U.S. ENVIRONMENTAL PROTECTION AGENCY (EPA)

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FEDERAL INSECTICIDE, FUNGICIDE AND RODENTICIDE ACT SCIENTIFIC ADVISORY PANEL (FIFRA SAP)

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REEVALUATION OF THE HUMAN HEALTH EFFECTS OF ATRAZINE: REVIEW OF EXPERIMENTAL ANIMAL AND IN VITRO STUDIES AND DRINKING WATER MONITORING FREQUENCY

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DOCKET NO.: EPA-HQ-OPP-2010-0125

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MONDAY,

APRIL 26, 2010

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The Panel convened at 1:00 p.m. in the Hamilton Ballroom of the Hamilton Crowne Plaza Hotel, located at 1001 14th Street, N.W., Washington, D.C., Steven G. Heeringa, Ph.D., Chair, and Kenneth M. Portier, Ph.D., Session Chair, presiding.

FIFRA SAP MEMBERS PRESENT:

STEVEN G. HEERINGA, Ph.D., Chair
KENNETH M. PORTIER, Ph.D., Session Chair
JOHN R. BUCHER, Ph.D., DABT
JANICE E. CHAMBERS, Ph.D., DABT, ATS
GERALD A. LeBLANC, Ph.D.
DANIEL SCHLENK, Ph.D.

FQPA SCIENCE REVIEW BOARD MEMBERS PRESENT:

SUSAN F. AKANA, Ph.D.

RICHARD H. COUPE, Ph.D.

KENNETH BARRY DELCLOS, Ph.D.

PENELOPE A. FENNER-CRISP, Ph.D., DABT

ROBERT J. GILLIOM, Ph.D.

RICHARD GREENWOOD, Ph.D.

WILLIAM L. HAYTON, Ph.D.

STEVEN D. HOLLADY, Ph.D.

TERESA H. HORTON, Ph.D.

KANNAN KRISHNAN, Ph.D.

HERBERT K.H. LEE, Ph.D.

KEVIN T. O'BYRNE, Ph.D.

NU-MAY RUBY REED, Ph.D., DABT

JEAN F.L. REGAL, Ph.D.

DANIEL J. SELVAGE, Ph.D.

CARMEN J. WILLIAMS, M.D., Ph.D.

LINDA J. YOUNG, Ph.D.

ALSO PRESENT:

JOSEPH E. BAILEY, Designated Federal Official

C-O-N-T-E-N-T-S

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

1:09 p.m.

MR. BAILEY: Good afternoon, everyone. My name is Joe Bailey, and I'm with the FIFRA Scientific Advisory Panel staff. I'll be serving as the Designated Federal Official for this meeting, and I want to welcome everyone, this afternoon.

We have a three and a half day meeting set up here, and I'm very pleased to kick this meeting off this morning -- this afternoon, and get it underway.

As the DFO, I serve as a coordinator between the Panel, the Agency and the public, in putting this meeting together.

The FIFRA Scientific Advisory Panel only provides advice and recommendations to the Agency. All decision making and regulatory decisions are left up to the Agency, and they take the advice and recommendations as they see fit.

We have worked with all of the Panel

members, in reviewing ethics requirements. They have all submitted financial disclosure information that we, at the Agency, have reviewed and made sure that we -- that all of the ethics requirements have been met.

Part of the requirements, under the Federal Advisory Committee Act, is that this is a public meeting. We do provide an opportunity for public comment, and the -- that is set aside for tomorrow morning.

So, if anyone has a desire to present public comments, please, let me know. If you haven't registered ahead of time, I ask that you keep your comments to five minutes or less.

There is a public docket that we've set up for the meeting. It should be on the agenda, the docket number. Everything that is presented in this meeting will be in the docket.

The presentations that EPA will be putting forward today are current -- they should be available in the docket, as of now, and any public -- any additional public comments or other

materials that come of -- become available during the meeting will also be placed in that docket.

I'm very pleased to introduce Dr. Ken Portier, sitting to my right, who will be serving as the Chair for this meeting, and without any further adieu, I'll turn the mic over to Dr. Portier.

SESSION CHAIR PORTIER: Good afternoon. I want to welcome all of you to this meeting of the FIFRA Scientific Advisory Panel on the Reevaluation of the Human Health Effects of Atrazine Review of Experimental Animal and In Vitro Studies and Drinking Water Monitoring Frequency.

I'll give a special thanks to our audience. I think they have ordered a few more chairs. We're going to accommodate everyone.

As many of you know, this is kind of the second in a series of three meetings that the Panel is having on atrazine this year.

We've put together -- the SAP staff has put together a very good panel of experts, a

mixture of experienced panelists, with some new people, some new voices.

So, at this point, I'd like to introduce the Panel. I'll start with myself. I am the Director of Statistics with the American Cancer Society National Office in Atlanta. I'm a bio-statistician and a permanent member of the SAP Panel, and I'll move to Dr. Heeringa here, on my right.

CHAIR HEERINGA: Good afternoon, everyone. I'm Steve Heeringa of the University of Michigan. I'm presently the Chair of the permanent FIFRA SAP and look forward to this three and a half day session, and I want to thank my colleague, Ken Portier, for agreeing to Chair this.

DR. KRISHNAN: Good afternoon. I'm Kannan Krishnan from the University of Montreal.

SESSION CHAIR PORTIER: What's your expertise?

DR. KRISHNAN: I'm an agriculturist and toxicologist by training. My expertise is in

PBPK modeling, risk assessment of drinking water contaminants and chemical mixtures.

DR. GREENWOOD: I'm Richard Greenwood from University of Portsmouth in the United Kingdom, and I've got a broad background in pesticide toxicology and also in pharmacokinetics.

DR. FENNER-CRISP: I'm Penny Fenner-Crisp. I'm currently a consultant and live in Charlottesville, Virginia, a one-time employee of EPA, including the Office of Pesticide Programs for a few years, and the newest member of Virginia's Pesticide Control Board. So, I'm back in the regulatory milieu.

DR. REED: I'm Nu-may Ruby Reed from California Environmental Protection Agency. I do pesticide risk assessment and address pesticide risk assessment issues for our group.

DR. LEE: I'm Herbert Lee. I'm a statistician at the University of California, Santa Cruz.

DR. YOUNG: I'm Linda Young. I'm

Professor and Associate Chair of the Department of Statistics, University of Florida.

DR. COUPE: Richard Coupe, I'm with the U.S. Geological Survey. I've worked on fate and transport of pesticides or agricultural chemicals.

SESSION CHAIR PORTIER: Dr. Selvage?

DR. SELVAGE: Yes, I'm Dan Selvage, Idaho State University, specializing in HPA axis and how gonadal hormones affect it.

DR. O'BYRNE: My name is Kevin O'Byrne and I'm from King's College London and my interests are in the effect of stress on fertility.

DR. AKANA: I'm Susan Akana. I'm from the University of California, San Francisco. I'm a physiologist and my area is the stress axis and its interaction with energy balance.

DR. HORTON: I'm Theresa Horton. I'm from the Department of Neurobiology and Physiology at Northwestern University in Evanston, Illinois. My specialty is reproductive

neuroendocrinology and maternal fetal interactions in the developing neuroendocrine system.

DR. DELCLOS: I'm Barry, or Kenneth Delclos from the FDA's National Center of Toxicological Research and I'm working on reproductive and chronic toxic effects of endocrine-active agents.

DR. WILLIAMS: I'm Carmen Williams. I'm at the National Institute of Environmental Health Sciences in North Carolina, and I'm in a reproductive endocrinology and infertility training position. I do research on reproductive track development.

DR. REGAL: Jean Regal, Professor of Pharmacology, University of Minnesota Medical School, Duluth, Immunotoxicology and Allergy.

DR. HOLLADAY: I'm Steve Holladay. I'm from the University of Georgia. I'm an immunotoxicologist. Most of what we've done is developmental immunotoxicology.

DR. LEBLANC: I'm Gerry LeBlanc and I'm

head of the Department of Environmental and Molecular Toxicology at North Carolina State University and my area of interest is in endocrine-toxicology.

DR. SCHLENK: Good morning. I'm Dan Schlenk. I'm a Professor of Aquatic Eco-Toxicology in Department of Environmental Sciences, University of California, Riverside. I'm a member of the permanent Panel and my expertise is on fate and effects of pesticides on aquatic organisms.

DR. BUCHER: I'm John Bucher. I'm the Associate Director of the National Toxicology Program. I'm a member of the permanent Panel and with a background in general toxicology.

DR. CHAMBER: I'm Jan Chambers. I'm a Professor in the College of Veterinary Medicine at Mississippi State University. I'm a pesticide toxicologist with emphasis on neurotoxicology and metabolism, and I'm a member of the permanent Panel.

SESSION CHAIR PORTIER: Thank all of

you for being here. A couple of, kind of, notes.

The agenda has been published, but just note that the times that are published are approximate. We're going to proceed through the agenda as it goes, and my goal today is to get through the EPA presentations before we leave this evening, hopefully, depending on the number of questions the Panel has on the presentations.

The second thing is, tomorrow morning, we're going to have a session on public commenters and if anyone wishes to speak before the Panel tomorrow, please, let Joe Bailey know, at this point, so we can schedule you in. If you have any materials for that public presentation that you want to make before the Panel, give it to Joe. It will get onto the docket.

All of the public commenters, as well as the EPA presentations are on the meeting docket on the internet.

At this point, I'm going to turn it over to Steven Bradbury, the Acting Director, Office of Pesticide Programs, to make his opening

remarks. Steve.

DR. BRADBURY: Thank you, Ken. I'd like to start off, first, by thanking the Panel, all the members, the standing permanent members of the Panel, as well as all the ad hoc Panel members for this week's peer review.

I greatly appreciate the time and effort it takes in preparing for these meetings, the time it takes while you're at the meeting and then, preparing the report, following the deliberations, and the Agency greatly appreciates your investment of your time and efforts and talents in our peer review process.

So, ahead of the week, thank you very much and again, I'll be thanking you, as we finish the week.

I'd also like to thank Joe Bailey and the staff that -- the Designated Federal Official who helped make these meetings happen. It takes a lot of work to prepare for the meetings and get all the logistics in place and I want to thank Joe Bailey and his colleagues, in all the work

that they've been undertaking to get ready for this meeting, as well as the previous meeting that we already had with atrazine.

I'd also like to, in advance, thank the public for their participation in the deliberations.

As Ken Portier indicated, tomorrow morning is our time for public input into this peer review process and we take the input of the public very seriously and it's a very important part of the overall peer review process that we're going forward with, and I can't stress enough, the importance of the public participation and transparency that an SAP review brings to the decision making process that we do in the Agency.

And it's a concept of public participation, transparency and the quality of science in the Agency's decision making processes. There are a couple of the very important principles that Administrator Jackson brings to the Agency.

And so, as we go through the Science Advisory Panel process, which our program has done for many, many years, it reinforces these important concepts of the new administration, in terms of ensuring that the science that we do that's a foundation of all the decision making that we make in the pesticide program is based on the best available information and the highest quality science that we can bring to bear, and that includes the very important part of peer review, to ensure that we're getting input from our colleagues in the scientific community, as we look at some very complex issues.

The other important aspect of a meeting like this is the openness and the public participation process, so that the public can participate and provide input to the deliberations and that the proceedings of the meetings and the notes from the meetings are all open and transparent, so that as people see us moving forward in our decision making process, the various components that are important to that

process aren't mysterious. It's not a black box. In fact, it's quite the opposite, very open process with a lot of lights shining on it, so everyone can see how we're integrating very complex scientific issues with the regulatory decision making that we need to do.

And in that context of sound science, high quality science and public participation, chemical safety also reflects one of the areas of emphasis of the administration, in both industrial chemicals, as well as with pesticides.

And so, ensuring, as we go forward in our pesticide decision making, that our decisions are those kinds of decisions that ensure safety to human health and the environment, as we go forward with our decision making process.

I'd like to spend just a couple of minutes reviewing the processes that we go through, in making our regulatory decisions and then how that connects with the efforts that we're undertaking with atrazine.

As we make decisions about new

pesticide products and whether or not they should be allowed to go on the market, there's a very extensive amount of scientific information that's required by the registrants, so that companies that own the products, need to bring quite a large amount of information to the Agency, everything from the fate and transport properties of the chemical, human toxicology, ecological effects, a wide variety of information needed to ensure that any decisions we make about the registration of a pesticide will ensure, again, protection of human health and the environment, including worker protection, including protection, if the product is in the food source, including that dietary exposures are safe, as well as any drinking water exposures.

The information that is brought to bear in the processes that we use are, in many ways, similar to activities undertaken around the globe. Much of the information and scientific processes that we use have been developed in concert with the European Union, our colleagues

to the north in Canada, as well as in Australia.

So, the information base and the processes that we use, while unique to our statutes and our approach, as also integrated or collaborative in nature in many ways, with our colleagues around the globe, at the federal level, and as well as with our collaboration with our partners in the state governments, that also undertake risk assessments and regulatory review steps.

So, the importance of science starts at the beginning of the life of the chemical, in terms of when we decide whether or not we're going to register a chemical.

We also have, through our statutes, a requirement that we periodically reevaluate all pesticides that are on the market, to ensure over time, that as the science change or policies change, in terms of the law or the science, that all the pesticides are current, in terms of the state of the science and the state of scientific approaches and policy.

And we have one system that we used in reevaluating pesticides, that ended in the 2006/2008 time frame and that reevaluation program, we termed the `re-registration program', and that's when we completed, between 2006 and 2008, a reevaluation of all the pesticides that were registered prior to 1984.

In 2007, we started our next round of reevaluation, which we call the registration review program, in which we're looking at all currently registered pesticides, to ensure that they're meeting the current scientific standards and policy standards.

In the case of atrazine, it completed its re-registration process in 2003, was the last time that atrazine went through its formal step of re-registration.

But I want to stress an important concept, in terms of the process that starts the chemical or a pesticide, in determining whether or not it would be registered and in this reevaluation process, which is on a specific time

frame, is that the Agency is always looking at all the pesticides, all the time, to ensure that if any new information comes to light, or new perspectives in interpreting the toxicology or the ecological effects of a pesticide, that at any time, the Agency can pause, take a look at the compound and ensure that the current science is such that it meets our continued requirements for safety, in terms of human health and the environment.

Now, in the case of atrazine and many other products, as these products went through re-registration, we required additional data or confirmatory data to come in after the re-registration decision, and in the case of atrazine, in particular, we were focused on drinking water and ensuring that we would have a system in place that would allow us to keep track of what was going on in drinking water sources, to ensure the concentrations of atrazine weren't exceeding our level of protection or our benchmark for protection of human health.

Just an example of many times activities that are going on with a pesticide on a periodic, sort of regular basis, a dynamic process, in terms of tracking the science and the status of a pesticide.

Now, as I mentioned before, atrazine completed its re-registration in 2003, and over the course of the last seven years, there's been quite a bit of research done on atrazine, looking at mode of action, looking at dosimetry.

There's also been a lot of information coming into the Agency, as a condition of re-registration, which is in part, a significant amount of drinking water monitoring data required of the registrant.

So, the Agency felt that over -- a year or so ago, that the time was right to take a look at the new science that had been emerging with atrazine and the context of the very full monitoring information that we had for drinking water sources, to do a check-in, to ensure that our science is current, that our risk assessment

of 2003 is still reasonable, in light of the new information, and to see if whether or not we need to make any adjustments in our current risk assessment for atrazine, in light of the new information that's come out over the last seven or so years.

And this Science Advisory Panel meeting is one of several, in which we're systematically taking a look at the scientific information regarding atrazine, as well as some underlying methodology, to ensure that we're current, in terms of the state of the science and our underlying risk assessment.

So, back in February, we had our first Science Advisory Panel meeting, which was actually broader than atrazine. It was looking at a number of issues, some of which are very relevant to our work with atrazine, and the February Science Advisory Panel meeting was taking a look at epidemiology data, incidence data, and asking some questions and getting some feedback from the Panel, in terms of a framework,

is how do you approach an integration of experimental toxicology data with epidemiology data, as one prepares a risk characterization, as one starts to understand and better express what the uncertainties may be, with the risk assessment.

And we brought forward some example atrazine epidemiology studies, as part of that process of understanding, getting some feedback, as to how to approach different kinds of epidemiology studies and to get some initial feedback, as to how to best integrate experimental data with epidemiology data.

The Panel's report was made available late last week, and we thank the Panel for their efforts in working on that first peer review that we had and getting the report out, and we're, of course, looking at it over the weekend and appreciate the input that we received thus far.

Today's or this week's Science Advisory Panel, as you know, is focusing on primarily experimental toxicology information,

both in vivo and in vitro, and taking a look at the non-cancer effects associated with atrazine, everything from mode of action to dosimetry, as well as beginning to get some feedback around that transition from understanding the dosimetry around the toxicology and how do you translate that into monitoring frequencies in drinking water, to understand what's the right time frame, in terms of looking at drinking water exposures in the context of the dosimetry evaluations coming out of the toxicology work, so that our exposure and that our effects information are properly integrated.

In today's -- this week's peer review panel will give us some important feedback, as we make a progress towards our September peer review, which will be taking a look, with a little modification that we had to do last week, will be focusing on non-cancer effects and looking at experimental toxicology, as well as epidemiology information in the context of non-cancer effects, and again, taking a look at

drinking water, sampling designs and frequency of sampling, to ensure that our interpretation of what's going on in drinking water sources can be properly interpreted, in terms of the risk assessment.

We originally had hoped to be able to also take a look at cancer effects, both experimental toxicology and epidemiology data in September and our goal for the September assessment was based on the National Cancer Institute completing some key studies that they've been doing in the agricultural health study, and our hope that it would be at -- those studies would be available during the spring, so that we could have a fall review on those studies.

Our colleagues at NCI haven't been able to hit that target, so, we're looking towards 2011, when the NCI studies would be available and then the 2011 time frame, we'll focus on cancer, looking at experimental toxicology information and epidemiology data, and

take a look at the cancer issue in 2011.

In the coming months, we'll be able to give you all a better handle on the time frame for taking on that specific task.

So, one other sort of broad comment, as I wrap up, is that clearly, the information that we're providing you all, and information we get from the public and then the feedback we'll get through the peer review process will be critical for our ongoing evaluation of atrazine and ensuring that our regulatory decisions are based on the most current and sound science.

But I want to point out that a lot of the topics that we'll be talking about over the course of the week, also, are very important for our broader program, in terms of how we undertake the emerging fields or issues in toxicology and risk assessment and exposure assessment.

And so, taking a look at mode of action and understanding toxicity pathways, how to understand relationships of perturbations across different levels of biological

organization, while clearly, very relevant to our discussions on atrazine, those concepts also play out in many of our risk assessments, and as we move forward in the context of the National Academy of Sciences report on toxicology testing in the 21st century and the use of toxicity pathways, to better understand what adverse outcomes may be associated with perturbations in biological systems, the feedback we get on atrazine will also be very helpful, as we think about frameworks and approaches for other kinds of chemicals.

As we learn more about how to integrate epidemiological information and experimental toxicology information with atrazine, we're assuming, and I'm confident we will, learn ideas and concepts that will be broadly applicable to the other types of chemicals we deal with and the broad activities that we do in our risk assessment process.

Looking at life stage susceptibility, obviously, critical for atrazine, but also

critical for many of our risk assessments in the scenarios that we deal with.

So, a number of broad range of applicability of the work we're going to be doing, and in the context of drinking water and looking at monitoring frequencies and understanding how to better capture information in source water and drinking water systems and how do you interpret that in the context of a risk assessment, it's not only very important for the pesticide program, but also, very important for our colleagues in the office of water, who implement the Safe Drinking Water Act.

And so, as we move forward in ensuring protection of source water and drinking water sources, the lessons that we'll learn today and tomorrow and over the course of the next year or so, are also very important for how we move forward as an Agency, in terms of drinking water protection and moving forward, both in monitoring designs, as well as in the science of doing toxicology and risk assessments.

So, not surprisingly, many of the individuals that you'll be hearing from over the course of the week aren't just from the Office of Pesticide Program and our Health Effects Division and our Environmental Fate and Effects Division, but also, you'll be hearing from our colleagues from the Office of Research and Development, who back in 2000, were instrumental in our thinking about atrazine and how to move forward in our understanding of mode of action, back in the early 2000s, but you'll also be hearing from a colleague from the Office of Water, in reflecting the interaction that we had with our colleagues in the Safe Drinking Water Office, in terms of how to advance the science, in terms of monitoring and linking that into risk assessments and toxicological interpretation.

So, while it's OPP and we're looking at issues of our statutes and how to ensure that our decisions around atrazine are bringing the best science to the table, again, the principles that we're learning broadly applicable and

reflect sort of, a broad Agency perspective, as we move forward with the state of the science.

So, with that, unless there's any specific questions, I'd like to turn it over to Dr. Tina Levine, who can introduce the whole team and provide some more detailed introduction into the activities over the next week. Thanks.

DR. LEVINE: Thank you, Steve. I'm Tina Levine and I'm the Director of the Health Effects Division in the Office of Pesticide Programs, a job that I will point out, Penny Fenner-Crisp used to have, when she worked in the Office of Pesticide Programs, and I would like to add my welcome and my thanks to everybody that Steve welcomed and thanked, including the SAP Panel, certainly, and the staff of the SAP, Joe Bailey, and the public, who are going to come here -- who are coming here to observe and comment.

Particularly to the Panel, we greatly appreciate your time and efforts. Your feedback is an important component of improving the

scientific foundation for our regulatory decisions, and we're particularly appreciative of your commitment this year, especially the permanent Panel, as we've been working hard to prepare for multiple SAP meetings on atrazine, and we understand the large amount of material you need to consider and we appreciate your commitment to the high quality science.

I want to acknowledge a number of people who have helped work -- are presenting today and have worked -- or have worked behind the scenes, the OPP team, first of all, Dr. Anna Lowit, who is the overall lead for our atrazine efforts, Dr. Elizabeth Mendez, Dr. John Liccione, Dr. Marquea King and Jessica Kidwell, among many toxicologists in HED who reviewed close to 100 new atrazine studies.

I also want to thank, from the Environmental Fate and Effects Division, Mary Frankenberry and Nelson Thurman, and from the Office of Water, Michael Messner. I don't know if they're doctors or not, I'm sorry, if I've

cited somebody without giving the proper titles. I should have stopped, and I'm just going to give people's names from now on.

Nelson Thurman and Mary Frankenberry, and perhaps, Michael Messner too, developed the paper and will be presenting the drinking water aspect of the meeting today.

I want, of course, to thank the Office of Research and Development, our ORD team, Ralph Cooper, Susan Laws, Tammy Stoker and Bob Luebke. Both Ralph and Bob will be speaking today. Our colleagues at ORD are vital to this ongoing reevaluation and we truly appreciate their time and talent.

As Steve said, the Agency signed the RED for atrazine in 2003. The human health risk assessment that supported the RED was based on the best available science at that time.

