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FIFRA SCIENTIFIC ADVISORY PANEL

(SAP)

OPEN MEETING

POTENTIAL DEVELOPMENTAL EFFECTS OF ATRAZINE

ON AMPHIBIANS

June 17, 2003

[8:30 a.m.]

Crowne Plaza Hotel Washington

National Airport

1489 Jefferson Davis Highway

Arlington, Virginia 22202

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DR. ROBERTS: The topic for this session is going to be the potential developmental effects of atrazine on amphibians. I would like to begin the meeting by introducing our designated federal official, Mr. Paul Lewis, and ask if he's got any announcements for us.

MR. LEWIS: I thank you Dr. Roberts. And I want to first thank Dr. Roberts for serving as our incoming chair for the FIFRA SAP, looking forward to working with him. And also to acknowledge our permanent panel members, Dr. Gary Isom and Dr. Steven Herringa. Dr. Handworker, also another permanent panel member, is unavailable at this meeting today but will be here in July.

I am Paul Lewis and I will be serving as a designated federal official to the FIFRA SAP for this meeting over the next four days. I want to thank both the members of the panel and the ad hoc members for agreeing to serve the next four days for what I think we'll find a very challenging and interesting discussion that we'll be having. Reviewing the potential developmental effects of atrazine on amphibians, we appreciate the time and the effort of the panel members in reviewing the materials and preparing their remarks, taking into account their busy schedules.

By way of background, the FIFRA SAP is a federal advisory

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committee that provides independent scientific peer review and advice to the Agency on pesticides and pesticide-related issues regarding the impact of proposed regulatory actions on human health and the environment. The FIFRA SAP only provides advice and recommendations to the Agency, that is, making implementation authority remains with the Agency.

FIFRA established what is called a permanent panel which consists of seven members. The expertise of the panel is also augmented through a science review board and science review board members serve as ad hoc temporary members of the SAP providing additional scientific expertise to assist in reviews conducted by the panel.

As the designated federal official for this meeting, I serve as liaison between the panel and the Agency. And I'm also responsible for insuring provisions of the Federal Advisory Committee Act are met.

The Federal Advisory Committee Act establishes a system for governing the creation, operation, termination of executive branch advisory committees. It highlights the consideration of federal advisory committees on the FACA as follows: The committees are chartered. They are governed by uniform procedures. They provide

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only advice, are open to public scrutiny.

In addition, FIFRA SAP meetings are also subject to FACA requirements. And these include public meetings, timely public notice of the meetings, and document availability. In that respect, we have documents for this meeting. The background paper, public comments, and the final meeting will be available through the Office of Pesticide Programs docket.

In terms of financial conflicts of interest, as the designated federal official of this meeting, a critical responsibility is to work with appropriate Agency officials to ensure all appropriate ethics regulations are satisfied. In that capacity, panel members are briefed with provisions of the federal conflict of interest laws. Each participant has filed a standard government financial disclosure report.

I along with our deputy ethics officials for the Office of Prevention of Pesticide and Toxic Substances, in a consultation with the office general counsel, have reviewed the report to ensure all ethics requirements are met. And a sample copy of the form is available on our FIFRA SAP web site.

The panel will review challenging science issues over the next several days. We have a full agenda and meeting times are

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approximate. This may not keep to the exact times as noted due to panel discussions and public comments. We strive to ensure adequate time for Agency's presentations, public comments being presented, and panel deliberations.

For panel members and public commentors, please identify yourselves and speak into the microphones provided since the meeting is being recorded. Copies of all presentation materials, public comments, will be available in the Office of Pesticide Program docket, as I mentioned previously, within the next few days.

For members of the public requesting time to make a public comment, please limit your comments to five minutes unless prior arrangements have been made. For those that have not preregistered, please notify myself or a member of the FIFRA SAP staff who are sitting just to the right of me here.

As I mentioned previously, there is a public docket for this meeting. All background materials, questions posed to the panel by the Agency, and other documents related to this SAP meeting are available in the docket. Overheads will be available in the next few days.

Finally, background documents are also available on the SAP web site. And the agenda for this meeting lists contact information

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for that type of material.

For members of the press, we have a press contact from our office, Mr. David Deacon, of the Office of Media Relations, is available to answer your questions. Mr. Deacon, please stand. Thank you. Any interested people who have questions from the press, please refer them to Mr. Deacon.

At the conclusion of the meeting, the FIFRA SAP will prepare a report as response to questions posed by the Agency, background materials, presentation, and public comments. The report serves as meeting minutes, and we anticipate the minutes to be available in approximately two to four weeks and will be posted on our web site and in the OPP docket.

Finally, due to unforeseen circumstances, this Friday's meeting will move from this location to the Holiday Inn National Airport, 2650 Jefferson Davis Highway in Arlington, Virginia. The meeting address for this Friday's meeting is noted on the meeting agenda outside this room, and you'll notice some placards in the hallway when you first enter the room. The Holiday Inn is approximately 8 to 10 blocks from our present location and parking is available. This meeting change will only be for this Friday, June 20. All other days, our meeting will be occurring here.

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We apologize for any inconvenience that may occur and are making special arrangements for this change of meeting location this Friday. If you require any assistance to attend the Friday meeting, including maps or shuttle service to the Holiday Inn hotel, please visit a member of the FIFRA SAP meeting.

Thank you. Dr. Roberts.

DR. ROBERTS: Thank you, Paul. The SAP staff has assembled an outstanding panel of experts to deal with this topic, and I would like to introduce that panel now and do so by asking each member of the panel to state their name, their affiliation, and their area of expertise. And I think we'll just go around the table counter-clockwise starting with Dr. LeBlanc on my immediate right and then for each member of the panel around the table to introduce themselves. Dr. LeBlanc.

