part of that, but the hazard piece was lacking. And that's the focus of this review.

We like your comments on our hazard conclusions. And we have sketched out three topic areas and a series of questions, the role of acetylcholinesterase in development, our interpretation of the animal studies on increased sensitivity of acetylcholinesterase inhibition caused by these OPs and what these animal studies mean in terms of risk to children.

I want to also point out a couple things about this review. We want you to focus on the common mechanism of toxicity. Namely, acetylcholinesterase inhibition.

And this is important to do because these compounds were grouped on that common effect and on that mechanism. And therefore, the

estimation of their joint risk is based on that premise. And that's why we apply a simple dose addition (ph).

It is also important to point out that the cumulative risk has its different scope and purpose. It is different from a single chemical assessment. But single chemical assessments are typically done before we conduct a cumulative.

And in those single chemical risk assessments we have considered all the mechanisms and all the potential toxicities of these individual OPs and have made separate FQPA decisions for the individual members of the class.

For example, chlorpyrifos has a large number of studies concerning its potential effects on the developing nervous system. All those effects were taken into consideration. And we retained the 10X FQPA factor in that case.

But because the scope of the cumulative assessment is different and were focused on the mechanism and the effects that can be associated with that mechanism, we have to revisit those

decisions for the group as a whole.

Although we're focused on the hazard piece on our paper, we did provide the exposure component of this analysis because it is important consideration in looking at risk to kids.

And what we have done is in the report provided a brief summary of important aspects that pertains to children's exposure.

There is a larger exposure discussion in the main assessment. But we have tried to summarize in this report again the key aspects. And when I present my risk characterization, I'll go over those again. I will also mention some of the updates that we have done.

Let's talk about the approach we took. It was important to consider the FQPA provision as well as some of the policy papers that we have developed.

And in terms of the approach it is, it is guided by the legislative language. What FQPA states is that in the case of threshold effects, we have to apply an additional 10-fold margin of

safety for children, for infants and children.

However, the administrator may use a different margin of safety for the pesticide chemical residue only if on the basis of reliable data such a margin will be safe for infants and children.

And what this means is that FQPA established a presumption in favor of applying an additional 10X factor. So unlike traditional uncertainty factors in risk assessments where you look at the data and you try to make a decision whether you need to apply an uncertainty factor, in this case you have a 10X and you have to look at the data to see if it is sufficient or you might be able to reduce that or remove that factor.

In this analysis, it is important to take into account, as stated in FQPA, the potential for pre and postnatal toxicity as well as the completeness of the toxicity and the exposure database.

We have two policy papers. Some of you

were actually on the panel when we took our FQPA guidance document. And we have a larger document that focuses on this analysis in individual chemical assessments.

And because the focus was on individual chemical assessments, we developed a smaller paper which we gave to you as a reference on how you look at those determinations and at cumulative risk assessments.

It is important to point out that the smaller paper on cumulative risk assessment does draw on many of the concepts and principles within the larger paper.

Again, both guidance documents, whether you are looking at a single chemical assessment or a multiple chemical assessment, is structured around these three areas of analysis.

This assessment was structured around these three areas of analysis, the completeness of the tox data, the degree of concern for pre and post natal tox, and the completeness of the exposure.

However, what is different in the cumulative assessment, again, as I mentioned earlier, is the analysis is focused on a common mechanism of toxicity and the associated effects in the young.

So this analysis is focused on the ability of these OPs to target and inhibit the enzyme, acetylcholinesterase. And therefore, the FQPA analysis looks at that information that pertains to that common effect and that mechanism.

Also, what is different in a cumulative assessment is that you can address or account for uncertainty in two different places in the risk assessment process and cumulative.

If the uncertainty pertains only to certain chemical members, in other words, it is not a shared characteristic of the group, you may use a factor to adjust the relative potency factors on a chemical specific basis.

However, if the uncertainty does pertain to the entire group, it's shared by the group, then you would apply a factor, as a group factor,

after you have determined the joint risk by developing the margins of exposures.

That's it for my background materials. I'll take questions on that. If not, we'll move on to the next presentation.

DR. ROBERTS: Thank you. Let me open it to the panel for questions or clarifications. Are there any?

If not, let's move on.

DR. DELLARCO: What I'm going to do is review the cholinesterase studies that we had available in immature animals. I'm also going to talk about some of the key questions that we asked of the data to work through our analysis.

Of course, the first question is why do we care about acetylcholinesterase in the young. What are the potential toxicities.

And it's not only cholinergic toxicity that may result in both the young and adult due to the accumulation of acetylcholine and hyperstimulation of the nervous system, but there have been several studies that have emerged over

the last several years supporting the notion that acetylcholinesterase and the neurotransmitter, acetylcholine, may play important roles in the development of the nervous system.

So it is important to look at cholinesterase inhibition from that perspective too.

Then the next question is what is the most sensitive and pertinent endpoint that we should focus our analysis on.

Given that acetylcholinesterase is the mechanism and would be the precursor to effects on the nervous system, that would be the most sensitive and pertinent endpoint.

And again, it's the focus of our analysis. When we have looked at all the available data that we have on OPs and the literature and the studies that have been submitted to us by our registrants, we have not seen neurodevelopmental effects in our animal studies that occur at doses below those which cause cholinesterase inhibition either in the fetus or the pup and/or the pregnant

dam.

And therefore, we're going to evaluate the sensitivity of cholinesterase inhibition to account for the potential pre and post natal toxicity that may occur on the nervous system in the young.

Simply, we're going to look at, will the young show cholinesterase inhibition at lower doses than adults. Or at the same dose will they be more inhibited.

This is just a table of all the OPs in our assessment. There were 30 OPs that were included in our cumulative assessment group. When I get to the risk characterization part of the presentation, you will see not all of these are contributors. I will point out who are the contributors of the total cumulative risks in each pathway.

In looking at cholinesterase inhibition in young, it is important to see if we have data that evaluates all development stages.

Our studies kind of fall into two types.

There are studies where there was gestational exposure. In this we have some information on the fetus. And there were also studies where they directly dosed the pups, or postnatal exposure.

Let me just go over the gestational exposure studies. We have data available on 10 OPs. And typically, the route of administration was in the diet via feeding, but there were some gavage studies too.

And the compound was administered the sixth day of gestation to day 20. And cholinesterase measures were made on day 20, typically. And all compartments are usually evaluated, the blood compartments as well as the brain.

And some studies looked at the postnatal rat at day four.

What we see from these studies is that, typically, cholinesterase inhibition does not -- you typically see more cholinesterase inhibition in the dam than you do in the fetus.

We can't really compare sensitivity

quantitatively because we don't know the absorbed dose or the delivered dose to the fetus. But the conclusion that we draw from these studies is that the fetus generally will not exhibit more cholinesterase inhibition than what is found in maternal tissues.

And this is not surprising because there are protective systems in the mother. The fetus is likely getting a lower dose. There are some studies on chlorpyrifos which suggest that.

And also, the young are really geared up for protein synthesis. So they are resynthesizing the protein or the enzyme much more rapidly, new enzyme, than the adult is.

So let's talk about the postnatal exposure studies. Again, this is direct dosing. And we have data on six OPs where we can do a comparison with the adult.

The route of administration practically has to be oral gavage, because baby pups don't start eating until about PND 21 totally. So these studies are gavage studies. There were a couple

studies that involved subcutaneous injection. There are single dose studies. There is repeated dose studies. Cholinesterase measures were typically in all compartments.

And we have data on PND on day 7 after birth, day 11. These are typically the acute studies and the measured cholinesterase inhibition at that time. And the repeated studies start around day 11 and end on day 21 and make the measurements there.

What we see from these studies is that some OPs do cause an increase in sensitivity in the young rat, but not all.

Therefore, an important conclusion is age-dependent sensitivity is not necessarily a shared characteristic of our OP cumulative assessment group.

This is just a table to qualitatively show you the responses. There are some OPs that you don't see any sensitivity with, like dimethoate and methamidophos.

And there are some that you can see

sensitivity after an acute study and a repeated study like methyl parathion and malathion. And chlorpyrifos, you only see sensitivity after acute dosing, not in the repeated study.

In the paper, we mention that towards the end of the analysis we attempted to model the data so we could look at the degree of difference between the pup and the adult.

And this is dimethoate. And this comes from a study that our registrant submitted to us. We have graphed here just the repeated dosing study. That starts day 11, measures on day 21. And this is female brain.

What you see is that there is not increased sensitivity. In fact, the adult is actually more inhibited than the pups. It looks that way from the graph.

But if you really look at those values, at the high dose, three mgs per kilogram, they are not statistically different. Their standard deviations are overlapping.

And if I had plotted the acute studies

in all compartments, in both sexes and a repeated study for the male brain you would see those dose response curves right on top of each other. So we don't see a difference with this compound.

Here is malathion. Again, this was a study that was submitted to us. And we did provide you the data entry records for these. You have that.

And here you do see a difference. And if those dash lines going down are the benchmark 10 responses, and it is about a threefold difference for malathion.

This is a repeated study. It is red blood cell, the male. That's because we see more of a response at the lower portion of the dose response curve for red blood cell.

Another thing that we see in these studies, if you kind of put them altogether, particularly there is quite a bit of data on chlorpyrifos, so you can look at different postnatal stages, but the degree of difference between the immature animal and the adult

diminishes as the pup's matures.

So you can see more of a response if you treat and measure at PND 7 versus PND 21.

So typically, in the acute studies you can see more of an effect because that's what you are dosing and measuring than you do in the repeated studies.

So this brings us to why. Why do we see this. Why do we see this sensitivity. How do we explain these results. So this will be very important in our characterization of the potential risk to children.

So I'm going to end my presentation right there and let you ask questions before Stephanie tells you about the biological factors involved.

DR. ROBERTS: Dr. Dellarco, it appears your presentation has generated quite a bit of excitement in the room next door.

Let me allow the panel to ask any questions they might have.

Dr. Hattis.

DR. HATTIS: Early on in your discussion, you made the statement that you have not seen developmental effects at doses that don't produce appreciable cholinesterase inhibition in either the young animal or the dam.

It is just that pharmacodynamic analysis of effects in relation to the cholinesterase inhibition that I think is going to be very helpful to document.

Can you elaborate more on your observations along those lines?

DR. DELLARCO: Right now we're only talking about the empirical observations. But we have DNT studies that not only have measured cholinesterase, but have looked for other neurological effects. Typically, we do not see those neurological effects occurring at doses in which we can see cholinesterase inhibition either -- there is a lot of racket here behind me (room next door) -- in the postnatal rat or in the pregnant dam.

You have to keep in mind you are going

to see a lower amount of inhibition in the fetus. But you will see that significant inhibition in the pregnant dam.

DR. HATTIS: I think that that is a key issue. I think it's a key issue that will be helpful to be informed by a quantitative analysis.

And If you had any quantitative analysis I would hope it would be --

DR. DELLARCO: That's what you're looking for, do we have any pharmacokinetic data on the absorbed doses that we may be seeing in fetal tissues? I'm trying to get at what you are --

DR. HATTIS: No. Essentially, the idea is that you have got comparisons at the level of cholinesterase inhibition.

But as I think as you observed, the cholinesterase inhibition is to some extent a precursor to the effects of real concern, and so it's very important, I think, to analyze what effects, what risks of effects do you see in relation to the cholinesterase inhibition, both

the magnitude of inhibition and, I think, the predicted duration of inhibition.

So either in cholinesterase inhibition in terms of a peak or a trough level or in terms of an area, the curve of the difference between normal and inhibited levels.

I think that that's sort of the key step in the chain of argument that I would hope perhaps Dr. Padilla, as well as you, could address.

The discussion at the beginning of the document that I suppose you are going to present has a lot of discussion of different kinds of influences of acetylcholine and cholinesterase and its inhibition on developmental processes.

But what we need to understand is the relationship of those things to the quantitative measures that we have that I think represent the kinetics.

DR. ROBERTS: Dr. Padilla, could you identify yourself?

DR. PADILLA: Stephanie Padilla. I'm with the EPA.

First of all, usually, people that measure the neurobiology often don't measure the cholinesterase inhibition. But if you read the papers, you can make a guess at what the cholinesterase inhibition is.

And you are usually working at concentrations that will produce cholinesterase inhibition if it's an in vitro kind of system.

In vivo, the papers that did measure the cholinesterase inhibition, usually, as far as I know, and I'm open to correction here, didn't see any effects on the neurobiology at doses.

Even the two new papers that I had sent you from Sloktin's laboratory where they were seeing effects at 1 milligram per kilogram in the dams that were dosed repeatedly, this is gestationally, the fetal brain did not show any cholinesterase inhibition. At 2 milligrams per kilogram there was significant cholinesterase inhibition. So you are in the cusp there.

But those dams, although he didn't measure, it has been measured in other tissues,

probably did show about 50 percent blood cholinesterase inhibition at that dose.

And if you look at other studies -- the other problem with measuring cholinesterase inhibition, especially in gestational studies and with the young animals, is measuring of the time peak effect because it is gone so quickly in those young animals.

And I know that Sloktin measured his at the right time. But some of these other studies waited 24 hours. And if you did, you are probably not going to pick it up. So that's the other issue. There's whether they measured it. Did they measure it at the right time and did they measure it in all compartments, including the dam.

From the papers that I have read, I cannot cite any instance where they did not see blood cholinesterase or any cholinesterase inhibition in the presence of effects.

DR. ROBERTS: Dr. Portier has a follow-up question. And Dr. Padilla, if this is something that is going to be covered in your

presentation, let us know.

DR. PORTIER: I specifically want to ask about the Sloktin studies.

Your comments confuse me a little bit. The first study by Sloktin where they measured the cholinesterase inhibition shows a nonsignificant effect at 1 milligram per kilogram per day on cholinesterase inhibition, roughly a four percent by sex in the two groups.

Yet, there is a significant effect on a number of developmental outcomes at that same dose later on.

DR. PADILLA: Right.

DR. PORTIER: So the statement that Dr. Dellarco made that says you are unlikely to observe effects at doses that you don't see cholinesterase inhibition confuses me in the sense that in some cases in this document you talk about statistical significance versus nonstatistical significance.

In other cases, you talk about biologically significant versus not biologically

significant. And then the sweeping statements about no effect, yes, in effect. And yet, the only papers we could find, at least I could find in here that actually give me that comparison, since I don't have the raw DNT studies in front of me, are the Sloktin papers which contradict that comment.

I'm completely confused in this issue.

DR. PADILLA: I will attempt to clear up the confusion with regard to the Sloktin papers.

The only compartment that they measured in those studies was the fetal brain cholinesterase.

If they had measured the maternal blood cholinesterase, they would have seen a highly significant cholinesterase inhibition.

So when he says, I think is what he says in those papers, that those occur in the absence of cholinesterase inhibition, he is only talking about the fetal brain cholinesterase inhibition. Whereas if they had measured blood cholinesterase inhibition in the dams, they would have seen

significant cholinesterase inhibition as is usually done in studies.

DR. PORTIER: I'll come back to this issue, then, again, but I'm going to put a place marker here and note that later on we're going to be talking about the relationship between cholinesterase inhibition in adults versus cholinesterase inhibition in the fetus.

And the fact that we're seeing at four percent cholinesterase inhibition in the Slotkin study significant changes in behavioral response later on concerns me about the fact that a 10 percent in the adult might not be equivalent to a 10 percent response in the fetus.

I think we have to come back to that issue.

DR. DELLARCO: I would like to point out something else about chlorpyrifos.

When we did the single chemical review on chlorpyrifos and looked at all these studies, the weight of evidence suggested that some of these effects that we were seeing on the brain may

not be due to cholinesterase inhibition. There might be other mechanisms occurring.

So you have that uncertainty with the chlorpyrifos database.

But again, aside from that, you would have expected significant inhibition in the pregnant dam. And the statement that we had on the slide is either in the pup and/or the pregnant dam.

DR. ROBERTS: Dr. Needleman.

DR. NEEDLEMAN: I think this is an extraordinarily important issue.

It is kind of the pivot of the whole risk analysis and the decision as to how much of a safety factor is required.

This is so obvious, I hate to bring it up, but the critical events that separate infants and children from adults is that the infant brain is developing and changing and the adult's is fairly fixed.

Now, you said quite categorically that there are no changes in brain effects without AChE

alterations.

Do you have studies to that effect showing -- measuring behavior, measuring synaptogenesis, measuring dendritic complexity?

DR. PADILLA: What I said was there wasn't anything that we could find in the literature.

DR. NEEDLEMAN: The absence of studies doesn't mean that there is no effect.

DR. PADILLA: I know that.

DR. NEEDLEMAN: One is the presence of studies and the other is the presence of effects.

DR. PADILLA: Right.

DR. NEEDLEMAN: So I don't think you can make that categorical statement, which is, the only thing one looks at in examining differential sensitivity in adults and children in the EPA's report.

And we do have these two studies which you distributed which you participated in which say that there are effects in behavior at leaven study at levels that do not affect AChE.

DR. ROBERTS: I feel like we may be getting ahead of Dr. Padilla's presentation a little bit. Why don't we go ahead and let Dr. Padilla make her presentation, which is on age-dependent sensitivity and susceptibility, and then we can continue with some questions if there are still issues that are unclear to us.

Is that all right with the other members of the panel?

Dr. Padilla, are you prepared?

DR. PADILLA: Yeah. I just thought we were going to have a break first. That's fine. I can do it.

DR. ROBERTS: We're running a little ahead of schedule. I thought we could go ahead and do your presentation before the break.

DR. PADILLA: I wanted to start off with some data. And I don't know if my presentation is going to answer all your questions, especially the last two that I have got.

Basically, what I'm presenting is work that we have been doing at the U.S. Environmental

Protection Agency and the National Health and Environmental Effects Research Laboratory looking at age-related toxicity to organophosphorous pesticides and trying to identify what might be the basis of that age-related sensitivity in hopes of looking and trying to figure out if it also might be the basis for humans.

This is some data that we collected. We started out studying chlorpyrifos and looking at the acute sensitivity to chlorpyrifos.

These are basically rats, male rats that have been treated with the same dose of chlorpyrifos.

These are 17-day old rats, 27-day old rats and adult rats. You can see, first of all, this is their control levels of acetylcholinesterase in their brain.

And you can see, first of all, that there is actually a developmental curve. And somewhere around 27 to 30 days, the animals basically achieve adult levels of acetylchlorinesterase in their brain.

These rats were treated with one dose of chlorpyrifos acutely. And we looked at their cholinesterase inhibition at the time of peak effect, which can be different for different ages, by the way, after dosing.

And you can see here that the adults only showed about 40 percent inhibition, whereas a 27-day old animal showed about 70 percent inhibition. And the 17-day old animal showed about 90 percent inhibition.

As Vicki mentioned, the sensitivity to chlorpyrifos actually decreases as the animal ages. It is not a punctate thing that happens. It happens gradually over the maturation of the animal.

This isn't true for all organophosphorous pesticides. Here is another one, methamidophos. This is arranged a little bit differently.

You can have males and females. This is, again, is one acute oral dose. And you have a dose response down here on the X axis. And

basically, control activity. You have both blood and brain here for the animals.

And what you can see, the salient point here is that these curves, the adult curves, which are red, and the green pup curves, again, these are PND 17-day old animals, basically sit on top of each other. There is no increased sensitivity in the young animal as compared to the adult.

So we began to think of why this might be true. And we looked, we divided our investigations into two camps, the toxicodynamic, which is basically what the chemical does to the animal, and the toxicokinetic, which is basically what the animal does to the chemical.

And we looked at the sensitivity that target enzyme. We looked a bit at differences in receptor responses, although, other people in the SAP panel have done a much better job of this.

We looked at the literature with regarding to increased activation. And then looked especially at decreased deactivation of the compounds.

And I will just sort of show this in a different graphic. Most of the OP pesticides, but not all, require hepatic activation to the oxon.

The oxon can inhibit acetylcholinesterase, which, of course, gives you your toxic response, or it can be broken down enzymatically by A esterases or combine stoichiometrically for the most part to carboxylesterases.

This is not true for all oxons or all organophosphates, but it is true for some.

This would be it in detail for one pesticide. So here is the parent compound chlorpyrifos. Chlorpyrifos can be detoxified by the P 450s in the liver or it can be activated to chlorpyrifos oxon.

The toxic reaction is binding to the acetylchlorinesterase, but it can also be detoxified by other B esterases, which would include -- I mean, it can be detoxified by the carboxylesterases, or it can be hydrolyzed by the A esterases.

So the first thing we looked at was whether the acetylcholinesterase, which is the target enzyme, is more sensitive to the pesticide in the young brain.

In this case, we took very young brain from four-day old rats and we compared it to adult rats. And what we did was basically construct an IC 50 curve. We basically exposed the young brain tissue and the adult tissue to different concentrations of the active metabolite of chlorpyrifos and malathion. We have aldicarb here for sort of an extra.

But the two OP pesticides would be right here. And you can see here that these two curves lies right on top of each other, which means that the acetylcholinesterase of a very young brain is not any more sensitive to the inhibition by chlorpyriphos or malaoxon.

And in fact, this parallels other data that have been collected in other people's laboratories which has always shown for every OP pesticide that has been looked at that the young

rat brain acetylcholinesterase is not any more sensitive to the pesticide. So this does not explain the increased sensitivity of the young animal.

The next thing that we have looked at is the receptor response. When you get acetylcholinesterase inhibition, you usually see higher levels of acetylcholine. And the receptors, basically presynaptic and postsynaptic, basically respond to those high levels of acetylcholine.

Here we have dosed animals, either adults or postnatal day 17 pups. You've got males and females here at two different timepoints with chlorpyrifos.

The adults received 80 milligrams per kilogram chlorpyrifos orally. The pups received 15 milligrams per kilogram orally.

These are equal potent doses in these ages, meaning they produce the same level of chlorinesterase inhibition. And you can see here that there was a lot fewer receptor responses in

the adult brain as opposed to the pup brain.

There are lower levels of receptors in the pup brain, which you would expect at this age, but there is also more changes, more down regulation in the receptor responses in these brains as opposed to the adult brain.

So let's summarize here. We have looked at some of the toxicodynamic factors. We can eliminate this, the target enzyme in the young animal is not more sensitive. That's not why the young animal is more sensitive.

There may be differences in receptor responses. And this is something that we should still consider.

And then we began to look at the toxicokinetic aspects. The increased activation we know from the literature -- actually, it's really interesting if you go back to some of the really old, 40-year-old papers, Burdur and Deboise (ph) really expected young animals not to be more sensitive to the organophosphorus pesticides because they knew that the liver was probably less

efficient at converting it to the oxon, and were very surprised when they dosed animals to find out that the young animal was more sensitive.

And as it turns out, the young liver is very deficient at activating, desulfurating and oxidizing the parent compounds.

So if these were the only two things that were going on here (referring to slide), the young animal should actually be less sensitive to OP pesticides. And it turns out that's not the case.

So we look here at the maturational profile of two of the detoxification enzymes in rats.

This is A esterase. In this case, we were using chlorpyrifos oxon as a substrate, which makes it chlorpyrifos oxonase. And you can see here that the young, the very young animal has very deficient levels of this enzyme, both in the liver and in the plasma.

And in about 21, 30 days of age, and this parallels work in other laboratories also,

they basically achieve adult levels, which is about the time of weaning in the rat.

If you look at carboxylesterase, you get a little bit of a different picture here. Again, it is deficient in enzyme. And as the animal matures, it becomes more and more adult like in its levels.

Basically, I have drawn these dotted lines here because other laboratories looking at this timepoint, 40 to 50 days of age in the rat, which is about puberty, have seen that the carboxylesterase levels basically achieve adult levels about that time.

So the thing to notice here is that there is a gradual increase in these detoxification enzymes, both carboxylesterases and A esterases in the rat, which suspiciously parallels the decrease in sensitivity to the pesticides.

So to summarize here what we have learned, we have the parent pesticide, which is hepatically activated and probably deactivated

somewhat in the liver.

And we would suggest that this is probably a deficient both activation and deactivation in the liver, which would not explain the age-related toxicity.

It gets converted to the oxon which inhibits acetylcholinesterase. The acetylcholinesterase in the young animal is not more sensitive to the pesticide. So if these two things were the only things that were going on here, the young animals would actually be less sensitive to the pesticide.

However, we know, at least in the case of chlorpyrifos at this point, the animal is actually more sensitive. So we began to look at -- we would hypothesize that it resides in the fact that the young animal is less able to bind the carboxylesterases or be hydrolyzed by the A esterases.

So then sort of faced with 30 pesticides, and it took us about six years to get to this point, we were trying to figure out how we

could begin to predict which ones might be more toxic to the young.

And so we developed an in vitro assay that would tell us -- the hypothesis was that one of the reasons that young animals may be more sensitive to the OP pesticides is because they basically lack the detoxification enzymes.

Therefore, if we could run these pesticides through an assay and figure out which ones were detoxified by these routes, we might go a long way towards predicting which ones might be more sensitive -- which ones in the young animals might be more sensitive to the toxicity.

So we basically did this with five pesticides, four of which we knew from the literature -- three of which we knew from the literature that young animals were more sensitive to, one of which we knew from our own work that young animals weren't more sensitive to and one that we didn't know. Sort of our unknown.

Fake data. It always looks so nice when you can draw it yourself. Basically, what we did

was we exposed acetylcholinesterase, very pure acetylcholinesterase to different concentrations of an inhibitor.

This is basically constructing an IC50 curve. So the higher the concentration, the less the activity.

Before we would do that, we would incubate the inhibitor with different tissues. We chose plasma and liver.

And those tissues would have the detoxification enzymes in them. If there were some breakdown of the pesticide during that pre-incubation time, then the concentration that the acetylcholinesterase saw when you put the acetylcholinesterase into the microtiter plate reader would be less. And therefore, you would have less inhibition.

A shift of this curve means that there has been some detoxification taking place. And we can separate this and look and see which are A esterase mediated and which are carboxylesterase mediated by the use of inhibitors. It turns out

that A esterase is calcium sensitive. It requires calcium for activity. It actually has two binding sites on it for the calcium.

So if you incubate the tissue plus E G T A and the inhibitor, then only the carboxylesterases are going to deactivate the inhibitor. And this is basically shown by a shift in this curve to the right. There has been some activation by the carboxylesterases.

Now if you do the same thing but throw in the calcium chloride, so you have whatever tissue, either liver or plasma, in calcium chloride you see a further shift of this curve, which means that both the A esterases and the carboxyl esterases are showing some sort of detoxification.

The other really nice thing about this assay is you are working down at very low concentrations of the inhibitor, concentrations that you would probably predict that you would see in the animal.

Many of the A esterase assays are

actually done at millimolar concentrations of the substrate, which will show us -- actually, it will be sort of fodder for conversation a little bit later.

So this is what we got when we did with chlorpyrifos oxon. The other slides look like this, and I'll sort of take you through this. This is the dilution of the tissue.

In this case, this is plasma up here with E G T A, so only the carboxylesterases are working to detoxify.

And here it is with calcium chloride. So both the carboxylesterases and the A esterases are working. This is liver tissue. This is the dilution of the liver tissue. And this is how long the preincubation was.

Basically, we used tissue from adult rats and young rat pups at about seven days of age. The tissue from the adult rats are the filled circles. And the open circles are the rat pups. And the blue line here is the acetylcholinesterase inhibition curve that you

would get if you didn't add any tissue during the preincubation.

You can see here that we have nanomolar (ph) concentrations, which are very low concentrations of the chlorpyrifos oxon.

So what this tells us here, we would be asking here, what is the detoxification profile of the plasma, plasma carboxylesterase. This tells us that in a young pup there is virtually no plasma detoxification at this concentration, whereas quite a bit in the adult plasma.