The science which supports the previous risk assessment, was reviewed by the SAP on at least three occasions and we relied heavily on your input to improve the science in our risk

assessments and to inform our decisions.

In this current year-long review, we are conducting an objective and thorough review of new science.

In this process, we continue to build on our belief, that by understanding how a chemical works in the body, its mode of action, we can better interpret dose response data and better understand life stage susceptibility, and that factors that lead to susceptibility.

In this SAP, we will also discuss the connection between atrazine's dosimetry revealed by the toxicological analysis and its -- the connection between this and potential exposure to atrazine, as revealed by drinking water monitoring.

We plan to use this information to determine the frequency of water monitoring necessary to ensure the continued protection of public health.

As you will hear from the next speakers, the literature review contained in our

draft issue paper represents a moment in time with respect to the state of the science.

We are aware of important new studies, which further elucidate the mode of action for atrazine, that are not yet available for review.

We expect some of these studies to become available, as we move toward preparing materials for the September SAP. We believe these new data will provide information to more confidentially establish the causal links between key events in the pathway to toxicity for the hypothalamic-pituitary-adrenal axis and the dose response of these key events.

Dr. Mendez will mention some of these key ongoing studies in her presentation later this afternoon.

So, I would like again, to thank you all for your commitment and your efforts, on our behalf. We look forward to your thoughtful deliberations over the next few days, and I would like to introduce our next speaker, Dr. Anna Lowit.

DR. LOWIT: The large green button, yes.

We're going to spend the next few days talking about a lot of very detailed, very technical, very sophisticated, very exciting science, about some fairly diverse topics, from drinking water statistics, all the way down to hormone regulation, which on the surface, could be -- you would not expect to talk about those same things at the same meeting.

But what I wanted to do, before we get into the technical presentations, is just take a few minutes and sort of start from the beginning, start from -- talk about where we need to be, how we're getting there, and really, what the goal is, over the next -- that we're in the middle of, and we'll keep working.

The 2009 science and decisions report from the NAS, one of the key messages in that report is that the Agency needs to do a better job at aligning risk assessment and risk management, and specifically, putting more

emphasis on problem formulation, at the very beginning of the process, beginning of analysis for the risk assessors and risk managers, to sit down and have very detailed dialogue about the goal of the analysis, what the purpose is, what the scope is, what the data available for analysis is, and what the risk management possibilities are, so that the risk assessors can work with those possibilities in mind, to put together a risk assessment that the risk managers can use, to make them much more -- improve utility.

In this example of what we're going to talk about today, and what this atrazine effort is really about, is exactly what the NAS wants us to be doing.

It's about taking a risk management need, which is drinking water monitoring, and ensuring safe drinking water and aligning that with a lot of very sophisticated science to get there. That's exactly what we're going to talk about.

So, the risk management goal here is really to have safe drinking water, whether it's all over the country or just in the Midwest. So, we're going to talk about two major topics.

We're going to talk about hazard assessments and the basic goals of hazard assessment are to think about sensitive populations and life stages, thinking about durations of exposure and dose response relationships.

In exposure assessment, we really want to think about the residues in the drinking water and how confident or uncertain we are about those values, because the relative confidence and uncertainty gets at how much monitoring we need to be doing.

So, we're on a very long process, you know, it's a very big process, it's accelerated. We started the SAP process in February. Some of you were at that meeting.

As Steve said, the report from that meeting just came out a few days ago, and I would

say my first read of the report is that the report is very consistent with what we heard of the meeting, that the Panel was overall, very supportive of the approach we're proposing, with some very appropriate versions, I think, in fact, they've already started making.

So, to the -- the meeting this week is about thinking about animal toxicology, in vitro toxicology and drinking water monitoring.

Originally, we had hoped in September, to do a cancer/non-cancer harmonization, in a weight of the evidence analysis, and to take that and to merge it with drinking water and have a new proposal, but as you heard from Dr. Bradbury, our friends at the National Cancer Institute will not have their studies from the Agricultural Health Study ready in time for that. So, that's been postponed. The cancer component has been postponed to 2011.

However, we are maintaining the schedule for September, on the non-cancer effects and largely, as I said at the beginning, this is

really the rubber meets the road analysis. At the end of the day, this is about drinking water monitoring and is the drinking water adequate, and largely we believe that that's going to be driven by the non-cancer effects, as they tend to be shorter duration.

The longer term cancer outcomes are much longer in duration, which would be less frequent monitoring.

Okay, so, a lot of what we're going to talk about over the next few days is about the hazard assessment component.

As you will hear from Dr. Mendez, this is -- what we're going to talk about today and what's in the Agency's issue paper is really a snapshot in time.

People are doing atrazine work all over the world, all over the country. People that are in labs are continuing atrazine work. It's really a snapshot in time, of where the state of the science was at the end of January.

We know that between now and the

summer, there are going to be more studies, and in fact, probably some important studies to think about dose response and life stages, and Dr. Mendez will talk to you about those later.

But our goal for September is to take our current issue paper with the revisions that we hear from all of you, the suggestions that we get, the comments we get from the public, and to blend in that new analysis with all the new data and to do a formal weight of the evidence analysis, like that we proposed back in February, at the February meeting.

Where we will take the animal toxicology data, the in vitro, the modeling, the epidemiology and put it together in a mode of action framework, to think about key events leading to toxicity, dose response and temporal concordance, do explicit evaluation of life stages pre, post and perinatal effects, and we're going to talk about human relevance of those effects, that the qualitative and the quantitative difference between the animals,

particularly rodents and humans, and all these effects, and that's largely where the epidemiology will come in.

On the exposure side, on the drinking water, you will hear us talk almost exclusively about drinking water, because that is the pathway of concern for this chemical, and as we talk about the rubber hitting the road, on this analysis, it's the thinking about the duration, the temporal aspects of the toxicology, that will drive those frequencies, and there are a variety of ways to think about this, single day peaks, or you can do multiple day averages of any of duration you choose, basically.

I believe we'll show some examples of shorter ones, maybe three to seven days. The 90 day rolling average, it was done in the last 2003 risk assessment.

A large component of the drinking water analysis has to do with really understanding the confidence and the data, the data that we have and the data that could be

collected if there was a different frequency.

It's important to think about it, both temporally and spatially, and you'll hear Nelson Thurman talking about that later.

You'll also see this map later. Nelson will talk about it quite a bit in a little while, but I think it's a nice illustration that, as you look at the blue and the dark-blue and the red areas, that the areas in the middle part of the country, particularly those where large row crops are grown, are really the areas where levels of atrazine -- we're concerned about in drinking water.

Okay, so, in light of the new science that we'll talk about, particularly on the hazard assessment, in the feedback we hear from you, the feedback we get from the public, we're going to do two things.

We're going to decide if a full-blown risk assessment has to be done, and what that means in practice is that right now, the focus is on updating the literature review, deciding of

the points of departure, i.e., the line in the sand where the assessment needs to move. The uncertainty factors need to be changed. The duration needs to be changed.

If one or all of those need to change, based on the new science, we'll think about that, if a new risk assessment needs to be done, and we'll also decide if the drinking water monitoring requirements for Syngenta need to be changed, and that's all I've got.

SESSION CHAIR PORTIER: Before we go on to Dr. Mendez's presentation, are there any questions from the Panel, any clarifying questions?

(No audible response.)

SESSION CHAIR PORTIER: Okay, we'll move on. Dr. Mendez is going to be talking on the human health effects.

DR. MENDEZ: Good afternoon. Before I get started, I want to apologize. I started coming down with a cold over the weekend. So, if my voice breaks in and out, or if I start

coughing, I apologize for that.

So, the goal of this presentation is really to give you a very brief overview of the review of the mammalian toxicity data that's been conducted, as part of this reevaluation.

I'm going to talk a little bit about our peer review history. I'm going to talk a little bit about the 2003 risk assessment, to put this evaluation into context, and then talk a little bit about the current reevaluation.

I'm going to defer the really technical talks to our colleagues from the Office of Research and Development, but I just wanted to set the stage, so you know what's coming, over the next few hours.

Atrazine is very widely used in the United States. It's approximately 70 million pounds of the active ingredient per year. It's used primarily on corn. It has been registered in the U.S. since the 1950s, and it has been the subject of numerous SAPs over the years, both on topics relating to human health and ecological

effects.

Today, what we'll be doing is, we'll be concentrating on human health effects. Review evaluation of ecological effects will be coming in the next year.

All right, so, a little bit about the peer review history. We had first review of the rat mammary gland tumors. We were seeing tumors in the Sprague Dawley rats and so, we came to the SAP, the first time around, to talk about that.

The SAP, at that point, pointed to the Agency, that we should consider the possibility that there was an endocrine component to this, and to try to further evaluate what the mode of action for this might be.

Back in 2000, the SAP evaluated the mode of action on mammary gland tumors, reproductive and developmental findings in the rat, and also weighed in on the human relevance.

In 2003, we had the evaluation of the prostate cancer and the epi studies and the relationship between atrazine exposure and

prostate cancer in workers at an atrazine manufacturing plant, and as Dr. Lowit just mentioned, in 2009, we had a very brief meeting for the permanent members of the Panel, telling you about our plans to re-open or to start the evaluation of the atrazine human health effects evaluation and in February 2010, we had -- we presented to the Panel a draft framework for incorporating epidemiology and human incident data into the risk assessment using a couple of epidemiology studies from atrazine as part of our case studies.

So, very briefly, the 2003 human health risk assessment, and the points of departure for the 2003 risk assessment are based primarily on our understanding of the mode of action at the time, the perturbation of the hypothalamic-pituitary-gonadal axis.

In 2003, there were a series of key events that were identified, the hypothalamic effects resulting in changes in catecholamine function and regulation of the pulsatile release

of GnRH, leading to the attenuation of the LH surge, sensation of ovulation with ensuing persistent release of estrogen, increased prolactin release of the pituitary as a secondary consequence, resulting from the elevated estrogen levels, and the prolactin in estrogen induced proliferative processes in the mammary gland leading to tumorigenesis.

So, basically, what the HPG axis perturbation told us is, we had attenuation of the LH surge, which led to our mammary gland tumorigenesis that we were seeing in the rats, as well as the delayed puberty onset.

When we came to the SAP back in 2000, what their -- the recommendations of the Panel, or what the input of the Panel was, that these mammary tumors were not relevant for humans, and the reason for that is that the processes that lead to these tumorigenesis in the rats are very different from what would happen in the human.

What we are seeing with the LH surge is that we have a constant stimulate, with the

attenuation of the LH surge, is that we have a constant stimulation of the mammary gland by the elevated levels of estrogen and prolactin, and that is what leads us to that mammary tumor development.

In turn, in the humans, that's -- this entire process of reproductive senescence, which is what's happening in the rat, is not happening in the humans.

However, the Panel also recognized that these effects may be relevant to assess potential non-cancer adverse reproductive effects in the humans, and that's how our 2003 risk assessment is based on.

So, these are the non-cancer effects. The acute exposure, which for the Agency is one day exposure, is based on delayed ossification in rat fetuses and decreased body weight gain in dams. We do not believe that that is the result of the LH attenuation.

It has an NOAEL and an LOAEL of 10 and 70 milligrams per kilogram per day. These short

exposures are based on the delayed puberty onset in males, with an NOAEL of 6.25 and LOAEL of 12.5 milligrams per kilogram, and the intermediate and long-term exposures are based on the attenuation of the luteinizing hormone surge.

Okay, so, our current analysis, what are the objectives? And some of these, Dr. Lowit had mentioned.

We wanted to conduct an objective and thorough review of the new data, integrate new information with existing data, to evaluate the hazard ID and the dose response relationship, determine the extent to which this new science leads us to believe that we may need to develop a new risk assessment, and reconsider, if appropriate, the frequency of drinking water monitoring conducted by Syngenta, with the atrazine registrar.

The scope of the current analysis, we restricted this analysis to mammalian toxicity and in vitro studies. Three databases were searched, EMBASE, PubMed and Medline, and we

actually cast a very wide net in an attempt to collect all relevant information generated since the IRED was signed in 2003.

A database query yielded close to 300 articles. However, some 200 were excluded because either the studies were conducted on non-mammalian species, the articles were commentaries or letters to the editor, they were abstracts that lacked sufficient detail in methods and results, or they were topic reviews that did not present original work, or they were epidemiology studies that we will be looking at, as part of our epidemiology data analyses.

In addition to all of that, we also -- of the peer reviewed articles, we've also included some studies submitted by the atrazine registrant, and those have been placed in the docket.

So, now, to give you sort of an "upcoming attractions". The presentations that you will hear this afternoon, Dr. Ralph Cooper will be speaking about the mode of action

analysis. Dr. Bob Luebke will be talking about the immunotoxicity analysis. Dr. John Liccione will be speaking about the neurotoxicity analysis.

At the end of their presentations, I'll be coming back to sort of give you a summary of what that -- those analysis lead us.

Then, our colleagues from EFED and the Office of Water will be coming in. Dr. Nelson Thurman will be talking about a purchase to evaluating water sampling strategies.

Dr. Mary Frankenberry will be talking about evaluating the performance of sampling strategies, and Dr. Michael Messner will be speaking about artificial neural network modeling, and one little bullet that's not included there is, at the end of the day, Dr. Lowit will come back and sort of, wrap mammalian toxicology and the drinking water evaluations together.

So, if there are no other questions, I will turn the mic over to Dr. Cooper.

SESSION CHAIR PORTIER: Dr. Chambers?

DR. CHAMBERS: Let me clarify one thing that you said there. On slide eight, you talk about the NOAEL and the LOAEL for acute. You really mean just one exposure, don't you, and not per day? That wasn't an extended exposure under acute, was it?

DR. MENDEZ: No, for acute, we assumed one exposure.

DR. CHAMBERS: One, so, it's not per day, just --

DR. MENDEZ: But it's from a study that has multiple exposures. It's a developmental toxicity study on the rat, where animals are exposed -- parental animals, maternal animals, are exposed, typically from gestation day six through gestation day 21.

It is the Agency's policy that we assume that developmental effects of that nature, because of a rapidly changing environment during development, may be the outcome of a single exposure.

SESSION CHAIR PORTIER: Dr. LeBlanc?

DR. LeBLANC: In your discussion of the literature review, you mentioned that non-mammalian studies were not included, and I was wondering, were they excluded, with respect to evaluating mode of action?

DR. MENDEZ: Well, we looked at them briefly, but they were not -- to the extent that they did inform the data, we considered them. But primarily, we were trying to concentrate on the mammalian toxicity.

SESSION CHAIR PORTIER: Okay, let's move on to Dr. Cooper, who will be talking on the MOA of atrazine.

DR. COOPER: Thanks, Liz and Anna. My presentation this afternoon is divided into, essentially, three parts, where again, this time, I'm going to do a little historical review of what we considered the mode of action, back in 2003, that atrazine alters the hypothalamic-pituitary-gonadal axis, and do a more pictorial than Liz did, but with some added details that I

think will help us interpret some of the more recent findings.

The second part of this presentation will look at the rationale and studies that we've been looking at over the last several years, indicating that atrazine may have an effect on the hypothalamic-pituitary-adrenal axis, clearly showing an activation of that axis, and trying to fit that into the mode of action and then, the last part is a brief attempt to do some synthesis with the effects that we've observed and that literature has reported of both these different regulatory systems.

I put some background slides in here, just to get everyone on the same track. This is the way we look at the estrous cycle in the female rat.

Most of the studies, I'll talk about in the adult animal are in the female, and the regulation of the LH surge, of course, was the key event, endocrine event, that was used for interpreting the mode of action, back in the last

Science Advisory meeting.

The rat has a four-day reproductive cycle, four-to-five-day reproductive cycle that's readily identifiable and measured by just simply following the vaginal smear of the animals and see if distinct changes take place over the course of those four or five days.

The key day is, to us -- the endocrinologically key day, anyway, is the day of vaginal proestrus, which is right here, and I've got in cartoon fashion, the indication that what happens on that day is in response to rising levels of estrogen and subsequently, increases in serum progesterone, coming from the ovary and perhaps, the adrenal gland, there is a triggering of a massive outpouring of luteinizing hormone from the pituitary gland that occurs at a very distinct time on the afternoon, approximate -- and peaks approximately two hours before dark.

That event is what then leads to ovulation and over the evening hours, the animal is sexually receptive. So, the timing of

ovulation synchronized with the behavior of the animal.

Because the rats are spontaneous ovulators, there's a lot of homology between the neuroendocrine regulation of the rodent reproductive cycle, ovarian cycle, and the human.

This is another cartoon of how we see the different parts, and again, to address the mode of action of the HPG, I have the rat brain, the hypothalamic, the hypothalamus, which is the origin of the -- the neuropeptide is secreted from the brain, through the portal system that bathes the interior pituitary gland, GnRH stimulates luteinizing hormone, luteinizing hormone then stimulates the maturing follicle to rupture.

Part of this whole system is that you have other hormones, FSH and prolactin. We've shown in the past that prolactin also appears to be a target of atrazine disturbances, and then this whole process involves feedback from the ovary itself, the steroid hormones estrogen and

progesterone, feeding back onto the brain, to further regulate -- regulate regulation and reproduction and behavior.

This is the kind of data that you see when you measure luteinizing hormone on the vaginal proestrus.

This particular slide was taken from a series of studies that we did, looking at the intact female that was dosed with what I guess now, we can -- based on the earlier work, relatively low doses of atrazine, where we sought to find a low LOAEL and NOAEL for this compound in the intact animal, and you can see a dose-dependent decrease in luteinizing hormone occurring on the afternoon of proestrus at 1800 hours, which as I mentioned, was approximately -- is two hours prior to the time lights are out in these studies, and that was the only time that you see the suppression and importantly, the suppression of the surge isn't a complete blockade of the surge. It's lowered in a dose response fashion. But these animals may, at this

time, since they're young adults, still be able to ovulate.

Again, by way of background, these are data taken from an earlier study that we did, showing that atrazine did block the LH surge, in two different strains of animals, the Long-Evans and the Sprague Dawley rat.

This first slide makes the point that in the Long-Evans rat, if we dose one day with increasing concentrations of atrazine, this is all by gavage, you can see that a single exposure really doesn't do a whole lot to the secretion of LH.

What I have here is the dose response on the side. These are animals that received the different doses at 16, 18 and an hour before and an hour after lights out, and even up to 200 milligrams per kilogram per day, we're still getting an LH surge in the animals, to 300. You seem to see suppression.

If we dose those animals, however, for three days in a row, you see a dramatic decrease

in the amount of LH that's secreted, the control animals here, being in the open bars and the different doses you see there.

There was the lower dose, an apparent delay in the peak, where it occurred at 2000 hours, an hour after lights out, and I should add that other work we've done showed that those higher doses, those animals don't peak, even if you test them or evaluate them later on, in the dark phase of the cycle.

If you look at that figure on the right, the three day dosing in the Long-Evans rat, it brings up another interesting thing.

First, of course, the main point I wanted to make with this slide, is that as the duration of the dosing increases, the effect that the -- the effective dose decreases.

But the other point was that when you compare strains, three days later, there's -- we've ran Sprague Dawley rats in the exact same study that you're reviewing here with Long-Evans, and you'll see that when you look at those

animals, which I have plotted on the right figure over here, there was still no effect in the Sprague Dawleys, even after three days of dosing.

These are ovariectomized, estrogen-primed animals, given the compound for three days, whereas the Long-Evans were affected at three days, the Spragues kept a pretty good size LH surge.

However, if we continued dosing both strains for a longer period of time, then eventually, you would see that this compound had an effect on the peak amplitude in all the different doses in both strains, and the way this works is that over here on the right panel, when we dose for 21 days, we only looked at 1800 hours. We're pretty convinced that at those doses, we'd see the peak there.

So, these are animals that were killed at that time, control animals at 1800 in both strains, and then you dose response down.

So, again, the longer the duration of dosing, the more effective the dose would be.

Another series of studies that we did back in 2000, were to look at whether or not the disruption of the LH surge was dependent upon changes that were occurring in the brain or the pituitary or the broader axis itself, and in those studies, we took apart the system, essentially.

First, ovariectomized the animals, thus removed the ovary. We still saw effects on the LH, so we sort of thought we were ruling out the ovary at that time.

Other studies we did is, we took the pituitary out of the animal, that was dosed, and looked at whether or not it could still release luteinizing hormone the same way control animals would do, and what you have here is a perifusion system, where the animal's pituitaries were placed in a bath where the medium was flowed over the tissue for a period of time, went to baseline, and then we dosed with gonadotropin-releasing hormone, which is the peptide that normally stimulates luteinizing hormone.

Pulsed it once, saw a response, pulsed it again, pulsed it -- I'm sorry, then we looked at KCL, but the point is, is that there was no difference there, which indicated to us that these pituitaries taken out of animals that were dosed for four days, were perfectly capable of responding to the natural releasing factor.

Another study that was done by Dr. Lee Tyrey, who was a visiting professor from Duke OB/GYN, working in our lab, looked at the pulsatile release of GnRH and here, what we have is a 28 day ovariectomized animal who will start to spontaneously surge -- show LH pulses that occur at a frequency of about two per hour, and in the control animals -- and these are mean data, so they're not really as pretty as what we really had, but he puts it together through a program, and can show you the means.

But control animals, you'll see these pulses, which reflects the activity of the GnRH neurons in the brain. We can measure the LH and every pulse of LH is reflective of increased

activity in GnRH.

When we increase -- expose the animals to 50 or 200 milligrams per kilogram -- I can't read that, I hope it's 200 -- 50 and a higher dose, from my eyes, I'm sorry, immediately prior to this time, you can see that the 50 had a little disruptive effect, but with the pulsar analysis that they did, we didn't see any real statistical difference, but that higher dose totally flattened out the pulses.

What this meant to us is that atrazine, in some way, was disrupting the brain's control of the release of the hormone itself.

The next study, or in another study, later on, in the same series of studies, where we were looking at the intact animal, we measured the amount of GnRH in the basal part of the brain, where the neurons -- or the basal part of the brain, where you have gonadotropin releasing hormone neurons, and we saw that the concentration of GnRH tended to go up in the atrazine-treated animals, which would be

consistent, again, changes in the brain occurring and would be consistent with the decreased pulses that we saw when we looked at them in the longer ovariectomized model.

And then finally, we did a study where we blocked the LH surge in ovariectomized animals with a very high dose of atrazine and went in and injected IV gonadotropin releasing hormone, and it showed that the pituitary was perfectly capable of responding to the neuropeptide, again.

So, these kinds of things told us that it appears as though atrazine was working on the central nervous system to disturb the pulsatile release of GnRH, and I haven't showed it, prolactin release, and in that way, we thought that it was disrupting the ability of the animal to maintain normal ovarian cycles.

Another key point, and again, Liz may have touched on it, was, what's actually happening is that as we dose these animals, we were lowering the LH surge and, in a way, that was analogous to what you see during the aging

process of the animal.