DR. LEBLANC: Thank you. My name is Gerry LeBlanc. And I'm a professor in the Department of Environmental and Molecular Toxicology at North Carolina State University. My area of expertise is awake toxicology.

DR. KELLEY: I'm Darcy Kelley. I'm professor of Biological Sciences and a member of the Center for Environmental Research and Conservation at Columbia University. And my area of expertise is

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sexual differential of the amphibian Xenopus laevis.

DR. KLOAS: My name is Werner Kloas. I'm professor for endocrinology at University of Berlin. And I'm also heading the Inland Fisheries Department of Leibniz-Institute of Freshwater Ecology and Inland Fisheries. My working group is the research focused on awake disruption in amphibians, especially addressing sexual differentiation and also the thyroid system.

DR. GREEN: My name is Sherril Green, and I'm an associate professor in the Department of Comparative Medicine at Stanford University. My area of interest and expertise is in laboratory Xenopus laevis, Xenopus laevis and Rana pipiens specifically as a laboratory animal model.

DR. COATS: My name is Joel Coats. I'm professor of entomology and toxicology at Iowa State University. My areas of expertise are in pesticides, especially environmental toxicology and environmental chemistry.

DR. DENVER: My name is Robert Denver. I'm associate professor of molecular, cellular, and development biology at the University of Michigan. And my area of expertise is developmental neuroendocrinology of amphibians.

DR. ROBERTS: Let's jump over to Dr. Gibbs.

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DR. GIBBS: My name is James Gibbs. I'm an associate professor of conservation biology at the State University of New York's College of Environment Science and Forestry in Syracuse, New York. And my area of expertise is amphibian demography and population dynamics.

DR. RICHARDS: My name is Carl Richards. I'm a professor of biology at University of Minnesota Duluth and director of the Minnesota Sea Grant College Program. My expertise is that of an aquatic ecologist and landscape ecologist.

DR. DELORME: My name is Peter Delorme. I'm a senior risk assessor with the Canadian Government working on risk assessments of pesticides. My area of expertise is aquatic ecology.

DR. SKELLY: My name is David Skelly. I'm an associate professor of ecology at Yale University. And my area of expertise is population and community ecology of amphibians.

DR. MATSUMURA: My name is Fumio Matsumura. I'm at the Department of Environmental Toxicology. My area of expertise is molecular toxicology. I'm interested in the frogs, too.

DR. THRALL: I'm Mary Anna Thrall. I'm a professor of veterinary pathology in the College of Veterinary Medicine at Colorado State University. And my area of expertise is veterinary

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clinical pathology.

DR. ISOM: I'm Gary Isom, professor of toxicology at Purdue University. And my area is neural toxicology and neural degenerative diseases.

DR. HEERINGA: I'm Steve Heeringa, biostatistician and director of the statistics design group at the Institute for Social Research at the University of Michigan. My specialty is population-based studies and design of population-based studies.

DR. ROBERTS: And I'm Steve Roberts. I'm a professor with joint appointments in the College of Veterinary Medicine and College of Medicine at the University of Florida. I also serve as director of the Center for Environmental and Human Toxicology there. My areas of expertise are mechanisms of toxicity, particularly involving the liver and immune systems and also methods of risk assessment.

I would now like to welcome Mr. Merenda who is Director of the Office of Science Coordination and Policy. Good morning, Mr. Merenda.

DR. MERENDA: Good morning, Steve, and welcome to all of the panelists as well as all of those who are participating in attending this session in the audience.

Within EPA, the concept of independent, external scientific

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peer review plays a very important role in our evaluation of decision processes. And so this kind of event, while complex to organize, is a very important part of our job. In fact, it's an important part of the job of my office, the Office of Science Coordination and Policy in EPA's Office of Prevention Pesticides and Toxic Substances.

We find that these kinds of meetings with both the permanent panel members and a number of expert ad hoc members who are selected specifically for your expertise on the subject atrazine and is extremely valuable to the Environment Protection Agency in helping us to better understand where we've done things well and where may have missed some points or where we need to look further and dig deeper as we evaluate the data available to us and make risk management and regulatory decisions.

This is going to be, as Paul said, a very full program. Over the next four days, there are a number of complex issues for us to deal with. And we are quite pleased to have the expertise that is being brought to us by all of you and we're quite thankful for your willingness to take time from busy schedules and other commitments to spend these four days with us in helping us better understand these problems.

So welcome, and just to reemphasize what Paul said, that if you

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have any needs for logistics or assistance with making this meeting work better or making your own arrangements work out while you're here at the meeting, please, do not hesitate to contact a member of our staff on the FIFRA Scientific Advisory Panel.

DR. ROBERTS: Thank you very much. I would also like to extend the panel's welcome this morning to Ms. Anne Lindsay, who is the Acting Deputy Director of the Office of Pesticide Programs. Good morning.

MS. LINDSAY: Good morning. You've taken my first line away. I was going to say I was Anne Lindsay, Acting Deputy Director for Programs in the Office of Pesticide Programs.

I'm actually here on behalf of Jim Jones who is our relatively new office director, though not new to the Pesticide programs. He is actually dealing with family responsibilities this week and asked me to welcome you to Washington, and in particular, to thank you for agreeing to serve on this scientific advisory panel and the sort of extensive meeting that we've got set up for the week. I'd actually hoped I was going to welcome you to a sunny Washington. But that doesn't look like that's going to happen.

The topic of this meeting, the Potential Developmental Effects of Atrazine on amphibians, is one that has generated an extraordinary

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amount of interest since the first data appeared suggesting a link between atrazine exposure and development effects in frogs. It's not only a topic that's drawn a lot of interest, I think it's fair to say, that it's actually been extremely controversial.