However, if you add calcium chloride, you see much more shifting of these curves. There is actually some A esterase detoxification in the pup. But the adult in 30 minutes manages to basically -- that tissue manages to completely detoxify any chlorpyrifos oxon that is in that preincubation.

The liver is very interesting. Because these look alike, we actually did not see significant A esterase detoxification at this concentrations. Probably if we had taken it out

longer or used more tissue, we would have seen some detoxification.

But the take-home message here is there is detoxification of chlorpyrifos via both carboxylesterases and A esterases and there is more detoxification in the adult tissues than there is in the young tissues.

The next compound that we looked at was methamidophos. Methamidophos is one of the compounds that we found was not more toxic to the young. And when we tried it in this assay, we got absolutely no shift in these curves. Which would indicate -- we didn't even try it with the young tissue, which would indicate that we didn't see any detoxification of methamidophos via carboxylesterases or A esterases.

From the literature, we know that young animals are more sensitive to parathion. So we thought we would try paraoxon.

This one was quite interesting. We did get detoxification via carboxylesterases in the plasma and in the liver, but there was no change

when we added the calcium chloride, which showed to us that the only detoxification that was taking place was via carboxylesterases and not A esterases. And there was very little detoxification in the young tissue.

So there was more detoxification in the adults than there was in the pups. And it didn't seem to be taking place by A esterases.

Well, this is kind of strange, because another name for A esterase is paraoxonase. We were getting at reasonable concentrations, sort of environmentally relevant concentrations, at least for toxicity, we were getting no detoxification by A esterases.

Well, when we went to the literature, which is probably what we should have done before we did the assay, but when we went to the literature we found out that -- we looked at some of Clem Furlong's work.

Clem has actually created mice that have no A esterase. And one of the things that he has done with these mice is challenge them with

pesticides and ask if they are more sensitive to these pesticides. In a way, it is a model of age-related sensitivity.

And he found that when he challenged them with paraoxon, that they didn't show any difference in their sensitivity, which would cause him to conclude that basically the A esterases weren't acting or the paraoxonases weren't acting as a significant detoxifier of paraoxon at reasonable concentrations.

In fact, when you go back and do the catalytic efficiency of the enzyme, you find that it has very little affinity for paraoxon at nanomolar concentrations, but does have quite, a high affinity for paraoxon at millimolar concentrations.

The next one we tried was malathion. There is a lot of really nice literature showing age-related sensitivity to malathion.

And if you incubate tissues with malaoxon, you see what you would expect from the literature. Malaoxon is not supposed to be a

substrate for A esterases. And we did not see that.

Both the plasma and the liver did a very nice job of detoxifying malaoxon.

In fact, it is supposed to be an enzymatic detoxification via carboxylesterases. Not a stoichiometric detoxification.

So what we see is these two look the same for liver and plasma. There is detoxification taking place in both tissues. And there is more detoxification taking place via the carboxylesterases in the adult tissue than there is in the pup tissue.

This was our unknown. We didn't know what we would find -- we did not know if young animals were more sensitive to diazinon. But we tried it in vitro first.

And we saw that in the plasma there was basically at this concentration of plasma from the young and the adult there was basically very little detoxification via carboxylesterases, which is basically true for the liver also.

However, the plasma, when you add calcium chloride, there was quite a bit of detoxification.

Again, there was more in the adult than there was in the pup. In fact, the pup really didn't show any detoxification of diazoxon by either route. But there was only A esterase detoxification here, anyway, by diazoxon.

This would have predicted that the young animals are going to be more sensitive. We tried this out by dosing young animals and adult animals, PND 17, animals and adults who were 17, five milligrams per kilogram of diazinon orally.

And what we saw was much more inhibition in the young brain. Much more acetylcholinesterase. About 80 percent, 75 percent in the young brain as opposed to the adult, which was about 40 percent.

From this, we would conclude that the young were going to be more sensitive to diazinon.

So to summarize, we have five pesticides, some which are more toxic to the young, some which aren't. And we have different

methods of detoxification.

Some like chlorpyrifos are detoxified both by the A esterases and the carboxylesterases. Some like parathion and malathion are probably only detoxified by carboxylesterases, either by being bound up to the carboxylesterases or being hydrolyzed. Then some like diazinon, which may be basically detoxified more by the A esterases. And some like methamidophos where there is no detoxification by either route, which correlates with the compound being less toxic to the young.

To summarize, we basically have -- these are different little signs here. I don't know what those are. They look like file drawers. They are supposed to be checks. But that's okay. It doesn't make any difference. We have basically toxicodynamic and toxicokinetic factors.

It is not the sensitivity of the target enzyme. It could be differences in receptor responses that would explain the increased sensitivity. It is not the increased activation. And I'm assuming that the hepatic deactivation,

the P 450, sort of goes along hand in hand with that, although I found very little data on that.

But it probably is the decreased deactivation by the carboxylesterases and the A esterases which contribute significantly to the age-related sensitivity of the young animal.

I want to spend a minute in talking about the repeated dosing conditions. We have sort of gotten on that already.

What would add to -- because the data I think that you have been given have been both acute and repeated. There is some extra consideration when you come to the repeated dosing conditions.

One is the differences in recovery of the cholinesterase activity. And also, during the repeated dosing, which is traditionally for most of the data that we receive between 11 days and 21 days postnatally, you actually have the animal maturing.

You have a moving baseline. So as you are dosing the animal, the animal is actually

maturing in its ability to handle the anticholinesterase. I have a little bit of data to show you on that.

This is an all fake data, actually. We had conducted quite a few studies on the sensitivity of the fetus to chlorpyrifos toxicity. And our usual dosing regimen was between 14 days and 18 days gestationally.

When we basically collected tissues at 18 days at 2, 5, 10, and 24 hours after the last dose, we always saw that the dam brain showed much more inhibition, 80 percent inhibition, as compared to the fetal brain, which usually only showed about 25 percent inhibition.

The fetal brain then recovered very quickly, so that by 24 hours after the last dose there was no inhibition in the fetal brain and the maternal brain was very slow to recover.

What we were interested in finding out, sort of the same question that you all have been asking, is this really because the fetal brain is less sensitive to the dose? Or is it because of

some sort of other thing that is going on?

So we actually took some animals at 14 days of age and dosed them with one dose of chlorpyrifos and sacrificed them at the time peak effect and measured their cholinesterase inhibition.

What we found out was that the cholinesterase inhibition was exactly the same in the fetal brain and in the maternal brain. So one dose produces the same degree of cholinesterase inhibition.

Multiple doses produces a diversion. Basically, the dam always shows more cholinesterase inhibition in the brain than the fetus does.

So our hypothesis is that the fetus is able to recover between each dose. So by the time you get to the second day and you give the second dose, the dam has only recovered a few percentage points, and now you have brought her down to 80 percent inhibition.

This is the fake data part of it. I'm

not too sure exactly what happens. But I'm pretty sure that in between each dose the fetal brain, because of its increased metabolism, is basically recovering between each dose. Whereas, the dam brain, because she is less likely to recover, is being brought down.

The same sort of thing is going on when you are dosing animals postnatally and you are comparing it to adult animals.

There is much more recovery in between each dose as compared to the adult, if you are using exactly the same dosing timing, which most studies do.

Next slide. This is not repeated dosing, but this is just to convince you that the young animal, even postnatally, recovers quicker than the adult animal.

This is the study where we gave the young or the postnatal day 17 animals received 15 milligrams per kilogram of chlorpyrifos orally, and the adults -- we got both males and females on this, this is time after dosing, received 80

milligrams per kilogram which are equal potent doses.

They produce the same degree of brain cholinesterase inhibition in these animals. But you can see that the young animals recover much faster, this is brain cholinesterase, than do the adult animals.

So even if are you doing this study between 11 and 21 days, you would have to expect the young animals to recover more between each dose than the adult.

Our conclusions are that acute dosing with some organophosphorus pesticides produces some, not all, produces more toxicity in the young as compared to adults.

This is most likely due to the immature detoxification of the pesticide.

During repeated dosing, the immature detoxification systems of the young rat may be maturing. And also, young rats, either pre or postnatal, recover more quickly from a given dose than do adults.

DR. ROBERTS: Thank you. Are there any questions for Dr. Padilla to clarify issues presented during her presentation?

Dr. Brimijoin and then Dr. Sultatos.

DR. BRIMIJOIN: I have a couple questions. First of all, I think I -- I should say that I'm very impressed both with your model, your ratchet model, but also with your clever assay which has a lot of promise for really enhancing the understanding of how different pesticides are working here.

But I have one sort of a small, factual question. Then another one about applications of this type of analysis.

The factual one is, I noticed in your slide number 59 when you were presenting the diazinon data, one peculiar feature of that slide, which may have to do with the time course, but I would like you to comment on it, is that this seems to be an exception to a fairly general rule that is emerging, we're searching for general rules here, about rapid recovery in the younger

animal. I see here, if anything, slower recovery in the neonate.

Can you comment on that?

DR. PADILLA: These weren't treated with equal potent doses. These were treated with the same dose. They were both given 75 milligrams.

DR. BRIMIJOIN: So in fact, in other -- in assays which would be done with equal potent doses, you would expect this chemical would also show -- the inhibition by this chemical, would also show a faster recovery --

DR. PADILLA: I would expect so. I don't know that's --

DR. BRIMIJOIN: You would expect that, but you don't know that.

DR. PADILLA: I don't know that's true, but I would expect so.

DR. BRIMIJOIN: The other questions I have -- they are of the nature of, have you done this. Of course, the answer is going to be no, but the implication is that you should do them or somebody should do them.

I think this panel -- one of the issues we're going to be grappling with here is the extent to which our developing understanding of age-related differences in sensitivity based on animal studies, and by animal studies we mean really exclusively the rat with a very rare exception, how well those will apply to the human case. That's all we're concerned about now.

And so first of all, this in vitro assay that you have used to characterize one important aspect of age-related differences in sensitivity is immediately applicable. You could tomorrow go into the laboratory and get blood samples from babies and old folks and everybody else and you could tell us before the end of the week whether, in principle, whether the kinds of mechanisms that you have identified apply to people as they almost surely will but may be with very different quantitative implications.

DR. PADILLA: That's right. We thought of that.

DR. BRIMIJOIN: I'm sure you have. I'm

just making that comment.

The other one is less specifically focused on your presentation and this specific analysis, but again, getting to the issue of the applicability of the concepts that have emerged from the rodent studies to humans. There are two key concepts here that I see. One is potentially huge differences in age-related mechanisms for detoxification. There we have the A esterases and the carboxylesterases.

And the other is this fairly consistent or maybe even universal observation that the cholinesterase will recover faster in the young because it is being renewed by resynthesis.

And that's a more difficult issue to settle because it is hard to conceive of appropriate resynthesis studies being conducted in human volunteers, even in adults, and certainly not in children, but I would like to put in a comment, a plea, even, that this issue is in fact so important, conceptually and practically, that it is time for us to break out of the box here and

--

This is a case where we should move beyond the rodent and straight into primates. This is a defensible study to be conducted in primates. The closer to humans, the better, to establish whether there is some comparability there.

DR. ROBERTS: Thank you.

Dr. Sultatos?

DR. SULTATOS: Stephanie, you didn't say anything about methyl parathion, but I wonder if you have any thoughts about what might be happening with that.

Methyl paraoxon is not a substrate for A esterase. According to the report, it has limited interaction with carboxylesterases. Yet, it shows a fairly striking age-dependent sensitivity when you compare it to things like chlorpyrifos and parathion.

Do you have any thoughts about what is happening there and how that fits in with the kind of testing that you are doing here with A esterase

and carboxylesterase?

DR. PADILLA: First of all, the context of what I was trying to do was just make a quick cut through them and begin -- to have some way to predict which ones may be more toxic to the young. I'm not pretending to have every possible variable covered there.

I'm trying to remember back about glutathione deactivation and P450 deactivation for methyl parathion. Would that fit into there?

DR. SULTATOS: The reported pathways are really pretty similar to parathion or chlorpyrifos oxide. The glutathione-dependent detoxification probably is not very important, except maybe at very high levels.

But it seems to me that it is somewhat inconsistent with the notion of A esterases and carboxylesterase and that it still shows the age-dependent sensitivity.

DR. PADILLA: Right.

DR. SULTATOS: To me, that perhaps implicates some other factors.

DR. PADILLA: I haven't tried it in the assay. I think that would be kind of fun to do.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: Thanks. That was a very interesting presentation.

I had a couple of questions. Let's start at the back and work our way forward.

54, which is the slide showing the recovery after exposure to chlorpyrifos --

DR. PADILLA: No, you don't mean slide 54.

DR. PORTIER: I guess it's 64? Yes, that one.

Cholinesterase levels are going up in the brain as the animal is developing through this period. Did you correct for that in this calculation?

DR. PADILLA: Oh, yes. In fact, we have -- in each one of these time points, we have concurrent controls.

DR. PORTIER: Yes. But the original dose was at a point, say, a week earlier. And so

as you go against a concurrent control, the inhibition you are seeing actually is going to recover faster simply because you are adding to the pool.

DR. PADILLA: That's exactly right.

DR. PORTIER: Did you subtract out that added to the pool?

DR. PADILLA: No.

DR. PORTIER: So that the recovery you might be seeing here is simply the fact that you've got increased esterase activity as the animal is growing older.

DR. PADILLA: That's exactly right. And --

DR. PORTIER: So you are not actually clearing it from the brain faster.

DR. PADILLA: No.

DR. PORTIER: You are actually putting more esterase into the brain during the period of time.

DR. PADILLA: That's exactly right. And I'm glad you made that point. You are diluting

out the effect. That doesn't say the effect has gone away. But you are diluting out the effect.

DR. PORTIER: You provided us a summary of the general results with IC 50s for five of these compounds, but only in the young, I guess -- these are not fetal responses; these are young animal responses -- and note that they are more toxic to the young in four of the five cases than they are to the adults.

Do you have the IC 50s for the adults?

DR. PADILLA: These are the IC 50s for recombinant AChE.

Basically -- the IC 50s from the adults and the pups probably wouldn't be any different. I know for chlorpyrifos oxon that's true. I know for malaoxon that's true. I know for paraoxon that's true from the literature and from our work.

But this IC 50 is just for the acetylcholinesterase that we were using as an indicator here.

DR. PORTIER: In terms of a comparison

in evaluating whether or not the fetal brain or the young adult brain is more sensitive, it would be very useful to have seen an actual direct comparison of the IC 50s in those two groups with the oxon applied directly to those cells so that we could make that comparison.

DR. PADILLA: That has been done.

DR. PORTIER: But it is not in my -

DR. PADILLA: Actually, there is a graph. I don't have it in front of me. It's about the fifth or sixth slide.

DR. PORTIER: That didn't give me the actual numbers. That just gave me a graph. I want to see the actual IC 50s as calculated from these so I can make a comparison.

DR. PADILLA: I can get those to you.

DR. PORTIER: The plots are on a log scale and it is on dose. And it is really difficult on a log scale for me to decide whether this is a threefold difference or a no difference. It is too close together on a log scale for me to be able to tell.

DR. PADILLA: There are multiple people that have done this, and everybody that has looked at a comparison between young brain acetylcholinesterase and adult brain acetylcholinesterase has seen virtually no difference in the IC 50s.

DR. PORTIER: Again, I would love to see that. It is important to this discussion because it gives us some idea directly at the cellular level.

DR. PADILLA: No problem.

DR. PORTIER: The other question I had, has anyone done radial labeled studies of delivery into the brain? We haven't discussed that issue here.

It would have been nice to have seen tissue concentrations in the brain in the fetus versus the adult or in the young versus the adult and compare that against cholinesterase inhibition, especially if you could go back and do a G C mass spec and find out whether it is the oxon or not that is in there so that you can

verify that in the in vivo situation delivery doesn't -- once you deliver, you get the same effect.

DR. PADILLA: Right. There isn't a lot out there. The best way to do that experiment if you are going to do radial label is to have a P 32 label. You want to have the business end of the molecule labeled.

The second best way is to have the ethyl/methyl groups which don't leave when they bind to the acetylcholinesterase.

Some people have done -- I'm trying to think. I know they have used radial labeled DFP, but not in young animals. And then that gets us to other studies that have looked -- they have used radial label, but unfortunately, the molecule has been labeled in the wrong part of the molecule, which is the leaving group.

There have been a few studies on the distribution of the compounds. The problems here are the oxons are usually there in very small concentrations and they are highly reactive. So

they are quick to actually bind to any serene esterase. And you are really interested in the oxon. That's your acetylcholinesterase inhibiting moiety.

We have actually done a little bit of work looking at the distribution of chlorpyrifos in the fetus in those figures that I showed you. We also looked at the distribution of chlorpyrifos in the fetal tissues and the maternal tissues.

We were unable to detect chlorpyrifos or chlorpyrifos oxon at those doses that we gave. Not being able to detect chlorpyrifos oxon is not surprising for just the reasons that I have just given you.

And usually, people only detect chlorpyrifos at high levels of dosing and very fatty tissues. We were, however, able to detect trichlorypyridnol (ph), which is a leaving group from chlorpyrifos.

And we saw in the fetal brain and the maternal brain at a time when you saw less cholinesterase inhibition in the fetal brain than

you did in the maternal brain, we saw actually twice as much trichlorypyridnol (ph) in the fetal brain as in the maternal brain.

And there has been other studies, I think, done in other laboratories looking at concentration. I believe it was chlorpyrifos in fetal plasma. They actually saw less chlorpyrifos in fetal plasma than they did in maternal plasma.

So if you put those two studies together, neither one studies the compound that you are interested in, but is the compound that could be detected.

So I don't know if that gives you any additional insight.

I think those were cited in the paper, the two distributional studies of chlorpyrifos were cited in the document that we gave you.

DR. ROBERTS: Yes, Dr. Matsumura.

DR. MATSUMURA: I enjoyed your presentation. That's a good way to go. Fundamental questions are being asked.

My question is you went pretty quickly

on the subject of the receptor, muscarinic receptor

Of course, the finding shows that the pups showed much less level of the receptors.

Now, you are just merely studying binding of the chlorpyrifos to the receptor. So you are not really asking questions whether those pups having a less amount of the receptor would be more susceptible to real ligant (ph).

Like if you give nicotine, would the pups become more susceptible? Assuming that same amount of ACH being a problem, the question must be that -- whether having a less amount of the ACH receptor would be affected more or not.

So that means -- I see some decrease, particularly in the males at the earlier time point. And I do not know whether it is significant or not.

Chlorpyrifos oxons, they are not really strong ligant to the muscarinic receptor.

One question remaining in my head is whether that's, the active site being affected by

cholinesterase, would the effect be the same in the adults and the pups or whatnot.

DR. PADILLA: Actually, you have on your panel an expert on this much better than I am. Dr. Pope has done an awful lot of work in that area.

DR. BRIMIJOIN: First of all, there seems to be a misconception here because that's not a slide showing the binding of chlorpyrifos.

DR. PADILLA: No, it shows --

DR. BRIMIJOIN: It is Q and B binding sites. Yes?

DR. PADILLA: Yes. So it's just the number of binding site.

DR. ROBERTS: Dr. Eldefrawi?

DR. ELDEFRAWI: Just a quick comment on receptors. The response of nicotinic -- postsynaptic nicotinic receptors is immediate because they change conformation quickly and shut their central ionic channel.

On the other hand, the muscarinic receptors take time to desensitize. They are not

that fast like the nicotinic receptors because they don't have that ionic channel. So there is a different time sequence for the response of the receptors to anywhere in the body to organophosphate insecticides and inhibition of acetylcholinesterase.

Then there is something else, I have a slide that I will discuss a little bit later on. And that's the presynaptic receptors.

DR. ROBERTS: Dr. Portier and then Dr. Pope.

DR. PORTIER: We still haven't addressed the question that I raised earlier about percent inhibition in the brain versus long-term effects in the fetus versus the adult.

Are we going to address that in later presentations, or is this it and we need to talk about this now for me to get some clarification on this?

DR. DELLARCO: We're not going to address that directly. Because again, the analysis is focused on evaluating age-dependent

sensitivity. And the premise in the assessment is we realize that these OPs can operate by different mechanisms.

But we have only been able to group them on their ability to inhibit this enzyme. Therefore, this is the precursor event. If we account for age-dependent sensitivity, then we are accounting for any pre or postnatal effects that can occur in the offspring, so the analysis is primarily focused on the inhibition and not trying to draw relationships.

DR. PORTIER: So my concern here is that there is inherent in that an assumption that a 10 percent reduction in acetylcholinesterase in an adult is equivalent -- this is a biomarker. This is not a toxic effect.

So a 10 percent effect in an adult is equivalent to a 10 percent effect in a fetus.

And I think we need some discussion of that issue before we accept the fact that equal reductions lead to equal risk.

And that's basically the premise of what

is going on with the cumulative risk assessment. And I think that's a very serious issue to discuss.

DR. DELLARCO: I am going to talk about the pod a little bit, so we can bring it up again when I get to that point.

DR. ROBERTS: Dr. Pope.

DR. POPE: Just a remark about the question from Dr. Matsumura.

Some of the oxons are actually fairly potent and interact with some of the muscarinic receptor subtypes. For example, in cardiac receptors, chlorpyrifos oxon could have an IC 50 of about seven nanamor (ph). That's relatively potent.

But the data that Dr. Padilla was presenting was changes in muscarinic receptor binding as pointed out by Dr. Brimijoin. Not some kind of interaction between the oxon and the receptor itself.

In that regard, we actually published a paper several years back suggesting that the

adults really showed, depending on when you looked, more receptor binding changes than the neonates did, which is kind of opposite of what she was showing.

DR. ROBERTS: If there are no further questions, thank you very much, Dr. Padilla, for your presentation.

Let's go to break now. Let's take a 15-minute break. Reconvene at 5 until 11.

We'll begin then with Dr. Dellarco's presentation on risk characterization.

(Thereupon, a brief recess was taken.)

DR. ROBERTS: Before we proceed with Dr. Dellarco's presentation, Dr. Eldefrawi has a slide that she would like to use to make a point or clarify an issue.

DR. ELDEFRAWI: This slide is not mine. It was produced by colleagues in my department, including the chairman, Dr. Edson Albuquerque. He does electrophysiology, patch clamping, the most minute things he can detect.

So what he discovered was, on the top

left, you see a neuron. This is the end of the neuron. And it is releasing a transmitter. Then the second neuron, the big one, receives that transmitter and response.

The idea here is that the left one there is located at the synapse, the pre-synapse, there is located receptors, nicotinic receptor, that when it receives the acetylcholine, it releases -- it gets activated and releases the transmitter.

In this case, the transmitter is glutamate and gaba, two different types of transmitters. That means two different receptors are responding.

But the initial one that gets hit -- this is a work done on the hippocampus on brain slices. It reflects the communication between different receptors and how one can inhibit the other one even within very short distances.

His work is all patch clamping and electrophysiological.

DR. ROBERTS: Are there any questions?

DR. HATTIS: I want to say I think it's

a gorgeous slide. But the other issue is -- relates to, we have been treating brain acetylcholinesterase inhibition as if this were one thing.

This slide suggests that subsets of neurons, you know, have different functions and it is not absolutely obvious that inhibition will be entirely uniform within the brain.

Is there any evidence on that point?

DR. ELDEFRAWI: Definitely, there are effects on other -- the other neurons are affecting what is released or not released. And then the end result here is gaba and glutamate.

So do we know in live animal studies, let's put it that way, where acetylcholine is working? Is it direct or indirect effect via other receptors -- anticholinesterase (ph), I'm sorry.

DR. HATTIS: I think that's an excellent point. But the other issue, it seems to me, is that measuring the regeneration of overall brain acetylcholinesterase is a very useful start.

DR. ELDEFRAWI: Definitely. I don't disagree.

DR. HATTIS: But I'm not certain that the regeneration can be counted on to be uniform among different kinds of neurons with different properties either by having second messengers or otherwise.

DR. ELDEFRAWI: Yes. Also, the receptors are different. There are so many nicotinic receptors. This one is the same subunit called alpha 7 subunit. Nicotinic receptor made of only alpha seven, alpha seven, alpha seven... makes the nicotinic receptor.

So there are many different receptors.

DR. ROBERTS: Dr. Pope.

DR. POPE: I don't have a beautiful slide to show, however, there is another receptor that is, I think, potentially of more importance here, and that is the presynaptic muscarinic receptor that acts in a negative feedback manner to inhibit further release of acetylcholine when there is excess of acetylcholine in the synapse.

That is a process we have studied for a number of years now.

And similar to the detoxification pathways, there is a correlation between the maturational expression of this receptor system and sensitivity to the OPs.

DR. ROBERTS: Thank you for adding that comment.

Let's go ahead and proceed with Dr. Dellarco's presentation on risk characterization.

DR. DELLARCO: This is the last EPA presentation. And what we're going to do is put the hazard and exposure pieces, bring the hazard and exposure characterization pieces of our assessment that supports our decision on the FQPA safety factor.

And determinations concerning the FQPA safety factor is informed by the risk characterization conclusions. And that's why I'm going to present them for you.

It is an integral approach when you make this determination. So you have to weigh the

strengths and uncertainties and the hazard along with those in the exposure analysis.

Next slide. These are the hazard conclusions. I'll start with the conclusions first and then go through our reasoning and rationale behind these.

As you know, we have used a database uncertainty factor to account for the incompleteness of the toxicity data base concerning cholinesterase in the immature animal based on the biological evidence that you have heard about from myself and Stephanie.

And we feel when we have done that and accounted for the potential for age-dependent sensitivity, there are no additional concerns for pre and postnatal toxicity that is the result of the inhibition of acetylcholinesterase.

Lets go through the reasoning. The issue that we have in this assessment with respect to this provision of FQPA completeness of the toxicity data is that our relative potency factors for the OPs and the points of departure for

methamidiphos were based on adult brain rat data.

And we have incomplete data for cholinesterase activity in the young for many of these OPs. I went over the data that we did have.

And the question that you ask simply because you have missing studies doesn't mean you necessarily need a data base uncertainty factor. You have to evaluate the likelihood that the absence of these data can change the outcome of your overall cumulative risk assessment such that you may understate risk to children.

Again, we're looking at the possibility of cholinesterase inhibition occurring at lower doses or exposure levels in children compared to adults.

We have concluded that there is a potential for these OPs that are missing data to show age-dependent sensitivity. We realize some may not and some may, but we can't predict which ones will.

And because we don't know how they are all detoxified and whether these esterases are

involved in their detoxification, but because they are involved in some OPs, there is a possibility they can be involved in others.

And we consider this a pertinent and relevant issue to humans because these pathways are present both in rats and humans.

Next slide. This is just a table that Stephanie showed you kind of correlating these two pathways with the absence or the presence of age-dependent sensitivity.

So the approach that we're going to take with respect to addressing this issue of completeness of the toxicity data base is going to adjust the relative potency factors, because not all OPs show age-dependent sensitivity.

So we don't need to do that adjustment, for example, dimethoate, methamidophos and chlorpyrifos. We'll adjust those values for those that do show it and those that we don't know about.

Then the issue comes, okay, we have made the determination that we need to account for this

data limitation for the group. So the next issue is what should be the size of that factor.

Again, we're going to look at the biological evidence to make that judgment. And again, when we look at these uncertainties, we're trying to use the data that we have available, the understanding that we have available to guide these determinations and judgments.

And therefore, what we want to look at are the developmental stages that may be sensitive and the degree of sensitivity that we see. We have already gone over this, that the early postnatal stages are likely to show large differences in sensitivity.

In fact, in our database, we can see differences up to about ninefold or so with chlorpyrifos. With repeated studies because we are looking at a later developmental stage, we see smaller differences.