Normally, the female rat, under a four-day cycle, has an abundance of LH secreted between -- just prior to time of lights out. But actually, to get ovulation, you only need a small amount of that.

Back with the old reference prep, it was about 2,500 nanograms per mL for a peak, and the people that work showed that you only needed about 200, or less than a tenth of the LH, to actually cause ovulation, which used to lead them to speculate what the rest of that LH is doing.

But as the animal ages normally, or if the animal is treated with atrazine, sufficient doses, what happens to the LH secretion is that the peak lowers and actually, the onset of the surge occurs a little bit later, sort of like what we saw with that 50 milligram per kilogram dose.

It can still cycle, again, because just like those animals I showed, even though they're statistically bound, they can still

function to cause ovulation.

As the animal continues to age further, it goes below that threshold. The animal doesn't ovulate anymore, which I show up here in this picture that everyone loves, my symbol for ovulation. Those animal's eggs are retained in the follicles and those follicles are secreting estradiol only into the serum, but not progesterone.

Another thing that happens, and again, as Liz mentioned, because of that increased estradiol, you end up with diurnal and a nocturnal peak of prolactin being secreted.

So, you have an endocrine milieu, that's totally out of bounds with what it should be, and the hypothesis that we worked with on that MOA was that atrazine, like aging, causes a decrease in this LH surge, which leads to an early reproductive aging in the animals, and when the animals show the persistent or constant estrus then, it will lead to the early appearance of mammary gland tumors.

So, again, that's just it, in bullet form. And the summary is that atrazine disrupts hypothalamic control of gonadal function and it contributes to early reproductive senescence, producing that hormonal environment, and again, a key point is, it's well known that reproductive aging in the rat, as I have up there, is driven by brain.

We could argue that. I know the work of Tuck Finch and Brower argue that ultimately, it's the amount of estrogen that that brain sees over its lifetime, but really, the events taking place within the central nervous system are what's disrupting the ability of the animal to produce the LH surge and it still has plenty of follicles left in its ovary, so that when the ovary stops cycling, there's lots of estradiol that's put out there into the blood.

Reproductive aging in the human is quite different. It's apparently driven by a depletion of the follicles in the ovaries.

In turn, you have a situation where

with aging, you have a relatively low concentration of estradiol and different endocrine milieu altogether.

So, that's where the Agency concluded that the mode of action for mammary gland tumors in the rat was not relevant to, or would be predictive of anything that would happen in humans.

But what happened in those studies, when we were doing that work, was, a number of different outcomes showed that there were clear other relevant reproductive effects that were occurring when the animals were dosed with estradiol.

Some of those are briefly mentioned here, and we talk about different life stages and that kind of thing. This is part of what was driving some of that.

Atrazine will delay puberty in animals. There was a study done by Tammy Stoker and Susan Laws, in the male and female rats, respectively. Those studies were replicated by

other laboratories. Those studies were -- or similar studies were conducted with the validation of the pubertal assays in the -- for the endocrine disrupting screening program.

So, it was a robust effect that was repeatable, that delayed puberty does occur when you dose animals with atrazine.

Other adverse outcomes -- and we know the toxicity pathways for some of these were prostate -- prostatitis, if the dam is dosed during specific developmental periods.

We know the disruption of ovarian cycles occur, and I should mention that the registrant did a very nice study, looking at the age-dependent changes in ovarian cycle, or estrous cycles in the rat and again, showed that the reproductive cycle does decrease earlier. It wasn't just a hypothesis, that in fact, these things occur.

And more recently, there's been a number of studies looking at testosterone synthesis that I think again, are going to become

-- maybe, we can interpret them easier, once we understand what's going on with the adrenal axis, but I think -- in the background paper that you received, I think there was four or five studies that have been published in different strains of animals, saying that atrazine, for an extended period of time, relatively high doses, will impair testosterone synthesis in the developing -- - most of the studies, in the developing male rat.

So, again, where we were with the 2003 SAP, there was general agreement that this pesticide or herbicide was working through a disruption of CNS-mediated decrease in LH and that was the relevant mode of action, and the key event in this whole process was a decrease in luteinizing hormone that contributed to those adverse outcomes, of course, with the exception of the prostatitis studies, which were linked more with changes in prolactin availability.

That's what the considered mode of action was, but yet, there were still some

nagging questions that existed, at least, in my mind. One of them was that delay.

When you dose animals and you disrupt the LH surge, we've worked with a number of different environmental chemicals, and you do this, usually, it's a very -- if it's working through the brain, it's a very rapid, almost -- you know, within hours kind of effect that you see, and that business where we saw, it took several days to start to see, there is this trade-off between dose and time that tended to have an effect on the LH surge, always was kind of intriguing to me, and I -- I just couldn't put together what it was that was responsible for that.

Secondly, in Dr. Stoker's study, where she looked at everything that she could get her hands on in the pubertal male, she looked at the concentrations of estradiol that were present in those animals, and found a significant increase in the serum estradiol in the males treated for 21 days. That's peripubertally. They start the

treatment on PMD-21 and kill on PMD-42.

Susan Laws -- we asked the question whether or not that was true in adult animals as well, because you really see, at the right dose of atrazine, an increase in estrone and estradiol in these animals, and Susan Laws replicated that study here, with only a four-day exposure.

And what she found was an increase in both those estrogens, in both the developing animal and in the adult, and you know, a high dose and -- it was something that we, you know, really didn't know what to make of, at the time.

But, at that time, there were a number of other studies reported, either in vitro or in vivo, looking at atrazine and the enzyme aromatase, which was the enzyme that's responsible for the production of estrogens from androgens.

So, that led us to look at little more closely at what might be going on, both in vitro and in vivo, with aromatase and estrogen, and whether or not that might be part of some of this

-- something that we missed, in terms of the mode of action.

So, at that time, because of the in vitro studies, we were looking at -- we were looking at aromatase or one of the cytochrome P450s, which converts the androgens to estrogens.

Aromatase is present in a number of different tissues in the body, some there listed, the brain, the gonads, fat and placenta. In the brain, it's responsible or plays a key role in sexual differentiation of the rodent brain.

Its regulation is different in the different tissues looked at. You have different promoters and other regulatory factors that are involved, whether you look in the ovaries or the placenta or the breast, and then there is alterations in -- it's been shown that alterations in gene expression that disrupt the estrogen availability are going to eventually impact not only reproductive development, but a lot of other normal functioning of different

tissues.

So what we want to do over the next few slides is to review the evidence that atrazine does alter aromatase activity, both in vivo and in vitro, and I think set the stage for why a lot of the things that we're looking at, if we stick with this one enzyme, wasn't really necessarily making sense, that we may have to broaden our scope of investigation a little bit bigger, to look at the whole process of steroidogenesis.

This is the study, including -- actually, the second in a series of studies by Thomas Sanderson, that -- looking at the chlorotriazines, and put everyone onto the idea that atrazine and its metabolites might be affecting the production of estrogens, and what Sanderson did is he used this cell-line up here, the H295R human adrenal carcinoma cell-line, which is kind of a neat and unique cell-line because it possesses all the key enzymes, P450s that are necessary to produce a number of

different steroids, right on out to estradiol. So, it does contain aromatase.

What Sanderson showed was that at, you know, fairly decent concentrations, around three micromolars -- decent, I'm saying compared to what some of the studies I've seen in the literature show, around three micromolars, you start to see an increase in what he termed the activity of that enzyme.

You saw an increase in the production of either tritiated water or the estrone itself, if -- in those cells.

But what was also interesting is that he found that only atrazine, the two intermediate metabolites that I call them, that's isopropyl and desethylatrazine, increased aromatase activity.

Sanderson also looked at the ability of atrazine to change the message in these cells and reported that there was a modest twofold increase in the message, along with that, indicating that exposure to these compounds, to

these three metabolites, anyway, could increase the overall production of estrogens. And importantly, and we'll come back to it in a moment, DACT had no effect on these endpoints, and that's the one there in the square blocks, in red, if you can see it.

So that's the -- one of the -- if you look in the blood of the rat, anyway, that's one of the really prominent metabolites, that that had no real effect on aromatase activity in this -- in the H295Rs, and one of his colleagues replicated these studies, looking at the JEG cells.

What that did for a time was put a lot of focus on aromatase itself. The light was shining there, and a number of different studies, looking at changes in estrogen production, all came to the conclusion that you could see increased estrogen synthesis, and again, this cell-line was used for the validation of some of the in vitro tests, and I think nine labs replicated the effects of atrazine on aromatase,

so -- or, the production of estrogens.

But one of the interesting things that we found was that in addition to just estradiol alone or estrone alone in there, you could see increases in other hormones, and recently, a paper published by Higley said that not only does atrazine increase estradiol or estrone, depending on what you're measuring, it also increases testosterone.

And in our own lab and others have also shown that some of these other earlier steroids in that pathway are increased when you stimulate with atrazine, or certain of the atrazine metabolites, which suggested to us that it's not just aromatase that is the target here, but in fact, increased steroidogenesis might be really what the whole series of changes that are taking place is.

There are some other studies focusing on SF-1, as a target site for this SF-1, as a co-factor involved in the regulation of aromatase. Others have said that that's not necessarily the

case. You may see changes in SF-1, but in fact, that's not necessarily the only target, that in fact, you do see a broader increase in a number -- - activity of a number of different singling pathways, and that's what these different studies down here show, and again, these are in the background document.

But the point is, is that in addition to aromatase per se, there is this overall effect on steroidogenesis that seems to be the "mode of action" within the cell. We're not sure exactly what all the molecular events are.

There is an argument that put forth, actually earlier on, by Sanderson, that atrazine, in some way, increases cyclic AMP activity, perhaps through a decrease in phosphodiesterase. That's something that can be -- has been substantiated by other labs, but the papers aren't published yet.

There is a study that I just read, where they block protein kinase activity and that reversed the effects of atrazine on the

activation of steroidogenesis in Leydig cells.

So, that paper is going to be out shortly, so I guess it's okay if I tip my hat on that one, but the point is again, somewhere upstream, a broader effect on signal transduction seems to be the cellular toxicity pathway and that the aromatase per se is not necessarily the sole target for these chemicals.

In vivo, again, as I mentioned, we were interested in whether or not atrazine can affect aromatase in vivo.

Walter Modic, who was a student with Dr. Laws' lab, did a series of studies looking at male rats to see if he could identify changes in aromatase activity or the message for aromatase, after being treated with atrazine at relatively high doses for -- in several different ways, and to make a long story short, it's nice to have a lot of slides, but when you get no effects, you end up with a slide like this that says, "Yes, you can induce increases in serum-estrone and estradiol in the male rat."

This is a different study that we did, showing that in the ovariectomized female, you can see increases in estrone activity at relatively high doses of atrazine, but nowhere was there -- were they able to identify changes in aromatase, either looking at microsomes taken from testes of animals treated, or, could they see changes in the message, looking in brain testes or adipose tissue.

So, it just -- if it's there, it's real difficult to find any specific change in aromatase activity.

This summarizes that series of studies, so we still have increases in those hormones. It increases the estrogens. There was little or no evidence to support the idea that it was working specifically through aromatase, either increasing the activity or the message.

What we had, and I don't want to get into it, because there's a lot of controversy over whether or not the adrenal hormones can produce -- the rodent adrenals can produce

androgens, such as androstenedione, and this literature is -- depends on which side you want to take, but the point is, is that these animals did have increases of estrogen in them, and we were thinking that perhaps one of the sources of this was from the adrenal gland.

And that led us to ask the question whether or not atrazine might have some kind of direct effect on adrenal steroidogenesis.

Here it is, 2007, and we've been looking at this for all this time and it seemed a little odd that no one else would have reported that.

There were dribbles and drabs in the literature and there was a couple of elegant studies by Dr. Pruett's lab, that said in mice, at the time, that atrazine does increase steroidogenesis from the adrenals.

So, we decided to look into this axis, arguing about whether or not the adrenal hormone secretion would be altered by atrazine, whether or not that effect would occur because of some

kind of central targeting of the compound or whether or not the adrenals may actually be targets themselves, since we know in vitro, there were effects on steroidogenesis.

So, Susan Laws published this study, some time late last year, demonstrating that there is really a clear effect of atrazine on both ACTH secretion and corticosteroidal -- or I should say ACTH secretion and a number of different adrenal steroids, and this is from that paper, where she gave doses ranging from five to 200 milligrams per kilogram orally, and looked at the production of atrazine -- I'm sorry, the production of ACTH and corticosterone at different doses.

And I should just mention here that these animals are all dosed for five days prior to testing. They're dosed with the vehicle carboxymethyl cellulose, that they're all dosed at the same time of day, at the nadir, I think it's nine o'clock in this study, when the concentrations of corticosterone and ACTH are

low, and so, when you look at the control animals there, the actual dosing procedure itself seemed to have minimal effects on the secretion of ACTH or corticosterone, which speaks well for the ability of that lab to conduct these studies.

But what happens is, as you increase that dose though, there is a clear increase in both ACTH and corticosterone, as well as progesterone and we're -- I'm pretty confident that this is adrenal-derived progesterone.

It's in male rats, and if you look at the testosterone that's being secreted, this is probably from -- or more than likely from the test, as you can see a significant increase there, as well. The time course is a little later. The dose required is a little higher.

Another important thing that Susan found was that when you dose with DACT, you saw no comparable increase in ACTH or corticosterone, which was sort of like what Sanderson had reported for the H295R cells.

Now, for some reason, that particular

metabolite of atrazine wasn't active, in terms of increasing the adrenal axis, and I have their DACT and ACT and atrazine on the right side, just so you can compare, there were significant changes, if you, as Liz likes to say, worship at the altar of the star, but I don't think they were really as dramatic as what you see over here with atrazine.

And the other thing is that the timing there, I don't think there was a delayed response in that, but again, nothing like what you see with the parent compound itself.

Another thing that Melanie Fraites is working on in our lab, did, was a series of studies to further get at whether or not this was a chemically specific activation of the pituitary adrenal axis or it was still some artifact that we were introducing, that we didn't know about, and we thought every -- tried to think of everything that we could to get at that, but up to this point, what we've done so far is, Melanie has shown that if you do things like cut the

vagus nerve, it doesn't interfere with it.

So, it didn't look like atrazine was getting down into the gut and causing some type of intestinal distress that was going to feed back.

There didn't seem to be any change in some of the endocrine components of feedback from the gut to the brain, and I think, to me, anyway, one of the most convincing things that you can get around the -- any signals from the gut being the cause -- the root-cause for this increase in ACTH, is her observation that you can get the same response if you give the chemical IV.

So here, you have an animal that's sitting there with an in-dwelling catheter, you dose with atrazine and you see the same immediate 15-minute increase in atrazine -- I mean, in ACTH and corticosterone.

Another thing that Dr. Laws did was look at not only atrazine in DACT, but the other two intermediate metabolites, not shown here, is DEA, but both atrazine and DIA do cause the same

type of adrenal response.

In this case, I think it's important to note that the LOEL for that study was 10 milligrams per kilograms, which is the equimolar dose to 12 and a half milligrams per kilogram of atrazine, and the other interesting thing in this study was, it wouldn't go away.

The increase in corticosterone -- now, she only measured corticosterone in these animals. She didn't measure ACTH here, but in these animals, that response persisted. It was there at the higher dose at four days and even out to 21 days, there was a significant elevation of corticosterone.

And this is something that was indicated in the female, in a study by Dr. Fraites, who showed that one dose of atrazine -- again, in this case, she used 75 equimolar dose of DIA, caused an increase in both ACTH and in corticosterone, but then, if she dosed for four days, an interesting pattern was present there, that we're still working on, in terms of

interpreting it.

But if you look at this, this is a single dose, and this is ACTH. The green here, the darker green, is the same animals. This is one dose; this is four doses. These are intact animals that are dosed on the day of vaginal estrus, diestrus one, diestrus two and the morning of proestrus, and they're killed 15 minutes after dosing. So, that response, that ACTH response there is markedly attenuated.

But if you look at the cort response in these animals, it's almost the same as it was on the first day. That's what you see when you restraint-stress the animals.

So, it's a response that's seen, at least in this case, to persist, and the other thing is, in these animals, I wanted to point it out, was that when she dosed with both DIA and atrazine, that lower dose is 12 and a half milligrams per kilogram, or 10 milligrams, the equimolar dose for DIA, and again, there was no real clear effect with this on -- with that. It

didn't change the adrenal axis.

And in those animals -- or in another population of animals treated the same way, she looked at the LH surge and showed that again, as we showed, I think, on the first slide, that there is a decrease in the LH in the afternoon at the same dose as the 12 and a half milligrams per kilogram for atrazine and the 10 milligrams for DIA.

There was a suppression of the surge with DACT. It wasn't significant in this figure; it was significant in others. The point is, is that DACT, I think you can get an effect, but you have to go longer and higher, to see inhibition of the surge, or a suppression.

So, in summary, in this series of studies in the male, both ACTH and adrenal steroid hormone production increased within -- as early as 15 minutes following a single injection of atrazine and DIA and DEA.

The dose response for the male study, at the moment, with that data that Dr. Laws had,

the lowest dose was five. Her next highest was 50. So, it was somewhere between 50 and five, but the LOEL that she found for DIA was at 10 milligrams per kilogram.

Compared to atrazine and DIA, the increases in ACTH and the adrenal steroid hormones is markedly less when you dose with DACT in equimolar doses.

That, in the male, corticosterone release was sustained for up to three weeks and in the female, we only went out to four days, we started to see a decline in the amount of ACTH secreted in those animals, suggesting, again, that there may be some direct effect of atrazine on the pituitary gland itself, in addition to that major increase in corticosterone release, in response to ACTH after the first injection.

And again in the female, we have a low or no NOEL, of 12 and a half milligrams there, and it's important that I mention this, and we just only have -- I'm already going on too long, but atrazine also induces comparable changes in

adrenal progesterone secretion in the female. Take the female's ovary out, you can see -- unmask those kinds of effects that are significant, both at one and four days of exposure.

Comparing those effects with what you saw, and I'm going back to what we saw with the effects on the LH surge, either in the ovariectomized estrogen-primed animal or in the intact animal, what we found was that in those earlier studies, which is the first three bullets there, with different LOELs, you have a single dose. You needed more than 200.

With three doses, we dropped it down to 50 and in an intact animal, we could get it down even lower.

It still indicated that you needed a number of days to see an effect on the surge, and we did a lot of studies early on, looking only on the day of vaginal proestrus, trying to either block ovulation or decrease the amplitude or timing of the -- often, the timing of the LH

surge, and we couldn't get anything, again, with a single dose up to 200, and if we gave 300, the animals went pseudo-pregnant on us. So they were still ovulating, apparently, or appeared to be ovulating.

So, the timing of this and the fact that you see these instantaneous changes in the pituitary adrenal axis, and given the literature, arguing that the adrenal axis is involved in -- or could be, if there is hyperactivity in the adrenal axis, could be involved in the disruption of LH, it suggested to us that we see changes that precede and may likely contribute to the disruption of the LH surge.

And so, that's what I tried to put together on this figure, where we have our hypothalamic-pituitary-gonadal axis, wherein we know that, time and again, people have published that when you expose in vivo to atrazine, you get a decrease in the amplitude of LH, you end up with, eventually, acyclic animals and the disruption of reproduction.

I have over here, the adrenal axis, where we know that -- and we're not clear, exactly whether or not -- obviously, I think, there's got to be a central effect, either hypothalamus or pituitary, given the rapid response that you see with AC -- and the response that you see with ACTH.

However, you see that sustained response in the adrenal. Whether that's due to direct effect of atrazine on the adrenal in vivo, we don't know, but I'd bet a dollar that you'd see it in the primary adrenal cells. And, given that effect and the speed with which we saw that and the timing for the pituitary effects, and the rich literature on this stuff, it argues that one of the primary effectors in -- or one of the events responsible for the decrease of the LH surge, may be that feedback of the adrenal steroids onto the brain and pituitary and the ability of it to regulate the LH surge.

Another thing that I think this kind of conceptual framework argues is that, if you

look at the high dose 21-day exposure of male studies, that seem to be popping up in the literature time and again, that that decrease in testosterone, in the gonads of the males exposed, that that effect may be due to a direct action of corticosterone on the Leydig cells.

Again, there is a literature, quite a strong literature, that shows that Leydig cells do have glucocorticoid receptors and when activated, and they're regulated by a -- a process within the Leydig cell itself.

But when there's over-stimulation of those glucocorticoid receptors, there is actually a down regulation of the enzymes involved in the production of testosterone, including the key one like STAR and some of the other CYPs, so -- and that's well recognized.

So there is another pathway that may explain the data that we see in the male rat, and then, finally, there is the data that shows that there is perhaps, a direct activation -- or I'm sorry, a direct inhibition of the GnRH pulses by

CRF in the brain, wherein, stress and other events that increase the activity of that neuropeptide, which acid increases CRF -- or, I'm sorry, increases the activity of CRF and ACTH, that you get a direct innovation -- inhibition on the pulses.

So those things make me argue, or feel, that a key part of this whole mode of action on the gonadal axis is this increase, at least early on, increase in the activity of the pituitary adrenal axis, and that's what's summarized here.

Again, it has a clear, acute effect on the HPA. We just don't know, haven't well characterized the longer-term effect, increasing both ACTH in the adrenal steroid hormones, that the temporal aspects of atrazine induce changes in the pituitary adrenal axis, and the pituitary gonadal axis, along with the similarities in the dose response -- that's what I was trying to get at there -- for four more days, suggest that the changes in the adrenal axis contribute to the

disruption of LH secretion and impairments in reproductive function.

And so, the current studies broaden our understanding of this mode of action for atrazine, incorporating those changes that we've already identified and others, in the pituitary adrenal axis, and those that likely contribute to the disruption of LH in the rat. Questions?

SESSION CHAIR PORTIER: At this point, do we have any questions from the Panel? Comments? Yes, Dr. Horton?

DR. HORTON: Yes, actually, I have a lot, but I'll save some.

I'll start with another possible model, besides the reproductive aging and the reduction of the LH surge, and ask, do you have measures or does anyone have measures, on what the LH levels look like on diestrus or metestrus, for the rats?

DR. COOPER: Yes.

DR. HORTON: And what are they?

DR. COOPER: The one thing we've seen

consistently, even on the morning of proestrus too, is no change.

DR. HORTON: They're not elevated?

DR. COOPER: The majority of studies that we've personally done, when we look at it, it appears as though baseline hangs in there.

If we really increase the concentrations of atrazine up to 200, I'd even argue 50 and above, it's lower.

DR. HORTON: Okay.

DR. COOPER: But we haven't seen any indication -- well, I have seen one, we do have one set of data that says we can get an increase, but it's in the afternoon, when we looked at very low doses. So, I don't know whether it's --

DR. HORTON: Okay. The reason I'm asking is, I'm wondering if it might fit a PCOS, a polycystic ovarian syndrome model; and also, has anyone looked at the body weights or body compositions in these rats?