Now for those of us who work at EPA on Pesticide regulation, such controversy is actually often or frequently part and parcel of doing our job. It's not that we want it to be that way. But that is often how it is. And I think that's the case because the issues we deal with are usually very complicated and often, as I think the subject of this meeting, really falls at the cutting edge of science so the answers may not be clear cut.

We find ourselves dealing frequently with situations in which the answers are not obvious. And different groups also have strongly held views and strongly held contrasting views. Over the years, we've developed a deep appreciation, therefore, for the value of science as the basis of our work and to have that science guide our decisions.

And that's really, frankly, where all of you as a panel and as experts in your various areas come in. Your job is to help us figure out what the scientific information actually does tell us, where we can trust information, where there are questions about it. We want your advice to guide us as we move forward making our regulatory

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decisions.

I've spent probably not only the largest part of my career in public service maybe the largest part of my life at this point in public service. I think when you do that, you have to be able to have a great deal of pride and draw a great deal of satisfaction in contributing back to the community and the country that you come from. And that is certainly, I think, how I and the other EPA folks who will be presenters here today feel about our work.

We hope that you, too, since at this point as members of the panel, you're part of the public service in effect, will take that sense of satisfaction for making a very real contribution to the civic life of our country. There's no doubt in my mind that the work that you will do as panel members has enormous value to us at EPA. And because of that, I believe it will have enormous value to the citizens of this country. So I want to thank you very much for making that contribution of your time and expertise.

I'd like in particular to acknowledge EPA's thanks to Dr. Roberts, the chairman of the SAP. This is actually, I believe, your first meeting as chairman of the SAP. But you are well-known and a highly regarded scientist who's served for a number of years on our panel and has presided as session chair at some of our most

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controversial meetings with firmness and grace. So we're looking forward to a continuation of that firmness and grace. We thank you for contributing on the panel with these new responsibilities.

We'd also like to extend our appreciation to the other new permanent members of the panel who I think Paul has introduced but Dr. Steward Handworker, Steve Heeringa, and Gary Isom. It's a delight to have Dr. Heeringa back with us after serving as an ad hoc panel member on multiple occasions and also we welcome Dr. Isom as a permanent member of the SAP.

In addition, my thanks to all of the scientists who have agreed to serve as expert advisors on the atrazine issues. Your willingness to contribute your knowledge and expertise to sorting out pressing scientific issues is invaluable.

And then finally, Paul, I want to thank you and the other members of the SAP staff. You do a great job taking care of the panel members and a great job running the meeting. And for that, we're deeply appreciative.

So, finally, let me wish you the best for the upcoming meetings and we look forward to receiving your report.

DR. ROBERTS: Well, thank you for your kind remarks. They're very much appreciated.

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Our discussion of the scientific issues is going to begin with presentations by the Agency. And the first presenter is Dr. Steven Bradbury of the Office of Pesticide Programs. Good morning, Dr. Bradbury.

DR. BRADBURY: Good morning, thank you. I'd also like to extend my thanks to the SAP staff for helping to organize the meeting and to the panel for the discussions we'll be having over the next several days. Your input and advice will be greatly appreciated and very important part of the scientific analysis that we've embarked upon with the white paper.

What I'd like to do in my presentation is go over a few of the issues you'll be hearing in more detail from Tom Steeger and Joe Tietge later this morning. And to review a bit why we're here and what we hope to accomplish over the next several days, as I've mentioned in my opening remarks, we're looking forward to obtaining your recommendations on our analysis to date regarding the potential developmental effects of atrazine on amphibians. And as we go through the morning's discussions, we'll be walking through several topics of particular note.

One will be the integration of the available information as we've summarized in the white paper. We'll also be discussing

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aspects of that information in the context of how it allows one to formulate risk hypotheses about the potential effects of atrazine on amphibian development. And from those risk hypotheses, establishing a conceptual model for potential effects. And ultimately, then taking a look at an analysis plan as to how to move forward in the context of that available information.

To provide a little background as to where we've been and how we've got here, let's go back to January 31, 2003. That's when EPA released an Interim Reregistration Eligibility Decision, an IRED. And that document included an assessment of human health risk assessment issues as well as taking a look at ecological risk assessment issues.

In the January '03 ecological risk characterization, we followed sort of the basic tenets of the Agency's guidelines for ecological risk assessment and took a look at exposure issues and took a look at the physical chemical properties of atrazine and compared both modeling and monitoring and find general consistency between monitoring studies and the classes of watersheds and uses that atrazine is associated with.

The effects analysis or effects characterization in that document focused on the integrity of aquatic communities or the

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stability of aquatic communities structure and function as a risk assessment endpoint and came to the conclusion the likelihood of adverse effects would occur at approximately 10 to 20 micrograms per liter over recurrent or prolonged periods of exposure. And this analysis was similar to a somewhat independent analysis going on in the Office of Water as they develop their draft water quality criteria for atrazine.

In the document, we also discuss some of the uncertainties that are associated with this risk assessment and all risk assessments have different levels in context of uncertainty. Then we discuss some of the exposure characterizations uncertainties, including some data gaps in terms of being able to predict the atrazine concentration patterns and attributes across a full population of water body types in the United States. And we also discussed some of the uncertainties in terms of the spatial and temporal variability of atrazine and the ability to predict or model those patterns.

In terms of effects characterization, we discussed the challenge of converting steady state atrazine exposures to fluctuating or transient atrazine exposures and how to work through the dosimetry of that kind of an exposure scenario. We talked about the issues in terms of quantifying aquatic community recover or resistance to

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repeated exposures to the compound. And, finally, we also discussed the uncertainties concerning the potential developmental effects of atrazine on amphibians.

At the time that the IRED was being prepared, there were several studies that were addressing the potential effects of atrazine on amphibian development and were being published at about the same time or during the period of time that we were preparing that January '03 document.