And the PND 7 11 rat will be generally equivalent to a human, a newborn, in terms of its brain growth development and its maturation

profile for these esterases. A PND 21 rat will be more similar to our one and two year old age group in the assessment.

So that's why it is important to and that's why we have questions on this issue to look at the maturation profile. Stephanie already told you what we understood about the rat.

For A esterase, it rapidly increases from birth and it reaches adult levels around postnatal day 21. For carboxylesterase, it increases as the rat matures reaching adult levels some time after puberty.

So what do we understand about the human situation? There are some human data on A esterase or Pon One (ph).

We have a couple papers from the early literature. We have made copies for you in case you would like to look at the data in these papers, Augustinsson and Barr and Ecobichon and Stephens.

And there is some recent work that Dr. Clement Furlong has done in looking at not only

the maturation profile for OPs, but the genetic variability. And he has actually provided you with a commentary. Since we didn't go into the genetic variability that much with our paper, we asked him to go ahead and provide a review in his perspective.

Again, this is an independent review. It is not our review.

And this is what we understand about A esterase in humans, that if we put all these data and studies together, that it appears that the human infant is very limited in A esterase, but after birth, it rapidly increases during the first six months. And it appears to plateau certainly by the age of two.

But some infants may not reach their mature level or adult level until six months of age. And some infants may not reach it until 12 months of age. And some infants may not reach it until 15 months or a little after.

So there is some uncertainty whether all children in the one-to-two-year-old age group will

have reached mature levels. They are coming up. That was an important issue in our assessment.

With respect to carboxylesterase in humans, we do not have any information on its maturation profile in children. The only thing we do know is that there are high amounts of this enzyme in the rat versus human.

We can give you those citations. I don't think we have that in our report.

Now, what we have to do is look at the different age groups that were considered in our risk assessment. And the one-to-two-year-old age group is our most highly exposed group.

This is when kids begin to eat uncooked fresh fruits and vegetables. This is where we tend to see our residue levels. They are more highly exposed than the infant or the kids less than one.

So we feel that the relative sensitivities that we do see for certain OPs in the rat studies, repeated studies, where they are measuring at PND 21 will better reflect or

approximate the maturation profile of this one-to-two-year-old age group.

These are our considerations for the 3X factor adjustment of the RPFs versus maybe a 10X. Again, it is the biological factors. The detoxification by these esterases has been identified as one major factor. We realize there may be other factors. But that's one that we have data on. One that has been correlated with the sensitivity.

And it is also based on just the empirical findings, the degree of difference that we see between the adult rat and the pup.

For the six OPs at the relevant age that matches that one to two year old, the sensitivities in the rat ranges from one X, in other words, there are three OPs that we see no difference, up to approximately a threefold difference.

Now, we realize that there will be greater sensitivities for earlier developmental stages like the newborn, but this is a relatively

low exposure group.

We did do analysis where we made a 10X adjustment of that age group in our cumulative assessment. And its risk still did not exceed that of the one-to-two-year- old age group simply because it does have lower exposure. That is why the one-to-two-year-old age group is the focus of our analysis.

A consideration -- while we felt we could just use a half-log of the 10X, the 3Xs, there is a possibility there can be a difference between adult humans and one-to-two-year-old children.

But it is not expected to be great. Human children will rapidly reach adult levels at some point. But we felt there was still some uncertainty about whether all would reach mature levels within that age group.

And therefore, this is what we have done. We have put a 1X adjustment on those OPs that do not show any age-dependent sensitivity. A 3X on those OPs that do show increased

sensitivity. And a 3X that had no data, all the other OPs in the assessment.

The next question is in bracketing those OPs without data with the 3X, how certain are we? Can six OPs represent a reasonable subset to make that decision. And we think that it is a reasonable subset. We're not really looking at 30 OPs as you will see when I present the exposure component. And we feel that the ones that we do have data on represent the different structural and pharmacokinetic characteristics of this group, albeit they don't represent every characteristic.

We have data on something like a very small molecular weight OP like methamidophos that has no ring structure. It doesn't require activation by the liver to cause cholinesterase inhibition. It is not detoxified by the A esterase or carboxylesterase. It is just something like methyl parathion that does have a ring structure, does require liver activation and can be detoxified by the esterases.

What we have done in summarizing the

hazard characterization is the ideal situation would be to have pharmaco specific data on children. And take a P B P K modeling approach to address children's risk. But as we have talked about in previous SAP meetings with you, we just don't have sufficient information to take that sophisticated approach.

So we have taken a semiquantitative approach by applying an age adjustment factor to the relative potency factors.

By doing that, we have addressed FQPA's provisions concern for the completeness of the toxicity database and also the potential for pre and postnatal toxicity as a result from that inhibition.

Next slide. This is just a slide, we have some new members here. This is what an RPF is. It's simply all the OPs to determine their toxic potency or contribution to the cumulative risk. They are all related to an index compound, which was methamidophos. So an RPF is simply the ratio of a benchmark dose 10 for your compound

compared to the index.

So the bigger your RPF is, the more potent that OP is compared to the index compound. The smaller the RPF is, the weaker it is, and the index compound, of course, would be one.

That allows us to sum the exposure and account -- normalize the data and accounting for different potencies.

What we have essentially done with that 3X adjustment is to make most of these RPFs three times more potent relative to methamidophos. Except, again, for those OPs that do not show sensitivity, like dimethoate.

This is a risk equation. You are familiar with this. Cumulative risk is expressed as a margin of exposure. Again, it's just the distance between where you estimate exposure and your point of departure. Again, that's a benchmark 10.

It is important to point out that the margin of exposures are evaluated in consideration with the 10X factor for interspecies variability

and the 10X factor for intraspecies variability.

Let me talk a little bit about the point of departure, since everybody's potency is being scaled to methamidophos' dose response and therefore its points of departure is being used to extrapolate risk. Again, methamidophos did not show age-dependent sensitivity. We had excellent data support modeling for the BMD 10 for all three routes of exposure.

And the central estimate and the lower limit on dose are nearly the same.

An issue that came up at the February SAP meeting was why use the benchmark response of 10? How did you determine that? How did you make that judgment?

So Dr. Setzer went back and did a power analysis. He analyzed the power to detect various degrees of rat brain cholinesterase inhibition. He looked at 1 percent response, 5 percent, 7.5 percent.

And he found in conclusion in his analysis that 10 percent brain inhibition is

indeed the low end of detectability, at the edge there of the background level. So that's why in the revised assessment we have maintained the use of the BMD 10.

Let me just make a few comments. Dr. Portier raised the issue about the 10 percent response in the brain, in the adult brain versus the young brain and what is our understanding about the quantitative relationship between a change in cholinesterase activity and a potential adverse outcome in the offspring.

We don't have that kind of sophisticated information to look at that in a very quantitative way. The only thing we can say in response to that is that we have looked at the literature and some of the studies, albeit, crude measurements of neurological structure and function, we do not see effects on the nervous system occurring in the absence of cholinesterase inhibition. Even when you look with more sophisticated tools like Slotkin has done, again, we do not see effects occurring in the absence of inhibition.

In that case, there was significant inhibition in the pregnant dam.

Next slide. These are just the points of departures that we have used. I put these up here because I'm going to give you some data on where we see exposure occurring so you can see the difference between that benchmark 10 response and where we estimate exposure. So for example, for the oral route, it is .08 milligrams per kilogram per day.

Next slide. That's the hazard conclusions. Let me move on to the last area of analysis concerning the exposure. In the report we provide a brief summary of the exposure. And that's because this report is actually a chapter and the big assessment, which is -- I don't know how many pages it is. I think it is over 1,000 pages and there are detailed chapters on each pathway.

What we have tried to do with this chapter is summarize the pertinent information with that section in our document.

Our conclusion here is that there is no additional concern that we are under- stating exposure to children and that our analysis is based on a very comprehensive and data specific assessment.

I'll go through that. I'll try to point out some of the key revisions that we have made since February as I summarize the important characteristics of the exposure database.

Next slide. Again, this is just our risk equation, so this is the input in for exposure. We have identified three pathways of potential exposure. I have incorporated them in the assessment, exposure via food, drinking water and residential uses.

We have considered several different age groups. In fact, we were asked to look at more finer break-outs. We have done that in the revised assessment.

What we have done for all pathways and all regional assessments is we have consistently looked at the one and two year old age group and

three to five years old age group.

The reason we did this is these are the two age groups that you typically see as the most highly exposed ones in single chemical assessments including those for OPs. But because we have such comprehensive consumption data for food, we were able to do more finer break-outs. And so, for the food pathway these are the age groups that we looked at. We have also included children less than one year old.

And although we didn't do these refined break-outs for all the regional assessments, we did do it for the Florida region. That's our worst case situation, so we looked at all these age groups for that.

We feel that we have really covered the different age groups that may be exposed.

That's one of the changes we have made, more finer break-outs.

For the food exposure, it's a very highly refined analysis. We actually have data on food consumption in kids and we have data on

residue levels in food.

For the food consumption, as you know, we relied on the CSF data bases which was supplemented by the '98 children's survey. That greatly extended the number of children, giving us more data, particularly for the ages from birth to four years of age. This survey represents all the different eating habits across the U.S. for all times of the year. For the residues on foods, we had several monitoring data bases, for example the USDA PDP database. PDP gives us, actually, measurements of co-occurrence of OPs.

Also, what we have done in the revised assessment is also included tolerances exceeding residue levels. They were added back in based on your recommendation.

We have considered the OP residues in commercial baby food. What we have done -- what we did in the assessment represented to you in February in the revised assessment is we're assuming adult levels of residues in baby food.

But because major manufacturers of baby food restrict the use of OPs, we did a sensitivity analysis where we zeroed out the residues to see what the impact would be on the risk from food. What we found is that it didn't really impact the one to two year old age group because they are not really eating that much baby food.

But it did have an impact on the children less than one. Even though, it had a substantial impact on them when we zeroed out the residues, even with the assumption of adult levels, their exposure still does not -- their risk still does not exceed that of the one to two year old age group.

We have considered baby formula in our assessment. For example, we have looked at the components of formula, so residues that you may have in cow's milk, soybean products.

We don't have much information on human breast milk. So we couldn't address that quantitatively. But we have dealt with that qualitatively.

We have reviewed all the data that we have on animal studies and what we understand about the physical chemical characteristics and partitioning into milk. And our weight of evidence conclusion is that this is not likely to be a significant pathway of exposure.

We have also factored in the OP metabolites in the food pathway. We have considered those metabolites that would give you significant residue levels, like omethoate, dichlorvos, methamidophos.

Although we don't have extensive analytical data for other OP metabolites, based on what we do have in the FDA monitoring data bases, what we have from metabolizing studies, they are not expected to be an important contributor to the food pathway.

Next slide. Here are the findings. This is our one-day exposure estimates on a milligram per kilogram day basis for the different age groups that were considered and at the upper percentiles of the distribution. So you can see

at -- if you look at our highly exposed group, the one and two years old, at the 99.9 percentile, you are getting .0018 milligrams per kilograms per day of exposure.

If we go back and look at our point of departure, which was at .08, that is about a 43-fold difference. If we go down to the 95th percentile of the distribution, we have .0002. Again, comparing that to the .08, that is 400-fold difference. That gives you a sense of where exposure is estimated and where our point of departure is in the assessment.

Next slide. What this is, this is just showing you -- I mentioned I would show you who are the contributors in this assessment. These are the most significant OPs in the top .2 percentile of exposure for children, one to two year olds.

Dimethoate is our major contributor accounting for about 48 percent of the total exposure. And then the next top two contributors, azinphos methyl, about 27 percent of the total

exposure. As you know, we actually have some data on dimethoate for age-dependent sensitivity and doesn't show it.

Next slide. We'll move on to the drinking water pathway. Drinking water estimates were generated using simulation models that provided probablistic distributions of daily concentrations, which were reasonably comparable with actual monitoring data that we have for similar locations or nearby locations we were looking at.

This assessment included geographic and temporal variations that you would expect. And most importantly, it captures in the regional assessments the most vulnerable drinking -- the water sheds, so where we would see sources of exposures that would have the highest exposures and the highest potential for combined exposure.

We have also considered the metabolites in the drinking water pathway, and the assessment you saw in February we had already quantitatively accounted for the sulfoxide -- sulfone

transformation products.

We had not accounted for the oxons in that assessment, so here is another revision that we have done.

We don't have enough data to do that quantitatively, but again, we have done it qualitatively by assuming a hundred percent conversion of the parent compound who its oxon form and order of magnitude increased in its potency.

When we do that in the assessment, we do not really see a change in our drinking water estimates.

The reason why we don't see much of a change is the major contributors in drinking water are those OPs that were the transformation products, the sulfides and sulfones and not the oxons. That's why it didn't make a difference.

Overall, the water pathway is not a major concern for the total cumulative for risk. It is an order -- and generally, it is an order of magnitude away from the food pathway risk.

Residential, we used actual chemical specific data, again, daily probabilistic estimates. Again, the regional assessments covered the geographic variation throughout the U.S. It reflected climate and pest pressures.

We have also looked at the activity patterns of children that would result in significant sources of exposure, like hand to mouth activity as established by videotapes.

We have considered all remaining uses, residential uses in the assessment. I think that adds up to 10 that is in that slide. And of all these remaining uses, the only contributor to the cumulative risk is the one remaining indoor use for dichlorvos, and that is dichlorvos on pest strips and the inhalation route.

So this brings me to our summary of the exposure part. We have high confidence in the exposure analysis. It is comprehensive, data specific. It considers food, water, residential exposures probablistically.

We have used actual data so it reflects

realistic pesticide levels and uses based on pest pressures, weather activity, patterns, et cetera.

We have also estimated risk for the upper percentiles of the exposure distribution. We're trying to capture the highly exposed groups in the population, including children.

So this takes me to our conclusions of the completeness of the data, the concern for pre and postnatal tox.

These are the uncertainty factors that we have used in our cumulative risk assessment. We have the standard, 10X intra- species -- inter and intraspecies factor that is considered for the group in addition to the 3X database uncertainty factor that was incorporated in the RPF's.

We feel that by incorporation of that factor we have addressed the FQPA provision concerning the completeness of the toxicity data base. We have addressed the concern for pre and postnatal toxicity. We have no additional concerns for exposure. Therefore,

we have removed the 10X FQPA factor in light of these factors that we are using in the assessment.

Questions?

DR. ROBERTS: Thank you, Dr. Dellarco. I'm sure the panel members have many comments they would like to make on the risk characterization, but we'll have lots of opportunity to do that later.

I would like to ask the panel now if you have any questions for clarifications regarding the risk characterization, and also, since this is the last agency presentation, if you have other questions regarding the agency's interpretation of the data or their analysis, this would be a good time to ask them.

Let me open it now to the panel for questions.

Dr. Brimijoin.

DR. BRIMIJOIN: I think I have a relevant question. I'm not sure what we're restricted to here.

As I understand it, we're looking at data that suggests that some OPs will have a differential effect on the very young when they are administered acutely, but not in the repeated dosing model.

And EPA has made a decision, a recent decision to consider the repeated dosing model as the more appropriate standpoint to decide whether an FQPA factor is needed for that particular chemical because it's the situation that most closely approximates the anticipated risk from exposure.

A case in point is chlorpyrifos where there is a difference in acute exposure between neonates and juveniles, between juveniles and adults. But this goes away when you do repeated dosing of the neonates.

Also, EPA has made a determination that the most -- the group perhaps at highest risk is the one to two year old group. Certainly, when we're considering FQPA factors.

So those are effectively weanlings. I

think there is -- maybe the EPA is aware of this, but it seems to me on the basis of the information summarized in our document here and that I'm aware of from my look at the open literature, at least there are no citations here to the appropriate dosing regimen in the animal age group which is most relevant. We are going to extrapolate from animal to people. We would like to extrapolate from the most relevant group.

So I think we are maybe making an assumption that, okay, in the neonatal rat we have a heightened sensitivity to acute exposure, perhaps because of reasons Dr. Padilla so elegantly demonstrated for us, but when we go to repeated exposures, that disappears because, again, thanks to Dr. Padilla's elegant other model, there is a more rapid replacement.

And I think we're probably assuming that these two opposing factors cancel each other out in the remaining balance as you move from the neonate to the weanlings.

But I'm not aware of studies with

weanlings comparing acute and repeated dosing and determining whether, in fact, what we've got -- we might get into a situation where there is remaining acute sensitivity but we have lost the ability for rapid resynthesis.

And I just want to raise that point. And maybe you have some comment.

DR. ROBERTS: I was going to ask is there a question?

DR. BRIMIJOIN: The question is is there any data about this and have you thought about the data gap? Are you comfortable with the conclusion that chlorpyrifos shows no age-related sensitivity in repeated dosing in the most critical group, the weanling rat?

DR. PADILLA: So you would be looking for repeated dosing from 21 days on, basically, 21 to 30 days?

DR. BRIMIJOIN: Yes.

DR. PADILLA: Most of the repeated dosing is done between 11 and 21. You are right.

DR. ROBERTS: Are there other questions?

Dr. Hattis.

DR. HATTIS: You have made a particular mapping of humans of particular ages with rats of particular ages.

Can you just briefly review the data base that you used -- developmental signals that you used to make that map?

DR. DELLARCO: That map is primarily based on the maturation profile for what we understand for the A esterases.

And again, it is based on a couple of studies that we found in the older literature and some recent work that Dr. Furlong has done.

And what we're saying about that maturation profile from what we can see, and we have these studies here for you to look at too since we're going to discuss this tomorrow, is that we can't conclude with certainty that all children by one year of age will be at their mature level. And thus they may be slightly more limited.

We wouldn't expect the difference to be

as great for a newborn who would be more limited, but we still think there is some uncertainty around that. That's simply what we have done.

DR. ROBERTS: Dr. Needleman and then Dr. Matsumura.

DR. NEEDLEMAN: I think you said that the immature infant has less exposure or is not of concern because of the exposure. Are you aware of the study of Ryan Wyatt and Dana Barr of meconium at Columbia University?

DR. DELLARCO: Why don't you summarize that.

DR. NEEDLEMAN: Sure. 19 out of 20 had positive detections for DETP, and 20 out of 20 had positive detections for DEDTP. There was a very high prevalence of chlorpyrifos analyzed in that study.

It was in Environmental Health Perspectives about six months ago.

DR. DELLARCO: There has been quite a bit of risk mitigation efforts with chlorpyrifos, and that is factored in to our assessment.

I don't know, Bart, do you want to comment any more about chlorpyrifos?

MR. SUHRE: I think that's the point. The point is that there has been quite a bit mitigation on chlorpyrifos with respect to residential uses. I think Vicki's comments were primarily geared towards the food pathway.

DR. NEEDLEMAN: But residential pathway is included in your risk analysis.

DR. DELLARCO: But it also factors in the mitigation efforts, even the recent ones that we have made.

Randy, do you want to add anymore to the residential pathway? There is only 10 remaining uses --

DR. PERFETTI: There is only one indoor use. Two things, the study you are referring to gave 19 out of 20 positive results. Is that what you said, Dr. Needleman?

DR. NEEDLEMAN: That's right.

DR. PERFETTI: According to NHANES, most humans have residues in their urine of OP

metabolites. 19 out of 20, it would be -- I would rather consider the levels rather than frequency.

DR. NEEDLEMAN: I don't believe that was reported. It is just that a very high prevalence of children are born with chlorpyrifos in their body or in their stool. DR. PERFETTI: The NHANES data would confirm that most would have a basic body burden of OPs.

DR. ROBERTS: Dr. Matsumura and then Dr. Portier.

DR. MATSUMURA: This DDBP, some years ago, we had the SAP reveal and we saw data that in the carpet DDBP residue was pretty high at that time. I guess the SAP thought that should be really looked at, exposure to those small ones crawling on the floors, dust intake, should be looked at it more carefully.

I do not know whether your agency completed that kind of review for the exposure.

DR. DELLARCO: Randy, I don't know if you want to address the issue of some of the single chemical assessments that are still going

on, even for dichlorvos.

DR. PERFETTI: The only remaining as we said indoor use is for DDBP. It is the pest strips, which would be an inhalation exposure, essentially constant inhalation exposure during the year.

So the carpets, as I recall, the carpets would have referred to some of the indoor uses, crack and crevice uses around the home that have now been mitigated.

DR. MATSUMURA: I have a lot more question on the dimethoate.

As far as I know, it is a major degradation that is done by carboxyl amidase, which is pretty different from the carboxylesterase.

In your assessment, I don't believe that you studied that at all. If it is the high exposure scenario, one should really start to look at it. It is a different spectrum. I don't think it is the same as the carboxyl esterase.

DR. PADILLA: You are exactly right.

Actually, there is some evidence that maybe methamidophos may be also broken down by those carboxyl amidases.

We don't have any evidence of on carboxyl amidase breakdown. But we do know that the young are basically as sensitive as adults to both those pesticides, methamidophos and to dimethoate. It is not the same as carboxylesterases.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: I have three questions.

First of all, make sure I understand. Table one in your document gives all the relative differences.

That's really the summary of the data?

DR. DELLARCO: That's correct. It doesn't reflect some of the recent modeling that we have done that I showed in my slides.

DR. PORTIER: On the food exposure results, I guess that's slide 99, you show basically a twofold difference between infants less than one and children one to two. That's

across the board basically, it is about a twofold difference in exposure.

But your Table 1 in looking at the acute effects versus the effects in older neonates show roughly a ninefold difference in the acute exposure for the young neonates versus a threefold, give or take, in the older neonates.

That would suggest, in fact, that the correct factor there should be 4.5 instead of three since there is a 1.5-fold difference in sensitivity between those two groups versus a three and -- threefold difference -- threefold difference in sensitivity versus a twofold difference in exposure which would lead to an additional 1.5.

Have you considered that at all?

DR. DELLARCO: What we have done simply in the assessment, we did this recently, is we put a 10X RPF adjustment for that age group, children less than one. And even when you do that with a 10X adjustment and with a 3X adjustment on the one to two year old, you still don't see the infants

exceeding the risk of the one to two year old.

In the assessment what we did pragmatically, given the time we had to do the assessment, we made a 3X adjustment on all age groups, including the adults where you wouldn't put any X on because they don't have an increased sensitivity compared to kids.

That's simply because, again, we think that the children, the one to two year old age group is the age group that is most highly exposed in the assessment. I don't know if I quite addressed what you were --

DR. PORTIER: I guess I'm a little lost in what you just said. If you could explain it to me. If I do a 10X on less than ones and a 3X on one to twos -- and is that 10X across the board or is that 10X on specific agents versus -- like you did with the 3X on specific agents?

DR. DELLARCO: The 10X is just like we did with the 3X. We applied it to all the OPs except those that do not show sensitivity.

So the 10X was not done for dimethoate

and chlorpyrifos -- chlorpyrifos isn't really a major contributor in the assessment given the mitigation activities -- and for methamidophos.

DR. PORTIER: The 10X for chlorpyrifos would have been interesting -- I understand that now.

Next question. Again, going to your Table 1. When you talk about repeated doses of pups to adults, when you look at malathion, in fact, you have a fold differences uncertain. In fact, it is infinite.

If you really want to calculate the fold difference in this case, it is in fact infinity since you have an increase in the maternal and a decrease in the pups.

So now in looking at the -- and this comes to the question of three versus one. You have got a distribution of differences across the organophosphates. The range as have cited, when you can estimate it, is between actually .6 and roughly 3.2.

Yet, you have this one outlier that is

up in the infinite range.

Did you consider looking at this distribution rather than you choosing a specificity on a chemical by chemical basis, and what would that distribution tell you?

DR. DELLARCO: No, we didn't look at the distribution. But what we did for this study that you are referring to, where it says you couldn't determine the sensitivity, when we did this table, we had not modeled the data of Dr. Setzer's exponential model so we could have a better basis of comparison so we could drive the benchmark response and do a comparison

It was hard to determine the differential from just eyeballing the data.

That's why I showed the slide where we have now modeled the data. That difference in that study is not undetermined, but example, for the repeated study it's about threefold difference.

DR. BAETCKE: I think that was still on red blood cell. Not the brain.

DR. DELLARCO: The red blood cell you

were getting more of response in the red blood cell. That's why we chose what we thought was the more sensitive endpoint to model.

DR. PORTIER: Then the last question, I'm still going to come to this fetal sensitivity versus adult sensitivity in the brain. Let me make sure I have looked at all the data. So the question is clarity of looking at all the data.

There is the Slotkin papers, which clearly give you both aspects, and then there is a number of papers in there, but all of the endpoints in the other papers are histopathological developmental endpoints. Am I wrong in that?

DR. DELLARCO: No. That's correct. The Sloktin studies provide the most sophisticated measures than those other papers.

DR. ROBERTS: Are there any other questions from the panel members?

Yes, Dr. Lambert.

DR LAMBERT: I have a few. The first one is, you were discussing the metabolic pathway

in looking at P 450s and discussed them. How does the animal and the human compare, and how do they compare developmentally?

DR. DELAYS: Actually, there is a paper that we referred to that is by Ginsberg and Hattis. He might be able to summarize it better than I did. They went to the therapeutic literature to look at the differences between children and adults.

And basically, for the P 450s, after birth, they come up very rapidly to adult levels.

So I guess -- again, Dr. Hattis can correct me. It depends on what SIP (ph) system you are looking at. But certainly, by six months of age for the SIPs.

DR. HATTIS: I think that's fair enough. Essentially, we find distribution of differences in premature neonates and neonates relative to adults.

Premature neonates average something like a fourfold difference. But there are some extreme cases that go up tenfold or more in terms

of a half life in infants versus the adults.

In addition to that mean difference, there is also more variability among infants, in that -- in a transitional period when some infants have gotten switched to more adult patterns. But that's more in the range of one to six month period or two to six month period.

By the time you got one to two years, our data are pretty compatible with adult half life patterns, although there may be just a bit more variability, if I remember correctly. I have some slides on that that I can show.

But if you are talking about one to two year olds, I think you are talking from the data that we have analyzed, it is just all pharmacokinetic and whole body pharmacokinetic, we've got pretty similar patterns by that point, although, we do have certainly much more variability and much larger mean half lives right in the neonatal period.

DR. LAMBERT: I think if you look at it and look at the families, they are very, very

dependent upon which family you're looking at. For example, family one particularly you want a 2B0 (ph) expression in a new human newborn and fetus. Family three is going to be elevated significantly higher in the human newborn than the adults.

If I'm not wrong, family three is what we're concerned about here. Is that right?

DR. PADILLA: Actually, there is in family three. But also, there is some evidence that it's -- I think the 2 D 6 may also activate.

I think it is different for different OPs and there is not a lot of information.

DR. LAMBERT: Also, in family three, it is one of the -- probably the only P 450 family that is elevated in the newborn, in the fetus, as compared to the adult.

In the human, there is a family 3 A 7, if I'm not mistaken, which is the fetal form of cytochrome P 450.

It is much, much, higher in the human newborn than the adult.

So if that's the pathway to activation, that will raise a real concern. If you look at the differential expression of that family and subfamily, it is much more than three; it is probably with an extra digit thrown in.

So that's just a concern as far as the real applicability (ph) of the animal data to the human. That's a real area of major concern.

The second thing would be in your assumptions that if there is no data, you will take three, and that will be okay.

I think in human neonatology, human pediatric studies and use of drugs or abuse of drugs or misuse of drugs, we are replete with many examples when people have assumed that a drug will be fine, and when they give it to the human newborn, and then look retrospectively, find that they have not only -- did no benefit, but they have done serious harm to a child.

You can go back to the early '50s or the late '50s with even things as simple as antibiotics where caused brain damage and also

death, up to the more recent time of using steroids to treat lung disease in newborns and finding that their brains didn't grow very well even with some relatively short courses.