DR. COOPER: In the earlier --

DR. HORTON: In the females, yes.

DR. COOPER: When you do the longer-term studies at those high doses, they're down.

DR. HORTON: They're down? Okay.

DR. COOPER: Significantly, but when you do the -- like, the first slide I showed -- I mean, the early studies that were done were, I think, hitting them with a hammer --

DR. HORTON: Yes.

DR. COOPER: -- and you do see marked decreases in body weight, but when we look at the shorter-term, lower dose things, we don't see a whole lot of difference.

DR. HORTON: Okay.

DR. COOPER: I mean, we don't see a difference. It's the same.

DR. HORTON: Okay.

DR. COOPER: That's not -- I should -- I have to qualify that, because sometimes, you say you don't see a lot of difference and they're not eating. But no, that's --

DR. HORTON: Okay, okay, but that's in terms of body weight, but no one has actually

looked at body composition?

DR. COOPER: Not to my knowledge.

DR. HORTON: Okay, no one has done a glucose tolerance test?

DR. COOPER: We have, yes.

DR. HORTON: Yes, from what I --

DR. COOPER: I'm sorry, yes, we've measured glucose, and we haven't found any change with blood glucose over the four days that we did the study.

DR. HORTON: Okay, thank you.

DR. COOPER: So, we didn't do a glucose tolerance test, no.

DR. HORTON: Okay, thank you.

SESSION CHAIR PORTIER: Dr. LeBlanc?

DR. LeBLANC: There's a lot going on here. A couple of questions, for clarification first.

In these studies, have you ever looked at the adrenal mass? Do the adrenals enlarge in size?

DR. COOPER: Yes, I'm trying to think

of the -- again, we got into looking at the adrenals kind of late. So, I'm sort of embarrassed to say, we don't have a real characterization of changes in body weight, nor histopath, and they may have it in some of the longer term studies that were submitted for registration. I haven't had a chance to look at them.

But what we see is almost an immediate enlargement in the adrenal gland, dose dependent, and then it settled back down.

PARTICIPANT: Did you have one in the --

DR. COOPER: And in the pubertal assay, they were increased, the male pubertal. But, yes.

DR. LeBLANC: The 2009 study by Laws, these were done in animals that were not gonadectomized, is that right?

DR. COOPER: Right.

DR. LeBLANC: Because you said, you thought the testosterone came from the testes.

So, obviously, they had testes, and I guess I agree with that presumption.

In terms of where the progesterone is coming from, is that because most of the progesterone in the blood comes from the adrenal? You said you thought the progesterone --

DR. COOPER: In females, on vaginal proestrus, there is a number of studies. One of my PhD mentors, was his claim to fame, with -- showed that the majority of progesterone comes from the adrenal vein --

DR. LeBLANC: Okay.

DR. COOPER: -- on the day of proestrus.

DR. LeBLANC: Okay.

DR. COOPER: I wanted to just throw this up. This was a slide -- this is a gonadectomized slide, where the animals were -- again, it was a study that Laws did earlier, wherein, she looked at both androstenedione and testosterone, here on the right, in response to 50 to 200 milligrams, and the estrogen production

over here on the left, and what was -- and this was an intact animal.

So, this is your testosterone here, and this is androstenedione -- I'm sorry, this is androstenedione in the intact animal, and in the castrated animal, and this is testosterone in the intact animal and there is nothing there, in the castrated animal.

And we've actually looked at the adrenals of these animals, to see if we can find androstenedione using HPLC, and we see it, and you know, you're not suppose to have CYP17 in the adrenals, and there are papers that say under certain circumstances and during development, you can see CYP17, that there is a methylation silencing that's taking place, and I just don't understand it.

But the point is, is that those data, to me, argue pretty strongly, A) that it's something coming out of those adrenals, if nothing else, it's androstenedione, and over here, there is a production of estrogens that

persisted and if you look at the Y-axis, they're not that different, after castration in the male.

So, back to your progesterone question, I'm sorry.

DR. LeBLANC: One last point, for clarification.

The cartoon you had at the end, the conceptual cartoon, I thought it was very helpful, and in there, you had the inhibition of steroidogenesis by clinical steroids, at the bottom.

DR. COOPER: Yes, right there.

DR. LeBLANC: Right, so, over and above all of this, are you suggesting that there is something else going on, that's contributing to the increase in testosterone, that -- we're not seeing a great increase, but we are seeing increases.

DR. COOPER: Right, okay, good question. Number one, I think those studies, looking at decreased steroidogenesis are really the result of some pretty high doses, 50, 200

milligrams per day.

I know that there was one that went five, ten, 50, 75 and 100 milligrams per day, from -- in the perinatal, even. But most of them are in the intact animals, peripubertal or going on into adults.

So, they're high and I think what's happening there, if you do a single dose with this, you'll always see an increase in testosterone. You'll see an increase in testosterone, and if you take the Leydig cell out, and you stimulate the Leydig cell, you'll see an increase in testosterone, you'll see an increase in progesterone, okay.

That's been done at APA, with Gary Klinefelter and there is a publication coming out soon, that that's the case.

You put that cell back in the body and can expose it to the glucocorticoids for an extended period of time, increasing glucocorticoid activity by a number of different means, if you want to stress them, and you do

that really mean stress, where you put them in the restraint cage, and -- for an hour a day and shine a light on them, and you know, you really keep the cort up, that will overwhelm the protective mechanism within the Leydig cell and you do see this decrease.

And there is studies now, even showing that that process, that glucocorticoid activation, stimulation -- hyperstimulation, that receptor is even bad for the developing animal.

So, it's a biphasic or bimodal effect. Initially, what you see in vitro and what you see with a single dose is increased T, extended dosing -- and I'm hypothesizing through increased adrenal activity, you're seeing perhaps, a direct effect on the gonads, and then of course, what you're supposed to see is that when you decrease T, the compensatory increase in LH, and the studies that Susan and Walter Modic did, didn't really show a whole lot of decreased baseline LH in those males, and we can go back and look at that.

So, that's why I'm leaning a little bit, towards it being a direct gonadal inhibition there.

But again, it could be a central mechanism, but I think -- that's central -- you know, I -- there is a paper by Zerkin and Ewing, years ago, that said, "How much LH do I need to keep secreting testosterone," and so, they took the pit out and they put silastic capsules in, or somehow, they fed them LH at real low levels, and it is -- you'd be surprised how low they went, before they would see a decrease in T.

SESSION CHAIR PORTIER: Dr. Selvage?

DR. SELVAGE: Yes, I just had a question regarding the CORT/ACTH measurements.

Do you have any time points that aren't in that -- where you measured these, that aren't in that frame, right after the atrazine administration, in the chronic studies?

So, for instance, are you disrupting circadian rhythm or anything like that?

DR. COOPER: Yes, Melanie, in the

female, what we did -- in other words, if you perturb it in the morning, is this -- the afternoon is going to be altered --

DR. SELVAGE: Yes, or the -- yes, or was it -- before, you know, you would normally give it in the morning, and what would be CORT-level?

DR. COOPER: The data that we have in the -- it's a four-day exposure. We looked at the dose immediate responses, but then we also looked at the afternoon increase in progesterone, because if you -- we were thinking that maybe progesterone later on in the day was being impaired.

And there -- in CORT, and Susan -- well, number one, is that there -- I think it was the high dose -- Melanie, was the high dose of -- which one knocked down P4 in the afternoon?

Yes, the high dose DIA did decrease progesterone in the afternoon, but for the rest of the things that we treated with and doses that we treated, there was nothing there, which was

surprising to me.

DR. SELVAGE: How many hours was that?

DR. COOPER: That would be -- yes, let's see, that would be treat 9:00 a.m. and you're looking at an hour before. So, you're looking from three hours, one hour and one hour after lights out -- three hours before lights out, 1600, 1800 and 2000 hours.

DR. SELVAGE: Okay.

SESSION CHAIR PORTIER: Dr. Delclos, please, use your mic.

DR. SELVAGE: I'm sorry, but you don't have it like, at say, eight? You're dosing them at nine, right?

DR. COOPER: When we did the proestrus, we did the -- the day of proestrus, we measured every two hours, up to 2000, from nine to 2000, and most of the dramatic changes we saw were the early --

DR. SELVAGE: Right.

DR. COOPER: -- ones, because, you know, it's coming up, and one of the things that

Susan found in the male was that when you look in the afternoon, if you try to increase cort in the afternoon, that's one of the things she wanted to do, you could get it to go any higher.

There's sort of like, a ceiling effect there. She couldn't get it to go up.

PARTICIPANT: It was still up then.

DR. COOPER: It was still up? Right, yes.

SESSION CHAIR PORTIER: Dr. Delclos, I think was next, and then, Dr. Akana.

DR. DELCLOS: I just wanted to make sure I understood. Does this mechanism play into the delay of puberty at all? Where you take the DACT having effect on that, but not having effect on the -- it was something that confused me, as I read the document.

DR. COOPER: There's two questions there, and the answer is, I don't know, to number one.

I mean, boy, you read the pubertal -- and you guys are the experts, and you read the

pubertal and adrenal literature, especially earlier stuff, I was really -- it's really hard for me to understand it, because you get this -- when you do things like adrenalectomy and all this kind of stuff, you seem to see the -- you seem to have an impact on the body weight of the animal, that might confound this a little bit.

Conceptually, I could make that argument, and there have been some kind of sophisticated explanations, as to how, if you dose with particular things that either inhibit or activate the glucocorticoid receptor, you could impair -- slow down puberty.

But the question about DACT, it's a separate question, because I think DACT -- I'm almost confident that it has to eventually impact regulation of the LH surge, and when I say it has to eventually do it, I mean, by dose, and by duration.

If you look at the blood levels in the rat, and we have limited data for this, but when we look at it, DACT is present in such high

concentrations, even -- no matter what you give them, and then you give them DACT, this equimolar dosing thing is -- it's nothing like what you really see inside the animal, when you measure it. You have very high concentrations of DACT.

And that's even if you -- you know, if you dose them with atrazine, the amount of DACT is way up there.

So, why in the pubertal assays, you see that, I don't know whether there's another mechanism kicking in or what, but it is -- it is something over there, that you can't dismiss. It doesn't always fit the picture.

I guess, if you were really making the argument that the adrenal was involved, you'd want no effects, with DACT, but that's not what -- the way the system was designed, and it bothers -- or it's something that we can't explain at the moment.

SESSION CHAIR PORTIER: Dr. Akana?

DR. AKANA: Could you repeat that finding in the hypothalamus, in the Modic study?

What I'm thinking about is the possibilities of central action of atrazine, and there are aromatases in the hypothalamus and there are some nice papers showing very site-specific brain localizations, like BNST, and a lot of those sites are also very gonadal-steroid sensitive.

So, it seems the framework could be there for some central regulation.

DR. COOPER: If I understand your question right, do you mean in the adult or the developing animal, first?

DR. AKANA: Adult first.

DR. COOPER: Okay, Susan, you looked at -- go ahead, do you want to --

SESSION CHAIR PORTIER: Please identify yourself, if you come up.

DR. COOPER: This is Dr. Susan Laws, and she's the --

DR. LAWS: Hi, I'm Susan Laws, from EPA. The question you were asking, the Modic study, we were looking at the hypothalamus for

message, for aromatase CYP19, and those studies indicated that after they were treated in vivo, there were no differences in the message, and we looked at -- after a single dose multiple time points, we looked after three doses, daily doses, and then at -- I think, that had a couple of time points also, and we were not able to see any differences in that.

So, it's -- it's challenging to measure, so it's a no-effect study, is what we have.

DR. COOPER: But the reason I asked about the developing animal, of course, is then, that's a whole new ball game or a whole different ball game, because of A) the concentration. Susan tells me they're much greater in the neonate, and so, if maybe there is one opportunity where there may be effects that we haven't looked at, that might be important.

DR. AKANA: Also wasn't there also some discussion of a critical period, in development for the aromatase?

DR. COOPER: Yes, well, early on, I mean, that was one of the driving forces behind her actually measuring the younger animal, because during development, the aromatase was coming down and we figured if we -- what Stoker found was a reflection of the earlier increased importance of the animal.

But we didn't look at the peripubertal or anything that would -- we haven't, yet, looked at the peripubertal -- we haven't looked around birth. That's what I'm trying to say, not puberty, perinatal period.

SESSION CHAIR PORTIER: Dr. Williams?

DR. WILLIAMS: I just want to switch gears a little bit, to get you to clarify the comments you made several hours ago, regarding the mammary gland tumors.

You said with aging, the cycles become irregular, the LH surge doesn't happen and this endocrine milieu in the rat promotes a development of mammary tumors, and then in your summary slide, you said that reproductive aging

is driven by the brain in the rat and by the ovary in the human, and therefore, the mammary gland tumor development isn't relevant to humans.

I don't really see that connection, though. It seems to me that you should be more concerned about the reproductive endocrine milieu, potentially leading to mammary gland development in humans, whether or not reproductive senescence is an issue as a separate story. Can you clarify that for me?

DR. COOPER: Let me repeat the question, because that was a long one.

DR. WILLIAMS: Okay.

DR. COOPER: I want to make sure I get it right. You're asking me, you said that we looked at the aging animal, but you're talking about mammary gland development, or are you talking about mammary gland tumor development?

DR. WILLIAMS: So, this is directly from your own slides, the nine and ten.

DR. COOPER: Okay.

DR. WILLIAMS: So, basically, you said

that you don't get the LH surge, so you don't have regular cycling and you end up getting in the -- in the rat, loss of CNS regulation, and that this endocrine milieu causes development of mammary gland tumors, and then in your summary slide, you said, because reproductive aging is different in humans, then the endocrine milieu isn't really relevant to human mammary development -- mammary --

DR. COOPER: If I said that, I misspoke. The hormonal environment, I think, is important, in both species, I mean, obviously.

I think that what happens with normal aging, and the fact that atrazine appears to accelerate that aging process in the rodent, is different than what you would anticipate in the humans, okay.

So, in the humans -- I mean, in the rat, what we're doing is, we're driving a faster pace, disruption of regular cycles and earlier onset, and really, I think once that cyclicity is disturbed, the pattern of constant estrus sets

the clock for when you're eventually going to get a tumor.

How long you expose estradiol, how long you keep your other hormones out of balance, the more likely you're going to see the tumor over time.

When I said it wasn't relevant, from the standpoint of these studies to humans is, that humans don't go constant estrus when they stop cycling. They have not -- they have insufficient number of follicles to -- the reason they stop ovulate -- I'm sorry, the reason they stop cycling their menstrual cycle is because they lose the follicles, critical mass there, and there is a few still left in there, like, 200,000 or something.

DR. WILLIAMS: Right, but as Dr. Horton brought up earlier, in cases where women don't really ovulate, for example, polycystic ovarian syndrome, they actually do have elevated levels of estradiol that are fairly, you know, prevalent throughout the cycle, rather than the nice peak

at pre-ovulatory and then lower during the rest of the cycle.

And so, it seems like the hormonal milieu may be fairly similar and --

DR. COOPER: Oh, yes, if you want to go -- okay, now, I understand your question better.

Yes, especially in light of some of the recent papers in the rodent modeling, trying to develop a model for polycystic ovarian disease, wherein they argue that the induction of constant estrus in the rat is going to produce, not only the endocrine environment, but also, some of the histological changes down within the ovary itself, that are reminiscent of, you know, the persistent follicles that you see.

Earlier on, in that literature, people -- and where I did my turnaround was the work that Ojeda has done lately, you may be familiar with -- or not lately, but when he started publishing on it, I began to believe that polycystic ovarian disease may be something that might fit into this picture, with the rodent

literature.

Now, in that case, I don't -- I would agree, that that's an important consideration. How that plays into the whole atrazine thing, I'm not sure of, because it's different.

I mean, those studies would probably -- those studies with the persistent estrous animal are done in a young adult female, and I'm not familiar, if there are any where they started to look at that kind of homology, if you will, between what you see in the aging female rat and what you see in someone with PCOS.

But, yes, now I understand your question and I'm in agreement with you.

SESSION CHAIR PORTIER: Dr. O'Byrne.

DR. O'BYRNE: Point of clarification. You said that you've tried blocking the LH surge with atrazine, in an acute fashion, and failed miserably. With other compounds, it works.

DR. COOPER: Right.

DR. O'BYRNE: To block the surge.

DR. COOPER: Yes, we've done a number

of studies using things like dithiocarbamates, which -- or and other fungicides that works to specifically disrupt neuroadrenergic transmission, and usually, we give them, somewhere during the critical period, usually about 15 minutes prior, in our -- between two and four o'clock in the afternoon, and very rapidly, you see the decrease in neuroendocrine and then the decrease in pulses.

DR. O'BYRNE: Okay, so, you know the mechanism action is quite clear?

DR. COOPER: Yes.

DR. O'BYRNE: Just another point, if I may. I'm sure we're desperate for some break and some tea or coffee.

Your pulsatile data, I don't know whether it was yours or somebody else's, but averaging pulsatile data and then trying to describe it as pulses, is -- it's very difficult to appreciate.

DR. COOPER: Well, yes.

DR. O'BYRNE: But you said you had the

individual data and you put it through the pulsar program, and the 50 milligrams, which is a whooping great dose, had no effect. Is that --

DR. COOPER: In the mean, right.

DR. O'BYRNE: No, no, no, in the individuals, and I don't know how many animals were --

DR. COOPER: Yes, this model --

DR. O'BYRNE: Do you have access to that?

DR. COOPER: Do we have access to the data?

DR. O'BYRNE: I have not seen those individual profiles. I'm just curious, as to whether there was a suppression of frequency, which you can't read from this average data.

DR. COOPER: Right, boy, that was 1997. When we did DMDC, we did the -- these are 28-day females, you put the catheter in, beautiful pulses, okay, and what you see, in the individual animal -- and the question that you're asking is, you inject them with the dithiocarbamate. It's

pulse, pulse, boom, okay, like, some data I've seen, other people have pub, and it's immediate, you know.

You know, and then they'll hang down there for three or four hours until it clears and then, you can see the pulses resume.

For Dr. Tyrey's data, for the 50 -- I know -- I can tell you, what it looked like for the 200, okay, pulse, pulse, flat, all right, and then D-flat, and then you'd see an occasional burst-through with the different -- now, an individual animal, you would see this gigantic pulse, like the GnRH hormones have to dump, that kind of thing.

But the 50, I think with the individual animals, you'd see a broader -- the broader pulses. So, it's a slowing frequency, but the amplitude was not down all that much. They're still doing okay, and boy, that was an old memory trace that, to pull back.

SESSION CHAIR PORTIER: I think at this point, we are going to take a break. Dr. Young?

(Off the record comments.)

DR. YOUNG: So, I've been kind of looking at this, you have to understand, I'm a statistician, so, that's going to be my kind of question.

So, do you do analysis for each time period or do you synthesize and to one large analysis?

DR. COOPER: For which study?

DR. YOUNG: Well --

DR. COOPER: For the --

DR. YOUNG: -- pretty much, all of these? So, for example --

DR. COOPER: When we do this --

DR. YOUNG: -- the ones on page 20 -- on slide 26, the ones on slide 23?

DR. COOPER: Twenty-six and 23, boy, Stoker, I hope these are your data.

SESSION CHAIR PORTIER: So, you're talking about coming up at the little stars on the top?

DR. YOUNG: Yes, how do you get that

star?

SESSION CHAIR PORTIER: How do you get those stars?

DR. YOUNG: Yes, you're right.

(Off the record comments.)

DR. YOUNG: I saw, on one of your -- well, on -- I couldn't find the Tyrey paper, if that's the way you --

DR. COOPER: That's an abstract.

DR. YOUNG: Okay, I saw the abstract, but where I -- no, I couldn't -- no, I didn't see that.

DR. COOPER: We have it in the 2007, but I don't --

DR. YOUNG: Okay, I couldn't -- one, I couldn't find the 1997 one.

DR. COOPER: Yes, please don't take me to task on the pulsar, because --

DR. YOUNG: No, no, I'm just --

DR. COOPER: -- we can --

DR. YOUNG: I'm just trying to figure this out.

DR. COOPER: Right, but here is 25, is that what you want?

DR. YOUNG: No, no.

DR. COOPER: Oh, the LH surge?

DR. YOUNG: Something like that.

DR. COOPER: Right, these are -- who did this?

DR. YOUNG: So, did you do one at each time point, or did you do one analysis for the whole graph?

DR. COOPER: The whole graph.

DR. YOUNG: Because sometimes -- in the captions, it talks about by time point. So, my assumption was, analysis was by time point and -- but I'm not sure that's right.

DR. HOLLADAY: I think that is, for this.

DR. YOUNG: This is the time point --

DR. COOPER: She did the analysis, let her speak.

(Off the record comments.)

SESSION CHAIR PORTIER: Can I suggest

that we hold this and that you guys talk about it at the break, and then we'll come back with that question?

DR. YOUNG: Excellent suggestion. Thank you very much.

SESSION CHAIR PORTIER: I know it's a large group of people, and a 15 minute break is very difficult, but my watch says 3:15 p.m. So, we'll be back at 3:30 p.m., on the dot. Thank you.

(Whereupon, the above-entitled matter went off the record at 3:15 p.m. and resumed at 3:33 p.m.)

SESSION CHAIR PORTIER: I was ready to get started, but Dr. Cooper wasn't here, so, I can't.

So, we're going to continue on and see if first, EPA can answer Dr. Young's question about analysis, and then, I think Dr. Williams wanted to come back to her question, to see if we can get that clarified.

DR. YOUNG: All right, I --

SESSION CHAIR PORTIER: I remind the Panel, this is our time to get clarification on the presentations and the material on the white paper.

So, it's not quite right on the slides, but you've got something in the white paper you can point to, we can go with that, as well. So, Dr. Young?

DR. YOUNG: I think I got clarification in the break. Some of the more recent papers do look at the whole study, some of the older papers, even though an effort has been made to look for proper analysis, do do time-by-time analysis, and some, we don't have the data to even know one way or the other.

In addition, I think there was a slight misspeaking, in that, one study in which we talked about a rat, after one dose, in the same rat, four doses later, those were actually different rats. So --

DR. COOPER: They had to be different rats. They were killing them.

But the one other thing that's important is that these data aren't transformed, and usually, when they do hormone data, they do the transformation and so, it's my estimation that these are really conservative evaluations, and I may be wrong in that approach, that if we didn't do the transformations because of the transformations, the amount of variance that you see, may change. Susan has just re-did a data set, doing that.

DR. LAWS: My data was --

SESSION CHAIR PORTIER: Identify yourself.

DR. LAWS: Susan Laws, U.S. EPA. My data that you see the -- the ACTH and the CORT-data, those that had the different time points, they were first analyzed with analysis of variants in -- for time and time of dose interactions.

But however, there is heterogeneity of variance in those data. So, those data, we actually used a non -- rather than transform

them, do a live transformation, which is usually done with hormone data, or can be done with hormone data, we used a non-parametric test for those and we did do those by time, each time it was done, in between.