As a consequence, we really didn't have time to perform a rigorous evaluation of these data for inclusion in that January IRED. And consequently we agreed with NRDC that we would proceed with our analysis of this issue of the potential effect of atrazine on amphibian development during the time period that we're all in now. And we set course on a path to review the available information through February 28 of 2003 and then convene a panel as we're doing today to discuss the information at hand and to gain your insights and comments on the conclusions we've reached to date concerning that information.

More specifically, what we wanted to set out in terms of the agreement between January and October, was to take a look at the significance of the amphibian risk data and determine whether there

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was a need for additional data to characterized more fully atrazine's potential risks to amphibian species, and if so, what data should be developed to further reduce the uncertainties associated with this question.

What I'd like to do over the next few minutes is just provide a bit of a roadmap and some of the milestones for the rest of the morning's presentations by the EPA folks. And the white paper actually sort of sets up the problem. It helps formulate the problem before us. And I've used the words "formulate the problem" in the context of the Agency's 1998 ecological risk assessment guidelines. And the roadmap that we're going to use today to summarize the highlights of the White Paper to in fact use the Agency's guidelines as a framework for the presentations and the logic train that we went through in interpreting the information that's currently available.

I think you're all aware of Agency's guidelines, but I'd like to spend just a few minutes touching upon what I feel are some of the important aspects of the Agency's guidelines and the role of science in risk management and regulatory decision making.

Obviously, I believe, ecological risk assessments are the science part of the overall decision making process. And in our risk assessments, which is the box in that figure on the screen, is where

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the science of ecological risk assessment occurs. And through the development of an ecological risk assessment, clarifying the uncertainties, establishing what we know and what we don't know the assumptions behind the analyses helps inform the overall decision-making process which blends in, of course, to many other considerations other than the science.

So the key over the next few days is to take a look at the science associated with this issue and determine where our certainties are, where are uncertainties are, and how that can be helpful in helping to inform the process of overall decision making with regard to atrazine and its potential effects on amphibians. So the focus is on the science.

Over the course of the next several presentations, or my presentation, Tom's and Joe's, we're going to focus on especially the problem formulation phase of an ecological risk assessment because, in fact, that's sort of where we are right now. We're in the stage of formulating the problem, starting to define what we know, what we don't know, what types of risk hypotheses can we formulate with the existing information, and to develop a pathway for moving forward.

So we're in the process of generating and evaluating preliminary hypotheses about why effects may occur or could be

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occurring and to articulate the uncertainties that would be associated with moving forward in a risk assessment. I think it's also important to remember that the guidelines for ecological risk assessments acknowledge an iterative process. And that as the science evolves and risk management issues evolve, the risk assessments can evolve too. And so in a sense, we're in an iterative process of the overall risk assessment for atrazine.

As I mentioned earlier, in the January document we articulated one uncertainty that was associated with the potential effects of atrazine on the development of amphibians. And in a sense, we're going through an iteration of the risk assessment for atrazine; and we're at the stage of looking at problem formulation.

So what does that mean? What I've done in the next slide is expanded on the concepts of problem formulation and what some of the key aspects of this phase of a risk assessment entail. And this will be the focus, this is really the focus of the White Paper and it is the focus of the presentations we will be making today.

Through the problem formulation, one sets the stage for moving forward in the overall risk assessment. Outcomes of a problem formulation could include a decision that there's no need to go forward, that, in fact, there isn't an issue that requires a risk

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assessment. Another option, talking about broad pathways, one can move forward after problem formulation would be that, in fact, there is sufficient data to move ahead with the risk assessment with specified levels of certainty. And another option would be that, while it's possible to form risk hypotheses, there are certain uncertainties that are associated with the risk assessment; and the analysis plan, therefore, may call for or suggest additional data that could be needed.

The key in problem formulation is associated with box the on my left and the arrows that are going both ways. And that's the dialogue between the risk managers and the risk assessment team. The risk assessment is science, but it's not science in a vacuum. It's science to inform regulatory decision assessment and risk management. And so the decisions with regard to certainty and uncertainty certainly have a scientific basis, but they also have a context. And the context is in the context of how much certainty is required to make a regulatory decision with a specified level of confidence.

The role of the risk assessor is to provide the risk manager insights into the uncertainties, the certainties, the risk hypotheses, and to engage in a dialogue as to the pathway and moving forward.

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The White Paper is a problem formulation. The White Paper is designed to help inform all of us and you and gain your insights as well to inform the broader community as to the potential pathways that make sense given the information that we currently have.

The key products in a problem formulation are the items in the circles on the figure. The clarification of the risk assessment endpoints and the measures of effects is an important outcome of problem formulation as is the conceptual models which is essentially as series of one or more risk hypotheses as to how one may envision or hypothesize that atrazine, in this case, could have potential developmental effects on amphibians.

And then an analysis plan, given the current body of information, a plan in moving forward. How are we going to use the available information, if additional information could be gained, what kind of information would contribute to closing what knowledge gaps. And again, before moving forward into actual risk characterization dialogue with the risk management community to ensure that the regulator decision makers in the agencies have a clear understanding of the uncertainties, and the issues associated with moving forward with different levels or amounts of data. The risk assessment is designed to inform regulatory decisions; it doesn't make regulatory

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decisions.

Let me just spend a few minutes, then, with this roadmap as background, hit a few of the milestones on the journey we'll take over the next couple of hours in reviewing the White Paper.

As I indicated in that previous slide, one of the most critical steps actually in problem formulation is integrating the available information. What do we know, what don't we know, and how do we use that information to draw conclusions concerning risk assessment endpoints, measures of effects; how do we use that information to help establish risk hypotheses, and ultimately establish the analysis plan for moving forward.

Tom Steeger will be providing an overview of the key studies that were discussed in the White Paper in some detail. And as you'll recall, there were 17 studies available for analysis by the Agency as of February 28, 2003. Seven of these studies were laboratory-based studies; and 10 of the studies were field experiments.