I think I'm really concerned about making assumptions without data that we're going to be okay. Because pediatricians have done that in the past with not very good outcome.

The other issue is an exposure -- Do you want any comments on that assumption?

DR. DELLARCO: We believe that our 3X decision was actually based on data, not just simply the absence of data given what we understood about the sensitivity that we're seeing in the animal system via these detoxification pathways and what we understood about their maturation profiles in human.

We haven't been able to attribute the P 450s with an increased sensitivity.

DR. LAMBERT: I'm not sure of the P 450s with an increased sensitivity. I'm not sure what you are referring to there.

DR. DELLARCO: In the Ginsberg paper, and we make mention of this, there is actually a point, between six months and two years, where they actually may supersede levels. So they could produce little more of the oxon than the adult.

We just mentioned this for completeness. But that hasn't been correlated with what we see in the animal studies. I think Stephanie went over that point.

DR. LAMBERT: That's because in animal they are not there. You have different expression of P 450s in the animal, in the rodent, as the human. You have differential developmental expression.

In the human has 3 A 7, which is not present in the animal.

For all those reasons, you may have higher generation of larger amounts of active metabolites in the developing human as compared to the rodent or the adult.

The last thing is just on exposure assessment. As we're starting to be concerned, we

went from everybody's a 70 kilo. male, in both classic drug use pharmacology, FDA type things, and EPA, to looking at kids differently.

And now we have progressed into the idea that there are subpopulations of children that might be even more at risk than the average child.

And just an exposure, I think the TexMex studies of certain organophosphates in urine are developing in much higher levels of urinary organophosphates in that group of people than we have seen in the past.

I can get that information. I think there is an abstract on that. We're talking, I think, close to worker levels in the children that live in the rural area of the Texas-Mexican border.

The second thing is the concern that children all do not act similarly.

One of the things that we have been concerned about or starting to look at using the videotaping is the kids with, for example, autism who are known to have repetitive mouthing, and

they may have hand to mouth activities much, much, greater of several fold higher than the average child.

So there is a susceptible population with the brain CNS issue, and their brains may be more susceptible, we're not sure about that. But their behavior would expose them potentially to higher levels.

That's just a comment on the exposure assessment in children.

DR. ROBERTS: Was there a question?

DR. LAMBERT: No, just a comment.

DR. ROBERTS: Any other questions, clarifications?

Dr. Portier and then Dr. Reed.

DR. PORTIER: In the studies you presented, I had one question again for clarification. Groups one through four, which are the ones we're talking about postnatal day 7 to -- 11 to 21, exposure of those particular offspring, I'm not sure which dams they're tied to because group one, group two, group three, group four and

most of these studies consist of dams that are exposed G D 6 to G D 20 and some G D 6 to PND 10.

Are the PND 11 to onward animals only from the G D 6 to G D 20 dams?

DR. DELLARCO: You must be looking at not the actual report, but the data entry record we gave you.

DR. PORTIER: That's correct.

DR. DELLARCO: We have different phases of studies.

I don't have that in front of me. I can't see what you are looking at. I believe in the DNT protocol, Karl, correct me, that for gestational exposures, it is G D 6 through 20 and then they evaluate a PDN -- they evaluate a G D 20.

They may also evaluate B N D 4. Again, I don't have the study in front of me. DR. PORTIER: In all the reports you give us, the numbers you are extracting are -- for example, I'm looking at dimethoate, which is study 870-6300 -- and P N D 21 --

DR. BRIMIJOIN: What page are you on?.

DR. PORTIER: This is date evaluation record for dimethoate. Special study cholinesterase inhibition, MRID 45529702.

If we look on page, I guess it is page 11, down at the bottom of the page, they are talking about PND 21 male animals which are the offspring of groups one through four, which is what you're using.

Those are the animals that I believe you are using when you are talking about the chronic postneonatal exposure animals.

Am I right or wrong on that question?

And since it links it back to groups one through four, I'm wondering whether in groups one through four are we talking about the animals that only got gestational day exposures or also got greater exposures? I want to make sure I understand the linkage here.

DR. BAETCKE: I would have to go back and look at the experimental details to see the linkage. My assumption is these were the dams

that were exposed during gestation. Treatment was ceased and then started again. DR. PORTIER: Okay. For Dr. Padilla --

DR. DELLARCO: She stepped out.

DR. PORTIER: My question -- maybe one of you can answer it, on the assays using brainstem cells and direct cholinesterase inhibition in the cells of brains, did she do any ex vivo studies so that the fetal, the animals were exposed during gestation, then the brains extracted and the ex vitro -- ex vivo, in vitro study done? Or were these all in naive brains?

DR. DELLARCO: I can't answer that. We'll have to wait for Dr. Padilla.

DR. PORTIER: Thanks.

DR. ROBERTS: Perhaps we can pick that up later, the answer to that question later.

The last call for clarifications.

Dr. Reed.

DR. REED: I have several clarification questions.

With the accounting for the possibility

of a higher potency or toxicity of breakdown products, I think the final or the analysis presented here is that if you consider higher potency of breakdown products is tenfold higher than the A I itself, then what I have heard was that it will not make any impact if you applied that to drinking water. Am I correct on that?

DR. DELLARCO: Yes.

DR. REED: That was in the context of cumulative risk. Not just the water route by itself.

DR. DELLARCO: That was water pathway and the total risk.

DR. REED: My question is that -- so essentially, if you apply 10X to many of these pesticides to drinking water and it does not increase substantially on the drinking water exposure itself, that's a 10X. Isn't it?

DR. DELLARCO: It's a 10X.

This is Dr. Thurman. He did the water pathway and he did that conversion. I'm going to have him explain in detail how he did that.

DR. THURMAN: Ask that again so I can make sure --

DR. REED: My understanding is that you are trying to see if accounting for higher potency for breakdown products will make any impact or what kind of impact it will make on your total exposure. My understanding is that by applying 10X assuming 100 percent of conversion from the parent chemical to the breakdown product, you would essentially be applying a whole 10X to the concentration of a pesticide -- in many of these pesticides.

What I'm hearing is that it would not make substantial impact on the final analysis of the exposure.

I'm a little bit puzzled by that.

DR. THURMAN: Okay, we only -- the 10X was only for those OPs that formed oxons.

What we were finding is that when you looked in each of the regions and we looked at the OPs that tend to occur together, particularly the ones that drove or the ones that occurred together

to make it a pulse dose, what we were finding is those OPs that formed oxons did not tend to be in that pulse dose.

They were not the major drivers that were effecting what the water exposure was getting. There's two factors involved. One is they didn't tend to occur within a pulse dose. So when they did occur, and you add the 10X, you were getting an increase in your exposure, but it was at a low level. Fairly low level outside of the pulse dose.

The other thing because we were correcting -- we basically changed the relative potency factors, those OPs that formed the oxons tend to have much lower relative potency factors than the OPs that were forming the sulfoxides and sulfones.

I think that may be as much as anything else what was causing that.

DR. REED: Thank you.

So a related question is that the agency have not tried to do an analysis, similar analysis

for the dietary route. Am I correct on that?

DR. DELLARCO: We have done analysis for the food pathway.

DR. REED: Accounting for possibility of higher potency for the breakdown product.

DR. DELLARCO: For the metabolites that significantly occur as residues in food, we actually have R P F's for them. We have data. We have data on omethoate, dichlorvos and methamidophos.

Now, for the other metabolites based on other monitoring databases, we have FDA monitoring databases and metabolism studies. They are not expected to occur at any significant level in the food.

We can have Dr. Smith come up if you want some more detailed discussion on that.

DR. REED: No, that's okay.

My next question is that earlier on in Dr. Dellarco's presentation there was a table about margin of exposure calculated for one day of exposure.

My clarification question is that that was using the same point of departure, same relative potency factor, same FQPA factors, all of them were derived or determined, establish based on sub-chronic data base. Is that correct?

DR. DELLARCO: Exactly, yes, steady state cholinesterase data.

DR. REED: And the margin of exposure, that column for one day of exposure was much lower than the seven day or 14 day rolling average. Right?

DR. DELLARCO: Right.

DR. REED: What is the agency's sort of intent to use that particular column on one day exposure? Is that just for comparison or does it have some meaning in the final analysis of risk management?

DR. DELLARCO: Do you want to respond to that Marsha?

MS. MULKEY: What we said in our risk assessment and in our public presentation is that we believe that these captured the range of

exposures of concern, articulated ways in which we think the one day exposure represents primarily an overestimate, although we identified some factors that go the other direction.

Similarly, seven days you will, I'm sure, remember from earlier dialog, there were a number of factors that called into question the appropriateness of the seven days. We have treated this as bounding estimates. That's the way we have articulated it in the risk assessment and in our public presentations.

DR. REED: Thank you.

DR. ROBERTS: For the record, that respondent for the agency was Ms. Mulkey.

DR. REED: I have sort of a more general question. I would really like to get the agency's way of concerning this large database we have.

When we look at what is available in terms of age-related sensitivity and especially for the young ones, we're making comparison -- rightly so, I'm not having a problem with it, we're making comparisons not just on brain

cholinesterase inhibition or its function and all that, what I'm seeing is a gap, and I would like the agency's perspective on that.

We're going from there into deriving an FQPA factor for children, infants and children applying that to brain cholinesterase as an endpoint. And as an indication of some sensitivity of something. But the endpoint is really brain cholinesterase.

There is a gap jumping from that, the database that we have that we are comparing that is not limited to brain cholinesterase to applying of factors to brain cholinesterase.

DR. DELLARCO: Let me just summarize what you have asked. You are saying when we are doing our sensitivity analysis we are looking at both the blood and the brain compartment and coming up with that 3X uncertainty factor based on the differences that we see in the repeated dosing studies.

We are adjusting RPF's that are based on brain cholinesterase data.

If you remember from the earlier risk assessments that we have taken to the SAP, we made that decision because we had evaluated all, both the blood and the brain compartment, when we made the decision to go with brain, and the assessment we brought to you in February, it was based on the observation that for most of these OPs we didn't see much of a difference in response in the adult between brain and blood.

There were some exceptions, but they went both ways.

So there were some OPs that were actually a little more potent in brains and some that weren't. When we went to the sensitivity analysis, we thought it was important not to focus just on the brain compartment, we want to protect against both the central nervous system and the peripheral system.

Again, we went back and evaluated both compartments and collectively what we were seeing looking at both brain and blood was still about a threefold difference. We made those adjustments.

It is not a pharmacokinetic specific adjustment that we're making. It is more of an age-dependent default adjustment.

DR. REED: One more clarification question.

I want to make sure I understand it right. That the point of departure 10 percent brain cholinesterase inhibition is really based on the ability to detect a difference statistically and not considering the role of cholinesterase in its inhibition and so forth, in terms of how adverse it is, not concerning that or concerning the lack of information neurobehavioral assessment of what percentage of brain cholinesterase inhibition might be correlated to that.

DR. DELLARCO: That's correct. It is our power to detect. I think most of the experimentalists will agree with us. That's really at the lower end of the dose response where you can pick something up.

DR. REED: And that's based on quite a few numbers of studies not just -- if you look --

what I'm trying to say if you look at individual studies, less than 10 percent brain cholinesterase inhibition would be detected as statistically significant from the control. But that 10 percent came from a number of studies and is sort of general.

DR. DELLARCO: It is sort of weight of evidence approach looking at all the data.

DR. REED: Thank you.

DR. ROBERTS: Dr. Portier would like the opportunity to pose his question to Dr. Padilla now that she is back.

DR. PORTIER: First, I'll make sure that I add a point to it.

Most of these studies were based on eight pups in terms of brain cholinesterase inhibition. You are comparing eight against eight which has pretty low statistical power. Ten percent is driven by the sample size more than biological importance.

My question, which I asked earlier, was in your in vitro study using tissue samples -- I

gather these are actually slices, not loose cells.

DR. PADILLA: They are tissues that are taken from the animal. So it's homogenates of the liver and then just plasma diluted up.

DR. PORTIER: But the brain itself is that tissue or --

DR. PADILLA: That's just recombinant ACHE. I'm not using brain tissue. I'm just using that as a barometer of how much inhibitor is left. I'm looking at the shift in the IC 50 curve.

DR. PORTIER: I guess I misunderstood your talk then. I'm a little confused by looking at any of these plots where you have the recombinant as one of the points.

DR. PADILLA: The recombinant -- I believe it is blue triangles.

DR. PORTIER: Then you have adult.

DR. PADILLA: Right. That is adult plasma or adult liver adult.

DR. PORTIER: Not adult brain?

DR. PADILLA: No.

DR. PORTIER: And rat pup, it's plasma

or liver, not brain?

DR. PADILLA: Exactly. What I was looking at was not the target tissue but really the ability of the other tissue to detoxify the pesticide.

DR. PORTIER: In all those cases, then, are those naive animals used? None of them have seen any of the particular OP in advance of the tissue being removed?

DR. PADILLA: No.

DR. ROBERTS: I had one clarification from Dr. Dellarco in the exposure assessment. Previously we had commented on the desirability of perhaps including homegrown fruit and vegetable consumption as part of the intake.

I'm just curious whether the agency had a chance to respond to that.

DR. DELLARCO: Dr. Smith did the food pathway. That's not incorporated in the revised assessment.

DR. SMITH: We do not have any information on that type of residue information,

however, we have considered the range of data we do have from the P D P probably covers a wide variety of circumstances. That's the best we have in terms of the information now.

DR. ROBERTS: Were you planning on putting it -- is that discussed or do you have that sort of caveat mentioned in the report? Sorry, I haven't had a chance to catch up with it.

DR. SMITH: I think it is in the risk characterization section. I'll double check that now.

DR. ROBERTS: Anything else from the panel before we break for lunch?

Let's break for lunch now. When we reconvene, we'll get to the public comments. Let's gather again at 1:30. We'll have just a little bit over an hour for lunch.

(Thereupon, a luncheon recess was taken.)

DR. ROBERTS: An important aspect of the meeting is the opportunity for the panel to

receive comments and input from the public.

I would like to now open the public comments section of the agenda and ask public commenters as we call on them to come forward and will is a spot, I think, on this corner of the table that we have set aside for them and the microphone is on its way there right now.

I would -- while we have the afternoon set a side for public comment, I would like each of the public commenters to respect the time limits they have negotiated with the SAP staff prior to the meeting.

Let us go ahead and begin with the first public commenter that I have listed here, which is Dr. Jennifer Sass and she will be addressing the panel on behalf of the Natural Resources Defense Counsel. Dr. Sass, did you want to comment from over there?

DR. SASS: Well, the thing is, I have my computer here and the microphone is here, so...

DR. ROBERTS: Well, then let's -- why don't you go ahead. I think it would be probably

easier to have you go ahead and comment from there. Be sure to speak into the microphone so that the folks in the audience can hear you.

Let me ask you and this goes for other public commenters as well. When you approach the table, please introduce yourself to the panel.

MS. SASS: Good afternoon I'm Jennifer Sass with the Natural Resources Defense Council. I'm going to be going through some of the data from the Developmental Neurotox Studies and making an argument that I hope is convincing that a safety factor, an FQPA safety factor of at least tenfold is warranted.

First of all, out of the 30 OPs, DNT results have been received for only six. The DNT results are publicly available and if I'm considered the public and rightfully, through the docket, I was only to get two, dimethoate and malathion.

So, those are the ones that I will be presenting in my talk, but I want to make the point that nothing else is in the docket. I

haven't been able to get anything else. I assume the rest of the public can't get anything else.

So, of the two that we're able to look at and scrutinize, I will be presenting arguments that a -- certainly that there is an increase susceptibility to juveniles of the lowest doses tested.

For dimethoate, the DNT study reports a NOAEL observed adverse effect level for pups that is 30 fold lower than the NOAEL for adults.

However, the dimethoate DNT study data demonstrates pup effects at the lowest doses tested. Therefore, that's not really a true no-effect level.

The effects on pups are often more severe than on adults in the dimethoate that I'm referring to -- the fetal resorption in and pup death were the effects and there were not effects in adults. If one or a few OPs are determined to be especially toxic to immature systems then it is scientifically reasonable to presume that all of the OPs are more toxic to immature animals, since

they exert their effects through the same mechanism.

The EPA data evaluation is inconsistent and I believe that it is flawed. These are some of the numbers that the EPA is proposing for the pesticides here. Whether or not they had acute effects at the postnatal day 11 stage, this is the stage that the EPA regards as most like a six-month old child.

They said that chlorpyrifos did have effects but that in the repeat dose experiments, which now take the animal out to postnatal day 21, which the EPA says is most like one to two-year or toddler stage, that chlorpyrifos did not have effect here. Therefore, although in the individual chemical assessments, chlorpyrifos is given a tenfold FQPA factor. Now, in a cumulative assessment, chlorpyrifos is given a onefold.

The reason being I was told is because everything in the cumulative is relying on this column here, this repeat effects postnatal day 21

and everything in this column here is being ignored. That is acute effects on postnatal day 11.

The reason why these effects are being ignored, EPA tells me is because the postnatal day 11 is seen as most similar to a six-month old human and this is not an age of concern because they presumably eat less pesticides, whereas, the one- to two-year-olds are the only age considered relevant because their exposure is considered higher, in other words they eat more of the residues. Therefore, these postnatal day 21 animals are the effects that are being considered.

So, anywhere that they say, yes, in this column, that chemical gets a threefold. Here for malathion, you see it -- although it has effects both of the acute and the repeat because in its in the repeat it gets a threefold in the cumulative risk assessment, whereas in the individual risk assessment it only received a onefold. Why, because EPA didn't know about these effects yet.

Therefore, they should have used the 10 times FQPA factor. That's what it's for, it is for when we don't know or when the date is missing. This to me is an example where just because we don't know doesn't mean there is not effects happening.

Dimethoate I want to flag for you, because as I said, dimethoate and malathion were the only two in the public docket I was able to look at.

For dimethoate, the EPA applied only a onefold safety factor in the cumulative risk assessment. The reason being is that they said there was no effects in this repeat dose postnatal day 21. In fact, I'm going to show you that data from the public docket which will show that not only is there effects but there is effects at the lowest doses tested.

In other words, the study did not derive a proper no-effect level. For the repeat effects or the postnatal day 11, the dimethoate -- I'll show you data that the juveniles are about

threefold more sensitive that the adults.

For the acute, the ones being overlooked -- that was about seven- to ninefold more susceptible to juveniles.

So, this is the study I'm going to be looking at. I was told that the SAP had these studies. Am I correct? In other words, can you look through your individual handouts as I'm talking through it, because the data tables are small.

DR. ROBERTS: That is correct. This was provided to the panel before the meeting. They may not have them with them right now, but the --

DR. SASS: I'm going to show it anyway, but it's just small. So, I don't know how this is going to show in a big room. I didn't -- we don't have a chance to practice in a big room like this with my talk so I'm not sure how it's going to show up, but there are handouts available for everybody and there are extras for people in the room if they don't have any.

This is the dimethoate development

neurotoxicity study. In the executive summary you'll notice --

DR. ROBERTS: Dr. Sass, I'm sorry to interrupt. Dr. Portier, had a quick clarification for us.

DR. PORTIER: I must be looking at the wrong one. I have dimethoate study -- I see, okay. This is the developmental neurotox study not the cholinesterase inhibition study.

DR. SASS: But keep that one out.

So these were the conclusions in the executive summary that -- notice for the maternal -- and this is the developmental neurotox, so these effects were motor activity and pup death in the pups. For the adults there were effects.

So, the study determined that the maternal NOAEL was three. Three was the highest dose tested, but in fact, the maternal NOAEL was not identified.

In other words, there were no effects in the adults according to this study at even the highest dose tested.

So the NOAEL may be even higher, maybe outside -- higher and outside the range of the study. That's important because for the pups this study determined that the NOAEL was .1, which is thirtyfold lower than the maternal NOAEL and that's based on pup death and increased motor activity.

This study determined that the NOAEL was .5 and therefore, the NOAEL was the lowest dose tested in this study.

I will show you data that there are effects of this lowest does tested and if you are convinced when that data comes out in the study, then keep in mind that in fact, the difference between the adults and the pup is likely greater than thirty-fold, because the pup would have a no-effect level.

You lower the adult -- clearly has a no-effect lever, even higher. This was the small part, but I'm going to be reading the numbers and all have you to look at is the red boxes, because I was always taught never throw up anything on a

screen that looks like this.

In these red boxes, this is the motor activity. These are two different dimethoate DNT studies and it is measuring motor activity. And you see in postnatal day 17, which to me should fall in the range of -- interesting for EPA in that toddler-rat range, you see it the zero or control animals, you have a 12-- 12.3, plus or minus 16.3.

That is the standard deviation is higher than the mean number being presented here. At the lowest dose given to these animals, the males here had an effect that was measured at 25, plus or minus 38. That's 25.1 plus or minus 38.5.

Here the standard deviation is higher than the mean being presented. Now I would suggest to you that there wasn't enough animals in this study or this study didn't have the power to detect effects that in this case are doubled.

In other words, 25 in the lowest dose is double the 12 seen in the control. And yet, although the numbers are doubling, it is

considered not statistically significant because the standard deviations are actually higher than the averages, than the means that are presented here.

So, I would suggest that you either have to dismiss this data as not robust enough or you have to look at the means and say these things doubled at the lowest dose tested. In the females postnatal day 13 and postnatal day 17, you see in the control zero here, the numbers .3, plus or minus .9.

In the lowest dose tested, it's 1.2 plus or minus 2.8. Again, the standard deviations are higher than the means and if you take a .3 and add it to the .9, you actually get 1.2. So, in addition to the standard deviations being higher, I would suggest that that is borderline significance. .9 plus .3 is 1.2.

The postnatal day 17 you see the activity actually decrease. The controls were 46 plus or minus 56 and the females here in the lowest dose tested were 19.5 plus or minus .20.

There is an activity increase that is listed as 50 eight percent in these little numbers here.

Again, the standard deviations exceed the means, but I would suggest to you that from 46 to 19 should be either seen as indicative or dismissed, because the study didn't have the power.

But certainly, you cannot conclude from these data that you are not seeing effects at the lowest dose tested. This is clearly not a no-effect level.

This is a different motor activity test. This is the cage floor activity and here in the males you see at the control postnatal day 13 and postnatal day 17 the activity was 223.5 plus or minus 211.7. At least the standard deviations are getting closer to the means here.

At the lowest dose tested it is 162.8 plus or minus 140. So, you have from 223 to 162. I don't know if that's significant or not, but it is a big change and with the standard deviations, I don't think you can make conclusions.

Here at postnatal day 17 it is 171, plus or minus 147. The difference from 171 to the lowest dose tested here is 244 plus or minus 231.

The study listed as a change of 43 percent, and yet it is considered non-statistical, not significant statistically because the standard deviations are so high.

So, the power of this study to detect a change -- it can't even detect a change, it is doubled and yet the conclusion were that there was no effects at the lowest doses tested.

So this study concluded that this, .5 was the lowest effect level and .1 was a no-effect level.

Cholinesterase inhibition. Here we actually attain significance at the lowest doses tested. This is for dimethoate again and this one you don't have a copy of, but I will -- there's only a few numbers and I'll read through the important numbers.

This study wrongly concluded that with repeated exposures, the no-effect level for

cholinesterase inhibition is .1, the lowest dose tested, based on brain cholinesterase inhibition in adults and off springs. I will show you in the data that there are effects at .1.

Table 5 of the study, looking at cholinesterase activity in adults and pups. Here you see again the controls run here and the lowest dose in this column. Obviously, there's effects at higher doses I'm not going through them because I want to stress these low-dose effects.

For postnatal day 11 pups, you see the red blood cell, have you a 18 percent decrease in cholinesterase activity here. It went from 197 plus or minus 620 down to 1,000 -- I'm sorry, 1,997, plus or minus 620 to 1, 647, plus or minus 291. 18 percent cholinesterase inhibition is not considered statistically significant in this study.

I think that's a weakness in the study. 18 percent is almost double what the EPA considers significant. They have set it at 10 based on their detection levels.

Repeat exposures, those were acute. What that means is that the EPA would have disregarded them even if it had been statistically significant because it's a postnatal day 11 acute exposure.

For the repeated exposures, the ones that we are considering here, you see that in the gestational day 20 fetuses, in the brain there is a statistically significant difference.

That little asterisk next to that number means that the study found statistical significance.

The control animals here were 1781 plus or minus 175 and the lowest dose tested in this study, 1,569, plus or minus 173 was a 12 percent inhibition cholinesterase. Compared to the adults, which are up here, running somewhere around -- hovering around zero basically, three -- two, I think, makes the pups 12 times more sensitive than the dams at the lowest dose tested.

That's significant, there is an asterisk

there. And that's repeat exposures, but it is gestational day and the in uteric effects were ignored.

Postnatal day 21. The males statistical significant effects in the lowest dose tested, .1. That one has an asterisk. There was a 4 percent change. It's considered statistically significant. It is a low dose. It's in postnatal day 21 and it was ignored.

Here postnatal day on females, there is -- in the red blood cells you have a change of 15 percent, cholinesterase inhibition compared to controls and that was not considered statistically significant in the study, although you notice right next to it at the next dose here, .5, there is a 23 percent cholinesterase inhibition that is considered statistically significant.

There is an asterisk there. The interesting thing about this, and why I point it out to you, is that it -- interestingly enough in the malathion, which I will show you in a minute, both malathion and the dimethoate data show a non-

statistical 15 percent cholinesterase inhibition in red blood cells for postnatal day 21 females at the lowest dose tested.

And yet, for dimethoate, this was ignored because it didn't attain statistical significance. And for malathion, it was considered treatment related, although it still did not attain statistical significance, the EPA considered that at 15 percent there was a red flag and they would consider it treatment related.

This is malathion. Here at the lowest dose tested, which was .5, and I'm sorry, this is all cholinesterase activity, these are not behavior. This is continuing along with cholinesterase inhibition-type test.

At 5, the lowest dose tested, 5 milligrams per kilogram per day, the postnatal day 11, males, the ones that aren't being considered by EPA, had red blood cell levels where there was a 16 percent cholinesterase inhibition at the lowest dose tested that had statistical significance as indicated by the asterisk and the

malathion testing people actually bolded the statistical significance stuff.

So, you will note that it's in bold as well as asterisked. The malathion effects are sixteen times greater in the pups than in the adults, that is adults actually had no effects. Up around here, these adults weren't filled in.

At the lowest dose tested, EPA disregarded them because, of course, they are in postnatal day 11 pups.

Repeat exposures. These are the ones we're supposed to be paying attention to. You see that postnatal day 21 males in the red blood cells at the lowest dose tested had a 17 percent inhibition in cholinesterase activity.

It is deemed statistically significant according to the study. It has an asterisk beside it -- lowest dose tested, postnatal day 21 males.

Here the postnatal day 21 females -- also remember the dimethoate I just showed you, also had in the red blood cells a 15 percent inhibition in cholinesterase activity that was not

considered statistically significant.

There is no asterisk besides that but it is in bold and the EPA considers it treatment related. Therefore, for malathion, they considered that there was effects at the lowest dose tested for dimethoate, they considered there were not effects at the lowest dose tested.

This is also malathion. This is not in the docket. This was obtained by me under Freedom of Information Act and it is a document that was submitted under FIFRA 682, which is where industries have to submit information they have showing that their chemical might be hazardous.

I can't show you most of this because there are multinational corporations in the room, not that you could actually synthesize malathion from this information, but that's what I signed when I accepted this and what I want to only show you is the data that acute and low-dose effects occur and that they were ignored.

This letter that came onto this data that that was submitted to the EPA states as

underlined in red here, although the no-observed effect level NOEL, for cholinesterase inhibition is higher in adults versus pups given a single dose.