And so, it was the analysis of the variants and then, there was a post hoc test for those stars, that you see.

Recently, we've transformed some data -- used a live transformation, and actually, it looks like they -- we have lower doses that are significant, not in this data set, but another data set.

So, I think the non-parametric test that we used for that report was fairly conservative, when it looked at differences between groups.

SESSION CHAIR PORTIER: Dr. Williams?

DR. WILLIAMS: Just to try to clarify, the mammary gland tumor development in women versus rats, and the mechanism of action you were talking about, can you clarify, maybe not what's

on your slide, but what is in the white paper, in terms of causing -- causative concerns regarding atrazine, LH surge lag, the endocrine milieu and mammary gland tumor developments?

DR. COOPER: You mean, the -- essentially, the strain difference? You mean, essentially, the strain difference, if you will, in humans versus rats?

DR. WILLIAMS: Why is it that you don't believe that the LH and endocrine milieu changes wouldn't be relevant to human mammary development tumor --

DR. COOPER: No, no, no, that's -- if I'm giving you that impression --

DR. WILLIAMS: That's why I wanted to clarify it.

DR. COOPER: That's not true, okay, that's not what we're implying, or I'm implying, is that the -- that environment that you see in the rat, is what's conducive for the growth of tumors, hyperplasia and then eventually, the tumors.

The human doesn't get to that condition, is what we're saying that, during aging in the human, what you see is a depletion of the follicles, and therefore, that you don't develop the high levels of estrogen when they go through menopause. Menopause is a period of low estradiol, or estrogen.

DR. WILLIAMS: So, then potentially, the mechanism of action would be relevant to a non-menopausal human, though?

DR. COOPER: If you can -- yes, that was a question that came up during the break. I would assume that if you could get a constant estrous human, a polyfollicular human maybe, I'm not familiar with one, a model like that, but if it's the case, then I don't know why that wouldn't be the same endocrine environment, yes.

So, yes, I apologize if I mislead you. I'm not -- it's -- that hormonal environment is the consequence of differential patterns of aging, rat versus human, and that's why the argument that the relevance that we see for that

particular outcome, in the rat, it doesn't appear to apply to humans.

And so, there is a -- it's sort of like, you know, I guess we make assumptions and this was our -- that argument was what we tried to make clear, the last meeting, and I -- rushing through that, sometimes, you don't get the -- all the little key events, like that. They don't come out right.

But your question is on target from the comment that, if you create this environment, rat or human or even -- you know, no matter what the strain -- I know there's a mouse strain. They say that atrazine doesn't induce mammary gland tumors in mouse, but there is a mouse strain that they used in aging, that was -- showed similar patterns of pituitary and mammary gland development in the female, when she grows older.

They showed it as a consequence of altered ovarian cycles and perhaps, if that strain is -- you should see the -- if that was

what you see, then you brought about the same premature reproductive aging as the experimental -- the studies show in that mouse strain, that you would see the same thing.

It's how you get there, or can you get there, in a human.

SESSION CHAIR PORTIER: Dr. Fenner-Crisp?

DR. FENNER-CRISP: Ralph, back in the stone age, when we were working on the mammary tumor MOA, this -- one could only say that atrazine had some effect, not otherwise defined, with respect to direct or indirect. The work hadn't been done to sort anything out, specifically, yet, adequately.

Now, with this new body of data, describing the disturbances in the HPA axis, the inferences, although you haven't stated it explicitly yet, that -- that may have to proceed the disturbance in the HPG axis for the mammary tumors, which would be indirect.

What evidence exists for or against

the -- a direct pathway being at play, and what could you do or have you done, to separate the two, to show whether or not there's still an opportunity for direct effects?

DR. COOPER: For the tumors outcome? There is a study that the registrant did, I think, that might speak to it, is that if the adrenal gland was actually driving the development of the tumors, independent of altered gonadal function, then you would see it, if you removed the gonads and dosed with atrazine for an extended period of time, removed the ovaries, in this case, and didn't they submit that study?

(Off the record comments.)

DR. COOPER: That would --

DR. FENNER-CRISP: Maybe that would --

DR. COOPER: That's the statistician --

DR. FENNER-CRISP: Maybe then the registrar could speak to my question, tomorrow.

DR. COOPER: I thought that that study was done. I guess, I misspoke.

DR. FENNER-CRISP: Okay.

SESSION CHAIR PORTIER: I think there was a hand in the audience, indicating that tomorrow, probably the registrant will speak to that, if we can --

I think at this point, we've kind of picked through this part and we're ready to move on to the discussion of the immunotoxicity. Dr. Luebke?

DR. LAWS: Go back to the -- whatever it's called, the hard drive. Go back to the hard drive. Thank you.

DR. LUEBKE: Okay, so I'm going to talk about some atrazine immunotox studies and basically, just summarize some of the work that has been done. I will not be going into the level of detail that Ralph did.

And because this is the first time through for immunotox, for the SAP Panel, I know that we have a couple of experts here, but for the rest of you, I thought it would be a good idea, just to cover just a couple of very general topics first.

So, immunotox is essentially unintended modulation of the immune system, and this can take the form of suppression or enhancement of certain functions that the cells do, and that the body does, and this can result in an increase in allergy, it can increase the risk of hyper-sensitivity, or autoimmune disease, and in very general terms, these mode of actions includes things like an altered supply in the cells, that would be coming in to perform these different duties.

There could be altered maturation of the cell types. There could be a difference in how the cells function or in the case of autoimmune disease, which we see up there, this could be a failure just to control auto-reactivity by some fairly sophisticated processes.

And the proximal cause of immunotoxicity can either be direct, so there are effects right directly on the immune system cells themselves or on their supporting tissues, or

they could be indirect, where there are effects that might perturb some other system and the fall-out from that perturbation would actually change how the immune cell functions.

So, host factors, as we have been talking about for the last little while here, can also have a dramatic effect.

So, up here at the top, you see that during -- well, that doesn't work, okay. During development and maturation of the immune system, it seems to be one of the more critical times for adverse effects on the immune system.

During this maintenance phase, when everyone is young and healthy, we can still get effects, and do, and we see those in adult immunotoxicity studies.

Gender and genotype can also have a big effect. Females tend to make a more robust response than males do, immunologically, and genotype controls a wide array of things.

So, the wide array of what constitutes normal function in an individual, normal function

across a broad scale of the population, and it also, as we've heard for effects on endocrine disruption, with Ralph, it can also control how an animal responds to exposure to an immunotoxicant.

So, mice exposed to very small amounts of dioxin get immunosuppression and doses many, many times, 30 times that high, don't have much of an effect on rats.

So, why would we do immunotox hazard IV studies? Sometimes, there is some clues from general tox studies, and these can be things like a change in organ weight, input organ weights with the cellularity. These tend to be sort of observational snapshots captured at one in time and it tells you sort of like, what the immune system was like, right then.

There may be a known or suspected mode of action. So, if -- we know that the compound alters protein-synthesis or cell division or alters neuroendocrine effects, that may be a clue that we need to look for immunotoxicity.

In some cases, testing is actually mandated for certain types of compounds, and they may be planned, like the NTP would look for effects, and these are generally functional effects that people are going to be looking for.

So, in those assays, you'll have some of those -- these observational snapshots, but what you might not have are these functional assays and what you will not have in the general tox studies.

So, in these functional studies, we ask the immune system to do something. So, it's generally challenged with an antigen, and then we measure the response to that.

So, the critical mode of action for evaluation of the immunotoxicity of atrazine was obviously, endocrine function.

We know that there are a variety of industrial compounds and pesticides and other compounds that are endocrine-active compounds, and they also have an effect on the immune system.

So, this first study that I'm going to talk about was done in my laboratory, at EPA in Research Triangle Park, and it was done by a postdoctoral student, Andrew Rooney, when he came through my lab.

He came out of Lou Gillette's lab at University of Florida, where he had studied the Lake Apopka alligators. So, he was very interested in endocrine disfunction and he had measured some endpoints in the immune system, as well, and this seemed like a perfect project for him.

So, he picked a dose of atrazine that would be just above what Susan Laws had reported for changes in the onset of puberty in female pups, and for a reduction in the NOEL of prolactin, in Tammy Stoker's studies.

Rather than including a dose response here, we included a couple of pharmacological agents, because we wanted to see if we could produce the same effects by altering prolactin production and we chose our dose there, to be the

LOEL for prostitis, and we also included PTU, propylthiouracil, to see if there was -- if we could do this by altering thyroid hormone production.

So, after the moms gave birth, we took the pups away. We segregated them out by gender and dose and then we re-distributed five males and five females to all the -- all the dams. We didn't make any attempt to return animals to their biological mom or to not return them, and we also got weights on the animals, at that day.

Then on day 14, we took one pup from each gender and we got thyroid hormone -- we got thyroid hormone levels on those animals and we also took a spleen and a thymus weight at that day, to see if anything had happened to those immune systems organs.

Then, once the animals became immunologically mature, we asked their immune system to do something by immunizing them to have them make antibodies or to make a cell mediated immune response.

We also looked at the ability of cells to produce phagocytosis, which can be the first step in resistance to certain sorts of infections, and we also evaluated natural killer cell activity, which is something done by specialized lymphocytes that kills tumor cells.

And we did these -- the assays -- the functional assays at a couple of time points, to see if the effects were persistent, and if you look at these data here, you can see that there was a real increase in the percent mortality.

So, two-thirds of the atrazine litters actually lost pups, and the numbers of litters that actually lost pups was also increased for bromocryptine. This didn't happen for PTU.

As Ralph mentioned earlier, we know that changes in prolactin levels will alter maternal behavior and we think, but don't know for a fact, that the reason that we had this increased mortality in the atrazine pups was for the same reason we did in the bromocryptine pups, because maternal behavior had been altered, and

they just weren't as good a mom.

We looked at the pup weights, to see how well they did during development, just as sort of a general measurement of pup health, and as you can see, we didn't really have any effects at all of atrazine in these animals at PND2.

We did pick up what was a statistically significant effect in the males, on PND7, but if you also look at the n value there, you can see that we had a lot of pups who -- this was a very large study and because of this, the one gram difference between the controls and the atrazine pups showed up as being statistically significant.

So, the bromocryptine and PTU had some effects as well, particularly on PND7.

Now, this one, I know is sort of busy. I'm going to try and show you here. These are body weights, going across here at the top, and you can see, there was no effect on PND14, in the body weights of the offspring, in the atrazine group, although the bromocryptine and the PTU

animals were still suppressed.

We also looked at the spleen to body weight ratio, sort of a somatic index, and spleen weights were not affected, nor were thymus weights affected in the atrazine-exposed animals.

They were, in the thymus -- I mean, in the PTU and the bromocryptine groups as well, but those were transient effects and PND62, they had all recovered.

So, this was a functional assay, where we immunized a large group of mice and then, we bleed them and we measured antibody titers, and what we found was there was actually a suppression in the male mice, but not in the female mice that were exposed to atrazine, and these effects were not re-capitulated by bromocryptine or PTU, and then a sub-group of those animals was given another dose a week -- two weeks later, of the same antigen, and we measured the IgG response and in this case, there was on effect on males or females.

We did a cell mediated immune assay,

where we had the animals respond to the swelling response to an antigen and what we found again here, was this sexually dimorphic effect, where there was suppression in the males, but not in the females.

This occurred at nine weeks, and I should mention here, on the antibody response, we looked again at six months and the effects that we saw on the primary antibody response were no longer apparent at six months, just at the -- at eight weeks of age.

So, here, we see that there was suppression of cell mediated immune responses at nine and 12 weeks. This did not persist out to six months.

So, in both of these cases, where IgM was suppressed and cell mediated immune function was suppressed, although it recovered, there was a time period there where the suppression could have rendered the animals more susceptible to certain sorts of infections, and these include viral infections and certain types of bacterial

infections.

There were also a number of negative effects that we had here. There was no effect on maternal weight gain, so, these effects, we don't think, were due to any sort of overt toxicity on mom.

There was on effect on phagocytic activity of these phagocytes at all. These phagocytes also do other things, like participating in antibody responses. So, we don't know whether they were responsible at all for suppressed antibody responses, but their phagocytic activity was not affected.

Natural killer cell activity was not affected and at least, you think these things might be refractory to any sort of developmental effects. Diethylstilbestrol given to neonatal female mice will suppress natural killer cell activity almost for life, in these animals. So, it is possible for an endocrine disrupting compound to change that.

We didn't see any effect on the

relative or absolute spleen and thymus weights, which suggests that there wasn't any sort of effect on the supply of cells that these animals had to work with, but clearly, somehow, their function had been altered, and we didn't seem to reproduce effects whatsoever, by using these pharmacological inhibitors.

So, this made us think that low levels of prolactin and low levels of thyroid hormone were not really to blame for the effects that we saw in the immune response, and so, since Andy had a background in endocrine disruption, he came up with the idea, before he talked to Ralph Cooper, that the GnRH may have something to do with this.

We know from a variety of different subjects, different studies, that this is sexually dimorphic, GnRH expression and the protein and the receptor expression are set early in development. They're sexually dimorphic.

Antagonists that are given to primates actually do suppress cell mediated and humoral

immunity in the males, but not in the females. The agonists, ironically, up-regulate effects on cell populations, not on function, but on cell populations in the female offspring, and we know that once this receptor is bound by its agonist on the lymphocytes, this leads to increased antibody production and it leads to increased cytokine production.

So, now, for the caveat, we did all -- we did those studies that I just described twice. Each time we did them, the data came out pretty much the same. Then, we moved about half a mile down the road, to a new facility, and because we didn't have a dose response, we went back to generated dose response, which we were unable to do.

We did the study three or four times. The same people were involved, that did the studies both times. We had used different lots of atrazine in the first study, and it didn't have any effect. So, I don't think that could have any -- you know, out -- bearing on the

outcome.

We did monitoring for parasites, the normal sentinel animal stuff in both facilities. There was no difference in infections.

I have a whole lot of confidence in both data sets, because of the things that I've just told you about. What I don't know is what this unknown environmental factor is, that in one case, the animals were suppressed, but in another case, at the same dose, they were not.

It could be something, just sort of an additive thing, with cage movement or something like that, some change in the way they were cared for, but we have not been able to figure out what that was.

So, there was another developmental immunotox study done. This time, it was done in mice and these mice were done for again, going back to these host factors that we talked about earlier, to see if there were differences in genotype.

The exposure here was quite a bit

different because they used a little implant that constantly delivered a trickling dose of atrazine, the same amount within a 24 hour period, and so, when they started dosing, they were at about our same level. They chose these doses, based on the studies that we had done.

But this had gone down to about 23 milligrams per kilogram per day, at the time that the animals were born, and then, they weaned the animals a little bit later than we did, at 28 days and then they assayed them, once they were mature.

They also found sexually dimorphic effects here, but what they found was that humoral immunity, the antibody response, was actually increased in the mice, where we had seen suppression, and it didn't matter how you -- how they expressed the data, whether they standardized it to the number of cells in the spleen or the number of cells in the spleen that were precursors to the antibody-forming cells.

They also found that there was an

effect on cell mediated immunity. They used different assays than what we did. The one on the left actually has -- causes cells to proliferate in response to foreign antigens. The one on the right, specifically armed cytotoxic T-cells kill target cells.

But just as was the case with the humoral immunity, what they found was increased reactivity in the mice.

And so, we talked about suppression in our animals, leading to a greater risk of infection. There are a lot of animal models out there that are shown. Compounds that cause this unwanted increase in immune reactivity can also make auto-immune disease worse or can lead to hyper-sensitivity and allergic effects in susceptible individuals.

They found the same sort of negative effects that we did. So, mom wasn't really affected in her weight gain, pregnancy rate, litter rate, things like that, were not really affected. There was no effect on offspring

weight gain, when they looked at it, at a single time period, so, the pups weren't particularly sick or anything like that, and because they didn't find any effects on the spleen weight, or they looked at the distribution of lymphocytes from within the spleen, for various types of cells. They didn't see any effects there, which would suggest that the supply of cells was not altered, but once again, the function of the cells was clearly altered because of what the outcome was.

So, what might have caused all of this? Well, we heard a great deal, a few minutes ago, about how much of a dose and for how long a dose, and what effects that might have on the outcome.

Rowe et al. put their doses sub-Q, and so, that means that not the same proportion of parent and metabolite is going to be produced in those animals.

But also, from the work that was done by Ralph Cooper and Tammy Stoker, it looks like

we might be looking at some sort of a qualitative -- I mean, quantitative effect there, by the amount of atrazine that was given, metabolite that actually reached the brain, but not a qualitative one.

So, that doesn't seem to explain the difference. The functional assessment end points were well recognized, and I think that they were really measuring the same thing that we were.

So, it comes down to species, differences in mouse strains, and we've already talked about how we can have very different effects, depending on what's the species of exposure.

The mice that they used were sort of prone to make antibody and allergic responses, but that might explain some of the effects that they saw, but not all of them, because both cell mediated and humoral immunity were up-regulated in those.

So, then the question is, are those results really in conflict? What are these

results telling us?

Well, we know that the effects on -- of gender were constant and the supply of cells seems to be pretty constant. It's genotype that's sort of, out of phase, and we can't really explain this.

However, we've realized, in the immunotox community, in the last maybe five to 10 years, that the thing that we really need to think about is immunomodulation and not really worry about whether we've suppressed the response or whether we've enhanced the response or whether it's allergy or what have you, we've caused something to happen that we didn't intend to happen, and so, immunomodulation is the thing that we tend to focus on.

So, those were sort of full gestation studies. There was one study that looked at just the late part of development. This was a study that was done in C57 black 6 mice, and you can see the doses that were used there.

They exposed these mice, starting at

four weeks of age, and these animals become immunologically mature around seven or eight weeks of age. So, what they did was expose the animals, just for this last period of the immune system development.

But rather than spend a lot of time on the details of the study, we can summarize it by saying that most of the effects that they saw, particularly at the lower doses, say, 25 and five, were sort of ephemeral and they were gone by a day.

Even at the higher doses, most of the effects had resolved by seven days, and all of the data that they used -- unfortunately, all of the data that they collected here were observational endpoints, and that can give you some really important information, if you know that there is a defect, and it will help you maybe choose what path to go down, to figure out what the defect is.

But all we know right now is that something happened in these animals and they

recovered fairly quickly.

There have been a number of adult exposure studies here, and probably the most complete one that has been done to date was one that was contracted by the National Toxicology Program, and was carried out by a contractor up at Medical College of Virginia, and they looked at a whole bunch of these different functional endpoints and observational endpoints, and they found very few effects in these animals at the lower dose group.

The one thing that is sort of interesting is that second bullet point there, where they found a 35 percent increase in antibody production, at 25 milligrams per kilogram, but it was perfectly flat across the rest of the spectrum, and so, I'm not really sure what that might mean.

They challenged the animals with tumor cells. They found that at the top two doses that -- the resistance to this tumor cell challenge was decreased in these animals, but when you go

back -- they also went back and looked at the primary effector cells in these animals, and those cells weren't really suppressed.

And so, I'm not exactly sure how to interpret those data either, but they were -- they occurred at some pretty large doses.

And they also had a lot, as I mentioned, these observational endpoints -- and none of those things really pointed to any sort of a clear picture of immunotoxicity.

They did look for antibody -- changes in antibody production, using the same assay that we did, and didn't find anything. They challenged the animals with bacteria that would -- that live inside cells and take a response of their adaptive immune response T-cells to get over it. They didn't really see anything there.

And so, we don't really have any clear picture in the NTP study, even at doses up to 500 milligrams per kilogram.

There was another study that was done in C57 black 6 mice and here, they based their

doses on percentages of the LD50, and you can see that the -- on the second line, the next to the highest dose, that should be 433, not 4,300. I think those animals would have been dead, without any questions, if you could have gotten that much into them.

So, there was really no effect on body weights in these animals. They had sort of a mixed bag, and I don't mean to be insulting, when I say a mixed bag of changes, but that's essentially what it was. There was no clear arrow pointing and even suggesting even one direction.

They didn't seem to have any effects whatsoever on the supply of cells, because they did the same sort of thing where they looked at spleen cellularity and the distribution of these cells, and there was no effect on the phagocytic activity of the animals.

They also took spleen cells out of these guys at different time points, and they immunized these spleens in vitro and then they

looked at the number of antibody producing cells that resulted from that, and it was sort of interesting there, at their lowest dose, at 25 milligrams per kilogram, they were initially suppressed and then, as they were allowed to recover out over time, over a period of about three weeks, that lowest dose came back to about control levels.

But at other doses, you didn't see that picture at all. The very highest dose jumped right back up to control levels at 14 days and then sort of dove back down to being suppressed and then went back up to being enhanced by 21 days, and the doses that came in between there were also somewhat mixed, like that, and again, I'm not really sure what to make of these studies, other than, most of the effects that they saw tended to be at the higher doses, with these effects on the antibody response, that I just don't understand.

Then, there were a number of really excellent studies that were done by Steve Pruett

and his co-workers, and they chose large doses of atrazine and large doses of ethanol, and large doses of some other compounds that they knew would induce a stress response, that they knew would really increase corticosteroid production, and their goal here was not really to do a hazard ID study for whether or not atrazine is toxic to the immune system or not, but rather, to see whether they could use this rate of production of corticosterone over time, as a predictor for how they would affect a variety of endpoints, including antibody production and things like that.

I happen to see that Steve Pruett is here at the meeting and I -- he may be talking about some of this tomorrow. He may not be, I'm not really sure. But these studies -- we know that at these levels, that corticosterone levels will definitely be up.

The interesting thing about one of Steve's studies was that he never did habituate the cort response after 28 days, the restraint

stress habituated alcohol habituated, but this didn't. This was sort of in keeping with what Ralph was talking about a few minutes ago, where you continue to see, when you expose the system to atrazine, that there's an increase in cort production.

So, if I was to try and sum everything up here in just one sentence, it would be that, we have pretty clear evidence that it's the developing immune system that seems to be the one that's more sensitive than the adult immune system, and it seems to be working through what might be similar mechanisms.

We never got a chance to pursue the GnRH thing, because we applied for funding, for Andy to continue his postdoctoral fellowship and that did not work out.

So, the other thing that I can say is that we really don't have any evidence to suggest that the developing immune system is any more sensitive than the neuroendocrine system, at least from the studies that we've done.

Then, just to sum it up, there's one more type of study that's been done, which is an in vitro study, and this is where they put different amounts of atrazine or the same amount of atrazine on the spleen cells, over time. They harvested those spleen cells and then they sent them onto one of these natural killer cell assays, and as you can see in the graph on the left, that the more atrazine that was given, the more suppression of the natural killer cell activity went on in vitro.

In the graph on the right, the dark black bars there -- I know that they're sort of hard to see, and I apologize. I copied and pasted out of a PDF, and it did not really work out well.