The White Paper describes how we looked at the study attributes, experimental designs, the various protocols that were used, and how we looked across those studies to take a look at the body of knowledge, looking at the consistency across the studies, how the studies as a whole provide insights into the strength of cause-effect or

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dose response relationships, the extent to which the body of knowledge provides insights on mechanistic plausibility concerning the potential effects, and issues regarding ecological relevancy.

And, of course, throughout the White Paper and throughout the process of developing a problem formulation, one keeps track of the certainties and uncertainties as one integrates and evaluates the existing information.

Tom, in his talk, will go over the body of the information and sort of a synthesis mode. Tom's not going to go through each individual study because the White Paper provides that level of analysis. And rather Tom's going to summarize the synthesis and the integration of the information. And so as he talks about study protocols and design, it's designed to be reflective of the entire body of studies and not necessarily a specific comment for a specific study. During Thursday or Friday, if you'd like to talk in more detail on specific studies, of course, we'd be happy to do so.

One of the outcomes of problem formulation, which I mentioned previously, is establishment of the risk assessment endpoints; and they draw in part from the available information, but they'd also have to be connected to the Agency, in this case, EPA's mission. What is it all about in terms of protecting the environment and human health.

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And so you have a train of logic that needs to connect environmental management goals to a risk assessment endpoint and then to the measures of effects which will be used to estimate how those risk assessment endpoints may change based on different exposure scenarios.

So in this context, we're talking about environmental management goal which is the viability of anuran populations. So the analysis of the studies that Tom and Joe will talk about, we're focusing on a risk assessment endpoint which involves the reproduction and recruitment of native anurans.

Again, a risk assessment endpoint needs to be a ecological entity and the attributes of the entity. So the entity is anuran, native anurans of North America, native anurans, and the attribute reproduction and recruitment.

Through the analysis of the existing information that Tom will describe as further highlighted in the discussion that Joe will provide, there's a whole family of measures of effects that have been reported in the literature and it can be useful in terms of estimating how the risk assessment endpoint may change based on different atrazine exposures.

And on the slide, I've listed many of the measures of effects

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that were included in those studies; and Tom will be talking about the interpretation of that information in the context of estimating the risk assessment endpoint behavior.

A second major output of the problem formulation stage and of the White Paper is the conceptual model. The conceptual model is a way of pulling together the lines of evidence, the information that's available, to formulate risk hypotheses and to provide insights into how we need to go forward. As I said, I'm going to provide some of the milestones on the path; and Tom and Joe will provide more of the details of how we got to some of the milestones that are shown on this slide.

We went through the available information and as we discussed in the White Paper we concluded that the lines of evidence did not show a consistent, reproducible effect of atrazine across the exposure concentrations in the amphibian species tested. But we also noted that there were issues concerning the study protocols, the experimental designs, and inherent uncertainties in the issue at hand to make it difficult to fully interpret the information. And consequently and as a result of the strength of the studies, we did come to the conclusion that the available data is of sufficient quality to establish a risk hypothesis that atrazine could cause developmental

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effects in amphibians.

Between Tom's talk and Joe's talk, we'll review the White Paper in the context of how we reach that risk hypothesis and establish in more detail the conceptual mode of atrazine's mechanism of proposed mechanism of action and how that could lead to developmental effects or reproductive effects in the context of the risk assessment endpoint that I mentioned previously.

And, finally, the last major product from a problem formulation and as discussed in the White Paper, is the analysis plan. The analysis plan, again, is a series of options to have a dialogue with the risk managers in the Agency in terms of where to move forward. And here, again, I think it's important to come back to the interface between risk assessment and risk management.

In an analysis plan, options are created based on the scientific certainties and uncertainties available in the information. The decision as to how much uncertainty or how much uncertainty one can make a decision is part of that interface between the risk assessor and the risk manager. And so the analysis plan lays out concepts for future studies, lays out a roadmap for future studies. But they're in the context of whether or not greater certainty is required to make the regulator decision.

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There's some science and then there's policy in FDM that goes on. And the White Paper is discussing the scientific certainties and uncertainties that EPA feels exists in the current body of information and provides some thoughts and some concepts and plans as to how those uncertainties could be closed if they need to be closed to make a regulatory decision.

So the analysis plan then, based on our risk hypotheses and the conceptual model, are designed to enhance or improve the clarity of potential causality in terms of atrazine's potential effects on amphibians as well as to further characterize the potential dose response relationship between atrazine exposure and developmental effects.

In further phases of the analysis plan, it talks about making connections to mechanisms of action as well as ecological relevancy.

I think that's another important point to bring out is that, as I mentioned earlier, risk assessments can be iterative, they can be phased, they can be tiered, and in fact, an analysis plan in an ecological risk assessment can lay out a phased or tiered approach to reducing uncertainties, incremental gains in knowledge as a basis of needs for informing the risk managers in the decisions they need to make.

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And as you'll note in the White Paper and as we'll summarize this morning, the analysis plan lays out a phased or tiered approach to looking at specific uncertainties in sort of cascade approach.

Again, the decisions to move through the phases in the analysis plan would be tied to risk management decision criteria. But how is the science in terms -- what's the state of the science in informing the risk managers for different decisions they may need to make.

So in conclusion from my talk, I want to stress that the White Paper reflects our conclusions to date based on our analysis of the information and our interpretation of this information in the context of the Agency's risk assessment guidelines. Now we're at the important stage of gaining insights and advice and counsel from our scientific peers in terms how we've taken a look at the data, how we've integrated the available information, gain your insights and advice and counsel on how we evaluated the studies, how we characterize the available studies, and the conclusions that we drew from the available body of information.