So, that's to understand that at acute doses there are definitely differences between pup and adult sensitivity. Pups are clearly more sensitive and this is recognized, that the NOEL of 5 milligrams per kilogram is the same for a adults and pups given multiple daily doses here. They are repeats.

Therefore, they do not believe that these data provide any basis for concern and I do. So this is the data that was in that document, submitted under FIFRA 682.

I want to make the point with it that the EPA disregards data on postnatal day 11 pups because they most closely resemble the six-month old babies and not the one to two-years-olds who consume more pesticides and that, in fact, there is a lot of effects here that we should be concerned about.

Some of these numbers you will recognize from -- this was the data that made up the study that the DER -- that I just went through, that the EPA went through consisted of.

So, this is the same data that went into the DNT, the cholinesterase inhibition that went into the public document that I just showed you. It is just in a different form.

And you will see here these are males, pups and adults the females, pups and adults. What you have to do across the -- going horizontally here the different doses, five is the lowest dose tested -- everything is compared to control.

The controls aren't shown here. You have to compare the pup plasma with the adult plasma. The pup red blood cell with the adult red blood cell. I have drawn a few red arrows to guide your eyes so you can compare relevant columns by relevant column.

What I want to show you here is, first of all what I showed you before from the DNT's

that the EPA presented at the lowest dose tests here, five that are -- effects that are statistically significant. I have circled it.

The pups that are postnatal day 11 have 16 percent inhibition compared to control pups and that's asterisked. It was considered significant by the study.

You see some of these other numbers I want to point out for you. Here at 150 in plasma, there is a 36 percent inhibition of the pups, so, 1 percent inhibition in the adults.

Here in the brain at the highest dose tested the pups had an 84 percent inhibition. The adults had a three percent inhibition. That one got two asterisks. It is very statistically significant -- .05 level.

You see in the females at the highest does tested in the brain, the pups had an 81 percent inhibition, the adults also a 4 percent inhibition.

This is the males here and females again. Cholinesterase inhibition, following 11

days. This is the repeat. This was the acute; this is the repeat.

You see at the lowest dose here even with repeat exposures, there is a 17 percent inhibition in postnatal day 11, it is considered statistically significant.

At some of the higher doses you get much bigger differences between the pups and the adults. Here are 16 percent inhibition in the brain of the pups at the highest doses and 1 percent inhibition in adults at the highest doses.

You can spend as long as you want going through these numbers, but there is differences. That's the end of the data section.

So, now I think I want to make the point and we have made this point over and over again, but this is worth making in this context, that children from agriculture areas are exposed to a greater degree of pesticides from more sources than other children.

The way the EPA cumulative assessment is done, it does a random probability distribution

and it captures the random American population.

These children are at very high risk and some data here is supporting that -- Atozine (ph), an outdoor herbicide was detected in one hundred percent of the houses of an Iowa farm-family study during application season and four percent of the nonfarm houses.

Neurotoxic organophosphate pesticides have been detected on the hands of farm children at levels that could result in exposures exceeding what the EPA has set as safe levels -- this is a 1997 study.

Metabolites of organophosphate pesticides here used only in agriculture were detected in the urine of two-thirds of children of agriculture workers and in 4/10ths of children who live in agricultural regions, 1997.

Farm children under the age of six in Washington State fruit growing regions had urinary metabolites for azinphosmethyl at a meeting concentration that was fourfold higher than nonfarm children, same study as above.

On farms, children as young as 10 can work legally and younger children frequently work illegally or accompanying their parents to the fields and these practices have resulted in effects in poisonings and sometimes in deaths.

These are some photos by Earl Dotter and this shows a man applying pesticides 1994 in the Salinas Valley, apply pesticides while his son follows him through the fields. This is a 17 years old girl. She is operating a tractor that clearly needs to be updated. It is not enclosed.

It doesn't have air conditioning and this young girl is farming the family field with this tractor. All this stuff in the background is smoke. It doesn't come out well on the photocopy. This was taken in '96 or '97.

Every year, approximately 300 children in the US are killed and 23,000 are injured in agriculture related activities. Of course, these aren't pesticide data. This is from NIOSH, 1992, Farming is a Dangerous Occupation. We have a chance here to relieve some of that danger by

regulating pesticides and toxic chemicals.

Our DC encourages the EPA to either make the following improvements to the OP cumulative risk assessment or retain at least a tenfold safety factor to adjust for the underestimation and exposure and in risk in the current risk assessment.

The SAP made suggestions in the February meeting and it came out in their report in 2002, to use time-weighted rolling average which would account for the previous day's exposures. This isn't being done.

It is important -- this is a quote from the SAP report, "It is important to consider not what a population is typically exposed to, but the probability that an unusual exposure might occur."

I would add to that SAP quote, or normal exposures in an unusually exposed population such as children in agriculture communities.

To characterize the exposure via drinking water for certain defined populations

such as infants, bottle-fed formula, made with powder and tap water -- I think this is important with the infants, although the EPA assess them as taking in less pesticides.

Since breast milk is not included I would consider that to be a data gap at the least. The SAP asks the EPA to consider the potential effects of spills and non-agricultural OP uses to drinking water. If the EPA cannot do this, then this certainly should represent a gap in the data base.

The SAP asks the EPA to consider adding a consumption of homegrown vegetation, exposure from drift and inhalation exposures to volatile active ingredients to lawn scenarios and particularly, to children for these applications.

Now, the EPA has done some numbers with drift and what the EPA has shown is that drift is insignificant compared to runoff in areas where there is high runoff, in areas where there is not high runoff into the water drift become as lot more important.

I would suggest that rather than comparing drift to how significant it is to something else, just add it in. Drift should be in this.

This particularly applies, of course to agriculture children, families. The risk assessment process must depend on a full evaluation of the toxic potential of individual products and not simply be tied to a single endpoint. This was a SAP recommendation in the last report. Cholinesterase inhibition is a marker, it's not an endpoint. We don't really know what the effects of these chemicals are at low doses. Where we do know, we should consider certainly consider that and where we don't, we should consider that in a gap in the data base.

The Agency must clearly recognize that a cumulative risk assessment based on a single endpoint does not address all the potential risks. This was brought up by SAP and NRDC recognizes this. The published literature recognizes this

and if it can't be incorporated, it should be considered a gap in the data base and warranting an FQPA factor.

The EPA is required to impose a tenfold safety factor. The law says, "An additional tenfold margin of safety shall be an applied for infants and children to take into account potential pre- and postnatal exposure and toxicity to infants and children.

Under the law, EPA may adopt a margin of safety lower or higher than tenfold only if, on the basis of reliable data, such margin of safety for infants and children can be shown. I would suggest that in fact, the data that the EPA presents here shows that a factor of at least tenfold is warranted on their data alone.

The EPA has ignored the increased susceptibility to mature animals to low doses, particularly, EPA has ignored published literature on the effects below detectable cholinesterase inhibition and EPA has ignored the malathion and dimethoate demonstrating effects in the postnatal

day 11 rat pups after acute doses, even those effects at the lowest doses tested.

The OP cumulative risk assessment is based upon cholinesterase inhibition data for adult rates, that is the relative potency factors are set that way and in the absence of data from immature animals. While the Agency said it will consider differential toxicity data for infants the versus adults, EPA has DNT data for in fact, just six of 30 OPs and publicly available comparative cholinesterase data for just three.

In the dimethoate DNT study, pups are 30 times more susceptible to cholinesterase inhibition than adults based on the no-adverse effect level for motor activity and pup death. That is, pups are dying, adults aren't having an effect at all.

This dimethoate study in rat pups does not show a proper NOEL for pups, only a NOEL. EPA has not used the SAP recommended time-weighting average to account for previous days exposures. Therefore, an NRDC would support the one-day

average.

Anything else will reduce the impact of acute exposures, which clearly are important and of great impact to infants as demonstrated by EPA's DNT data.

Data for some OPs shows that the young are far more vulnerable than adults, so it is scientifically reasonable and obvious that we should assume that all OPs acting by the same mechanism are also more toxic to the young by at least a tenfold as demonstrated by the malathion data.

EPA has repeatedly under estimated exposure by among other things failing to consider over 1 million children who live on farms, for whom data show are far more exposed to pesticides.

EPA has also failed to properly consider exposure from air drift. I say properly, because they have shown it is significant and then they have mooted other it out by comparing it to runoff in wet places.

EPA has failed to consider exposure from

homegrown foods, from U-Pick Farms and from over 1 million Americans who shop at farmers's markets and other highly exposed subpopulations.

Despite serious indications of much greater cholinesterase inhibition in the young than in the adults and despite important absent toxicity and exposure data for young animals in fetuses, the OP cumulative risk assessment applies only a threefold safety factor for most OPs and no additional safety factor for three of the OPs, including dimethoate and chlorpyrifos.

The EPA lacks the required reliable data on pre- and postnatal exposure and toxicity for infants and children to warrant the imposition of a safety factor lower than tenfold, in fact should be the safety factor of over tenfold based upon the data they do have.

I want to quote the SAP report again from the February meeting over confidence limits, the general under estimation of uncertainty and/or assigning confidence.

The general under estimation of

confidence limits that are too narrow is one of the best documented phenomena in risk assessment. I would caution us not to make this obvious mistake here. We are being warned.

The bio-monitoring from NHANES suggests that more than 80 percent of the American public have urinary metabolites indicating possible exposures to OPs.

The cumulative risk assessment that we're looking at here suggests that almost no one is being exposed. In fact, only very few people are being exposed.

The reason why the EPA has told us is because they've built into their assessment the mitigating changes and chemicals that have been dropped off the market or uses that will no longer be allowed -- that's all built-in, so, the future is built into this.

Those phase outs and cancellations will happen, some of them immediately, some of them over the next four to five years. And what the EPA has not built-in is all the other

chemicals, the tier two organophosphate chemicals that will obviously come up to take their places.

What this particular talk didn't have, because I had to use my zip drive, because my computer wouldn't hook up to this thing, is a list of the organizations that are supporting the use of at least a full 10 times FQPA factor for this cumulative risk assessment.

The NRDC, Consumers's Union, Farm Worker Justice Fund, World Wildlife Fund, Children's Environmental Health Network, The Northwest Coalition for Alternatives to Pesticides, Physicians for Social Responsibility, Northwest Science and Environmental Policy Center and the New York State Attorney General's Office, Environmental Protection Bureau.

I stand here with the permission of the leaders of those organizations who represent millions of members in this country and I tell you that we will not accept toxic chemicals in our food, in our water, in the air we breathe and we will not accept anything less than at least a full

10 times FQPA factor. Thank you.

DR. ROBERTS: Thank you, Dr. Sass.

Are there any questions from members of the panel for Dr. Sass?

DR. BRIMIJOIN: You are obviously a brilliant individual, but are you confusing the ratio between the magnitude of effects with the kind of safety factors that we're talking here?

Surely you realize that if you had as small a difference as maybe a twofold difference in actual sensitivity, measured on something like a ED 50 and you went down to the bottom of the curve and you could measure things with absolute precision, at the low end of the curve you could get any ratio you want, and get down to where one of them has an effect of 0.1 percent and the other still has an effect of 3 or 5 percent, you can get a ratio of 100 or 1000.

So, I think your presentation confused these two factors when you are looking at tails and the curves. I think we should be keeping in mind that we're really trying to estimate where

the position of the whole curve is.

DR. ROBERTS: Do you want to respond?

DR. SASS: No. I understand what you are saying. I really have only this data. I mean, there are only two chemicals that are publicly available on the docket that I can look at and this is what we have to look at. These effects I think are dramatic. I think the effects at the lowest dose tested is important.

I think that we really don't have a good sense of that curve because we don't have a no-effect level for these chemicals and we don't have data on the other chemicals to look at.

DR. ROBERTS: Dr. Portier?

DR. PORTIER: Dr. Brimijoin is right, obviously, that looking at details it is going to be more variable, but I will remind you that when we make a comparison at the EC 50, our assumption is that those curves are parallel throughout the entire dose response curve when we apply that to the lower dose region in assessing whether in fact it is constant across the entire dose response

curve is an important consideration in this evaluation.

DR. BRIMIJOIN: That's also true. But again, we would still be wanting to know what is the horizontal distance at the bottom end of the curve.

Go down the -- go up the curve as far as we need to say that might be biologically significant and then what is the horizontal distance at that point. Not what is the vertical distance from curve A down to the curve B.

DR. PORTIER: Again, looking at it mathematically if you are believing the assumptions that go into a comparison of DC 50s, it won't matter whether you look vertical or horizontal.

The ratios of difference should be the same mathematically under the assumption of the analysis that makes EC 50 make sense.

If the shapes are not the same, then comparison at the 50 percent point makes no sense at the 10 percent point.

So again, an assessment of the parallelism of these curves is an important characterization. I had a different question. You raised an issue that I had not noticed and so I will ask the question.

In looking at the DNT studies where you noted that the variances are in fact, equal to the means and that the means are increasing and the variances are increasing proportional to the means, as a statistician that immediately makes me want to worry about doing a log scale transformation or some other type of transformation on the data before I do my test for statistical significance.

Did you in fact, do that? I'll follow up with that question to EPA. Did you in fact, or did the person who presented the data to you in fact, do a log scale transformation first?

DR. SASS: I didn't.

DR. DELLARCO: I can't answer that. I will have to talk with the people who did the modeling.

DR. BRIMIJOIN: One more question for Dr. Sass.

Dr. Sass, you raised rather dramatically and pointedly the case of children that are -- let's say, children of agricultural families and their heightened risk of exposure because of such practices as allowing them to apply pesticides or a accompany parents who are doing that.

What I wonder is, although that strikes me in fact as a very significant issue and certainly one of health policy and maybe for OSHA and other agencies to consider, from the EPA's perspective, what do you or what does your group consider would be the impact on these children of anything that stops short of simply banning pesticide use out right?

So, if they are applying, let's say, half as much pesticide or do you think it would make any real difference in the types of exposures that children in that special situation would be encountering?

DR. SASS: For some scenarios, I think

it would make a difference and for some it wouldn't. I mean, the drift is an issue. If there is less drift, then that would be an improvement.

As far as tracking in and, you know, dad's clothes and dad driving the kids to school in the farm truck, which is not only going to the field, it is the same farm truck that mom delivers the lunch out in to the field workers and this kind of stuff, I don't know it would make a difference. I do know there is data out there.

I mean, I know that there are studies been done that have found pesticides in the homes, on table tops and counters and rugs and curtains and I would suggest that the law has to protect those children too, that they don't fall beneath the law.

DR. ROBERTS: Any other questions?

DR. REED: This is sort of a follow up with the short discussion between Dr. Brimijoin and Dr. Portier and this is my curious question to Dr. Sass and also to the Agency.

In your comparison of the -- between the postnatal day 11 and 17 to the adults, you were comparing at certain points and I think what Dr. Brimijoin mentioned, I think is important to me, that I think it is probably better if you compare it based on benchmark-dose type of approach so you get the whole dose response, and then you can pick your point -- 50 percent 10 percent.

Have you done that? I think the Agency had done some of that with some of the data set. My understanding or my recollection is that it will not change the picture that you are looking at by doing that.

But my question then to the Agency is: Have you done that with every single data set or this particular data set?

But Dr. Sass, have you tried that approach in terms of comparing it based on --

DR. SASS: No. You know, when these went into the public docket was last week, about Tuesday, I think. I can't remember and the malathion I got even later.

So this is -- I have only had access to this data for about five days.

DR. DELLARCO: First I want to make a clarification about the DER's and then I want to address the comment that was made.

These DER's were developed for the purpose of single chemical assessments. That's why there may be discussion about LOAELs and NOAELs, because the purpose of the single chemical assessment is to try to identify a no-observed adverse effect level.

We provided these to the SAP because it was a record that we had to at least show you the cholinesterase data that we were looking.

But again, we were looking at it from the perspective of not NOAELs and LOAELs levels but the compared sensitivity between the pups and the adults.

We did model the data for our chemicals, except there were some studies you couldn't model. For example, the study that Dr. Stephanie Padilla did for dimethoate -- and that was a one-dose

study, so, you can't model that.

So, we just had to report the difference between adults and pups for response of that single dose and where we had data we tried to model it. So, we could model all malathion and dimethoate, methamidophos.

Actually, Ginger Mooser (ph) provided those response modeling and benchmark responses for that and then the Xie paper that we looked at that came out of Dr. Pope's lab, they also had dose response data where you could look at an ED 50. So, it depended on the data whether we could do it.

DR. REED: But you did all the ones you could do?

DR. DELLARCO: Yes.

DR. REED: Thank you.

DR. ROBERTS: Dr. Hattis.

DR. HATTIS: I wanted to follow up on that.

Have you provided us with --. I wanted to get in front of me, if you could point it out

to me in the document or elsewhere, if you could supply it, a comparative of BMB 10 determination for each of the chemicals where you have the data in the dams and the -- either the fetal or postnatal day -- whatever exposed animals?

DR. DELLARCO: Let me just clarify. Where we could model data, we only did it with postnatal exposures, okay, so, we didn't do it for the gestational exposures, because you don't know the dose there. We did that at -- we did that -- it is preliminary modeling, it's not in the document.

There was only a paragraph, but what we could od is -- I can go back to the office and see if there is any spreadsheets that we can give you to look at. We showed some graphs in the presentation.

DR. HATTIS: It would be helpful for us to be able to make a distribution from whatever individual chemical data you have for different comparative ages.

DR. ROBERTS: Thank you.

Dr. Portier.

DR. PORTIER: This is my lack of preparation here, because I -- in looking at the table of the individual chemical safety factors, uncertainty factors versus cumulative assessment uncertainly factors, it had occurred to me to do the same table, but I actually didn't go back and get the individuals.

So, I might be putting you on the spot here and I am apologizing in advance for doing that, but can you tell us the differences between these -- for instance, the 10X chlorpyrifos, why is that one 10X? What is the endpoint that is driving the 10X? The 3X methamidophos, what is the endpoint for methyl parathion -- is that possible?

DR. DELLARCO: In single chemical assessments?

DR. PORTIER: On the single chemicals, yes.

DR. DELLARCO: For chlorpyrifos, I can tell you that one, because both Dr. Becknick (ph)

and I were involved in that one in addition to Dr. Fadia (ph) and what drove that 10X decision was not so much the differential that you saw in the cholinesterase response, but it was a body of data that was coming out in the published literature, particularly Dr. Al Slotkin's laboratory about these other effects that he was seeing in the brain, effects on proliferation, signal translation pathways.

A lot of these studies were not done with a route of administration where you could identify a NOAEL level.

It was -- is it IVDNSO -- sub QDNSO and in the research study where the purpose wasn't trying to identify effect NOAEL effect either -- and further more, there was suggestion in the literature that these effects may not -- you may not be related to acetylcholinesterase inhibition, that there could be another mechanism going on leading to these effects.

And therefore, we felt that using cholinesterase inhibition would not be protected

for these other effects which may be operating by other mechanisms that we couldn't quantify and that was the basis for the 10X.

DR. PORTIER: And that is true pretty much across the board in terms of, there is other mechanistic information that suggests an independent effect.

DR. DELLARCO: Right. There are some studies, some OPs that -- again, I can bring that back for you. I have to go to the office and get that, but it may have been due to some sort of developmental effect in the teratology test. There were different reasons for them.

So, all toxicities were looked at in these individual assessments.

DR. NEEDLEMAN: Could we see that data? I would very much like to see that data.

DR. DELLARCO: That would be a -- that would be a heroic effort, because it would be -- it would involve pulling all the individual assessments together.

But the point is that -- I said in the

morning, the purpose of the cumulative is very different than a single chemical assessment where you are looking at all those toxicities where you are trying to identify the lowest effect, the most sensitive endpoint.

In the cumulative the whole basis of this assessment is based on this common effect. So again, that was the focus of the analysis. Are any effects that could be linked or tied to that?

DR. ROBERTS: For the record the information request was from Dr. Needleman.

Any other questions?

DR. MATSUMURA: I need some clarification. Under this FQPA, aren't we looking at those -- let's say illegal exposure by using the child label in the agricultural field or are we limited to food, drinking water some household exposure? Maybe Ms. Mulkey can answer.

MS. MULKEY: Well, I will try.

I don't want to give you a precise legal response, because I just don't have the mastery, but essentially, the aggregate exposure to be

considered under the statute is, all sources other than occupational sources of exposure.

So, that we aggregate food, drinking water, residential sources, if we had information that there are very small number of chemicals that have non- pesticidal uses and so forth. So, it is the non- occupational sources of exposure are to be aggregated.

Now, whether -- if you had an ability to measure and consider exposure of children in fields who were legally working versus not legally working, we have not sort of fine tuned this issue of exactly what constitutes an occupational exposure.

But the basic answer to your question is we aggregate -- we also consider under the pesticide law occupational exposures and have to make -- reach a determination that there are no unreasonable adverse effects taken into account -- benefits, basically.

So, it is not like we ignore occupational exposures, but they are not part of

the aggregate. I hope that was enough to answer your question?

DR. ROBERTS: Thank you Ms. Mulkey.

I believe Dr. Sass wanted to -- did you want to respond to that question?

DR. SASS: I just want to add very quickly, the law has actually decided that those are not considered occupational exposures when children follow their mothers into fields or when pregnant women work in fields or when children live next to fields or when children live on migrant housing that's in the fields or next to the fields, that those are not occupational exposures because those children are not considered supposed to be working.

MS. MULKEY: I didn't mean to imply that we felt that they are, that those particular children --

DR. SASS: So, we think they should be covered under FQPA because they are exposed environmentally.

DR. ROBERTS: Dr. Hattis, I believe had

a question.

DR. HATTIS: I wanted to further clarify from Dr. Dellarco.

Are you urging us not to consider these results from the Slotkin papers that we have because the effects are likely to be due not to direct cholinesterase inhibition or are we to gather from Dr. Eldefrawi's comment that the effects by way of other transmitters might be secondary to the acetylcholinesterase inhibition itself.

DR. DELLARCO: We would like the panel to focus on acetylcholinesterase inhibition and how that behaves in the young versus the adult versus sensitivity and with respect to other effects, those that can be linked to that.

We did discuss the Slotkin papers in our report because we're trying to give an overview of the literature and the understanding and the effects that they see in that study. We really don't know what the basis is.

DR. ROBERTS: We have a pretty sizeable

list of public commenters. So, I think it is best if we move along. I would like to thank Dr. Sass for her comments. It certainly stimulated some discussion here.

I would also like to invite the next public commenter Dr. Rudy Richardson from the University of Michigan to approach the panel and Dr. Richardson is here on behalf of the Sound Science Policy Alliance.

MS. DUGGAN: Actually, Dr. Roberts, I am going to introduce the presenters. I've discussed it with Larry Dorsey. It's not on the agenda though.

My name is Angelina Duggan. I am Director of Science Policy for Crop Life America. I have the honor of introducing the public commenters today on behalf of my colleges, Crop Life America, for the FQPA Implementation Working Group and the Sound Science Policy Alliance.

Actually, my slides will be coming up shortly. so, I'll just hold off for a minute. While they are being projected I'll tell you a

little bit about the different organizations the Crop Life America represents the manufactures and formulators of products for science solutions for agriculture in the United States.

The Implementation Working Group is the coalition of grower groups and manufacturer of crop protection products involved in FQPA implementation. The Sound Science Policy Alliance is a coalition of manufacturers of cholinesterase products.

We have various presenters today in three separate areas. The first set of presentations will address EPA's FQPA questions. We have broken them out as to the questions and issues.

First will be Dr. Rudy Richardson from the University of Michigan. He will discuss the various parts of question one, related to issue one.

Secondly, Larry Sheets from the Bayer Corporation, Bayer Crop Sciences now will discuss issue two. Issue three will be covered by Dr.

James Gibson from East Carolina East Medical School from East Carolina University.

He will discuss issue three and then Dr. Sheets again, will get up and summarize the positions and issues that have been covered in these presentations.

We'll also have a presentation on modeling and exposure assessment and Jack Zabik from Dow Sciences will present that part of our public comments. And then finally, Ed Gray from -- representing the Implementation Working Group will discuss the Agro Science Policy and provide concluding statements in regards to the OP cumulative risk assessment.

So, with that brief introduction, it is my pleasure to thank EPA for the opportunity for myself and my colleagues to address the panel this afternoon and I turn the presentation over to Professor Richardson.

DR. RICHARDSON: I'm Professor of Toxicology of the University of Michigan. As was announced, representing the Sound Science Policy

Alliance. I'm going to be addressing specifically, question 1.1 that is before the panel.

My expertise by the way, is in the chemistry and toxicology of organophosphorus compounds and other inhibitors.

The question, I'll quote it for you, before us is: "Does the scientific evidence support the conclusion that perturbation of the cholinergic nervous system during development by inhibiting acetylcholinesterase, AChE, can potentially lead to deficits in the structure and function of the central and peripheral nervous systems."

What we're talking about here is AChE inhibition and possible connection of that to neurodevelopmental abnormalities.

I'm going break this down into three parts looking at the overall question of acetylcholinesterase inhibition by environmental levels of organophosphorus compounds or OPs and have the premise that this does not lead to

neurodevelopmental abnormalities. It based on three points that I will address in turn.

By way of introduction, I will state them here, that the EPA CRA exposure levels or orders of magnitude below those required in postnatal rat studies for cholinesterase inhibition.

I'm using the abbreviations as defined in the EPA document for today. AChE refers to studies that include AChE and BChE. Where it is not defined in the test system whether you are looking at both activities or one or the other.

Also, studies showing a link between AChE inhibition and neurodevelopmental effects are based on in vitro systems or doses that are much higher than the EPA CRA exposure levels.

Finally, I think the most interesting, most fascinating aspect of this is the recent model that has been developed by Roxanna Lockridge (ph) and colleagues, of the acetylcholinesterase knockout mouse. Where, if you had both wheels where you have knocked out the enzyme

completely -- this is referred to as the minus, minus knock out, the total knock out -- that these actually show normal development of CNS and PNS in the recent paper by Mesulam that I'll cite later.

The heterozygote animals, as opposed to the enzygotic (ph) animals, the plus minus animals have exactly 50 percent AChE activity throughout the peripheral and central nervous systems. These develop normally. They undergo all the normal developmental milestones, despite a chronic 50 percent deficit AChE.

So, you have here a peer system where you haven't had to add a chemical, but you have genetically deleted half of the enzyme activity.

This indicates, I suggest to you, that as much as a 50 percent decrease in AChE activity during development is not injurious.

You seen these data before. Dr. Dellarco presented them this morning to us, so, I won't dwell on them. You seen how they are derived. These are the OP CRA food exposure levels that have been derived by EPA's model.

I focus your attention, as they have done, on the second line down under age group of children one to two, where if you look at the highest level, the 99.9 percentile, that the estimated exposure here in milligrams per kilogram per day is 0.0018. The next slide -- contrasting that with results of animal studies, this is summation of literature information on postnatal rat OP testing where we have various OP compounds under consideration. The reference there is given to the literature.

Most of these are cited in the EPA document for today and the dose producing cholinesterase inhibition either acutely or by repeated dosing. I have indicated there where in a couple of the studies on repeated dosing, the dose was administered sub-Q as opposed to by the oral route.

You notice overall, particularly focusing attention on the repeated doses, that all of these are at least a couple orders of magnitude higher than the 0018 level that I showed you in

the previous slide.

So that looking at -- the point of this is to contrast the animal studies producing cholinesterase inhibition with actual exposures with what people are really being exposed to and asking the question, do we expect an effect from these actual levels of inhibition.