But the dark black bar is the atrazine on the right and as you can see there, the longer you put those cells in culture with 10 micromolar atrazine, the more suppression of the natural killer cell activity there is.

But if you look at the graph at the

bottom, these are the natural killer cell. This is natural killer cell activity from the mice in the NTP study, and what you can see there at the bottom is -- if I can make this arrow appear, right here is this one little blip, and this is at 250 milligrams per kilogram per day, and it is at the very lowest effector to target cell ratio.

So, in this assay, what you do is, you mix more natural killer cells in there with the numbers of target cells that you have in there, and so, at the very lowest level of natural killer cell activity, at this medium dose, they found this one in vivo effect on natural killer cell activity, which sort of makes me dismiss the other ones as it was -- they were interesting studies, there's no doubt about it.

Rowe found, in these studies, that he did, that there was a specific defect in the natural killer cells, that prevented them from delivering a lethal hit to the target cells.

But these two studies don't tell us a whole lot more about the effects, potential

effects from natural -- of atrazine on natural killer cells, because all of the animal studies have tended to point to no effects on natural killer cells, particularly at rational doses, and that's all.

SESSION CHAIR PORTIER: Why don't we open it up to any questions? Dr. Regal?

DR. REGAL: Yes, do you know in the Rowe study, with the mice, was there any mortality in the litters?

DR. LUEBKE: No, according to their paper, they didn't see -- they didn't even see any effects on pup numbers or weight gain or anything, in those.

SESSION CHAIR PORTIER: Any other questions? Yes, Dr. Holladay?

DR. HOLLADAY: Yes, Steve Holladay, good overview. I think we've all been there before, where something works well and then for some reason, something changes and it doesn't quite come back, but you know you did it well the first time, and not much more Balb/c mouse work

has been done. The B6C3F1's and the C57's are more T-helper 1 strains on the Balb/c, T-helper 2.

You commented in rats that a much higher dose of dioxin could be used before you see the immune changes in mice, and it made me think of Ralph Smialowicz in that. What do you use, like one-tenth of a microgram per kilogram, and suppress the DTH?

DR. LUEBKE: That was a developmental study. Now, in that study, the developmental exposure to rats caused life long suppression of DTH in the offspring, and the male were a little bit more sensitive than the female, and that was in rats, and the same sort of thing happens in mice.

I was talking about an adult study. So, the ID50 for suppression of the antibody response in a C57 black 6 mouse is .7 micrograms per kilogram of TCDD, and doses in rats with STARS and F344's, up to 25 or 30 micrograms per kilogram, don't do anything, and once you get

above 30, you get some enhancement of the immune response.

DR. HOLLADAY: I guess we've got automatic shutoff switch here.

Some of the differences, I think, are undoubtedly strain related. The rat is not a mouse. You have the Balb/c as a T-helper 2 skewed strain, or that's likely the case.

It makes me think too, that we're seeing more and more with different compounds, when we realize where we need to look.

I can remember when we said DES produced transient effects on the immune system of mouse and then we realized, if we looked in different places, that these were actually permanent changes.

Then the same became true with dioxin. We described them as transient in the early 90's, and then they became permanent shifts, and with your different mouse models, are different models of rodents.

In one case, you're describing immune

suppression and in the other case, immune enhancement is being described, and I'm wondering if we look in the right places, in the same or different models, if we're going to see that actually both occur and they co-exist, which is kind of a strange phenomenon, but we're finding out that in fact, it's true, when you treat an animal with dioxin, they are both immune suppressed and skewed toward immune enhancement. It just depends on which area you're looking, what part of the immune system you're evaluating.

So, I don't know, I wonder if some of this is going on with atrazine and that's part of why we're seeing these differences and -- between the models. What do you think?

DR. LUEBKE: Okay, so, that was a question. So, yes, I think that it's strain related and that was the first thing I thought, when I saw Rowe's antibody data.

The problem is, it would be interesting to go back and challenge these animals with a dust mite allergen and see what

happens, because that's the sort of model that you're talking about there, and it's not just endocrine disruptors that do that. We know that heavy metals and a variety of things do that.

So, you're right, you have to use the right assay, to see what's suppressed. Hexachlorobenzene is a perfect example, where you have some effects going this way and that way and the other way, and they're auto-immune and it's a real mess.

So, we may not have done enough assays on these animals, and you know, if -- in terms of immunotox hazard ID, I think the best way to look at this is that we had unintended modulation at the same dose in two different strains of rodent, that behave immunologically different, somewhat, but that's still unintended, and I think that's probably the crux of the matter here.

DR. HOLLADAY: Something else too, that I was curious about. In your mice, the thymic weight didn't go down, but T-cell function was changed. Do you think it's corticosteroid

independent, based on that, or is it too early to say that?

DR. LUEBKE: Well, we didn't measure cort levels in these animals. From all the work that's been done by our colleagues over in the reproductive tox group, I would have to say that cort is involved here, some place. It just --

I mean, even if it's going to be alteration of GnRH, you know, that seems to trace back to an initial effect on -- you know, on the HPA axis.

But I don't think that this is the sort of sledge-hammer effects that you see, when people start talking about, "Well, that's not immunotoxicity. It's generalized toxicity," because you have huge levels of cort.

I almost wish now, that we had gone back and measured cort levels on these, because it would give us some idea early on in those pups' lives, what their cort levels were like, how long it took for them to smooth out, how long before they came back to normal and whether that

-- you know, there are a number of studies that have shown that if you do restraint stress on an animal and then you come back and challenge that animal later, if you -- I'm sorry, if you stress mom, and you look at her offspring later, that they make a much more pronounced stress response, and that can have real consequences, just in resistance to infectious disease, when you challenge those animals with bacteria and viruses.

But I wish that I could connect that, I wish that I could tell you what prolactin levels were like. I wish I could have, you know, but I can't.

SESSION CHAIR PORTIER: Dr. LeBlanc?

DR. LeBLANC: What happened to thyroid hormone levels in the Rooney study?

DR. LUEBKE: I can show you the -- no, I can't. Yes, I have that on my laptop. They were suppressed. They were non-detectable in the day 14 PTU animals.

DR. LeBLANC: Okay.

DR. LUEBKE: Surprisingly enough, there were some papers that showed that if you compromise thyroid function, in -- particularly in adults, you also compromise immune function.

That certainly did not happen here, and I was, frankly, surprised, when -- you know, when we did the repeat study, we got exactly the same thing, that there were -- you could tell that those PTU treated animals just weren't quite right, as they say, but their immune function was fine.

SESSION CHAIR PORTIER: Yes, Dr. Akana?

DR. AKANA: Just a small comment on the troublesome sets of data that didn't repeat.

We lost a whole year of research and it was because our university decided to do construction, unplanned, all around us, and it had an amazing effect, but we did publish a paper afterwards called "Beware Construction."

So, unplanned, unpredictable vibration, which they like to do at the night, so they don't bother the investigators or the

patients, is just -- it was deathly for us, and my other personal observation is, when we repeat an experiment and we shift to the new barrier facility, with the filtered air flow, that had a decided effect on HPA.

So, small things, environmental, that are not atrazine conducing.

SESSION CHAIR PORTIER: Okay, I don't see any additional questions. We're going to move on to Dr. Liccione and discussions of neurological effects of atrazine.

DR. LICCIONE: Good afternoon, everyone. In this presentation, I will be discussing new studies that have been published since 2003 on the broader effects of -- the broader neurological effects of atrazine, and I will be presenting our assessment of these studies.

Okay, four new studies since 2003 have been identified and were reviewed. The first two pertain to monoamine systems in the brain and the third one was reported on potential effects on

the brain somatostatinergic system, and the last one was on neurobehavioral development.

I will just go briefly through these studies. The Rodriguez study examined, in part, the broad effects of atrazine on various monoamines that included dopamine, serotonin and norepinephrine in the hypothalamus and various extrahypothalamic areas of the brain.

However, the focus primarily in the study was still on the nigrostriatal dopaminergic system.

This was a six month dietary study to several doses of atrazine. Monoamine levels were measured only at the end of the study. Locomotor activity following and acute D-amphetamine administration was monitored at one time, and then spontaneous locomotor activity was monitored at several points in the study.

The number of dopaminergic and non-dopaminergic neurons in the substantia nigra and the ventral tegmental area were measured and micro-dialysis was included to examine dopamine

release from the striatum.

Coban and Filipov focused specifically on the dopaminergic system, and in particular, the nigrostriatal dopaminergic system and the ventral tegmental area.

This was a 14 day oral gavage study, and included various doses. Dopamine and its metabolites, as well as the number of dopaminergic neurons were measured in this study.

The third study, Giusi, reported effects of atrazine on the mouse somatostatinergic system in the hypothalamus in various extrahypothalamic areas.

Dams were given atrazine in corn oil solution and the rats were actually trained to self-administer the solution from drinking it from a syringe, and that was administered on gestation day 14, through postnatal day 21.

The authors were trying to simulate what they refer to as environmentally relevant concentrations of atrazine as has -- that had been detected in the drinking water.

The dams were removed, the F1-offspring were continued with treatment. Then they were sacrificed at postnatal day 60 to 65, and the measurements in this study included the various somatostatinergic receptor sub-types. There are five receptor sub-types, and neuronal degeneration.

Belloni, who was actually a co-author to Giusi study, investigated the potential neurobehavioral development in the mouse, and dams were given atrazine as was done in the Giusi study, on post-day -- on gestation day 14 to postnatal day 21.

Various neurobehavioral parameters were measured. This included writing reflex, cliff aversion, four-paw grasp, auditory startle. They also look at pup ultrasound vocalization. Auxiliary temperature and body weight were also measured.

This slide summarizes our overall assessment of the monoamine studies. The brain monoaminergic studies indicated potential broad

effects of atrazine on dopamine, norepinephrine and serotonin, as well as a decrease in the numbers of dopaminergic and non-dopaminergic neurons in the substantia nigra and the ventral tegmental area.

Only one study, that's the Rodriguez study, actually provided some evidence of altered behavioral responses. The overall findings from these studies, however, are inconclusive because of the number of limitations that I will discuss briefly in the next slide.

Okay, limitations of the study include the very small sample size that were used for the stereology, the reverse transcriptase polymerase chain reaction and the immunohistochemistry experiments, to illustrate the impact of this.

In the Rodriguez studies, the results of the stereology were based on a very small sample size, and there was high individual variability in the count data, when you looked across all of the groups, and the decrease count that was reported at the high dose, reflects a

decrease in the counts in a few individuals when you're considering the variability range.

They only presented median values. Other statistical data, such as the mean and standard deviation, coefficient variation, interquartile range were not available, that would help interpret the statistical dispersion that you can see in the data.

The high variability raises some questions in the precision of the stereological estimates.

There was limited information on the sampling procedure that they used and there was no statistical data that would give you a clue on the biological variation and the coefficient variation, because these estimates, along with the coefficient of error would give a better assessment of the precision of the study, which is very important in stereological examinations, and insight that will give you an idea of the biological variation between individuals and the variance of the estimates at the level of the

respective individual brain regions.

There was also a lack of a dose response in these studies and there was also overall, minimal alterations in the monoamine levels.

The minimal changes in the dopamine was also noted in the Rodriguez's micro-dialysis experiment, which they commented on, when they tried to interpret the lack of the change of dopamine metabolites, DOPAC and HPA.

And the lack of dose response was also evident in the count data for the dopaminergic neurons, that was reported in the Coban and the Filipov study.

There's also a lack of supporting evidence. For example, in all of these studies, there were either no photomicrographs on the reverse transcriptase polymerase chain reaction and immunochemistry results or only a few that were of very poor quality.

Both studies also relied on positive tyrosine hydroxylase immunoreactivity in those --

as well as the stereology, to assess the actual changes in the number of neurons, and Coban and Filipov expressed this very well, the limitations of this, where he acknowledges that it is unknown if the decrease in the positive tyrosine hydroxylase immunoreactivity is actually due to neuron mortality or the loss of the positive tyrosine hydroxylase phenotype.

Other quantitative approaches, for example, morphological methods, would have been useful to support their conclusions, given the limitations, such as the small sample size, the variability, the minimal changes in dopamine, the lack of a dose response and limited or no behavioral measures.

Now, to discuss a little bit about behavior, I want to just elaborate, just a little more.

In the Rodriguez study, it was not possible to fully correlate the reported increased locomotor activity with the levels of dopamine levels, which were measured only at the

end of the study.

A rationale for D-amphetamine selection was not given and only one dose was examined.

The persistent locomotor activity that was reported as puzzling and is uncertain, as to what -- how to interpret this.

There was no inclusion of any direct dopamine receptor agonist and antagonist, as well as any kind of receptor binding assays in the study design.

All of these limitations are important to consider because the authors concluded that the altered behavioral response to D-amphetamine may be due to an up-regulation of the striatal dopamine receptors by atrazine, yet, they provided no support for this critical hypothesis.

Their alternate hypothesis, that the response may be due to dopamine release produced by atrazine is actually not supported by their own data, and that of Coban and Filipov, and in Rodriguez's micro-dialysis study, it actually

shows that atrazine actually attenuated the basal and high potassium stimulated release of striatal dopamine.

Coban and Filipov did not include any behavioral measurements at all, to assess any of the relevancy of the reported changes in the dopamine and the metabolites in the dopaminergic neuron counts.

This is important because in the Coban and Filipov study, the reported loss of dopamine neurons did not correlate well with the dopamine and metabolite changes at the various time points that were measured. In addition, there was no inclusion of dopamine receptor agonist and antagonist, or any receptor binding assays that would clarify the observations.

And another problem with the Coban and Filipov study is although they studied the total neuronal population that would include some of the non-dopaminergic neurons, at various time points, they did not actually present this data with very good stereological estimates and that

is another limitation of the study.

Okay, finally, another important point that I want to address is that the authors, in particular, Coban and Filipov, suggest a link between atrazine exposure and human neurological disorders, in particular, Parkinson's disease. But the data did not support this for several reasons.

Although it is limited, the Rodriguez study suggests potential non-specificity of atrazine effects on monoamine levels in neuronal populations other than dopimangeric neurons, which contrasts with the clinical picture of Parkinson's disease and what was known with animal models of Parkinson's disease, which reflected clearly a selective degeneration of nigrostriatal dopaminergic pathway.

More over, the results of the Rodriguez study suggest potential effects on hypothalamic serotonin, as possibly more sensitive than dopamine, and -- but no additional investigation of this was actually performed.

This is important because the authors, in turn then, concluded that the effects on the hypothalamic serotonin are consistent with atrazine's neuroendocrine alterations, which we know to date, are the most sensitive effects of atrazine.

They did not explore this further, but simply focused on the nigrostriatal dopaminergic system.

In addition, the Coban and Filipov study suggests that there is a maximal limit in the loss of dopaminergic neurons, which again, in contrast what is known with the clinical picture of Parkinson's disease, which involves a progressive loss of neurons, which can culminate in greater than 50 percent of the neuronal loss.

There is also a lack of histological behavioral correlates that you see with Parkinson's disease. Neither in vivo study demonstrated any significant increase in dopamine turnover rates, which would be typical of the clinical picture in Parkinson's disease and what

you see in -- with animal models of Parkinson's disease.

None of them included any clear positive controls for Parkinson's disease, like 6-hydroxydopamine or MPTP, that would help to support or -- their claims.

The limitations of the studies, besides the limitations that I mentioned, another limitation that would impact any conclusion on Parkinson's disease relates to the lack of the examination of neuronal loss in the striatum, per se. The focus was only on the substantia nigra pars compacta and the ventral tegmental.

Now, since Rodriguez' study indicates non-selectivity of neuronal loss, this would be important and had they looked at this, this might clarify some of the potential effects that they're seeing, and also, it would be a good comparison with what is known with Parkinson's disease.

Finally, the epidemiological evidence itself, to date, does not support a link between

atrazine exposure and Parkinson's disease or any other neurological disorders.

Okay, moving onto the brain somatostatinergic system, the Giusi study reported altered expression of specific somatostatin receptor sub-types in the hypothalamus and various extra hypothalamic regions of the brain in the males and female mice.

However, the findings are inconclusive because of serious limitations that limited its use in risk assessment.

Limitations of the study include the small sample size that they used for the staining to assess neuronal degeneration, the reverse transcriptase polymerase chain reaction and the in situ hybridization experiments.

There was a lack of supporting evidence. For example, somatostatin, per se, was not measured, that would have supported the neuronal degeneration and possibly provide some insight into the up-regulation and the down-

regulation that they were reporting in the various receptor sub-types.

No in situ hybridization photo-micrographs were provided for support. There was no functional assessment of the reported changes, for example, behavioral tests, growth hormone measurements, which was one of the rationales for looking at the study, or any other hormonal measurements.

There was no -- the study design also did not include any specific somatostatin receptor antagonist, that would help to explain all of this up-regulation and down-regulation that is very confusing to understand.

There were some discrepancies between statements made in the text, that were not supported by the reference citations. There was a lack of details on the age and body weights of the mice used in the source and purity of atrazine.

The study also did not include any measurements of systemic toxicity that might be

confounding factors, and the photo-micrographs of the amino cupric silver staining were of poor quality. This is important because this is the evidence that they used to support the neuronal degeneration.

Finally, there was actually no quantification of the neuronal degeneration, especially in supporting an important conclusion by the authors who state, in the dentagyros, the neurons showed a rather significant overall reduction in the total number of neuronal cells.

But yet, no quantitative data were provided with appropriate statistical analysis to support a very critical conclusion and no photo-micrographs of the dentagyros were actually provided.

And all of these limitations would help to help in assessing the -- what is going on, because it was very difficult to correlate the neuronal degeneration with -- consistently with the changes in neuronal expression of the somatostatin receptors, as an example, in the

high dose female, you will see some hippocampal degeneration, but no change in the neuronal expression.

Okay, now, for the neurobehavioral development study, the Belloni study reported alterations in writing and grasping reflex and also, ultrasound vocalization in mouse pups. They also reported decreased auxiliary temperature.

The findings, we think, are inconclusive because of the serious limitations of the study.

Limitations include inappropriate statistical analysis. The authors relied primarily on the pup as the statistical unit. The failure to count for the litter effects can result in an increase in Type-1 errors and overestimate any of the effects, thus, reducing the validity of the findings.

In addition, there was no convincing statistical evaluation of the potential interactions, for example, sex, age and

treatment, which the authors claim.

There were two -- there were a number of discrepancies in the reported number of pups that were evaluated in the tables, which raises questions about the accuracy of the results that were reported.

There was no clear dose response relationship for the reported effect on the number of ultrasound vocalization and auxiliary temperature. These effects were noted only in the low dose mice, but not in the high dose mice, and the authors did not provide any support for their hypotheses that were put forth, to explain the lack of the dose response.

They hypothesized effects on aromatase modulation and hormone levels, but no measurements -- such measurements were actually made to support in -- at their conclusion.

In addition, Belloni acknowledged that they cannot exclude the possibility that the hormonal alterations in the dams may have resulted in altered maternal care and

consequently, in altered pup behavior, and as I mentioned, those -- the study did not examine hormonal levels, which would clarify this as a possible confounding factor.

And another important point is that the spectrographic analysis of the ultrasound vocalization were very limited. It's not clear to me, why considerably less number of pups that were evaluated for the number of ultrasound vocalization were subsequently examined for spectrographic analysis, and this was an important measurement to assess the various specific parameters of the ultrasound vocalization, especially since the authors conclude that lower atrazine exposure was more effective than the higher dose in modifying behavioral response.

There was also a lack of objective and validated behavioral methods. The authors relied on methods published in 1965 and the final point was that there was no neuropathology that would help to clarify some of the results.

In conclusion, the recent neurotoxicity studies of atrazine on the brain monoaminergic and somatostatinergic system and neurobehavioral development, because of the many numerous limitations, are -- did not provide any definitive conclusions, and the effects on neuroendocrine function, we think, are still the most sensitive effects of atrazine.

SESSION CHAIR PORTIER: Okay, any questions? Dr. Chambers?

DR. CHAMBERS: John, I was trying to assess the dose levels of these things, and I was confused when I read the Rodriguez paper.

Since that was a dietary study, how did they go about assessing that those were the dose levels?

Not the rationale, it's just, I didn't get a sense of how they assumed it was five milligrams per kilogram per day, when it was a dietary study. They didn't describe that very well, did they?

DR. LICCIONE: No, they did not

describe it. They reported the results at five milligrams and 10 milligrams per day, but they don't provide any information to verify how that was derived.

A lot of these studies don't give any information on quantification of the samples, any analytical measurements, food consumption. I think that would have helped to support their conclusion.

So, I agree with you, that that's another limitation that one could actually add to the study.

SESSION CHAIR PORTIER: Dr. LeBlanc?

DR. LeBLANC: Do you have any idea if the Giusi and the Belloni studies represent the same experiment, with different analyses performed?

DR. LICCIONE: Well, the study design is the same, but there are -- there were some differences in a sense, that the Giusi study -- they removed the dams -- then the F1s were treated for a longer time, but as far as I can

tell, they were not part of the same experiment, but one wonders, if they were two overlapping studies, if that's what you're asking.

DR. LeBLANC: So, in the Belloni study, the pups weren't treated?

DR. LICCIONE: Right, that's true. They were trying to look at lactational and it -- in gestational lactational exposure to the pups only. In fact, they actually state that they prevented the pups from drinking from the syringe.

So, there are differences in the study.

SESSION CHAIR PORTIER: Yes, Dr. Reed?

DR. REED: I think what the Rodriguez study -- I think -- I thought they restricted the diet, so that they can do the calculation. They didn't just let the animal eat as much as they want. So, that was how they calculate the dose. I think there was --

DR. LICCIONE: It wasn't clear to me, from --

DR. REED: But I have a separate question. I agree, then when I was looking at these studies, they're not -- you know, they have holes and gaps.

When I see your conclusion, saying that the other, you know, endpoints were still more sensitive, so, my question to you is, would you be thinking of -- because some of the deficiencies are reporting deficiencies and so forth, that you might be able to go back to the authors and say, "You know, if you have something, some information, some protocol and study design," you cannot.

DR. LICCIONE: Right.

DR. REED: The litter effects and how they called the litters, and stuff like that.

DR. LICCIONE: That's an interesting question, because give this some thought. If you look at the Rodriguez study, which is a very interesting -- is that the very first initial results that they report are on brain norepinephrine, dopamine and serotonin,

indicating a non-specificity of effect.

Then they jump in to the nigrostriatal dopaminegric pathway, without any good rationale. The only thing I can think of is maybe the lab is comfortable with that, they're specialized in that. Same thing with Filipov.

However, given that you're seeing hypothalamic serotonin -- and Rodriguez actually acknowledges that, this may be related to the neuroendocrine effects, it seems like, to me, the more proving experiment would have been to study that, rather than focusing so much on dopamine and Parkinson's -- and trying to -- you know, with Filipov's study, I thought I was reading a new model of Parkinson's disease.