Of course, we're also looking forward to your thoughts and opinions in terms of the risk assessment endpoints and the measures of effects. And then ultimately the conceptual model and the analysis plan.

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So with that, I'll close. And I'll be happy to answer any clarifying questions at this point.

DR. ROBERTS: Thank you, Dr. Bradbury, for laying out the task in front of us. Let me ask the panel if they have any questions or clarifications for you.

I see no questions, so thank you very much.

Let's go on then next to Dr. Steeger's presentation on an overview of the atrazine studies. Good morning, Dr. Steeger.

DR. STEEGER: Good morning. Thank you for the opportunity to discuss some of the recent literature that has become available regarding the potential effects of atrazine on amphibian development.

As indicated, the Agency has developed a White Paper intended to review recent studies conducted on the effects of atrazine on amphibian development. This presentation will provide an overview of the study reviews and attempt to integrate the information to answer whether there is sufficient information to substantiate claims that atrazine exposure results in development effects in amphibians.

Panel members have had an opportunity to review each of the studies for themselves. This presentation will look at the studies collectively rather than focus on individual studies.

As early as 1998 and continuing through this year, a series of

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studies have been published indicating the variable lengths of exposure to atrazine is associated with gonadal effects in amphibians based on two studies published by Tevera-Mendoza in 2001, three studies published by Hayes in 2002, research described in a poster presented by McCoy, et al., in 2002 at the meeting of the Society of Toxicology and Enviornmental Chemistry; and a study by Carr, et al., in 2003; there is sufficient information to formulate a plausible hypotheses that atrazine exposure may result in development effects in amphibian gonads and that these effects may impact secondary sexual characteristics in these animals.

However, also based on these studies reported in the open literature, there is a lack of consistency in the type of effect produced and the concentration of atrazine required to produce that effect.

As part of a consent decree between the Agency and the Natural Resource Defense Council, the Agency agreed to conduct and review the available literature regarding the effects of atrazine on amphibian development. The Agency reviewed a total of 17 studies that were submitted as of February 28, 2003. As Steve indicated, 12 of the studies were sponsored by the registrant where 5 were drawn from the open literature.

Registrant-submitted studies received more scrutiny since more

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data were available. Although none of the studies were conducted under good laboratory practice conditions, many of the studies had standard operating procedures and some level of quality assurance in place. Additionally, on studies where raw data were available, the data were re-subjected to statistical analyses.

Since most of the published studies did not have standard operating procedures nor were raw data available for review on most of the studies, the open literature studies were evaluated at face value with the understanding that all these published studies would have been subject to some degree of scrutiny already through the journal's peer review process.

No formal guidelines existed for specifically examining the effects of atrazine on gonadal development in amphibians. Currently, there are no guideline studies for amphibians and the Agency relies on other aquatic and terrestrial test species to serve as surrogates for estimating risks to amphibians.

Additionally, many of the measurement endpoints examined in the recent studies differ from those regularly utilized by the Agency to estimate acute and or chronic risk. However, the Agency is not confined to using guideline studies to identify potential hazards. The Agency routinely relies on open literature to provide additional

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insights on the potential effects of pesticides and may use this information to request additional studies to address uncertainties.

The registrants Ingenta voluntarily undertook all of the studies submitted for the Agency review. The studies were prompted by concerns that atrazine exposure could potentially result in developmental effects in amphibians. Although over many years the registrant has completed both acute and chronic ecological effect testing on a range of species in both the laboratory and the field, we are focused today on the recently completed studies completed on amphibians.

As Steve indicated, a total of 17 studies were submitted by the agreed upon February 28, 2003, deadline. The deadline was imposed to allow sufficient time to review the studies and write a White Paper regarding the review for submission to this SAP. Seven of the studies were conducted exclusively in the laboratory, while 10 of the studies were conducted in the field. Field studies included Florida, Illinois, Indiana, Iowa, Michigan, Nebraska, Utah, Wyoming, and South Africa.

When studies are submitted to the Agency, data evaluation records are completed on each of the studies. Typically, data evaluation records detail how and why the study was conducted, the

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results, and what the study's author concluded from the data. The Agency then analyzes the study's raw data and attempts to draw its own conclusions from the data. Reviewers identify any inconsistencies in study methods and results and then summarize their interpretation of the study results.

As noted earlier, most of the open literature did not have sufficient detail of the complete in-depth data evaluation records. And, in fact, data evaluation records are not typically completed on open literature. However, evaluation records were completed for the five open literature studies to capture as much of the methodology, data, and results that were available in the published study.

All data evaluation records completed by the Agency undergo secondary review to verify the primary reviewers interpretation of the study. For each of the 17 studies reviewed in the White Paper, data evaluation records were reviewed by three secondary reviewers. Copies of the data evaluation records for the amphibian effects studies have been provided to the panel members.

Reviewed were the studies' protocols and quality assurance, the strength of cause-effect relationship, whether there was a dose response, whether the observed effects have a plausible mechanism of action that is consistent with what is known about the chemical, and

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finally, whether the measured effects are ecologically relevant.

A range of amphibian species were tested in the studies. Although the laboratory studies may have relied on non-native species, each of the field studies examined species within their native range; thus cane toads were studied in Florida, bull frogs were studied in Iowa, northern leopard frogs were studied in Wyoming, Utah, Nebraska, and Indiana, green frogs were studied in Michigan, cricket frogs were studied in Illinois, and the African clawed frogs were studied in South Africa. Although most of the studies relied on tadpoles, field studies examined both larval and adult forms.

Endpoints measured in the laboratory and field studies included time to metamorphosis, growth in terms of length and weight, presence of gonadal abnormalities, laryngeal muscle area, sex rations, plasma steroid concentrations, and brain and gonad aromatase activity levels.