Under the general rubric of AChE inhibition and neurodevelopment, Dr. Bigbee and colleagues have produced some fascinating results that bolster the overall case that I think has been well made for the entire -- an interrelated familiarly of proteins, some of them not having enzymatic activity at all. The tachtins (ph) for example, that have close homology to the acetylcholinesterases, showing they are involved in some sense in some systems in development.

For example, he has shown that neuro outgrowth does correlate with AChE expression in an in vitro system.

Where he has a cell culture system, where he can control the expression of acetylcholinesterase in

that system by either lowering it or increasing it and he gets a corresponding diminution or increase of neuro outgrowth.

Bear in mind this is in a system that is not complete. It is not containing glial (ph) elements but only neuro elements.

Then I want to highlight something from today's EPA document. This is a direct quote. "Adverse neurodevelopmental outcomes that are a result of the inhibition of cholinesterase should not occur at doses that do not inhibit cholinesterase.

This is essentially a tautology, but I think it makes a good point that goes back to the linkage that has to exist between the exposure assessment and the hazard assessment.

Here we come to what I think is truly fascinating. The AChE knockout mouse from Lockridge and colleagues -- in the total knockout, you have zero AChE, this animal is completely devoid of AChE. Before this experiment was undertaken, there were actually bets placed on

whether these animals would survive.

Most people thought they couldn't survive. This is highly conserved enzyme throughout the animal kingdom. Seems to be something that would be considered necessary for life and yet the animals do survive. If you feed them properly as she has shown, they survive into adult hood and ultimately achieve the developmental milestones.

They do show some delayed development in the total knockout. It is amazing that they live at all. But some of the gross developmental milestones such as the day of eye opening would be delayed. Ultimately, they grow up and in the latest study that has come out of collaboration with Mesulam, et al. -- that just came out this year -- they did a detailed microscopic analysis of the cholinergic nervous system and found that even in these total knockouts, the cholinergic nervous system is in tact and identical to the wild-type animal.

The function of the knocked out AChE

seems to be taken by BChE, which is not present in the in vitro systems used in Bigbee's experiments.

In some ways, even more interesting is the heterozygote animal. The AChE plus minus mouse that has exactly 50 percent of AChE activity throughout it's nervous systems and these show essentially normal development, behavior, health and reproduction.

Some of this is summarized in the paper that's also quoted in EPA document by Xie, et al., and this is also from Dr. Lockridge's laboratory.

The conclusion that I reach from this is that the current state of knowledge does indicate that AChE inhibition by environmentally relevant levels of organophosphorus insecticides does not result in neurodevelopmental abnormalities.

Even though you have this fascinating in vitro evidence, for a developmental role for acetylcholinesterase you have on the other side the studies of the AChE knockout that indicate the deficits in cholinesterase activity by as much as

50 percent are not deleterious to development, health or reproduction.

I have a concluding slide just for the record that shows the references that I cited in this presentation.

Thank you.

DR. ROBERTS: Are there any more questions for Dr. Richardson?

DR. PORTIER: In the knockout animals, how much is BChE OP regulated?

DR. RICHARDSON: This group reported in one paper that came out in -- I think it was recently, that they thought that they saw an increase in BChE, that there was actually an up regulation of BChE to compensate for the knocked out AChE.

In their latest work, they came out with Mesulam, et al., they don't find that. There seems to be the normal levels of BChE throughout the nervous system, but it becomes clear if you are doing histological staining for cholinesterase when have you knocked it out entirely, you can see

where the BChE is. They found there is a wider distribution than was once thought, but the activity doesn't actually seem to be increased. I think the J. Neurochem (ph) Paper was based on a different solubilization procedure that released more activity that might have been cryptic and not seen in earlier assays.

But they do believe -- to follow up, they do believe that the function of the AChE is somehow being taken over the by the BChE, which by the way, seem to be expressed mainly in glial cells rather than in neuronal cells.

DR. BRIMIJOIN: I want to add to that, because I was a coauthor on the J Neurochem Paper and actually, we didn't find any OP regulation of BChE.

DR. RICHARDSON: Oh, there wasn't? Okay.

DR. BRIMIJOIN: No, it's -- that's the amazing thing. It may compensate physiologically, but there isn't actually more enzyme activity.

DR. RICHARDSON: Thanks for that

clarification, because -- so, it actually wasn't a difference between those two papers and I had not cited the J Neurochem Paper in this presentation, so, I hadn't recently familiarized myself with that.

DR. ROBERTS: Is there a selection among the knock outs, do they have the same mortality experience or is there some sort of selection among those animals?

DR. RICHARDSON: I believe in the total knockouts, they have zero AChE. There is some increased mortality. So, there is some selection. I think the heterozygotes, they -- I don't think they have an increased mortality.

I would have to go back and check the data again, but the thing I wanted to point out is that they do seem to develop normally. There is no apparent difference in the 50 percent or the heterozygote animals compared to the wild-type.

DR. ROBERTS: Dr. Padilla.

DR. PADILLA: Stephanie Padilla. Actually, I can clarify that. In her very first

paper where she described the knockout, they calculated that there was a 25 percent in utero mortality of the complete knockout mouse.

DR. ROBERTS: Thank you, Dr. Padilla.

DR. PORTIER: You mentioned gross measurements of development. Were there any neuro behavioral assessments -- have there been any neuro behavioral assessments on these animals?

DR. RICHARDSON: I'm not aware of detailed neurobehavioral assessments. I think these admittedly have been fairly gross observations. Just looking for ordinary developmental milestones and ordinary behavior. I don't think they's done something that quantifies neuro behavioral paradigms.

At a seminar that Dr. Lockridge presented at the University of Michigan where she described some of these experiments, she was asked in particular about the heterozygote animals, which toxicologically are the most interesting.

She said, unfortunately, they have focused on either the wild-type versus the

complete knockout for most of their studies and they really haven't done the studies they would like to do as yet on the heterozygotes.

DR. LAMBERT: What is your opinion of using AChE for a sensitive indicator of potential neurological effects?

DR. RICHARDSON: Are you asking is it an indicator of toxicity versus a bio-marker of exposure.

DR. LAMBERT: Yes; that would be one. And the sensitivity and applicability.

DR. RICHARDSON: Well, I think looking at the normal situation where you have an intact nervous system, you don't have the knocked out gene for AChE -- actually, I think they knocked out -- what was it Dr. Brimijoin?

May I ask for clarification? Was it for the five oxons?

DR. BRIMIJOIN: There virtually is nothing left of it. It is not one of these things which just is a -- enzymatically null -- it's -- the protein is just not there, just a tiny

fragment is left.

DR. RICHARDSON: Other than that unusual situation and for dealing with -- even though you might speculate there might be heterozygotes in human population, where we only have 50 percent AChE, that hasn't been demonstrated.

I think there is ample evidence to indicate that the common mechanism of the organophosphorus insecticides is inhibition of AChE and therefore, I think we should use that.

That's what is on the table now, I think even though the knockout experiments are fascinating and it opens up a whole lot of questions, where there is obviously, some sort of a compensation that can occur where you don't have even any AChE -- if you do the total knockout, of course these animals aren't completely normal, they are more sensitive to organophosphorus compounds than an individual that -- the wild-type individual.

So, I think we would still use it as -- certainly as a bio-marker of exposure. And if it

is in the nervous system, it is at least the prelude to the actual toxicity. That is, we accept that the common mechanism is AChE inhibition and the toxicity proceeds from the excess acetylcholinesterase that accumulates as a result of that.

DR. LAMBERT: But as you indicate, the knockout would speak against that at least decrease the sensitivity or...

DR. RICHARDSON: Well, no, it doesn't really because you do have this compensatory mechanism where now BChE, which is -- it is a very promiscuous enzyme, it can hydrolyze a wide variety of substrate structures including acetylcholine. No acetylcholine seems to be the candidate.

DR. ROBERTS: Dr. Hattis?

DR. HATTIS: In cases where as organophosphate exposure during life you have a transient depression of AChE, you have any evidence on the dynamics of compensatory responses that we should be expecting?Obviously knockout

is a situation where you have a constant loss of enzyme activity, either the he to or the homo sigh does. Whereas in the case of prenatal or postnatal exposure you would usually have some transient depression that might be some have somewhat different consequences.

Do you have sort of comment on either the difference between those two situations or the dynamics with which you could expect some adaption to occur?

DR. RICHARDSON: I think the point is he personally addressed in the EPA document for today's discussion where they do mention the paper of the knock out experiments.

And indicate a knock out model does have to be interpreted with some caution because of the kinds of adaptations that are you talking about over the course of development so you might presume that the knock out might be a good model for exposure during the entire developmental lifetime we do have, such as non regulation and receptors.

So that's perhaps a different sort of a adaptation that is going on in this model. The reason I site this is to provide a very interesting and rather extreme case of substantial loss of function of this enzyme under discussion today.

And contrast that with levels that people are actually being exposed to according to the EPA estimates one to two-year-olds 99.9 percent tile where you have levels of cumulative OP would not produce detect table AChE inhibition.

Here we have the contrast 100 percent or 50 percent knock out of the enzyme. And with 50 percent these the animals are apparently fine.

DR. ROBERTS: Dr. Reed and then Dr. Matsumura.

DR. REED: I had two questions, but I think one of it was the same as what Dr. Hattis was asking if other one is that in the total knockout, you mention that the survival rate if you feed them right. Could you expand on that?

How long do they survive both the plus

minus and minus minus?

DR. RICHARDSON: I don't want to speak for Dr. Lockhart's laboratory but from I know in communications with her because we have been looking into a collaboration using this model.

She has told me and also mentioned in her seminar she gave at University that in the total knock out by paying attention to new needs because early on they didn't seem to be feeding as well as the wild type animals, and so if they were taking care of a hand feeding, then they would live into adult hood.

I don't know how long now she has taken these out. And because Dr. Brimijoin is actually involved in collaboration with this. I think they have more information than I do to clarify that my impression is even the total knock outs can survive into adulthood.

I think the paper they have taken at this time out to 21 days. If I'm not mistaken. But the work is not yet published I believe it is into an adult hood.

DR. ROBERTS: Dr. Brimijoin can clarify that.

DR. BRIMIJOIN: You are actually, we had even just with ordinary feeding Mayo Clinic, at least my routine diet, we were getting some animals surviving to the age of three months or more, so, well into adulthood.

Roxanna has experimented in depth with the feeding schedule. She found she went to a high lipid fatty diet and I guess maybe a liquid diet she could get survival indefinitely. I suppose the mortality is lower. They will drop out as they age faster. But essentially, they will survive if you take care of them especially indefinitely.

The heterozygote sigh does show no deficit at all. We are working with these mice intensively. In fact we're doing some behavioral studies on them. We have data on more res (ph) and hetero standard of care outs, where we can compare these mice within the same genetic background.

I didn't bring any information. I don't want to say anything about that other than there is certain number no dramatic and may not be any observable. If you could continue.

Do you have to force feed? If so, is it because they are neurologically impaired and can't swallow.

DR. BRIMIJOIN: The total knockouts -- they are born at essentially the same birth weight. There may be 25 percent in utero, but they don't gain weight, in fact fall for a while, and gain much more slowly than their liter mates.

They are fed a typical diet, many of them die with what looks like some kind of congested GI system with tremendous stomachs that have milk in them. Actually, they are still nursing at that point -- and with tremendous bowels distended with air.

However -- so I think she is simply going to a different fed formulation. It is not a matter of eyedropper care, no.

DR. LAMBERT: Do you think it's a

neurological issue?

DR. BRIMIJOIN: I think it is related to the deficit of AChE very specifically. And the most likely place for that to be exerted would be in the nervous system but it may not be the brain. It could be the enteric nervous system. My enteric plexus I think, is actually a very interesting locus to look at.

DR. RICHARDSON: I would add a comment to that. It is important in looking at these knockouts to distinguish between the minus minus, the total knock out versus the heterozygotes, which seem apparently normal in everything that has been evaluated to date and you would expect some sort of deficit in 100 percent AChE knockout.

In fact, it is astonishing that they survive at all. I think it has opened up a whole new world for cholinergic neurobiology and toxicology.

DR. MATSUMURA: I have the same question regarding the heterozygotes.

Did anybody challenge those animals with

some poisons, some cholinergic poison or anything else?

DR. RICHARDSON: Yes. We looked at both in the total knockout in the heterozygotes. My recollection is that you get the expected result. If you have the wild type at some level of sensitivity the 50 percent knockout has some what increased susceptibility because you have already essentially inhibited half of the enzyme.

And the total knockouts are more sensitive still as you would expect.

DR. ROBERTS: Dr. Needleman and then Dr. Portier.

DR. NEEDLEMAN: Related to the previous question, did anybody challenge them with tests of behavior rather than just observation?

DR. RICHARDSON: Someone asked that question earlier. How detailed -- I think it was Dr. Portier, how detailed the behavioral tests were.

The ones that have been published so far, there has been very little that has been

published on the heterozygotes behaviorally. There are studies ongoing that Dr. Mesulam is actually involved with in part.

DR. PORTIER: Separating fact from hopeful fantasy, you list exactly one publication on the knockouts.

Is that the only publication available, because I'm going to go and read these papers this evening?

DR. RICHARDSON: The latest one I'm aware of is the one by Mesulam, et al. As far as I know right now, the main author to look for for sites on this would be Roxanna Lockridge and she's collaborating with several other laboratories and publications may be emerging.

But the two -- there were two cited, Xie, et al. -- and thank you for that pronunciation correction -- and the one by Mesulam, et al. There is one more that Dr. Brimijoin mentioned, J Neurochem. It is a new model. I don't think you are going to find a large number of publications as yet.

DR. ROBERTS: If there are no other questions for Dr. Richardson, thank you very much for your presentation.

Let's move on I believe I understand correctly our next presenter is Dr. Sheets, from Bayer Crop Science, whose is going to be talking about giving us some related to question two.

DR. ROBERTS: Welcome Dr. Sheets.

DR. SHEETS: Thank you.

I'm Larry Sheets. I'm a toxicologist with Bayer. By way of introduction I have been there about 14 years; I worked with OPs for about 20 years.

At Bayer, we have a number of organophosphates and through the years I have had direct experience working with all of them.

Specifically, related to what we're talking about here today. We have done -- I have been the study director for the adult neurotox studies and more recently study director for the developmental neurotox studies, with several organophosphates. Some of them have been

reported. Others are in various stages of progress.

I was also a member of the LC Working Group on the common mechanism for the OPs and have been involved with a lot of discussion since the date of call-in for the OPs come out on how to go about conducting the standardized guideline study to look for developmental neurotoxicity and at the same time address the issue of looking at the relevant sensitivity of the young animal versus the adult.

So I have been involved in discussions on the complement of studies that should be done to address that issue as a separate point.

This is going to be a pretty short and straight to the point presentation. We thought it was important for us to look at the issues that the Agency has posed to this panel.

And in this presentation I will just go through and state our position on the questions related to issue two. If I could have the next slide.

Those have been introduced already. What I will do is systematically then go through the -- for issue two, which is age dependent sensitivity to cholinesterase inhibition in animal studies. Questions 2.1 several points. Question 2.2 and 2.3. I'll describe those or read those specifically as we get to them.

So the first question is to asking for comments on the extent to which the report adequately discussed and summarized the current understanding of age dependent sensitivity to cholinesterase inhibition. The prevailing views in the scientific community concerning the biological factors involved and the role of esterases as a major factor accounting for potential increased sensitivity immature rat.

It is appropriate to begin by saying that we believe the document provides an excellent overview of the extensive scientific data base that is available for the organophosphate pesticides.

I know of no other group of pesticides

we know as much about. And there has been work as this document shows -- been work looking at age dependent or age-related sensitivity for 30, 40 years.

So it is not a new issue, and there is a tremendous amount of data to review in the published literature as well as from the proprietary studies done by the registrants. In this presentation, we think it is important to provide comment on the core scientific issues that pertain to this.

We want to emphasize the associated practical issues that must be considered for risk assessment.

Young animals -- one of the questions raised or points made -- young animals may exhibit higher levels of cholinesterase inhibition compared to the adults. We agree with that point. We emphasize some of the caveats associated with that that are very important.

As the Agency has established, this is a compound specific phenomenon. It is evident with

some OPs, it has been shown not evident with others. There are a number of OPs where we have a limited uncertainty. It hasn't been looked at specifically.

We'll get to the issue in a few minutes about the reliability or suitability of the compounds that have been tested for extrapolation to the ones that have not been tested. That's why we say we see some limited uncertainty.

One of the things we want to emphasize is the issue that the difference in sensitivity is very much dose related for compounds where you do see a difference. You don't see parallel dose response curve.

You see a divergence of high dose levels relating to the mechanism of kinetics or limited metabolic capacity.

As you move to lower dose levels, the young animal is better able to accommodate the exposure and does a better job of metabolizing the compound down to low dose levels where you see little or no difference in sensitivity.

I think it is very important to point out the issue that we do understand the mechanism and it has been linked to limited metabolic capacity of young animal.

It is important to note that there has not been or there has been shown that there is not a difference in the sensitivity acetylcholinesterase itself in the fetus and neonate compared to the adult.

The next question is from our perspective critically important. That is not just is there a difference in sensitivity but is there a difference in no effect level. So, that is getting at levels that are used to really make determinations to establish safety.

We agree there are cited cases that indicate a difference in no effect level. We'll point out an example and would ask the panel and others to look at the data more carefully to determine whether the differences that are cited there are realistic or whether they are somewhat overstated.

Like I said, we'll show an example where we believe it is. There are two things that contribute to the over statement or exaggeration of difference in sensitivity. It is not unique to this particular circumstance.

Differences can in some cases are due to a declaration at a given dose level that you have an effect in a young animal and you have a marginal or no apparent effect in the adults.

So based on statistics or criterion level one is declared an effect level the other one is not an effect level. If there is 3 or 10X difference in dose levels that are tested at the next level dose then that no-effect level says there is a 3X difference in no effect level and it is inferred there is a 3X difference sensitivity.

The next slide shows example with methyl parathion. These are data that the panel has. So in this study they were establishing low effect level or no effect level for brain and erythrocyte acetylcholinesterase activity.

You can see in yellow there, a dose of

.3 milligram per kilogram, the effect that it had in the day 11 male pups and the adult males and the female data are also included. I have just shown one here provide to provide as an example.

In this particular case, that dose level produced 14 percent inhibition of brain cholinesterase and 20 percent inhibition of erythrocyte. In the adults, it didn't produce a statistical or a biological change in brain cholinesterase in the activity in the adult. It produced 17 percent decrease in erythrocyte.

The conclusion from that was you have an effect on pups and not in the adults. So, they tested a lower dose level and it was a no effect level in the pups.

Comparing those no effect levels, one sees .3 milligram per kilograms in NOAEL in the adults, .11 milligram per kilogram in pups. That would support or suggest a 3X difference.

If you look at the data more critically and start thinking in terms how robust a phenomenon do we have here?

If you were to repeat that experiment twice, what confidence do you have that those results would exactly repeat themselves or is that 17 percent difference -- I should qualify the word different.

It wasn't statistically significant -- is that 17 percent lower cholinesterase measurement -- something that if you repeated the study would be effect level etcetera, or if you were to a test dose between point 11 and point 3, would you get a much more comparable no effect level.

It is just raising the question of, one can use data like this and come to a conclusion somewhat over states the difference in effect level.

Speaking of the issue of repeated exposure in animals, we agree with the conclusions in this document, that there is more rapid recovery of acetylcholinesterase activity in the postnatal and fetal rat. That has been shown to be due to more rapid resynthesis or replacement of

the inhibited enzyme.

We agree with the point that this would make the young animal more resilient or tolerant of repeated exposure than the adults.

The document points out that there is a relative lack of information regarding the occurrence of this phenomenon in people. We agree with that point, but would say there is -- we know of no reason to expect people would respond differently than animal models. We feel there is reason to be confident that the same phenomenon would occur in people as occurs in our animal models.

Speaking to the issue of the biological factors involved in age dependent sensitivity to acetylcholinesterase inhibition, including the role of esterases, we agree that metabolic enzymes including esterases express relatively low activity at birth with rapid development to approach adult levels at weaning.

This immaturity may contribute to increase the sensitivity of a neonate to some OPs

but differences at low levels of exposure are modest or absent.

This slide raises the question 2.2 asking for comment on the timing of administration, ie, the developmental stage treated and the differential sensitivity between adults and the young animal.

We agree with the conclusion of the document that differential sense activity is associated with the development of metabolic enzymes kinetic factors not an inherent difference in acetylcholinesterase sensitivity.

We make the point that development of metabolic enzymes in the rat we agree with the point that occurs rapidly from birth to weaning and is generally associated with age-related sensitivity to high dose levels. So, toxic dose levels in the neonate is more sensitive than juvenile and juvenile more sensitive to the adult to these higher dose levels.

The question of developmental stage versus differential sensitivity. The first point

relates to the metabolism. We agree that with the conclusions in the document, that rats are equivalent to the newborn infant, around postnatal day 11 and approach the adult circumstances at around day 21.

That's an important point I think we raise later is that tests involving the treatment of rats younger than 11 days old are really more comparable to the human fetus in the third trimester.

So, to try to model neonatal exposure in the rat to the human infants you shouldn't be dosing those animals before about 11 days of age.

The second point deals with exposure. We agree with EPA that breast milk is not a significant source of exposure, so dietary exposures as not likely until six months of age or later.

However -- I don't think I should say however, it is not appropriate -- we believe it is not appropriate to treat rats. You could strike however off of that. That doesn't make sense.

The point is we believe it is not appropriate to treat rats to determine age dependent sensitivity until around postnatal day 14 to 17. That's when the young rats start getting into the feed. Their eyes are open, they are mobile, they are getting into the feed, playing with it and beginning to eat some feed.

By the time they are 21 days old they are totally weaned. We agree that repeated exposure is more relevant than acute exposure for risk assessment also.

This is a question 2.3. Comment on the extent to which cholinesterase data on the six OPs may represent a reasonable subset of structural and pharmacokinetic characteristics to define an upper bound on differential sensitivity with other OPs.

We believe that the data for the six OPs are suitable to define an upper bound upon the differential sensitivity and think the Agency has done a good job in the document of explaining the reasons for that. Finally, we think the threefold

database safety factor is sufficiently conservative and protective.

As we've argued, we think 3X may over estimate the differences in sensitivity. In some cases that for the reasons provided in the document we believe that document provides a good basis for using a threefold database safety factor to protect infants and children.

DR. ROBERTS: Thank you, Dr. Sheets.

Let's see if the panel members have any questions for you.

DR. PORTIER: I wasn't going to have any questions but you said something that got me a little interested.

In your second to the last slide you noted postnatal day 11 is equivalent to newborn. Prior to postnatal day 11 you feel is like a human fetal exposure. If that's the case, then I guess I would argue the opposite of what you.

In fact I would argue that one needs to dose up to postnatal day 11 to match the human fetal situation, since between birth and postnatal

day 11 you have argued -- EPA has argued there is almost no exposure through the breast milk in the rodent, that you have actually got a window three of zero exposure that if you tied it to the human situation should be in utero exposure.

So am I missing something here? .

DR. SHEETS: No, I think you detected one of the limitations of a model we're working with. It is really impractical to try to model an in utero exposure by lavaging the pups or by other means that someone could imagine and so, in terms of trying to model that, you need to take into consideration exactly what you are doing, whether that's relevant to the human circumstance.

There has been a lot of discussion about working with -- developing a new model for developmental studies as we're talking about where the fetus is maintained in the uterus longer to a more mature state so that you can model whatever is going through the placenta and into the circulation of the animal are you working with then would be relevant to the human fetus.

One can maximize exposure obviously, by getting a bolus dose to those young animals. The question though then is how does that relate to fetal exposure?

If I could ask one more question. The issue noted earlier on the dimethoate, I guess --

DR. SHEETS: The table?

DR. PORTIER: The table where the means and the variances increase at the same time. This is the DNT study on dimethoate. I gather you were associated with this study also?

DR. SHEETS: No, I wasn't.

DR. PORTIER: I asked EPA and I asked Dr. Sass if they had redone the analysis on log transform data. I was going ask you since no one from industry was here to answer that question for me. So, that's okay, thanks.

DR. SULTATOS: Just a clarification. You said on postnatal day 14, I think it's the metabolism is similar to the child? What do you mean by that? Are you talking about all the metabolic roots and pathways and enzymes and the

equivalent?

DR. SHEETS: I think the only thing I mentioned about day 14 is that's when the young rats start getting into the food and start having --

DR. LAMBERT: You have a metabolism. You used the word metabolism is similar --

DR. SHEETS: So -- yes thank you. The point there is that we believe that the rats, as they are getting into food, starting to consume feed, moving away from milk is better suited to try to model the human infant as they are starting to consume food as well.

DR. LAMBERT: But you are saying the metabolism is similar between the 14 day old rat and the human infant?

DR. SHEETS: Well, I think the day 14, day 17 rat is in the range where we should be working rather than day 4, day 11 and realistically, in the day 14 through day 21 I think is the time frame that I think -- in the rat is best out to try to model.

DR. LAMBERT: I know it is best suited to try to model, but you said that the metabolism is similar. I was wondering if that's true. There is a difference between the best model and are they similar.

DR. ROBERTS: Dr. Hattis and then Dr. Sultatos again.

DR. HATTIS: You also identified -- a rough you a rough equivalence between human infant at -- human newborn and postnatal day 11 in the rat. I asked EPA this question a little bit -- exactly what data leads you to that identification?

DR. SHEETS: It is obviously not my data. It's based, as I understand it and from what I've read, it is based on the stage of brain development as well as some of what is going on with the metabolism.

DR. HATTIS: Metabolism is one thing. What about the stage of brain development gives you that analogy?

DR. PADILLA: I can attempt to handle

that. You owe me thanks for this.

The paper that most people that most people refer to is a dobbing (ph) paper where they looked at the brain growth spurt in different species and attempted to equate them on the same X axis.

What they saw was that humans were born basically at the peak of that brain growth spurt. Whereas, rats had already been born and then around 10 days of age, 9 to 10 days of age you saw the peak in the brain growth spurt.

And that I believe in my searching through the literature is all they are looking at, is the brain growth spurt.

DR. ROBERTS: Thank you Dr. Padilla.

DR. SULTATOS: I just have one quick question.

With your example with methyl parathion, is that a single or repeated exposure on your slide?

DR. SHEETS: That was a single exposure.

DR. ROBERTS: Are there any other

questions for Dr. Sheets before we move on to the next speaker?

If not, thank you very much Dr. Sheets.

Our next presenter is Dr. James Gibson from East Carolina University. He will be addressing issues related to question three.

Welcome Dr. Gibson.

DR. GIBSON: Thank you, Mr. Chairman, ladies and gentlemen. My name is Jim Gibson. I'm research professor of pharmacology and toxicology at the Burdie (ph) School of Medicine at East Carolina University.

My comments will be restricted to issue three, the relevance of the animal findings to children. I will comment on each of the three questions posed by this issue.

First, though, I want to commend the office of Pesticide Programs for the U.S. Environmental Agency for their excellent work culminating in the report entitled in part, evaluation of sensitivity and susceptibility to the common mechanism of toxicity

acetylcholinesterase inhibition.

I believe the Agency used good science and good judgement in reaching their conclusion that, "the scientific assessment of organophosphorus pesticide food safety strongly supports our confidence that the United States has one of the safest food supplies in the world.

Now, with regard to question 3.1 and the maturation profile of A esterases and what should be assumed in humans, especially children aged one to two years, given the animal data and what science understands in general about detoxification maturation profiles, I will offer this by way of example.

That is chlorpyrifos and several other organophosphorus pesticides are metabolically activated to the corresponding oxon. The oxon selectively and strongly inhibits acetylcholinesterase in cholinesterase synapsis resulting in accumulation of acetylcholine and subsequently cholinergic hyper-excitation.