So, I think the right experiment would be to look at this, especially since the serotonin was reported, it looks like it may be more potential, but the results are limited, because they only did a preliminary assessment of these monoamines.

It seems like to me, that the

neuroendocrine in serotonin thing might be more fished out, rather than relying on dopamine.

DR. REED: And that was actually one of the questions that I was curious about, in that given all the gaps and deficiencies these studies have, my question was since -- you know, your conclusion is that they're still not the most sensitive endpoints, but apparently, you have some idea of how the study could be conducted, as you were just describing.

Would you be interested in designing something, just to see, you know, what the endpoint is going to fall out to be, because for example, I think most of them were very high dose studies --

DR. LICCIONE: Right.

DR. REED: -- but the Rodriguez study was, you know, five --

DR. LICCIONE: Five milligrams, the lowest dose.

DR. REED: And they were seeing some effects. So, my question to you, so, it's

getting close to the endpoint issue, in terms of what is most sensitive. Are you thinking of designing something that could get the answer that you were looking for?

DR. LICCIONE: I haven't given --

DR. LOWIT: John, if we could answer that. Liz, do you want to jump in?

SESSION CHAIR PORTIER: That's Anna Lowit.

DR. LOWIT: Yes, sorry, and I've been moving around chairs. That's a good question. I think the short answer would be, we're not sure of the value of what those studies would be, and if there is an argument that those studies would be valuable, with respect to keeping in mind, that at the end of the day, we're asking risk assessment questions, and if you could design an experiment that will lead you to something of a lower endpoint, of a shorter duration, that's in a separate direction from where the HPA, particularly new data, is leading us, which we think was going to really drive many of those

regulatory decisions.

So, you're a regulator, you know how it goes. If it's something that's going to -- that you believe would change the direction of the assessment, I think those would be valuable. But that's the question, how valuable would those experiments be?

SESSION CHAIR PORTIER: Dr. Horton?

DR. HORTON: I have more of a comment than a question here, because I think, in response to your question, in terms of the development of the neuroendocrine system, with respect to both the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal axis, both of those are in play, at this point.

Anything that's going to influence the development, and since things seem to be pointing back to the developing axes, we do know that in the development of those, both the BNST and the brain regions that we're talking about right now play important roles.

Also, the hippocampus plays important role in the regulation of those areas, and that if there's anything that's going to have a toxic effect, or not even a toxic effect, but alter the developmental pathways of those in a plastic way, we need to be aware of it.

And so, if there's anything in this data that suggests that that might be there, then it's probably worth going back and evaluating those under-properly controlled and properly designed studies.

DR. LOWIT: Just one follow up and we can probably close this.

To the extent that, you know, that there is science to support those things, we would encourage it to appear in your report, or in your deliberations over the next few days.

I just want to be weary, that -- and in the days of limited resources and moving towards less animals and not more, that we design those experiments in a way that we get the most out of them.

SESSION CHAIR PORTIER: I think we will go on. I would like to say, I'd like to see one of these studies done with adequate sample sizes, though.

We've seen a lot of neurotox studies and they're always under-powered, for the kind of results they're looking for, and it's very frustrating, because I think we all kind of agree, that this should be important, but why aren't we putting the effort into those studies?

Dr. Mendez, you have a summary.

DR. LOWIT: And we're doing a computer pass, as we speak. Well, maybe Liz can start talking, since we're running really late.

SESSION CHAIR PORTIER: How many mics can we drop? He's got it untangled. Now, watch the water jug.

DR. MENDEZ: I sure require a lot of assistance, don't I? Just give me one second. Okay, I don't think this is mine.

All right, I promise, I'm going to try and move quickly, and pick up the pace here.

So, what I wanted to sort of do is bring us back, wrap things up, as to where we are and where we hope to go, between now and September.

The status of the reevaluation, we have reviewed approximately 100 studies that have a wide range of topics, from mode of action, reproductive and developmental effects, immunotox, neurotoxicity and carcinogenesis.

You'll notice that we did not talk about the carcinogenesis when we were putting this paper together. We were hoping that we would be able to address cancer issues in September.

As that is not the case, we've chosen to defer that conversation so that we can look at both the animal and the epi data together.

We still believe that the effects of neuroendocrine function are the most sensitive effects, based on the data that we have in front of us today.

We continue to evaluate additional

studies that have become available since February, and studies that will become available, hopefully, in the spring and summer.

Having said that, where are we right now? Mode of action, our understanding of the mode of action is expanded.

In 2003, it all hinged about the perturbation of the HPG axis and that is still applicable. The activation of the HPA axis, however, has come into play over the past seven years, and we think that it may precede the effects on HPG axis, and Dr. Fenner-Crisp actually alluded to that a little earlier, in one of her questions.

We see changes in ACTH, corticosterone and progesterone, within minutes of exposure. However, the toxicological significance of these adrenal hormone changes per say is still unclear to us. Were those 15 minute blips sufficient to cause an adverse health outcome?

From the data that Dr. Cooper discussed earlier today, we believe that there is

a general stimulatory effect in steroidogenesis, not necessarily a direct effect on aromatase.

We see the delays in puberty onset in both genders. Prostatitis is related to decrease in prolactin and atrazine exposed dams during lactation, and we didn't talk a lot about that today, but it's described in the paper in some detail, and the mammary gland tumor development, based on the findings in the -- and the conclusions of the 2000 SAP and what we have in front of us today, still do not appear to be relevant to humans, in terms of the mode of action that we're addressing today.

Oh, this is a little skewed, but this is a schematic that appears in the paper, about the mode of action. Can I have the pointer, please?

And what -- I'm not going to go through each one of these steps. I think that what's important to keep in mind is that we had some of these steps back in 2003. We had the GnRH pulses going down. We had the decreases in

LH and prolactin, and we had the gonadal changes that led us to the altered reproductive function.

As important as the understanding of the additional steps are, as they pertain to the HPA axis, one of the things that I find very important about this, one of the reasons why I like this particular schematic is, to my mind's eye, this almost looks like a house, and the idea that in 2003, we had this mode of action data, and the foundation of that house is still solid, seven years later. It still stands, and that's important, that's an important message to take home, in my mind.

Immunotoxicity, I'm going to really fly over this. Immunotoxicity exposure during development may lead to altered immune function of the offspring. However, that does not appear to be more sensitive than the atrazine induced effects on neuroendocrine function, and as Dr. Luebke alluded to, during his talk, it may be related to some of that GnRH changes, that we're seeing.

Immunotoxicity, we've just had a discussion about that. Based on the data that we have in front of us, it does not appear to be more sensitive than the atrazine induced changes in neuroendocrine function, and it does not appear to be related to Parkinson's disease.

So, where does that leave us? Well, that leaves us with the understanding that the toxic effects are due to multi-level interactions of a variety of systems, neuroendocrine, reproductive, nervous, immune.

At the activation of the HPA axis is followed by the disruption to the HPG axis, which then leads us to the most sensitive effects attributed to atrazine exposure, but what is still not clear is the extent of HPA activation, both in terms of duration or dose level, that meet -- that are needed to elicit the decrease in GnRH, and subsequent LH surge attenuation.

All right, so, I just told you where we are, as of today, and we sort of give you the coming attractions.

The next steps in preparation for September, as we want to apply the mode of action framework to analyze the data that we have in front of us. We want to further characterize the HPA/HPG axis interactions, describing the temporal relationship between HPA axis activation and adverse health outcome.

We want to better describe the dose response relationship between atrazine exposure, HPA axis activation and adverse health outcomes. We have some data that we hope will be coming in, in the spring and the summer, that will help us address these two issues.

Once we have that data, we intend to conduct benchmark dose analysis, to better characterize the dose response curve for the effects on HPA and HPG.

Dr. Cooper alluded that some studies we had in NOAEL-5 and in LOAEL-50, we'd like to be able to define that a little bit better.

We have additional review of data that is coming in, in the spring and summer. Again,

data available between February 2010 and July 2010, in the mammary gland development, which is a separate issue from the tumorigenesis.

We have decided to postpone that conversation because we are aware that there are data that will be hopefully published very, very shortly, that will really help us have a fuller picture and a fuller understanding of that issue, and we felt that it was best that rather than roll it out piecemeal, to bring it all together.

We need to start thinking the duration of exposure. How long must somebody be exposed, before an adverse outcome occurs?

Life stages, we all know that response to chemical exposure may vary through life stages. How do we do that, in terms of atrazine?

We've seen that we have a variety of effects, whether we have animals expose pre-natal, perinatal or postnatal. So, we need to start thinking about that, as we think about the risk assessment.

What is the life stage that we really

need to be protecting and therefore, will protect all other life stages?

We are going to review the available non-cancer epi data. We're going to start working on a weight of evidence analysis integrating the experimental tox data and the epi data.

If warranted, we intend to propose new points of departure. We will reevaluate the FQPA safety factor and we will reconsider the drinking water sampling strategy, in relationship to the toxicological duration of concern, because we all understand that the shorter duration of toxicological concern may lead us to more frequent sampling.

So, as I said, we're going to apply the moral framework. Let me tell you where we are, within that process.

Postulating the MOA, can the MOA be established in animals? Well, we already had the HPG axis that's been going through review, so, we have the MOA in the animals.

We're going to describe the key events. We've been starting to do that, as we talked about the changes in ACTH, the changes in cort, changes in progesterone. That's where we are at this point.

We're going to start working on establishing the dose response concordance and establish the temporal concordance.

Once we've narrowed that down a little bit further, then we'll proceed with the analysis, talking about the strength and consistency of the finding, the biological possibility, whether other MOA's can be ruled out, and the statement of confidence analysis and implications.

So, additional data that we expect in the spring and summer, sort of wet your appetites for what's coming.

We have studies coming on in effect of four day atrazine exposure on ACTH, cort, progesterone and LH. Effect of atrazine on steroidogenesis in rat granulosa cells, and that

study has actually been submitted for publication.

Effect of atrazine exposure on GnRH and kisspeptin neuron regulation. Effect of gestational exposure to atrazine, an oral gavage immunotoxin and hormone evaluation study for the effect of atrazine in male rats, and male prostate inflammation on low dose metabolite exposure, and that manuscript is in preparation.

Other data that we expect is oral study of effects of atrazine on estrous cyclicity and the estrogen induced hormone surge in female rats, comparison of effects of atrazine on pulsatile LH release and the LH surge in non-adrenal-ectomized compared to adrenal-ectomized female Wistar rats, and I believe that somebody in the Panel asked about that. We know that the study is underway, we just don't have the data yet.

Use of a PBPK model, to characterize atrazine by transformation to inactive moieties, in relationship to dynamic changes in hormone

levels, and potential adverse outcomes, and a dose response modeling and determination of points of departure for risk assessment and the validation methods for that.

So, what we hope to do with the dose response concordance is establish the extent of corticosterone changes needed to result in adverse health outcome, incorporate this data in the point of departure and determinations as needed, and with regards to temporal concordance, situation of changes in GnRH, ACTH, corticosterone and progesterone needed for an adverse health outcome, changes seen within minutes, sustain changes need to elicit the adverse outcome, one day, four days, one week.

We do know that atrazine and its metabolites are quickly absorbed and distributed in the body, so, we need to take that into consideration, as we move forward in our analysis.

And just a little bit about the mammary glands development. These are some of

the data that are currently available and it's the adverse effects. You can see -- you can read them as well as I can.

A number of studies are currently available in the literature, about potential adverse effects on mammary gland development and a few more are coming.

Atrazine dose dependent effects on mammary gland development in Sprague Dawley's and Long-Evans rats, the effects of atrazine induced mammary gland effects in Sprague Dawley and Long-Evans rats and carcinogen induced tumor incident, and an oral development study of atrazine in the rat, including cross-fostering and pair feeding.

The Agency already has that last study, but because it speaks to the mammary gland development, we have deferred the discussion to it for a later date.

And then the 2011 SAP on carcinogenesis, AHS epidemiology study on atrazine in human cancer publication, we hope that that will happen some time by the end of

this year. We integrate experimental carcinogenesis data and the epi data, AHS and other cancer epidemiology studies, and we will be seeing you then, in 2011.

So, if there are no questions --

SESSION CHAIR PORTIER: Any questions? Dr. Krishnan?

DR. KRISHNAN: A minor one, related to the -- your house pictorial, number five slide.

Actually, I was -- since it's more of a statement of the mode of action, I was looking for some level of statement about the dose metrics involved or the consideration of alternative dose metrics.

So, I know if it's a split-level or cottage, I could see that, but I don't see the final connects of this. So, if you just want to clarify what level of thinking has gone into that or is it only the pharmaco-dynamic, if you will, without potential toxic being captured in here? What was intended?

DR. MENDEZ: At this point in time, my

intent with the little house was to sort of illustrate the key events.

We are thinking about the PK aspects of it, but that's not at this point, in there, just yet.

SESSION CHAIR PORTIER: Dr. Fenner-Crisp?

DR. FENNER-CRISP: I guess my question earlier, goes to whether or not your roof is leaking. The point being whether or not the whole left side of the roof exists. That was -- I interpreted that step one as being the old direct effect.

DR. MENDEZ: So, your question was about step seven?

DR. FENNER-CRISP: No, one, one.

DR. MENDEZ: Okay.

DR. FENNER-CRISP: The roof. Does the whole roof exist? That was the essence of my question earlier. Is there evidence to support a direct effect of atrazine in this whole thing, or is it all going to be shown to be indirect

through initial disruption in HPA to get to the disruption in the HPG?

DR. MENDEZ: Ralph, you want to attempt it?

DR. COOPER: Yes, I think I understand the question. I think that right now, the -- I just told Dr. Levine, this looks more like -- it needs an arm out there, to be a children's swing set, and --

But one and seven, seven are the -- if we put it this way, I think based again, on the temporal aspects, at least for the CNS ACTH response, I'd argue that one is centrally up there. If it's not at the brain, it's so rapid, it would have to be brain impaired. So, that one still exists.

Whether GnRH is still the target, I think it's -- those shingles are still there, but seven, there's an increasing number of shingles on that roof, that says that seven is growing credence, if nothing else.

So, but so, that means then -- and you

know, we always think linearly, or we always think sequentially, and they say tox pathways paint us in these boxes that are lines, and this is more -- we understand, given the assumption, anyway, right now, that we're dealing with a very basic cellular mechanism, disruption of maybe cyclic AMP signaling, and we're going to have a lot of pits in the whole neuroendocrine axis.

So, I'm not going to place my bets on whether that roof is going to hold up under a lot of scrutiny, but seven seems to be winning out, right at the moment.

SESSION CHAIR PORTIER: Okay, is most of this team going to be available tomorrow morning, because I doubt if we're going to get through all of the EPA presentation today, at this point, since we're running about 45 minutes behind.

I'm just wondering if the Panel has questions in the morning, and we have kind of an open -- Anna, will any, or most of these people be available?

DR. AKANA: We're all here, all the meeting.

SESSION CHAIR PORTIER: Okay, good.

DR. AKANA: Including our colleagues from North Carolina.

SESSION CHAIR PORTIER: Okay, good. Okay, I think we need probably a few minutes, for you to change over to the hydrology groups and so, we can stand, but don't leave the room, unless you really have to.

(Whereupon, the above-entitled matter went off the record at 5:13 p.m. and resumed at 5:16 p.m.)

SESSION CHAIR PORTIER: Let's begin again. Just for the Panel's -- just so you know my thinking, I think we probably will try to get through six o'clock, but depending on the -- I guess, we'll ask the -- Mr. Thurman, when he wants to make the break, but I'm certain that we're going -- we probably won't get the summary and conclusion until tomorrow morning, and maybe -- you know, the water sampling summary, until

tomorrow morning and maybe the artificial neural network might be a good topic for tomorrow morning.

Okay, Mr. Thurman is going to be starting the discussion on evaluating the water sampling strategies.

MR. THURMAN: Okay, I thought I'd start out, because it seems odd to have water sampling in the middle of a toxicity SAP. So, I'm going to kind of remind you why we're here.

You know, in light of new science, we may end up looking at a different endpoint than we looked at for the 2003 -- based on 2003 RED, and if that's the case, the question is going to become, we had registrants do monitoring study in community water systems in vulnerable areas with a sampling frequency sufficient to characterize in 90 day exposure period.

They sampled weekly during that -- during the use and run-off season and biweekly in other parts of the year. If we end up with a shorter duration, the questions that are going to

be coming to us are, well, how well does that existing monitoring design characterize the shorter durations of exposure, if they don't, do you need to sample more frequently, in order to do that?

We want to be able to answer those questions when they come, instead of having to say, well, wait, we've got to go separately.

One thing I'm going to point out, this is a question that doesn't just apply to atrazine. It's the type of question we wrestle with, with other pesticides as well.

Atrazine is a little unique, and we're going to be focusing on atrazine because it's probably got more monitoring data than you're going to see in all of the other pesticides combined, and that may be a hyperbole, but the point is, atrazine may be one of the few pesticides where we're able to do drinking water exposure almost entirely on monitoring, rather than supplementing monitoring and modeling together.

So, that does make it unique, but we are interested in what may apply beyond this.

Very quickly, I'm going to provide you with some considerations, you need to take into account, both in designing a monitoring study and evaluating existing monitoring studies. Then, I'm going to provide an overview of some of the approaches we've been looking at, for analyzing monitoring data of varying sampling frequencies and in terms of applying through various durations of exposures.

Then, we will either get to one or both of these example approaches that are in the background paper we gave you, today, or split it between today and tomorrow, and then I'll come back and provide you with a summary.

The next few slides are going to illustrate the -- how -- the complex spatial and temporal patterns we find in pesticides in water. Some of the other -- these factors all -- from the temporal and spatial patterns, to the temporal autocorrelation, which means the

pesticide concentrations you measure on one day are related to exposures that you would find the day before, and the day after.

We also tend to find, when we talk about sensor data, we're talking about pesticide concentrations that are below a level of detection, and we often see infrequent sampling.

All of this provides us with challenges, both in terms of looking at a monitoring study design, as well as interpreting that, and so, I want to make sure we have that background, as we go forward.

You saw this map in Anna's presentation. I'm not going to spend too, too long on this, I hope. When you're looking at spatial -- and we know atrazine has widespread use, but at the same time, there's enough information on atrazine that we know that we can zoom into certain parts of the country, and that's where we expect to find community water systems with higher concentrations than in other parts.

When you're trying to address spatial variability, there's a number of ways of doing that. On one extreme, you could sample every community water system in the country. That's several thousand of them, and you're probably not going to get that with any level of intensity.

On the other end of it, you may look it -- and this is what we did for an ecological monitoring study that we brought to the SAP in the past. So, you've heard that, this one is focusing on drinking water.

We started out by using a model developed by U.S. geological survey, a watershed regression on pesticide, which was developed using atrazine monitoring data, to identify the watersheds that would be most vulnerable to atrazine exposure, based on that model, and that's what you see in the dark blue.

What we did in that case is, we basically had a study set up, that sampled watersheds that represent those most vulnerable areas and we looked at how far we could apply

that.

The drinking water is a little different than that, in that, what we did for this is, as a result of the 2003 IRED, atrazine is already being monitored quarterly at each of the community water systems, as part of the Safe Drinking Water Act.

We were looking at a 90 day exposure period, so, we said, you know, quarterly sampling probably isn't frequent enough to characterize a 90 day exposure period.

So, what we did is, we looked at community water systems that had annual average concentrations above a certain threshold of concentration, and those were put into more intensive sampling.

The interesting thing that I want to show here is that almost -- the vast majority of those community water systems fell into this area as identified as the most vulnerable watersheds using the WARP modeling.

So, that helps us know that -- we've

already a good bit, and we're working with what we've learned, but it tells us that we know we can zoom in on certain areas. Even within those areas, we're seeing that there are some water -- community water systems that tend to have higher concentrations of atrazine more frequently than others.

So, there is localized variability that further drives that. Some of this, we're learning and it is a result of ecological monitoring and some of the feedback we've gotten from the SAP on that, that we hope to pass on.

But because of the nature of the fact that we're looking at individual community water systems that get thrown into this because of the monitoring data, spatial patterns aren't as much of a challenge for us in this regard as the temporal patterns, and because we were looking at these individual community systems, each of which serves its own population, and each person -- each population potentially drinks the same concentration of atrazine, as their neighbors do.

So, I'm going to go into the temporal patterns. One of the things, when you're trying to show temporal patterns and try to show daily variations, you quickly find that there's not a whole lot of monitoring data out there, that samples daily or almost daily.

What I'm showing you here are some monitoring that was done with the ecological -- atrazine ecological exposure monitoring program. They tend to be a little farther upstream then what you would see for most of the community water systems, although there are a few community water systems that are in those same type of stream networks.

These were flowing water systems and what you would expect for flowing water body is, you expect to see a short, higher pulse moving through than you would for a reservoir, which would tend to dampen it out and spread it out.

But what I wanted to do is illustrate, this is -- when we're talking about seasonal variations, this is -- typically, what you'll see

is one or two short pulses, where you get elevated concentrations that decline fairly rapidly and a lot of times, either low in negligible detections.

This is in one year. The previous year at that site, the same site, we got a little different pattern, and a different pattern can result from different application periods, different rain fall events, different ways it's used.

There's a couple of things I wanted to show you. When we're talking about the auto-correlation, you can really see it here, where you see how the concentrations are related to the neighbors. You also tend to see a tail coming out, where you could -- a rapid, initial peak and then a tail, tailing out. This is common in a lot of our -- particularly, flowing water, but it is a common thing that we see, and just to throw in another one, when Mary Frankenberry starts talking about short exposures, longer exposure chemographs, shapes of the chemographs, this is

an illustration of one where you do -- what you actually have is multiple peaks running together, so that you get a longer duration of exposure.

This is interesting because it does make a difference on how frequently you have to sample. We're not always able to predict those shapes of those chemographs A-priority, but to the point that we can, it helps us interpreting the results of the monitoring data.

It's not enough just to worry about how much -- how -- variability you have in a season, but you do have year-to-year variability.

The first slide I showed you happens to be this year, and the second one was -- this was the double-peaks. What you tend to see from the year-to-year, and this is a six year period, is not only do you see differences in the magnitude of your exposures, but you also see different shapes in the chemographs.

And just to drive home the spatial variability again, this site is 50 miles south, same years, and you can see the difference in

your exposures from year to year, so that it does thrown in the fact that it's -- that a lot of these differences are also localized and it does present some challenges to us.

So, now, all we have to do is figure out how to take that into account, when you're looking at designing a monitoring study.

When we're looking at monitoring study proposal, a protocol that would be submitted to us, we need -- the questions we have is, how is it going to account for spatial and temporal patterns in exposure, and a lot of that is by targeting areas where the pesticide is used and when it's used.

So, you have a limited number of samples. Instead of spreading them out throughout the year, you would concentrate them in the times when you expect to find it. There are a lot of variabilities, additional variabilities, based on the nature of the pesticide itself, how it's applied, the weather patterns, it's mentioned some of the water body

types.