Gonadal abnormalities include misshapen gonads, for example, discontinuous testes or multi-lobe testes. However hermaphroditism was also observed. For the purposes of this presentation, the terms hermaphroditism, intersex, and ovotestes are used interchangeably to represent the co-occurrence of testicular and ovarian tissue either in the same gonad or individual.

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Effects on the amphibian laryngeal dilator muscle were also described. Although a variety of methods were used to document this effect, generally the cross-sectional area through the laryngeal dilator muscle was measured. Typically, male frogs have a larger dilator muscle than females.

No effort was made in his presentation to single out a particular study. Rather the focus is on issues that were identified in the studies collectively. This is not to say that all the studies exhibited similar difficulties. Some studies contained relatively few issues, while others may have contained several. However, no study was devoid of uncertainties and or inconsistencies.

Since each of the studies contained sufficient uncertainties and consistencies or inconsistencies that rendered the data of questionable utility, data evaluation records focus primarily on methodological issues rather than on a statistical analysis of the data.

As mentioned previously, there were 7 laboratory studies and 10 field studies. Most of the field studies had some laboratory analyses. Collectively, the following issues were identified in the laboratory studies: Atrazine contamination of the controls, poor water quality, poor growth and development and or survival, high variability in endpoint measures, lack of reproducibility, and the

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unresponsiveness to positive controls.

Atrazine contamination in the controls seemed to be a recurrent theme in several of the studies. Measured concentrations of atrazine exceeded the levels of detection by a factor of two and were at concentrations reported to cause effects in other studies. For example, in several of the studies, atrazine concentrations in control tanks was higher than 0.1 micrograms per liter. The concentration of atrazine reported by Hayes to cause developmental effects in frog testes.

Additionally, several studies suggested that animal feed used in the studies may have contained atrazine residues. However, separation techniques are not sufficiently developed to allow the researchers to verify and or quantify the concentration of atrazine in the feed.

Laboratory studies ranged from testing a single concentration of atrazine to testing a broad spread of concentrations. Although gonadal effects have been observed between 0.1 and 25 micrograms per liter, most of the studies did not sufficiently bracket these concentrations to verify whether atrazine at these concentrations can result in a consistent developmental effect.

Poor water quality was one of the most frequent issues

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surrounding the laboratory studies. Although the Agency does not receive many amphibian studies and does not have specific guidelines to conduct these studies, several sources, for example, the ASTM, exist that do provide guidance for conducting aquatic toxicity testing using amphibians. Unfortunately, high loading rates and frequent and incomplete exposure water changes resulted in diminished water quality as evidenced by high ammonia and nitrite levels coupled with low dissolved oxygen.

As a result of poor water quality, many of the study animals exhibited poor growth, low developmental rates, disease and high mortality rates that contributed to the tests' inability to differentiate treatment effects. In some cases, growth was negatively correlated with length of time to metamorphosis. Where Xenopus laevis typically requires 58 days to complete metamorphosis, in some studies larvae had not undergone metamorphosis by as late as 100 days. High mortality rates confounded some of the studies; and in some cases, required a proposed study methodologies be abandoned.

Several of the studies elected to measure plasma testosterone and estradiol concentrations and aromatase activity in the brain and gonad. Variability in measured steroid concentrations were so high that in some cases the study was unable to differentiate males from

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females. Aromatase activity in the gonads ranged from being below the level of detection to sporadic peaks in activity rendering within group variability so high that it would be difficult to differentiate any treatment effect.

With coefficients of variation as high as roughly 500 percent, study designs were insufficient to account for this level of variability and still be able to detect treatment effects. In some studies, measurement endpoints would have had to differ by roughly 80 percent before this study would have been able to detect the difference.

Many of the studies did not run positive controls. However, on some of the studies which did utilize dihydrotestosterone and 17-beta estradiol, low percentages of animals responded to the treatment. This response differed from other studies that indicated that the treatment of frogs with steroids would markedly impact sex ratios and the rate of hermaphroditism.

It is uncertain whether the lack of responsiveness to positive controls was due to animals genuine insensitivity to the steroid hormones or whether there was insufficient chemical present to elicit a response.

The Agency recognizes that field studies can be difficult to

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conduct since researchers are not able to control environmental conditions. Also the Agency recognizes the difficulty in identifying sampling sites that can be considered true replicates of one another.

However, of the field studies submitted, there tended to be considerable variability between sampling sites. Similar to some of the laboratory studies, atrazine, both the parent and its degradates, was present in reference groups. Additionally, other trizine herbicides and chemicals were present but not always well-characterized.

Where pesticides were characterized, their concentrations were in some case relatively high. And it is unclear what impact they might have had on the outcome of the study. In some studies, there were unusual environmental conditions that may have impacted the study. Unusually high rainfall and increased predation due to introduced species were problematic.

Similar to laboratory studies, variable hormone concentrations in aromatase activities were problematic. The variable plasma hormone levels may have been a result of collecting animals over a protracted period of time. In one study, animals were collected over roughly a six-month period where study animals were likely to be at different stages of their sexual cycles. Additionally, it's unclear

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whether housing Xenopus laevis in close proximity to one another following their collection influenced their hormonal concentrations and or aromatase activity of these opportunistic breeders.

In spite of all the issues identified in the available studies, the Agency believes that the laboratory and field studies have provided useful information. The studies provide sufficient information with which to formulate a hypothesis. They provide insight on the potential sources of variability and they provide insight on future test species and study conditions.

Although many of the studies did not demonstrate any effect of atrazine on amphibian development, there are sufficient data to suggest that atrazine may be affecting gonadal development. In six of the studies, atrazine exposure was associated with a range of gonadal effects across three species of amphibians. There are sufficient data to minimally formulate the hypothesis that atrazine exposure may impact gonadal development. However, there are insufficient data to refute or confirm whether atrazine is actually causing gonadal effects.