The oxon is hydrolyzed by A esterases as a

key detoxification step at high does, and I emphasize high doses.

The first line of defense is gut detoxification and P glycol protein exclusion of the oxon.

The second line of defense is hepatic metabolism. The third line of defense is binding of oxon to B esterases like butyryl and carboxyl esterase. When all of these defenses have been breached by high doses, then A esterase becomes important.

For lack of importance of the A esterase at low doses, I ask you to see Tim Chuck's report, which is entitled, "Montecarlo (ph) Analysis of the Human," chlorpyrifos oxalosis A esterase one, polymorphism using physiologically based pharmacokinetic and pharmacodynamic model.

This publication is a work in press in toxicology letters and is going to be discussed further the next session by Dr. Sheets.

For chlorpyrifos, the A esterases hydrolysis results in the formation of the

toxicologically inactive 3, 5, 6 trichlorophenol.

Rats do not fully develop the esterases needed to detoxify organophosphates until 25 to 30 days of age, which is nearly equivalent to a human child of 4 to 6 years.

Several studies show that human children are born with 25 to 40 percent of the adult protective esterases and have fully developed these esterases by three to six months of age.

Thus, in this case, the animal model does not serve as an appropriate surrogate for the human.

It should be added here that the lesser fetal or neonatal probability to detoxify high levels of oxon is more than compensated for by the greater fetal ability to synthesize acetylcholinesterase enzyme when relevant lower doses of the organophosphates are studied rather than the super high doses that have been used to find greater sensitivity in young versus adult animals.

In fact, several studies use doses as

much as 100,000 times greater than environmental exposures and overwhelmed the developing rat immature detoxification mechanism.

When studies are conducted using doses that do not overwhelm the young animal's ability to detoxify organophosphates, young animals are of similar sensitivity as adults.

To characterize risk properly, considerations of exposure are critical and exposure scenarios specific to infants children, and other potentially sensitive subpopulations need to be assessed.

A probabilistic model is used by Shirdit (ph) and others to determine the potential aggregate exposure that is the total dietary and residential exposure from all use patterns.

For chlorpyrifos, the estimated aggregate exposure was less than 1.2 micrograms per kilogram per day for infants and children which is well below the acute and chronic RFD values for chlorpyrifos.

Comparison of these result to actual

measurements of the primary metabolite trichlorophenol by the Centers for Disease Control for the U.S. population, showed that the highest exposure to chlorpyrifos is less than 1.4 microgram per kilogram per day. These factors must be kept high in mind when considering the relevance of data collected using doses that are many fold the actual environmental exposures.

Question 3.2 asks what can be inferred from animal and human information regarding the potential for different age groups to show increased sensitivity if exposed to cholinesterase pesticides?

Does scientific evidence support the conclusion that infants and children are potentially more sensitive to organophosphorus cholinesterase inhibitors? While at exposures of regulatory concern, the weight of the evidence support the conclusion that young animals will exhibit cholinesterase inhibition, that is, either less than or similar to that produced in adult animals.

For example, the fetus is less sensitive than pregnant adults to the cholinesterase inhibitors. Six different organophosphates that produce cholinesterase inhibition in pregnant dams did not inhibit fetal brain cholinesterase or produce embryotoxicity or teratogenicity in offspring.

In vitro tests showed that fetal and adult brain cholinesterase were equally sensitive to a variety of inhibitors indicating there are no inherent sensitivity differences in the cholinesterase enzymes taken from fetal or adult rats.

Moreover, young animals recover more quickly from the affects of the organophosphates in adult animals because they can synthesize replacement cholinesterase faster.

The rapid recovery of cholinesterase enzymes in the fetus is attributed to the de novo synthesis of the enzyme in the fetus compared to the mother.

Many studies of the issue of the

differential susceptibility of infants and children, relative to adults, have been conducted on a large number of organophosphate cholinesterase inhibitors. None of them affect fetal development or reproduction at maternally non toxic doses.

In contrast, the studies where maternally non toxic doses were used, are many inappropriate studies that have been conducted to assess relative sensitivity of young animals when compared to adults as follows. The roots of exposure used in animals were inappropriate as potential roots of exposure in human infants and children. Animal data generated was subcutaneous or intraperitoneal injections are not encountered with humans.

At least one laboratory dissolves the test pesticide and dimethoate sulfide "To provide rapid and complete absorption and is injected subcutaneously to dams in a volume of 1 mil per kilogram on gestational days 17 to 20, for example.

For studies of chlorpyrifos at doses selected by this laboratory were 1 or 5 milligrams per kilogram. The higher dosage is maternally toxic and is well above the maximum daily aggregate exposure 1.4 micrograms per kilogram.

In other recent studies from the same laboratory, doses of chlorpyrifos as high as 40 milligrams per kilogram per day were used.

Doses larger than could be fully observed by a neonate using an appropriate route of exposure are an unfortunate choice of technique and too many studies as well. The Society of Toxicology has advised that such studies be avoided for purposes of risk assessment.

Risk assessment approaches are crucial to making informed regulatory and policy decisions about chemicals such as pesticides. Decisions must be firmly based on scientific weight of evidence with respect to toxicity and exposure and especially sound science.

In this matter, the weight of the evidence using toxicity and exposure information

does not support the conclusion that infants and children are potentially more sensitive to organophosphorus cholinesterase inhibitors.

I have already discussed most of the points of question 3.3. The most salient point to this question is that in order for cholinesterase to recover, it needs to be sufficiently inhibited to elicit the symptoms of cholinergic stimulation.

In the context of regulation governing the sale and use of cholinesterase inhibiting pesticides, it would be a rare event indeed, to provide any meaning to question 3.3.

Unless these subject products are seriously misused, their margins of safety are wide enough to protect everyone with the potential to be exposed. I believe the weight of the evidence supports this conclusion.

Thank you.

DR. ROBERTS: Thank you, Dr. Gibson.

Let me ask the panel if they have any questions for you.

DR. NEEDLEMAN: Do you believe that all

of the toxic potential of organophosphates can be captured and measured by acetylcholinesterase?

DR. GIBSON: I believe that all of the relevant toxic endpoints can be measured by the inhibition of acetylcholinesterase, yes.

DR. NEEDLEMAN: If there are no other expressions of toxicity?

DR. GIBSON: Not that I'm aware of.

DR. ELDEFRAWI: I have a comment, but not directly related. It's still on the esterases on the brain on the children and the adults, etcetera.

DR. ROBERTS: I think -- let's just get clarifications from this particular speaker, and then you will have the opportunity to raise -- make your comment, I believe later on.

DR. PORTIER: Did you have a chance to read the entire EPA risk assessment on this? EPA gives a considerable amount of information on human incident information.

DR. GIBSON: Yes.

DR. PORTIER: Human incident cases,

especially pesticide poisonings in children. Somewhere around 5,000 exposures among children 6 to 19 years-old.

Are you suggesting that all of these are in fact accidental over exposures by improper use of the chemical when you say that proper use -- do you have evidence to support that fact based upon this database?

DR. GIBSON: Yes. There has been a very detailed retrospective analysis done these cases, mainly using various poison control center data bases where they have gone back and examined the source of the incident and divided the incidents into those which could truly be regarded as something that could have been avoided.

A lot of the accidents, I think ended up being attributed to events that had nothing to do with the exposure to cholinesterase.

I don't have the citation to that publication right at the at the tip of my tongue but there have been at least two publications in the last two years analyzing all that Poison

Control Center data.

DR. ROBERTS: Dr. Brimijoin.

DR. BRIMIJOIN: Did understand you right? If we really wanted to get an accurate estimate of the relative sensitivity of young organisms to OPs that we should be conducting experiments with dose levels that approximate the actual average calculated exposures?

Is that the essence of your argument?

DR. GIBSON: Well, the simplest way to answer that is yes, that is the essence of my argument, but I have nothing against studying high doses.

I simply would plead for any study of high dose to also include doses that are environmentally relevant so that the perspective -- to that the data could be put into perspective.

DR. BRIMIJOIN: But as far as we know, these calculated exposure levels are calculated -- I mean, EPA has been -- without maybe as much scientific basis as one would like it has been regulating things so that the actual probable

exposures are so low that we couldn't measure anything at all.

DR. GIBSON: Well, as a matter of fact, bio-monitoring has been used very extensively to measure actual exposures to a variety cholinesterase inhibitors.

DR. BRIMIJOIN: You could perhaps measure how much an inhibitor is taken in or how much metabolite you find, but we can't actually detect any biological effect from those levels because we've tried to avoid exposure levels where you could detect biological effects.

I just don't see how that's -- that seems to me a puzzle, how we could go about doing that sort of assessment.

DR. GIBSON: I think the answer is simply study dose response that is inclusive of doses that are environmentally relevant.

DR. ROBERTS: Dr. Reed.

DR. REED: I need some clarification. I'm desperate for your opinion too, because I think that this is an issue we been grappling

with.

In your handouts on the questions 3.2, the fourth paragraph, you said that many studies of the issue of the different susceptibility of infants and children relative to adults had been conducted on a large number of organophosphate cholinesterase inhibitors. None of them affect fetal development or reproduction at nontoxic dosages.

Are you mostly referring to the tradition of teratology and reproductive two generation, three generation reproductive studies?

What do you think DNT -- and I really would appreciate your opinion. As I said, this is something that we grapple with a lot.

What is sufficient in your opinion, what type of studies, what type of database would be sufficient to say that there is sufficient studies and it didn't show check any heightened age susceptibility issue?

DR. GIBSON: With regard to the first

part of your question, yes, I think by and large my comment refers to the more traditional teratology and multi generation repro studies. But as more and more DNT studies have become available, I think there is something to be learned from that as well.

As you know, the Agency and the Registrants continue to struggle with defining appropriate protocols for DNT studies.

Some of the protocols suggested are so costly as to be impractical and some of the particular protocol procedures are impractical and may not even be possible to do.

So it is a struggle to figure out what to do. It's also the possibility that the results can become compounded by variables not intended to be a part of the experiment such as various unintended stresses.

Studies to sort all this out really haven't been done as far as I know. I do know it is a struggle to decide on what an appropriate protocol would be.

As you know, of -- I can put it this way. Maybe no two DNT studies have ever been done exactly alike. Maybe that's not exactly right, but there has been a lot of changes and modifications as the development of -- development on neurotoxicity studies has evolved.

DR. ROBERTS: Dr. Lambert.

DR. LAMBERT: In these rural families that may be getting higher levels of OPs into the kids, do you know anybody who has ever looked at the kids who have been chronically exposed in the neuro behavior assessment?

DR. GIBSON: Neurobehavioral assessment? Well, no, but I'm aware of at least four studies that are ongoing now that are related to farm families exposures.

And again, the endpoint there simply being to measure what is the level of exposure on the farm for the farmer, the farm wife and the farm children.

I expect, as these studies evolve, they will move in to look at endpoint points such as

neuro behavioral effect. Obviously, there has been some literature like that. But it is probably some work that needs to be reproduced before one would be real happy with it.

But I think right now, I think very well conducted farm family exposure studies are just coming to conclusion, and will be very useful and I -- what little bit I'm aware of the preliminary results show that there really is little difference between exposure to farmers and farm families that children and spouses and that these exposures are happily well below what one might have expected.

So I think the generalization that farm families of farm children are exposed to higher levels may not be borne out by some of these studies that are ongoing right now.

DR. LAMBERT: I think there has been recent abstracts that suggest otherwise. Again, until we look at the kids to really look at the neuro behavior function --

DR. GIBSON: It's a work in progress.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: In your presentation, you referred to the Slotkin studies, which were the endoperitoneal injected studies. You refer to the highest dose as having maternal toxicity, which I actually would agree with you.

But the usual definition of maternal toxicity is not actually what you are seeing in the Slotkin study in the sense that you don't see a 10 percent change in weight gain over the course of the study. You see a very temporary change in weight gain and then they recover by gestational day 13 or 14.

My question to you is: Are we applying a double standard in the sense that when we look at it as acetylcholinesterase inhibition, even though we see a statistically significant finding, we are ignoring it because it is not greater than 10 or 15 percent?

Yes, here in the case where we see maternal toxicity statistically significant, but what has classically been referred to as not

biologically relevant, we are not ignoring it. Is there a paradox here?

DR. GIBSON: Probably, but you notice I didn't use Slotkin's name, but I didn't disguise my reference very well, I'm afraid. I suppose that there maybe something of a double standard, because 5 milligrams per kilogram does inhibit acetylcholinesterase but it doesn't inhibit it to a level of 70 percent which would elicit cholinergic symptoms. So there is clearly that difference.

I think the interesting thing about the Slotkin study is the fact that one milligram per kilogram caused effects and 5 milligram per kilogram did not.

That particular phenomenon, which he describes is an U-shaped dose response curve, which, of course is a big subject in the literature, is interesting. But in this particular case, I would like to see those studies reproduced to really understand that high dose no effect and low dose effect situation.

DR. ROBERTS: Follow up?

DR. PORTIER: To what endpoint are you talking about in terms of the U-shaped, since there were a number of endpoints in the Slotkin study that were, in fact, reduced across the board and some that were U-shaped. So, to which endpoint are you talking?

DR. GIBSON: To tell you truth, I don't recall which endpoints go with what. All I can do is generalize.

DR. ROBERTS: One last question from Dr. Matsumura.

DR. MATSUMURA: Thank you for that lecture.

I'm certainly interested in the lines of defense like you described, particularly with OP including the blood cholinesterase. They serve of the defense.

In that particular case, you should expect some changes in the slopes at the lower concentrations when you are overcoming at the very high doses and all of a sudden you start going

over that.

Do you detect in any of those cases in a change in the slopes of the in vitro -- let's say any endpoint LC 50, EC 50, measured in the brain versus dosing?

If you see those, did anybody run that kind of analysis in the pups versus adults?

DR. GIBSON: I'm sure someone has, but I can't recall specific literature to cite on that. I'm going to have to say, I don't know.

DR. MATSUMURA: Did you see low dose effects from some changes in the slope?

DR. HATTIS: That's the point of Woody Setzer's expanded model. And it is the model that is used for the determination of the BMD 10. I don't know the details, but there are significant appreciable numbers of the agents where that kind of commonality is detectable. I don't know how big it is, how often.

It does give you a linear response at the low doses but at a different slope than at high doses the. I don't know exactly where.

DR. MATSUMURA: The question is: In this particular case?

DR. HATTIS: I haven't seen it applied yet to the developmental studies. It may be that the data are too few to have done that, but it would be of interest to see that application.

DR. ROBERTS: Thank you very much Dr. Gibson. I appreciate your coming.

Oh, was there another question?

DR. ELDEFRAWI: I have a comment.

We are interested, definitely more interested in humans and we're using animals as our means of comparing the closest mammals to humans. But what I was thinking is we do have a lot of human cells in culture available.

We do have -- this made me think further that we can add, since we're looking at organophosphate cumulative risk assessment it has to be some thing that happens in the brain that applies to all the acceptable doses of OPs.

So why don't we use protonics and see brain extract if we can have human cell cultures,

brain cells in culture, it would be very helpful to know what the target is. I mean, which protein is affected?

Is it just a matter of like Alzheimer's, that you can push over parts of the brain, destroy them? Most probably, it is not because we haven't heard anything as big Alzheimer's in children.`

And also genomics, we can either do a cell culture or more appropriately in this case probably would be an animal model.

And then can take the brain of the animal, the rat or whatever and then a mammal and then see about the genes. Is it the genes that are affected or is it proteins that are affected and which ones? So, this can he eventually lead to therapy.

In other words stopping this from happening taking a certain drug if it acting as an agonist, the chemical, then we can add an antagonist, whatever. Anyway, these are futuristic ideas. I sometimes like to think about the future.

DR. ROBERTS: Dr. Sheets, let me suggest that we take about a 10 minute break -- short break everyone, to get -- kind of stretch, reenergize before your presentation.

DR. ROBERTS: Welcome back, Dr. Sheets.

DR. SHEETS: Thank you.

In the previous three talks we have spoken specifically to the questions that were addressed to the panel and what we want to do in this presentation is to look at all of the information that is covered in the document and approach it from the perspective of looking at cholinesterase inhibition and moving through the issue of relative sensitivity etcetera.

So what we hope to try to try to do is put everything in perspective and then we will end with revisiting those questions, although we might punt on that in the interest of time.

So in this presentation, I want to begin with the issue of acetylcholinesterase inhibition as the basis for the cumulative risk assessment and move through the issue of age-related

sensitivity and the factors we want to emphasize as being relevant with respect to the animal models we're working with and the circumstances of exposure.

We would like to return to the question that the SAP was originally asked to focus on, specifically the scientific evidence that the young may be more sensitive at some life stages than adults to the inhibition of acetylcholinesterase inhibition or acetylcholinesterase of OP pesticides.

We want to emphasis the consideration of suitability of the animal mode at the various ages and some of those points that have been alluded to already in particular the neonatal rat versus the human and realistic circumstances of exposure for risk assessment.

Next slide, please. We're in agreement that inhibition of acetylcholinesterase activity in nerve tissues is the common mechanism of toxicity for the OP pesticides.

And we agree that inhibition of

acetylcholinesterase activity is the precursor of antitoxicity and it is appropriate to use this as the basis for cumulative risk assessment for the OPs.

We also believe that inhibition of acetylcholinesterase activity is the most sensitive measure of effects.

We believe that a no-effect level for cholinesterase inhibition will protect for other effects.

The question is there evidence that exposure to OP pesticides pre and postnatally perturbs neuro development? We believe at low doses it is clear that the no-effect for cholinesterase inhibition is protective.

In the fetus, we agree with the points made in the document, that there are effects in the absence of cholinesterase inhibition in the mother. Any kind of developmental exposure study you can't loose sight of the fact that the fetus is not disconnected from the mother.

So, if you have maternal toxicity, you

can potentially have fetal effects. It may not be specifically related to a known mechanism. It is not possible to associate the two. You also can't account for the metabolism and what passes from the mother to the fetus.

You have a complex circumstance there when you see effects in the pup in the absence of cholinesterase inhibition in the pup doesn't mean there is no effect -- there is no toxicity there.

So, in the context of the fetal maternal unit, we believe that the fetus is protected by no-effect level for cholinesterase in the mother.

In postnatal studies, there are not reports of effects in the absence of acetylcholinesterase inhibition. We agree with that conclusion.

At high dose levels in terms of effects on neuro development, at high dose levels, the interpretation of results are more complex and some of the complexity is well summarized in the document.

We agree that the no-effect level for

acetylcholinesterase inhibition in the young will protect for potential effects on neuro development that might be a so associated with acetylcholinesterase inhibition.

And we would point out that there is additional protection for the young provided by using maternal no-effect level for acetylcholinesterase inhibition.

There is a section in the document dealing with human incident information discussing the accidental poisonings showing more severe outcomes in children in many cases in poison circumstances.

We agree with the conclusion that accidental acute exposures does not mean greater sensitivity. The differences seen in those poisoning cases do not apply to environmental exposure.

For example, in terms of -- you're typically talking about acute bolus dose there you have a transient high peak in tissue levels of cholinesterase inhibition and of the compound and

you compare that with the sustained low level dietary exposure, there are marked differences.

In that case, there was mention in those cases we typically don't know what the dosage is. So, the difference in terms of the severity of the cases in children versus adults can simply be a manifestation of greater dose that children receive on a milligram per kilogram exposure basis.

In terms of lab animal studies, gestational and lactational exposure, we'll start with the fetus. We see there is data with many OPs show that treatment of the pregnant dam induces more acetylcholinesterase inhibition in the mother than in the fetus. We agree with that.

In neonatal exposure, exposure to OPs under conditions that are relevant to environmental exposure also cause less acetylcholinesterase inhibition in young rats than in the maternal adult animal.

There we're speaking specifically in

terms of the neonatal rat that is exposed through the milk compared to the mother which is exposed directly.

The mechanism for this inhibition of acetylcholinesterase in the fetus and neonate, we agree the document makes good points there. The mechanism may involve less dose being transferred to the pup or an increased rate of synthesis of replacement of acetylcholinesterase.

We would point out that regardless of the mechanism the practical outcome is a no-effect level for acetylcholinesterase inhibition in the neonate -- I'm sorry, in the maternal or an adult animal -- will protect the fetus and the newborn under conditions that are relevant to environmental exposures.

Speaking to the issue of acute and repeat dose studies with OPs in the young animals versus the adult, we see a lethal or near lethal doses, age related sensitivity, must be examined on a case-by-case basis. Some OPs are much more toxic to the young than the adult at nearly full-

dose levels. Comparisons of no-effect level for cholinesterase inhibition provide examples where the young appear to be 1.5 to threefold more sensitive than the adult to some OPs.

We point out in terms of the neonatal sensitivity relative to the adult that is a compound specific phenomenon as we mentioned before.

Seen with some, not with others and unknown for several OPs has been associated with limited metabolic capacity with no difference in sensitivity of the enzyme itself and is dose related as we pointed out several times. It's primarily seen at high dose level.

This is a new paper that I would like to point out to the panel. You might not be aware of it. I just learned of it a couple days ago as we were trying to put material together to bring here. This was a paper that was presented at the American Industrial Hygiene Conference in June of this year.

The authors are Tim Chalk and others, working at Bechtel. They are working to develop a physiologically based pharmacokinetic, pharmacodynamic model for chlorpyrifos using neonatal rats as a surrogate for children.

This is work that is being funded at least in part by a grant from the EPA.

The objective of this work is to adapt this model, a model that was developed for chlorpyrifos to incorporate age definite in metabolism and esterase levels and to evaluate the model response against available data.

I believe they used EPA data in developing this model and challenging and testing to see whether their model agrees with actual data in whole animals.

They point out this is the first step towards development of an age dependent human PBPKPD model for chlorpyrifos.

I would like to show a couple slides of data from that presentation. In this one they graph the activity of the four enzymes shown here

as a function of body weight.

So obviously, at the left hand side, the low body weight, you have very young animals moving toward adult animals.

And as they point out in their paper, they have different enzymes involved with either bio-activation or deactivation. And they have three enzymes here.

You can see then, the youngest animals there is some activity present and in each case activity increases to a certain plateau level. Looks like it is 100 grams of body weight.

And so based on this, you have different possible outcomes which of these is having the primary effect, the one that are activating and making chlorpyrifos oxon, the toxic metabolite or is it the deactivation?

The next slide then shows data in which they measured the amount of chlorpyrifos oxon in the brain of the adult animal versus the postnatal day 4 rat and realize this is a very young rat.

And what they found is high dose levels

you have much more oxon present in the brain of the young animal as compared to the adult and as you move down the dose response curve, those functions come together to a point at -- I believe that's .5 milligram per kilogram -- there was no difference.

That is showing as a lot of in vitro data show that at high dose levels you really see the -- a much greater difference in sensitivity of the young than at lower dose levels. At least in this case it indicates you reach a point where there is no difference.

So, that gets to the point of the young animals's ability to accommodate a lower exposure. They are able to handle it. Just to point out, the middle bullet from their paper, the conclusion is, although the neonatal rat is more sensitive to acute high dose effects at low doses they say more realistic environmental exposures, the neonate appears to be no more sensitive than the adult.

Like I say, this isn't a thorough

discussion of the data. I think it is very important. I want the panel to be aware of it. I do have a copy of the slides that were presented at that much more detail.

I think there are a couple people at this table that know more about it in details of the work or the authors would be someone to talk to.

In terms of the practical circumstances associated with looking at age related sensitivity, this slides deals with the fetus. The no-effect level for acetylcholinesterase inhibition in the mother protects the fetus.

It is the conclusion drawn in the paper that we agree with. That's regardless of the route of exposure to the mother. The maternal protection to reduce fetal exposure is relevant to human circumstances, so it should be considered in risk assessment circumstances. It involves multiple mechanisms.

Obviously, the mother is expose directly and it is only through her system it passes to the

-- a fraction of her exposure passes to the fetus.

In terms of the neonate, the no-effect level for acetylcholinesterase inhibition in the adult protects the fetus -- I'm sorry the neonate, in terms of lactation. The potential neonatal exposure via milk consumption is not a significant route of exposure for the OPs.

It is appropriate to use this kind of a model. Other dietary sources, dietary consumption, beginning around postnatal day 14 to 17 in the rat compares with children when they begin to consume foods that may contain pesticide residues.

We agree that the optimum data to quantify relative sensitivity involves low doses, no-effect levels and low-effect levels for acetylcholinesterase inhibition involving brain and peripheral tissues and repeated exposure is more relevant for extrapolation. In terms of repeated exposure, as we said before, we agree. It is more relevant for establishing safe levels of exposure for people including infants

and children and agree replacement of the enzyme is more rapid in the young than as adults.

So, we can agree there is more rapid replacement reduces cumulative -- would tend to reduce cumulative effects in the young compared to the adult.

The next few slides, I'll quickly move through them to fit in that with our response to the questions earlier.

In terms of question 1.1 that Dr. Richardson spoke to, our bottom line is no-effect level for cholinesterase inhibition in maternal and other adult animals. Will protect for potential effects on nervous system development.

With respect to cholinesterase inhibition in animal studies, young animals can exhibit higher levels of cholinesterase inhibition at the same dose, but this has to be determined on a case-by-case basis, since we know that varies from OP to OP.

It's primarily evident at high-dose levels and the mechanism is understood to be

linked to limited metabolic capacity.

Under some testing circumstances young lab animals may demonstrate cholinesterase inhibition at lower doses than adult animals.

However, concerning the data to support this position we have reservations about the biological significant and reproducibility of the differences and believe the magnitude of the difference is overstated in some cases.

One of our bottom line conclusions is relevant to this panel meeting is we agree with the document's conclusion that a threefold data base safety factor is a sufficiently conservative and -- it is conservative and protected.

In concluding we say there is an extensive scientific database available to address the issue of whether infant and children may be more sensitive than adults to OP pesticides and we agree that inhibition of acetylcholinesterase activity in nerve tissues is suitable for cumulative risk assessment, since it is the common mechanism, the precursor of cholinergic toxicity,

the most sensitive bio-marker of effects and inhibition is directly related to human circumstances.

Second point is new information, maybe to some of the panel members. The registrants presently in the process of generating DNT and relative sensitivity data for the additional OPs, since related to the 1999 data call-in for the organophosphates that included the need for development of neurotox studies and tests to establish relative sensitivity.

And we believe that when these data are available, the need for safety factors should be reevaluated. That's it.

Thank you for your time.

DR. ROBERTS: Thank you, Dr. Sheets.

Any questions from panel members for Dr. Sheets?

DR. REED: This is more of a curious question I have in my mind I think for more than a month.

I think in page two you mentioned that

inhibition of acetylcholinesterase activity is the most sensitive measure effects.

Are you specific about CNS or brain cholinesterase inhibition and on the subchorionic situation, repeated exposure situation or is it just a general statement?

DR. SHEETS: I know of no case where we have had evidence of a compound related effect with an OP where you didn't have cholinesterase inhibition.

DR. REED: But you are talking about a cholinesterase inhibition not specific to brain cholinesterase inhibition or are you specific about brain cholinesterase inhibition?

DR. SHEETS: From my work we measure both in the CNS and the peripheral compartment. As you probably well know, some OPs have a preferential effect on the brain cholinesterase and some tend to inhibit peripheral cholinesterase activity earlier. So, in looking at this, we measure both compartments and do an overall -- an assessment of that.

I think I can still say that I don't know the case where there was no cholinesterase inhibition in the brain and we had an effect or there was no cholinesterase inhibition in the periphery and there was detectable effect with an OP. Does that answer your question?

DR. REED: Not quite yet.

I think we're together on some of the things and not -- I'm not sure about the other issue.