It's also important, as you've -- as we've heard referred to throughout the day, the exposure duration of concern is also very important, in terms of how frequently you have to sample, and it makes sense that you're going to have to have more intensive sampling, if you're looking at shorter duration exposure, than a longer term duration.

If we were to come out with an exposure period that's 90 -- similar to what we have now, 90 days or longer, we're probably okay with it, but we are okay with the monitoring data we have. But if it's a much shorter duration exposure, that's where we have some questions.

On top of that, we're also looking at the consequences of rather -- of failing to detect high exposure, exposures above our level of concern, when they do occur, or on the other hand, estimating an exposure that's above the level of concern when, in fact, it turns out not to be.

Because FTPA requires us to come to a conclusion of reasonable certainty of no harm, we tend to err on the side of false negatives, more so than false positives, but at the same time, we want to try to be as reasonable with our exposure estimates as possible. We don't want to end up with an outrageously high exposure estimate that really doesn't do anybody any good in that regard.

Finally, the reality is, we can't overlook the cost. It's prohibitively expensive to sample daily, at enough sites, in enough years, to give a full characterization of any potential exposure you're looking at.

So, we end up with less frequent sampling and less -- and fewer monitoring sites. In a lot of cases, we rely on modeling to fill in the holes, and sort of help us focus on more vulnerable areas.

What we're coming to you today isn't inventing a new wheel. It's building on things that have already been done in the past.

Shortly after FTPA was passed and we had to look -- and we started looking at drinking water exposures routinely, International Life Science Institute convened a pattern that -- a panel that provided some recommendations on how frequently you'd have to sample for -- for instance, in estimating peak exposures.

We brought some information to an SAP on what kind of monitoring study design you would need to estimate chronic exposures. So, we've built from that.

Charlie Crawford provided some -- evaluated some varying sampling strategies and you'll see in our paper, that we refer to a lot of a Crawford's work and tried to take advantage of what he found, to build on that.

And both of these particular authors noted that the number and frequency of sampling will depend on the time frame of concern. So, that's, once again, bringing it back to that, linking it together.

What I'm going to do is, spend a

little bit of time now, talking about how we analyze and interpret existing monitoring data, because most of the time, we're not designing new studies, but we're looking at existing monitoring data that's already out there.

In the case of atrazine, we're looking at several years of monitoring community water systems, and once again, we're going to be asked that question very soon, is that adequate for a new exposure duration?

So, we want to be able to get at that. I'm going to begin with some of the basic approaches and then walk through to more complex approaches, as we go along.

Let's go back to that chemograph we had, that I showed you earlier, that has two exposure peaks, one that occurs in late April and another one, about a month later, and let's say, for this particular thing, we know -- this is an actual -- the true chemograph that you would see, and let's say, we had monitoring that was done on a weekly basis, beginning April 1st.

So, those red dots you see are the actual samples, and what you see is, well, we did -- the timing of sampling happened to hit this first peak pretty well.

For the second peak, we're on the down-slope side of that, and what we ended up with is roughly about half of what the actual maximum concentration was, in that regard.

So, we already know that if we're looking at an acute exposure, that we're going to end up with an under-estimate.

We're still at the point is, if we're looking at an average concentration over another duration, exposure duration of concern, to get to that, we've got to figure out, how best do you fill in the holes in between? We've got these seven day snapshots. How do we fill in the holes?

Well, one way to fill them is to assume the same concentration on subsequent days, until you get your next monitoring, kind of a stair step approach, and you end up with a blocky

chemograph, which, if you blur it, a little bit, it's not too bad, but it's still kind of blocky.

Another one that's done, probably more commonly, is kind of doing a linear interpolation between the sampling points, it's like connecting the dots.

If you're looking at a short term exposure of concern, and particularly, if you're looking at duration of exposure that is less than your sampling, then there's a good chance, in most cases, you're going to miss your highest exposures.

If you're looking at longer term durations, that may not be as much a problem, because of the -- it will average out over time. In fact, if you look at some of the things that Mary will talk about later, that we do find that for the longer your exposure duration of concern, the less error we see in the estimates, but shorter duration, the more error we see.

Now, even weekly sampling is still kind of a Cadillac in a lot of monitoring

studies. Typically, we will see studies that may be sampled biweekly or monthly or quarterly. So, we're going to go back to our original site and instead of sampling monthly -- I mean, weekly, we're going to sample every other week.

And so, you see, we still hit that first peak, but suddenly, we're way down on the tail of the second peak. So, any type of -- no matter what interpolation you do, you see -- you have missed where the highest exposure is.

What we'd like to do is, try to figure out -- you know, at some point, the sampling is going to be so infrequent, that your exposure estimate is probably not going to reflect a whole lot of what we see out there.

Some of the questions we've been wrestling with over the years is, how do you know if you've missed this? Are the ways that can help us know when we may have missed the exposure point of long term concern?

We're still wrestling with that. Some of the modeling methods that we mentioned at the

end of our chapter are ways that we think might help us start to get at that, but it's still a challenge we have.

And so, the basic interpolation approach, as I just showed you, like I said, these are the ones that are commonly used. They're quick and they're simple and if you have a long enough exposure duration, and in particular, your sampling frequency is more frequent than your exposure duration, that may be fine, within incertain error bounds.

The disadvantages are, they assume no concentration is greater than the maximum that they measured. They're likely to under-estimate your peak or your short term exposures, and how well they characterize actual concentration profile depends on the timing of sampling frequency. The farther apart it is, the more likely those methods are to fall apart.

So, what if we, instead of trying to draw boxes or connecting dots, we want to try to fit that monitoring data with more some type of a

curvilinear pattern or something that may better reflect what you're actually seeing there, or we want to provide some type of confidence interval around our exposure estimates.

You're going to see some of this illustrated in Mike Messner's talk. He's going to talk about one way, one approach, using artificial neural networks, to try to fit that pattern, the pattern of the monitoring data, and some of the things that can be done, in terms of providing some kind of confidence intervals and -- and also, in terms of looking at how frequently you did monitor, or where do you have to set your threshold of exposure to kind of provide either balance between the false positives and false negatives?

Mary Frankenberry is going to present an approach where we think we can start getting us at providing some types of confidence intervals.

The next couple of slides I'm going to show you are ones that we did not provide an

example for you yet, and I put it in there because it is definitely something that we are considering and we've done a little bit of testing with.

If you've heard of kriging or nearest neighbor approach, you've probably heard it used in geo-spatial analysis. It's been a way to take a look at monitoring points over an area, in other words, monitoring points in space, and fill in, in between those sample points.

It's a way, essentially, the way you do it, is you have the points, you analyze them, you look for any correlation between the sampling points, which is what this variogram is one way that it's done, and then, you fill in, by waiting -- the waiting, based on the correlation between the points with the nearest neighbor type approach.

And so, most of the time, it's been used for spatial analysis. We've looked at this because I think, that same concept seems like it would also work for temporal analysis, and I have

seen -- I have been to some national meetings, where I have seen this approach tried out.

We had a couple of people, Jim Hetrick and Jim Wolf in our office, who looked at it and did give it a try, and so, what I want to show you is, very quickly, what they did is, some of their preliminary analysis, they looked at atrazine and they looked at a couple of other pesticides.

The basic approach is first doing a visual inspection of the data, and you noted from the atrazine chemographs I showed earlier, that you could see the temporal correlation -- auto-correlation in there. It was evident.

They did some variogram analysis and it did -- to quantify the temporal dependence, and what they found is that you did get correlation between measurements that ranged from 10 to 60 days apart.

The other thing they found is, it wasn't constant from year to year. So, it's not something you can apply to one year -- that you

can analyze in one year, and apply year to year.

So, it isn't constant from year to year, but it does find a way of pin-pointing that auto-correlation and they've done some cross-validation and what they have -- some of the preliminary analysis is they can do a conditional simulation with -- that can give you an imaging of the time series over years. They can use -- come up with a probabilistic generation of exposures, and do some stochastic simulations, so that we can get some confidence bounds on that.

So, in addition to what you're going to hear from Mary and Mike, this is another approach that we're going to be taking a look at.

The statistical approaches that I've mentioned, their disadvantages are complex. They take a lot more effort. It's not a quick analysis of your data.

But where we need to provide probabilistic distributions or where we need confidence bounds around existing monitoring data, that may be -- we see that as an approach

to take.

One of the other advantages, they don't necessarily assume that the greatest -- the highest exposure has already been detected, and that's one of the reasons for the confidence intervals on that, and they provide us with some non-subjective way of estimating missing values and the ways to account for temporal correlation.

They're more complex. The other issue is, they don't necessarily capture the underlying explanatory variables, or explaining what caused that spike to occur when it did, but they may have a utility in certain instances, particularly when we're looking at needing a confidence bound on our exposure estimates.

I've mentioned a little bit about modeling methods and we are getting -- you know, getting into a lot more detailed, involved procedures. WARP, I showed you how we've been using WARP and a lot is, in terms of identifying -- raking watersheds, based on vulnerability and then targeting areas of -- where more monitoring

would be needed.

But WARP has provided an estimate of selected percentiles, as well as an annual mean, an annual maximum -- and it's provided some moving average concentrations.

We look at it as a potential for use. A lot of it, as the authors and developers of WARP have said, it helps us screen areas where we need to have more targeted monitoring.

They have -- they do provide some estimates of these various exposure percentiles, with some confidence bounds on there, so, it's something that we may be looking at, as we go forward with this.

In a lot of cases, we've used deterministic models to provide ground water -- I mean, surface water exposure estimates. We've used pesticide route zone model, which simulates pesticide run-off from the field and we use exams with exposure analysis modeling system, which simulates the receiving body.

And we've used that to provide

multiple years of daily concentrations, varying with weather patterns, and so, again, it gives us a way of assessing the effect of different weather patterns, and we've used that in various tiers of our drinking water exposures, exposure estimates.

In the 2007 SAP, that we had on atrazine's ecological exposure monitoring programs, Syngenta investigated a potential for using PRZM, to fill in concentrations between measured data from the chemographs.

We've looked at that. It probably was a little bit more intense involved and complex than we were planning to go into, but it's something we were considering and have taken a look at.

I put this in, we really didn't include this in the paper, but we have seen some attempts to try to use stream-flow as a way to adjust pesticide concentrations in between monitoring events.

I've seen as many chemographs where it

didn't work, as where it did. There are -- it's not -- what we quickly learn is, it's not just the flow, but it is also the flow in relating to the timing of the planting, the timing of the rain fall events, all those together.

Some folks at the USDA's ARS research center and -- in Missouri, Columbia, Missouri, have looking at an index of trying to couple flow adjustments with the timing of planning of rain fall events, and we're keeping an eye on that, because that offers some promise, that we might be able to use that.

At this point, I'm finishing my presentation, and I'll open it for questions, then, we will move on to the next one or two of the next two examples. I'll let you folks figure that.

SESSION CHAIR PORTIER: Likely, one. Questions? Yes, Dr. Krishnan?

DR. KRISHNAN: I have two clarifying questions. First one is, in the graphs you showed, it was atrazine, but was that like, a

total, in terms of including also the other duration products, or do such figures exist for other duration products?

MR. THRUMAN: In that particular -- this particular illustrations, it was atrazine only.

One thing I just want to clarify, we're not -- at this point, we're not really doing -- presenting a drinking water exposure assessment. We're using whatever available data we have, to try to come up with approaches that we'd use to analyze, that we would then apply to the drinking water exposure assessment.

The monitoring data that we got from Syngenta on community water systems is total chlorotriazines. So, that would be what we would be focusing on, in that regard.

DR. KRISHNAN: Okay, my second one, in your initial slide, you refer to the 90 day rolling average, probably you or someone else can answer, as well, and you refer to the time frame of concern being the major reason for the choice

of 90 days.

I have seen some justification along those lines, as well. I'm wondering if we could get some further clarification on the choice of the 90 days, as to what went into that thinking? Is it based on the tox studies, during which --

DR. AKANA: I guess, I'll answer a question, and I'm not asking, just to make sure that we're on the same page.

The 90 day rolling average was what was used in the 2003 risk assessment. So, one of the major points on the table for reconsideration, as part of this analysis, is in a pretty straight forward question, is the 90 day still the right time frame, and that, hopefully, will get a lot of conversation over the next few days.

So, it's absolutely on the table, to move that 90 days shorter or longer, for that matter, depending on, you know, where the data move.

At that time, the 90 day rolling

average was selected by a match to the toxicology data, specifically, the LH data, specifically, a six month rat study. I don't know the exact author, if you really want to see it, but that's my --

DR. KRISHNAN: No, I think we're on the same page. At least, I understand, that's what I wanted to make sure, because 90 days, typically, in rodent studies, represents about a tenth of the life time, which is not what corresponds to what's typically the sub-chronic.

On the equivalent human, typically, it's a tenth of a duration -- a life time is considered to be about seven years. That's what people normally use. That's why I tried to see, but this clarification helps -- would help in other discussions.

DR. AKANA: It was done as an equal matching of the days, on an effect, the 90 days or short than the six months. So, that was a quite conservative choice on the Agency's part.

SESSION CHAIR PORTIER: Dr. Lee?

DR. LEE: I have a question and a comment. First, the question, do we know about the measurement error uncertainty, in taking these samples, from the water streams, and they're sort of presented up here, as stated points without any error.

Presumably, if you sent several people to the same stream and had them all take a sample and then go analyze them in separate labs, you'd get different answers.

MR. THURMAN: First of all, we probably can get a little bit of that information. They have -- they did do some fill-blanks and such.

I think the question you ask is complex in all kinds of different ways, because it does -- what you saw were daily chemographs, but they were basically sampling, for the most part, taken in one day, one time -- one incident a day, dipping graph sampling.

Now, a few of those actually were auto-samplers that sift at intervals, and then what you have for a daily is a 24-hour average

over that.

You still have sampling taking one point, across the stream. So, you will get some variation in that. We don't really have that characterized in this particular study.

DR. LEE: That could be a useful thing to know a little bit more about, if you're trying to make any sort of confidence interval. That's going to be critical for your intervals.

MR. THURMAN: Yes, that's true. Now, what I will say is that for the community water systems, it comes from the -- they have -- they've sample both source water and the treated water. We're focusing on the source water. It comes from the intake.

So, what you see is what is integrated at that intake, which takes some of that variability out. You still have a temporal variability that you're going to get in that.

In fact, sometimes, what we'll see is monitoring data is, where they've paired the source water with the treated water is, that

sometimes, you'll see treated water with higher concentrations than source water because it's differences in time, and so, it's part of the variability that's --

DR. LEE: That's natural variability.

MR. THURMAN: Yes.

DR. LEE: You can't get rid of it. The question is just can we quantify it a little better, and it's not that you need to sample it everywhere, but if you had some sort of global idea of what it might be like, that would be really helpful.

MR. THURMAN: I know we have a couple of USGS folks on the Panel, who hopefully, can provide some words of wisdom to help us figure that out.

That's one we've wrestled with, because we do know that --

DR. LEE: I figured it's something you must have looked at before, but I didn't see anything about it in the white paper.

The other comment I want to make is,

I think that the kriging Gaussian process type approach has a lot of potential here. I'm glad to see you folks are looking at it.

I did want to comment, that you talked about some variogram analysis, and they've noticed that it varies a lot from year to year. It's actually a very noisy process to try and analyze a variogram, and I'd expect that even if you sort of just took repeated samples, you'd get very different variograms within the same year.

And so, there is a lot more possibly going on there, and it may be that it actually doesn't change that much year to year, and you're just looking at variability, sampling variability.

MR. THURMAN: Well, I will say that their initial analysis was with a limited data set. We were going -- we're going to be looking at a lot -- hopefully, a lot more data -- sets of data.

So, hopefully, some of that will start shining in, but I appreciate that.

SESSION CHAIR PORTIER: I had a question, it was just a clarification. The hydrographs we're seeing here are for the raw water is raw water at the intake, or is this from the ecological studies that we --

MR. THURMAN: The ones I showed you were illustration of ecology studies, and the reason I showed you that is because -- and you see in the paper, we showed some of the chemographs with the drinking water, but those are weekly samples.

So, what I wanted to do, to try to -- particularly, when I started illustrating, you know, let's take seven day samples, I realized, that's not going to work for those -- you know, for those chemographs we generated before.

So, that's why I went to the ecological exposure monitoring, because it did have daily variations.

SESSION CHAIR PORTIER: I was thinking about intake water coming from -- well, we have a dendritic system, right, and you're up here, at

the ecological studies were high up here, level one, level two streams, and rarely do community water systems draw from that level. They're drawing three, four and five, right?

MR. THURMAN: Yes, that's true, although, as we've looked at this, I have found -- I have come across looking at maps and analyzing particularly, some of the monitoring sites and where it goes, I've found community water system intakes up in those same areas, and some of --

So, it can happen. We know that those are typically higher up in the watershed than what you're going to see farther down. I selected those because while they're farther up stream, they do show the shape of the chemographs that you're likely to see, even farther down stream.

SESSION CHAIR PORTIER: That's the confirmation. Dr. Young?

DR. YOUNG: I just have a basic question. I don't -- I think I'm missing some big

idea, because you talk about false positives and false negatives. So, then that -- I'm not clearly, exactly what you're trying to estimate, because if you get a value, it could have measurement error, but it may -- what if it's right on, but you take another value and it's right -- no measurement error, but you get different values?

Are you trying to estimate the mean for the area, a quartile? You see, I don't see what the foundation is for false positive and false negative.

MR. THURMAN: I think that's one where I think Mike Messner will be able to illustrate it a little bit better in his presentation, than what --

What we're looking at is, and just to try to give you -- which I don't know if it will help you or not, we get exposures -- monitoring sample -- data samples, say, weekly, and we've got to come up with an estimate of a duration -- of an exposure for a duration that's say, one

month.

And so, if we take those weekly samples, there's various approaches that you can take. I mean, if you assume that the same concentration for the next -- for a seven day period until your next sampling and average -- and use that to average those together, to come up with a one month sample, there are times when you may have hit, where if the one sampling point happened to hit your peak, and you carry that out seven days, when in fact, it drops pretty rapidly, you're going to end up with an over-estimate of that monthly average.

There are other times when you may be an under -- under-estimating, because you don't hit that peak. So, that's what I'm --

DR. YOUNG: I think, let me see if I have it. So, what you're trying to estimate is the average for the temporal scale of interest?

MR. THURMAN: That's correct.

DR. YOUNG: Okay.

MR. THURMAN: That's correct.

SESSION CHAIR PORTIER: Dr. Hayton, from the Ohio State University joined us. He wasn't here when we started, and he's got a question, and --

DR. HAYTON: Well, a couple of questions. One is, what -- you're talking -- these chemographs, what happens to that in the pipe? I mean, when I open my faucet, am I -- you know, if I took a sample out of my kitchen sink, once a week, would it look like what you're showing us, or does that get modulated in the distribution?

MR. THURMAN: It gets modulated. There are a number of factors that go into that. It goes through a treatment system. Depending on the type of treatment that the community water system has, you may have some non or all of the atrazine removed, depending on the treatment system.

Because you're going into collecting and holding tags of processing tags and moving through, it does get -- tend to get modulated as

you go through what's exposed.

However, I will point out that EPA's goal is to protect the source water. So, we're focusing on how do we protect the source water?

We need to generate exposure estimates that are going to reflect drinking water for human health, at the same time. If you look at the reasonable certainty of no harm, if we can get the source water concentration lower than our levels of concern, we can be certain that the treated water is going to --

DR. HAYTON: So, there's a margin of safety there, and then finally, what -- it wasn't clear to me, exactly how do you use those concentrations? I mean, obviously, protecting public health, but how -- does a bell go off somewhere, when the atrazine concentration spikes and the whole water system shuts down, or what happens?

MR. THURMAN: What we do is, we have a dietary exposure assessment and Dr. Lowit will probably be able to answer a little better than I

do, but you basically, you take your drinking water exposure estimate, with the average for whatever duration of concern it is, couple that with your dietary exposure and in cases where you do have residential exposure, you couple those together.

Atrazine, the dominant route of exposure, and maybe the only real route of exposure for atrazine is from the drinking water. So, we don't really have exposure in food that we would expect, and while there may be some long use in some areas, for the most part, we don't have residential exposure, that we look.

So, essentially, we are looking, in this case, at concentration in drinking water, but for other pesticides, it's coupled with the dietary exposure.

DR. HAYTON: But if you think it's too high, do you -- you know, what's the next step?

SESSION CHAIR PORTIER: I think Dr. Bradbury can answer that.

DR. BRADBURY: So, just let me touch on

your question and reaffirm that the conditions of the re-registration in 2003 are designed to protect the source water.

So, the monitoring analysis that you're going to hear about from the team, combined with whatever you decided the appropriate exposure window is, if we need to change it, the whole construct of the regulatory decision is that people are drinking the raw water, not the finished water, and their goal is to ensure protection of the source water, because 5th RED, we can protect the source water.

Safe Drinking Water Act, we use technology, if necessary, to get the concentration down to a safe level. Our approach is, we should be protecting the source water through a regulatory decisions.

Now, the conditions of re-registration in 2003 have this monitoring program in place in the community water systems, and if we get a level above our level of concern, we're aware of it quickly and I believe in our condition of re-

registration, if in two -- let me back up.

The community water system is monitored for five years. So, if it goes into the program, it's monitored for five years. If during the course of the five years, it never exceeds the level of concern, then it can get out of the monitoring program, but it could always come back in again, if it's safe drinking water sampling regime, which is once every quarter, starts to approach the three part per billion MCL and then it goes back into the sampling program.

So, it's an ongoing monitoring program. But five years in a row, you don't exceed a rolling 90 day average, then you -- it comes out of the monitoring program.

If it hasn't exceeded in two years, so, one year, it exceeds, it exceeds the next year, then atrazine is off-labeled in that drinking water schedule and it can't be used in that watershed.

So, we do have a bell that goes off and the bell that goes off is, you can't use

atrazine in that drinking watershed, if you exceed the level of concern.

Some of the dialog we'll have on the science is, is that 90 day rolling average changing, as we look at the toxicology? Do you have a monitoring program in place, if exposure window is variable? How should your frequency of sampling vary?

SESSION CHAIR PORTIER: Well, a bell has gone off. It's 6:02 p.m. by my ruling and we have about an hour's worth of presentation by EPA, that we're going to put off, I'm sorry, Mary, until tomorrow morning.

Well, I'll looked at your slides, and the statisticians over here could probably stand it, I'm sure this whole Panel would -- would fall off.

We're going to pick up in the morning. This will impact the public presenters, in the sense that they're going to be moved back another hour. If there are presenters for which this is going to cause a problem, please, let myself or

Joe Bailey know. We'll try to schedule you a little earlier. I know some people don't stay for all of this discussion.

We start again tomorrow morning at 8:30 a.m., on time, with EPA's continued presentation on the water.

(Whereupon, the above-entitled matter concluded at approximately 6:05 p.m.)

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