My question is still, since we're using brain cholinesterase inhibition as an endpoint and we're looking at age specific sensitivity, then your statement is that acetylcholinesterase inhibition is the most sensitive endpoint.

I'm still curious about -- are you referring to or are do you confining that statement to say subchorionic repeated dosing kind of a situation, because the reason I ask that is because in many acute studies you will see neuro behavior changes or effects at a level where you don't see brain come cholinesterase inhibition.

And that's why I was curious about what is the confine of your statement?

DR. SHEETS: I would question whether they actually measured the peak at the right time -- their cholinesterase activity, because with some OPs you can miss the peak by waiting 12, 24 hours after the exposure.

DR. REED: Right. but are you referring to again CNS cholinesterase inhibition or there could be peripheral cholinesterase inhibition that is not reflected -- refracted in brain cholinesterase inhibition.

Your statement is actually confining to certain situation, because -- and I'll give you the background as why is it important in my mind because I have been struggling with this -- is that in many subchorionic, say, FOB studies, I do see that brain cholinesterase is fairly "sensitivity," in that you don't see neuro behavior effects at a level whether you don't see 20 to 30 percent brain cholinesterase inhibition, but that same picture is not true for the acute

type of exposure.

DR. SHEETS: My take on that -- that's not been my experience. When we have any kind of a neuro behavioral effect, motor activity we have much more than 20 percent inhibition of brain cholinesterase activity.

DR. REED: Even with acute studies?

DR. SHEETS: Yes.

DR. REED: I did a lineup of all the OPs and brain cholinesterase inhibition and RBC and plasma and identification of neuro behavioral effects.

I certainly see cases where you don't have significant inhibition on brain cholinesterase, but you have FOB-type of effects being identified.

DR. SHEETS: Yes. That's interesting.

DR. ROBERTS: Any other questions for Dr. Sheets? If not, thank you very much.

Our next presenter is, I believe Dr. Zabik, from Dow Agro Sciences.

Did I get your last name right?

DR. ZABIK: Close enough. Jack works.

My name is Jack Zabik, from Dow Agro Sciences and I'm commenting on behalf of the exposure sub-team of the Sound Science Alliance and I should make this pretty quick.

In interest of time, buckle up. What I really want to do is first say that we recognize EPA has come a long ways in advancing the probabilistic risk assessment. There is a number of things listed on this slide up here.

One of the things I want to highlight is the last point, transparency stakeholder involvement and sound science have been key to this. And I really want to comment Bart Suhre's group -- he and his group for maintaining an open discussion on these things. We very much appreciate that.

Of course, with open discussion, there is always an opportunity to comment on some things.

The first thing we want to comment on is that the model output analysis should focus on

exposure metrics that are most biologically relevant to the tox benchmarks, being used to characterize potential health risk, ie, the repeated dose studies used to drive BMD 10s.

Therefore, a moving average on the exposure would make most sense for comparison. If you are going to look at the acute exposure, then it seems that moving to go an acute NOAEL would make most sense.

In addition, looking at the model outputs analysis should include moving averages, ie, 7 day through 21 day across a range of percentiles to characterize the various exposures to the public.

In addition contribution analysis is key to this effort, particularly with mitigation considerations that can only be based on creditable contribution analysis at adequate level of resolution ie, food and dietary and then products in residential.

Contribution analysis should be based on the moving average exposure assessments.

In addition, input sensitivity is extremely important in terms of key data, model inputs, the methodological improvements made to these models and model capabilities to refine assessments.

This is particularly important as a part of the EPA registrant dialogues regarding whether mitigation is necessary and if so what options should be considered. Those are my comments.

DR. ROBERTS: That was fast.

Any questions from panel members?

DR. LAMBERT: Just a general question. If we are so far above the levels of exposure where we're even addressing, like for the 10X factor, I mean, what is the relevance?

DR. ZABIK: I'm not sure I'm following.

DR. LAMBERT: If the current levels of exposure in the population are so much lower --

DR. ROBERTS: Dr. Lambert, I'm sorry. Can you speak into the microphone.

DR. LAMBERT: If the current levels of exposure are so low, putting in a 10X factor is

probably not -- versus 3X, what is the difference as far as industry, just out of interest?

DR. ZABIK: In terms of margin of exposure? I would defer that to the Agency on what they found in their comparisons.

DR. LAMBERT: I'm looking for industrial standpoint, which I shouldn't get into.

DR. ZABIK: Well, with the -- and I think is the tox folks have been talking about, with the conservatism of the tox endpoints and the safety factors, if you keep adding additional safety factors you will reach a point where they always drop below say 100, if that is in fact the kind of line being drawn.

DR. ROBERTS: Thank you very much.

My understanding, the last member of the Crop Life American team is Ed Gray. Welcome, Mr. Gray.

MR. GRAY: Thank you.

It's a pleasure to be here. I'm actually speaking on behalf of the FQPA Implementation Working Group.

I want to mention a bit about the role of cumulative risk assessment in the food and drug act as amended by the FQPA. It is really simply a factor to be considered as part of a lot of other factors -- along with a lot of other factors in aggregate risk assessments.

That's what the law says. It doesn't really say anything about how to do one. It doesn't go into anything like the detail that it does about aggregate risk on individual compound.

I think the Agency has done a lot of flushing out here, made a lot of policy decisions about how they want to do it, and I think they have done a good job.

I think they have looked at what the cumulative risk is and decided that there really isn't anything they need to take from it back to the aggregate risk assessments to make changes.

We agree with that and we also think that there is some conservatism built into this that haven't been talked about today that I just want to run over.

We think that it indicates that there is -- this is really a very time conservative risk assessment, and that there is good reason for the Agency's position.

Two of these assumptions are the use of tox data from the long-term studies, particularly when they are comparing quite short-term exposures. We have talked a lot over several panel meetings about this.

And I'm not arguing here that they have done it wrong. What I'm saying is I don't think they have given themselves enough credit for the conservatism of this thing, because -- when you do a one-dose one-day study, you need more of a dose to get to an ED 10 or any particular dose level than you would need if you dosed the same dose over a period of time like three weeks or a year.

I think we all understand that. We have argued in the past that it would be useful to compare the short-term exposures with a short-term toxicity endpoint.

EPA is bothered about that because they

are concerned about the possibility that there is a left over inhibition from prior exposures and we understand their concern.

I want to talk a little bit about the context about that. It is probable that, I don't think any of us know for sure about humans, but the way brain cholinesterase is replenished is almost completely by regeneration and not by -- at least with OPs not by reactivation of inhibited molecules. So, if you get enough of a dose, yes, there is going to be inhibition and it will carry over for a while until the regeneration can pick up.

But in view of the use pattern that will be in the future for the OPs, exposure would be mostly and perhaps entirely from the diet. It seems to us very unlikely that a person will ever receive a dietary exposure that would result in measurable brain cholinesterase inhibition under this regime.

We think the odds are small that a person that receives a relatively high exposure on

a given day would receive other high exposures in the past. I put these numbers up here as things you can think about.

You can think about -- I don't know if it's exactly statistically true -- probably isn't, but 99.9 exposure is something like one event in one thousand days.

In the 80th percentile and below those kinds of numbers are the things are the you are going to be exposed to most of the time. That may not be relevant except when you are thinking about accumulate accumulation, but it's pretty important, I think, when you are thinking about a cumulative dose.

I put this thing together. We all focus on the high-end exposures when we're looking where we should be regulating at. We sort of forget to look at, what are the exposures most of the time?

You can see from these numbers that most of the time most exposures are real low. If there is any anything like sort of random distribution to the way these exposures run in people's diets,

most of the time are you going to be getting diets that are essentially not going to cause any possibility of exposure or of an inhibition.

Every once in awhile you are going to get one that might come close, but are you still 100 times below the takeoff point except at the very top, except at 99.9. We already talked about that. I would like to focus on what is likely to be the case on most days, over say a two week period or a one year period.

If you are thinking about the typical or predominate situation being where you only have a very occasional high-dose and don't have much accumulation, then it is fair to compare that exposure to a one-day toxicity number. That's what these numbers up here are.

These are the inhibition numbers that came out of the methamidophos acute neurotox study that I think Dr. Sheets had a lot to do with. The way I look at it, it appears that somewhere around .4 in a female, you are probably going to get an ED 10.

That is important because it is five times higher than number that we're regulating off here now.

The other conservatism I want to touch on is the Agency's basis for using 3X database uncertainty factor. EPA has assumed that kids older than 12 months need to be treated as if they may still be more sensitive to cholinesterase inhibition from some compounds than adults are.

This is, they say because of differences in enzyme levels. And this is really -- this assumption is the foundation for using the 3X factor the way they have done. I think this is also a very conservative assumption.

If you look at the actual data, there are two published studies that are in the main source of the data on these esterase levels. I think you have distributed both of them, I'm not sure.

Then there is the information that is related by EPA from Dr. Furlow's recent collection of data.

The published data, as far as I can tell from reading them, indicate fairly clearly that when are you a year old you are an adult. This is one of these cases where a kid is a little adult for this particular purpose according to these authors.

And these guys I take it from reading, aren't considered slouches in the field. Neither is Dr. Furlow. He is considered to be extremely good researcher. I'm having a little trouble with the data on kids development of A esterases, though, because we have seen hardly any of it.

We have only seen two slides, two different kids and they have been measured over different periods of time.

One of them shows this classic pattern that goes back to these earlier studies, whereby the time you get to be six months, you are an adult.

And the other slide shows a bunch of measurements that look like they were all made

before one month and then a measurement at 25 months, I think it is, and a curve drawn between those but I can't figure out how you draw the curve.

So, it doesn't seem to me that from Dr. Furlow that we actually have anything that says this is different. Here are some people that are different. It may very well exist, but I haven't seen them and I don't think anybody else has outside his laboratory. Maybe they have, but that raises a question. I don't see any data there yet.

So, it seems like what we're saying is, the data we have say the enzyme -- A enzyme is developed. We can ask ourselves about other enzymes, the B enzymes.

One of the studies that is up there, the ECOBICHON study, has not for cholinergic esterase, but for three other esterases, a pattern. You can see that they all behave essentially the same.

From what little I can read, it looks to me like scientists, of which I'm not one, consider

carboxyl esterase to be in the same family. My suggestion it behaves the same way, and at we at least ought to consider that possibility.

I know there is a huge amount of data that has been done on plasma cholinesterase because of the concerns that anesthesiologists have about how it behaves. I think there is a Danish data base that looked into that extensively and has thousands of samples.

These are two things that I think show some conservatism in the exhibit that -- in the numbers that we have that go beyond what the Agency itself is saying. It seems to me they need to be taken into account. Thank you very much.

DR. ROBERTS: Thank you Mr. Gray. Are there any questions from the panel members?

DR. NEEDLEMAN: I just want to respond for the statement that a child at one is biochemically an adult. That may be true. I don't know that that is true.

Between one and two, the great brain is growing and getting complex -- a child is learning

10 new words a week or more.

Think what is going on in the child's brain. If you perturb a brain at that time, it may be fixed. So I think you can go from buy a biochemical phenomenon extrapolate to behavior.

MR. GRAY: I'm certainly not going to argue that kids are amazing little things that are developing for quite awhile. That's not really what I was testifying to. Simply what the data say about this one particular esterase and it's rate of development and when it reaches a plateau.

DR. ROBERTS: Any other comments or questions? If not thank you very much for your input to the panel.

The next public commenter that I have listed is Mr. Art Beltron. Welcome, I appreciate your patience.

MR. BELTRON: Thank you very much and thank you for the opportunity to speak before the panel, thank you. As you indicated, my name is Art Beltron and my wife and I have lived in Cheswick (ph) Virginia, just outside of

Charlottesville for the past 16 years.

During this time we have raised two children. One, now married, the other soon to be married. This past March we became grandparents when Christine Marie was born. Today I come before you a concerned parent and a concerned grandparent and somewhat frightened as well. Certainly, frustrated. Let me tell you why.

We lived live in an agriculturally zoned part of Albemarle County in the Charlottesville region. In the year 2000 an adjacent farm was made into a vineyard. Almost 50 acres are now planted in vines -- thousands of individual plants.

Last year as the vines grew pesticide spraying began, often once and sometimes twice a week. Sometimes it rained right after a spraying. When the operations manager of the vineyard was asked what was being sprayed, we received absolutely no answer at all, no response.

Because the area and other areas like it are zoned agricultural use, there are no

regulations by the county or the state regarding chemical spraying. And there are no regulations regarding the total size of the vineyard. This particular piece of land consists of 400 acres. About 50 are now planted.

It could become a 400 acre vineyard, almost a wine industry. There are only suggested guidelines from the Commonwealth of Virginia's Viticultural Office. Let me just read these to you.

As a matter of fact, I sent an e-mail to him asking him what regulations or what suggestions he could make to me if I wanted to establish a vineyard and this was his response.

"No fixed rules of separation or vineyard rose from surface water, however some fungicides and insecticides are very toxic to aquatics. Airborne drift would probably be the greater hazard, however. I would suggest -- he writes suggests all in upper case letters -- as a general precaution, that you remain at least 300 feet from surface water or neighbor's property

lines.

You can also do much to minimize spray drift by not spraying during windy conditions, erecting fast growing vegetation such as leland cypress, and using contemporary spray or technologies."

That was the response of Tony Wolfe (ph). With that --I don't have any slides, I'm sorry, but I do have two very large enlargements of spraying that was being conducted last year. I would like to submit this to the panel for their view and consideration.

I'll leave them here and leave them with the appropriate bodies at the conclusion.

In essence the vineyard operator and its owner, as we speak today, can do as they please. The operation, we have found is self policing. Spraying is often conducted during windy days with drift crossing property lines as is seen in these photos. The drift goes into ponds, streams, and hay crops.

Vines have been planted as close as 42

feet from property lines when it is suggested by the state viticulturist to stay 300 feet away and 12 feet from a pond or a stream.

Vines on hillsides are sprayed as well as on the flat lands with the residue wash downward to lower elevations. Drinking water in our area is by well, not by city municipal water.

Everything that is being sprayed and washes down after the rain is going onto the land that is around these wells and the wells are as close as 150 feet to the spraying.

This isn't just our residence, but it is the residents all around the 400 acre farm. Our community concern is protection, now and in the future.

Protection from those who defer from sound agricultural management practices who have no concern for the quality of the water, the air and people who are looking only at profit margins and who reject suggested guidelines and who in all likelihood will never the precaution read the precautions on chemical labels or follow them.

Please consider what is happening in communities like Cheswick, Virginia in your research and decision making. I thank you for listening.

DR. ROBERTS: Thank you for your comments.

Let me ask the panel if they have any questions for you. I don't see any.

Thank you very much.

We have another public commenter on our list, Dr. Judith Shriver, from the State of New York, office of the Attorney General. Welcome, Dr. Shriver.

MS. SHRIVER: Thank you. My comments today are going to talk about some clarification points I had for some of the comments that I have heard today.

The Office of the Attorney General in New York will be providing extensive written comments with regard to these issues.

** (5:00 p.m.) **

As I'm sure you don't have to be

reminded, FQPA really is a broad mandate for EPA to protect the health of infants and children in the United States and I'm happy to see that EPA has taken this charge seriously and really has done a considerable amount of extremely good work.

We're glad you are taking it seriously and we certainly are as well.

I know this esteemed panel does not need to be reminded that humans are a diverse group, genetically very dissimilar to one another in many ways and are not just a bunch of genetically similar rats to which we can be exposed and assessed. I know committee is aware of that.

In the interest of brevity, I actually will scuttle some of my questions since I see it is already 5 o'clock, but you are all staying awake rather ell, including Dr. Ruby, who came from California, who probably doesn't know what time it is at this point.

I wanted to ask how many of the DNT studies have been submitted to the EPA on the OP's that have shown effects and perhaps more to the

point conversely how many have not been conducted that the Agency still needs to see in order to have a complete data set with regard to neuro developmental toxicity?

I guess I would venture to say that until those studies have been conducted, this represents a serious data gap. Can anybody from EPA or the SAP respond to that?

MS. MULKEY: Perhaps it would be a good idea if the commenter went through all her questions and then we could see whether it's appropriate to try to answer some of them here or in another place.

DR. ROBERTS: We were just about to make the same suggestion. If you don't mind, Dr. Shriver, if you could go through the questions and then I guess we could sort of decide which ones are --

DR. SHRIVER: So, that one deal primarily with the fact or my estimation that that represents a serious data gap and how does the Agency intend to address that?

Also, it seems that it is not just the dose that makes the poison of course, but the timing of the administration of that dose, which is key.

And that, if the timing is neglected in terms of acute exposures, there certainly could be a critical window of vulnerability in the developing fetus or neonate that can result in effects that are life long, whereas in the maternal animal who may have some effects if perhaps is a transient effect perhaps a reduced weight gain or some other transient nature which is much different than what might affect the fetus does and the offspring in terms of human babies.

For example, alcohol ingested by a mother may have a transient effect on the mother but can a have a lifelong effect on the infant.

I was wondering whether the EPA and the panel would consider cholinesterase inhibition that results in profound changes in the fetus or perhaps even -- how did we call it before -- loss of production, fetal loss, which I guess in my

estimation is a much more serious effect than a transient effect of toxicity in the mother animal.

I would ask the EPA and the committee to consider why acute exposures are not being considered in this evaluation as a means of determining whether there is an effect from the OP and the correct safety factor to use with that.

Which really brings us to the issue of susceptibility. I think EPA has evaluated sensitivity of the off spring, but I think perhaps has neglected the susceptibility aspects.

In other words, perhaps the offspring are having effects or some sort of offspring effect is being observed at the same dose that causes an effect in the maternal animal, but the maternal animal's effects are transient and the fetal effects are permanent or long lasting.

I would say looking at other EPA documents, the other document on FQPA -- I'm sorry, yes -- on FQPA safety factor determinations clearly states that it is not just whether the

effect occurs at a lower dose, but whether the effect is different than or more profound than the effect on the adult animal.

And I think with OPs we have a lot of cases that support that. A lot of evidence that supports that situation that indeed there is increased susceptibility in children, although it may occur at the same dose that is causing a problem in the mature animal.

In terms of -- I'm a toxicologist and risk assessment public health person in my office but as you know, I'm with the Office of Attorney General and so I have come to think a little bit more legally about some of these questions.

And in reading the statutes, I think its very clear that the FQPA safety factor of 10 is the default, is the number that you must use if there is no information to the contrary to change it.

And I think the reliability of the data upon which these factors are based need to be completely -- there needs to be a complete data

set and exposure analysis in order to divert from the tenfold safety factor.

And I would say that the rational presented by the EPA in moving from a tenfold factor to a threefold factor does not appear to be justified.

Not only do the young have more sensitivity, they also have susceptibility. They also most likely have a greater exposure, but I would say the exposure is somewhat poorly characterized.

And I would like to hear something back either from the Agency or the SAP as far as for example the exposure data base -- the breast milk analysis I thought was really given short shrift and some hand waving went on about how it is unlikely that this would be a route of exposure but I don't believe the Agency did a very thorough evaluation of the likelihood of the exposure through breast milk.

Dermal absorption, I didn't hear discussed at all today. For example, children and

infants may have different absorption through dermal than adults and I believe the dermal exposure factor that is used by the Agency was based on adult studies not children.

So, right there you have a deficiency in the exposure assessment that I think really could be quite critical and represents a data gap.

Based on EPA's own criteria, there were three questions that were posed. Is the toxicology database complete? I would say, no, it's not. Is there concern about pre-imposed toxicity? Yes, there is. Is the exposure data base complete? I would say, no, it isn't.

In face of those three uncertainties, the tenfold safety factor must be retained. It's as simple as that, that's what the law says.

So, I think the determination of the 3X -- the decision making that went into the 3X factor, although there is a lot of discussion about various studies, I think in the end it is not particularly clear how the EPA determined that a threefold safety factor was the appropriate one

-- why not 5, why not 7, why not 10, which is the default number that is in statutory requirement.

I was very interested in -- I don't recall what slide number it was, I couldn't see it from where I was sitting, there was one slide that the margins of exposure were presented at various percentiles of dietary exposure, 95th, 99th, 99.9 and so on.

I was wondering what would happen to those margins if, instead of the threefold FQPA factor -- a full tenfold FQPA factor were applied. I would like to see that calculation or perhaps one of the members of the SAP could ask the EPA to do such a calculation.

Also, with regard to one of -- some comments made earlier, at the 99.9 percentile, unless I'm misunderstanding this, it means that .1 percentile of the population out there, the U.S. population eats more of a particular commodity or groups of commodities and ends up at an even higher level of exposure.

If you take .1 percent of the U.S.

population of 280 million, you have a substantial number of people who are actually are ingesting foods containing OPs at levels greater than what we have calculated the risks for. What about those children?

What about the child of the man who just spoke of it a moment ago who is living next to this grape orchard? The child in that household I'm sure gets a lot more exposure probably more than what the 99.9 percentile is.

So the mandate of the FQPA is to take into account and to protect children and infants in the United States. I think those children ought to be protected as well. There was one part toward the end -- again at the 99.9 percentile, where infants, and I just dotted these down quickly.

I don't recall now this was for a particular chemical or for the group, but it was listed for example, infants were exposed at 0.009 milligrams per kilogram per day, whereas the one to 2 years old were at 0.18 and the adults 0.005.

So, essentially, infants were exposed at about half the milligram per kilogram per day amount as were the one to two-years-olds. Hold that thought for a moment. But then, in terms of the toxicology, one to two-year-olds were found to be more sensitive by 9 times compared to an adult under the slide that was shown at the time.

So, if you combine those two features you find that not only should you have an FQPA safety factor of 10 to account for this ninefold sensitivity in children, but you really should also have a three times additional safety factor to account for the additional exposure, because exposure is also parts of what is to be assessed under FQPA. It is the toxicology database and the exposure.

So, you have not only an increased exposure between adults and children but you also have increased sensitivity of children. So, I would argue that you need a full tenfold safety factor for the toxicology database and the inherent uncertainties in it and additional

threefold factor to account for the increased exposures.

Finely then, one last point, is that it is my understanding and correct me if I'm wrong, that EPA removed from the exposure considerations the OPs that were being phased out or are under some sort of mitigation requirements.

So that those assessments are not included. And I was wondering whether EPA had considered the exposures and risks associated with OPs which are going to replace the products which are going to be phased out or mitigated?

I presume that if something is being removed from the market, there will probably be another chemical, perhaps an OP, perhaps something else that is going to be replacing it. Has that come into play in the EPA's assessment?

And that's all the comments and questions that I have today. As I mentioned, our office will be putting in extensive comments on this issue.

DR. ROBERTS: Thank you, Dr. Shriver.

Before I ask the panel for questions, Dr. Shriver laid out several questions that were really probably more directed to the Agency than to the panel itself.

Let me give Ms. Mulkey the opportunity to pick among those whichever ones the Agency feels would be appropriate for them to respond to here and maybe respond through another means to the other questions.

MS. MULKEY: Based upon my notes, although a number of things were posed as questions, really a lot of them were very much in the nature of public comments.

And I think really were more if you will, hypothetical or rhetorical, which is not to diminish in any way the message behind them, but just that -- I don't know that we would advance anything by attempting to go engage on them.

The first and last matter, however, did seem to lend themselves to an opportunity for us to provide some information now that might be helpful for the commenter.

The first had to do with a number of developmental neuro toxicity studies which we have or other studies which allow us to review comparative sensitivity which the document itself, of course, references and goes into a good bit of detail about the studies available on the six OPs for which we do have studies.

I think the document is the best source of that but the latter part of the question was, when did we expect to receive the required studies and the remainder of the OPs.

As one of the industry commenters observed, those are due to the Agency actually they are due over some difference in schedules depending on the compound, but the last one is due -- currently due in November of 2003. So, it might be helpful to add that.

Then moving to the very last question, which had to do with a question of whether the risk assessment -- whether and how it accommodated possible future shifts and use.

What we have done is where we know that

a chemical crop combination or a chemical use pattern has been by some formal legal instrument -- an agreement or otherwise, are removed from the market. We have taken the exposures associated with that out of the risk assessment.

However, take for example the dietary exposure data that we have -- are based on measured residues in the market place most recently about year 2000.

So, obviously, the measured residues do not reflect either changes in use that occurred as a result of regulatory actions that were less than complete removals.

For example, reduces in rates, of which there had been a number. Changes in application interval before harvest, of which there had been a number. So there are probably some ways in which the residues in the year 2000 overstate the future picture.

And they also do not obviously, accommodate for new use that might occur to OPs as a result of people moving from one of the canceled

to one of the new ones.

But I will say that it is not always the case that there is even the opportunity to make such a movement, that there is another OP registered for that use or practical for that use. So, perhaps that answers that question.

Otherwise, I don't think that there was anything that seemed obvious to me, but if the panel would like us to give some additional information on any of these we can try to. Does anybody on the team think we have anything more that would be useful to do.

DR. SHRIVER: Excuse me, Ms. Mulkey, what about the question about the acute -- maybe better put to the panel -- the question of the acute toxicity and whether that's considered in the assessment?

MS. MULKEY: That was, obviously, one of the questions we had put to the panel --

DR. SHRIVER: So, that will be taken up tomorrow?

MS. MULKEY: As I understand it, that's

part of the subject to address the panel which is why I didn't think we needed to engage in that at this point.

DR. ROBERTS: And the panel will provide their feedback on that in our deliberations tomorrow.

But let me offer the panel the opportunity to ask any questions they might have.

DR. REED: As I asked the previous presenters, I would ask you again, you mention that the database is not complete. Could you elaborate a little bit on what you would consider as a complete database?

MS. SHRIVER: Well, I think there is pretty standard protocols. In fact, a paper by the EPA about what is considered to be complete database for neuro toxicity.

I find it troubling to base opinions on cumulative risk assessment when you only have six neurotoxicity studies that can even begin to address the problems -- the neurological problems that could result from exposure to these

chemicals.

I think it would be -- I think the public, really, if they were aware of the Agency's putting so much stock on these six without regard to the studies that are not there, I mean the uncertainties in the database is in fact the reason why the tenfold safety factor is there, is to account for things we don't know.

And you can't possibly know it if the studies haven't been yet conducted or submitted. I would say that that's a really big problem.

DR. REED: So you are referring to DNT studies?

MS. SHRIVER: DNT studies and I'm not familiar with each and every one of the OPs, but I'm pretty sure there are other missing pieces if you went back through all the individual assessments.

I also have concerns about whether -- well, cholinesterase inhibition certainly is the common mechanism but are there other features that are related to the cholinergic pathway that also

could result in problems in off spring? And I think that that's a situation that really needs to be addressed.

DR. ROBERTS: Are there other questions from the panel members?

If not, thank you very much for your comments.

DR. ROBERTS: This concludes the list of individuals who have expressed an interest to us in advance to be willing to speak or address the panel.

Let me now open it to the audience. Is there anyone in the audience who would like to address the panel or make comments on these subjects before we close the comments period?

This is the only opportunity that the public has to address the panel so this is -- if there is comments that you want to make, this is the time to do it.

I see none. So, with that, let's close the public comment period.

Let's also close our session for Mr.

Lewis has an announcement.

MR. LEWIS: Thank you, Dr. Roberts. Before we adjourn for this evening, I want to thank the panel members for the diligence in working through the issues today.

I want to ask all the panel members if we can reconvene in about five minutes in our workroom to go over some administrative issues for this evening and for the rest of the work tomorrow.

Thank you, Dr. Roberts.

DR. ROBERTS: Thank you.

Our deliberations will begin again at 8:30 tomorrow morning. We look forward to seeing you all here bright and early and ready to discuss these questions. Thank you.

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[Whereupon, at 5:20 p.m., the meeting adjourned.]

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