The Agency believes that there are insufficient data to refute or confirm the hypotheses that atrazine exposure may impact gonadal development because of the collective uncertainties associated with the existing studies. Uncertainties include whether the cause-effect is

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real and can be readily repeated in different laboratories; what is the dose response relationship, the mechanistic plausibility that atrazine exposure is causing a given effect; the inability to readily extrapolate laboratory effects to the field; and the uncertain ecological relevancy of the measurement endpoints.

Without addressing these uncertainties, the Agency has no way to determine whether a particular effect can consistently be expected to occur at a particular level, whether the effect if real can be expected to occur in other animals, and whether the effect is likely to adversely effect an animal's reproductive fitness.

While gonadal development appears to be the primary effect associated with atrazine exposure in amphibians, a consistent measurement endpoint for the effect has differed. Atrazine exposure has been demonstrated to result in hermaphroditism in several studies and laryngeal effects in a single study. However, other studies have not been able to demonstrate similar effects. While males have been primarily affected, there are conflicting data on whether females are also impacted.

Obtaining a clear dose response relationship has been problematic for most of the researchers engaged in studying the effects of atrazine on amphibians. In some studies, atrazine exposure

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resulted in no effects; while in others, concentrations as low as a tenth of a microgram per liter resulted in hermaphroditism in the laboratory. Efforts by some researchers to substantiate these laboratory results were only successful at atrazine concentrations 250 times higher.

Additionally, data from some studies have suggested that following a threshold effect concentration, there is either a leveling off of a response or a diminished response at higher doses. Therefore, the existing data have not demonstrated a traditional monotonic dose response curve.

Several of the current studies have proposed that atrazine exposure results in up-regulation of aromatase activity and a subsequent decline in testosterone concentrations and an increase in estrogen that in turn lead to feminizing, that is, hermaphroditism, and demasculinizing, that is, decreased laryngeal muscle effects in atrazine-exposed males.

However, no study thus far has directly demonstrated that aromatase activity has indeed been up-regulated. And only one study has demonstrated that plasma testosterone has decreased in atrazine-treated males.

Although many of the studies thus far have examined plasma

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steroid levels and brain aromatase activity levels, it is uncertain whether the proposed mechanism of action is likely to be observed on the basis of the whole animal. Rather aromatase activity is proposed to increase in the testes where androgenous testosterone is converted to estrogen. It is unclear whether these localized increases in steroid conversions could be detected in blood plasma at all.

Out of the 17 studies, one demonstrated gonadal effects in both the laboratory and the field. However, in this single study, there was a clear lack of a dose response. Coupled with the variable effects that have been noted, even within the same species, extrapolating atrazine to potential field effects is difficult.

While intuitively it may seem that the presence of ovotestes and reduced numbers of spermatogonial cell mass in males and reduced numbers of primary and secondary oogonia in females, may impair the reproductive fitness of frogs and that reduced laryngeal muscle mass and secondary sexual characteristics may impair an animals ability to attract mates. There are not data currently available to the Agency with which to gage impaired reproductive function, recruitment, or survival.

Additionally, the current ecological risk assessment of atrazine identifies that some plants have exhibited resistance to atrazine.

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Some researchers have speculated that amphibians may also develop resistance to the potential effects of atrazine on amphibian development. The Agency is uncertain regarding the role of resistance and recovery from the potential developmental effects.

The primary criteria for conducting ecological risk characterizations in the Agency are that they be transparent, clear, consistent, and reasonable. Of these criteria, transparency is viewed as the principal value from among the four since it leads to clarity, consistency, and reasonability.

Consistent with the EPA's process for conducting ecological risk assessments, it has evaluated the available data following specific evaluation criteria. That included experimental design, the strength of the cause-effect relationship, the dose response relationship, the mechanistic plausibility, and the ecological relevancy. The Agency has provided these reviews to panel members.

Based on its review of the available literature, the Agency believes that there is sufficient information to formalize a hypothesis regarding the potential effects of atrazine on amphibian development. But because of the uncertainties surrounding each of the studies conducted thus far, the Agency is recommending that additional studies be conducted. The next presentation by Joe Tietge will

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discuss the Agency's recommendations for additional testing.

In conclusion, as of February 28, 2003, the Agency has reviewed a total of 17 studies examining the effects of atrazine on amphibian gonadal development. These studies have involved both laboratory and field work and have looked at six species of anurans. In each of the studies, the Agency has identified concerns regarding the study methodologies and or results that potentially limit the utility of the studies.

Based on all 17 studies, atrazine exposure did not produce consistent, reproducible effects across all species tested; therefore, the weight of evidence suggests that atrazine exposure does not impact gonadal development. However, there are lines of evidence from both laboratory and field studies that support the formulation of a plausible hypothesis that atrazine exposure may result in developmental effects in amphibians.

Although the current studies cannot be used to refute or confirm the hypothesis that atrazine exposure may result in gonadal development effects, the studies do provide useful information of the sources of variability. This information will be critical to the design of future studies.

There are insufficient data, as I indicated, to refute or confirm

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the effects of atrazine on amphibians. If risk managers, however, wish to reduce the current uncertainties regarding the potential effects of atrazine on amphibians, the Agency recommends that additional studies be initiated. These studies should build on the current body of information.

If additional testing is required, the Agency is proposing that a phased approach be used to examine the cause-effect dose response mechanistic plausibility, and the ecological relevancy of any effects observed following the exposure of amphibians to atrazine. Joe Tietge who will follow me will present what the Agency is proposing as follow-up studies.

Are there any questions?

DR. ROBERTS: Great. Thank you, Dr. Steeger. Before we move on to the next presentation, I think this is good opportunity for the panel to ask you any questions they might have on the Agency review of the 17 studies. Are there any questions among panel members regarding the Agency's review.

DR. KELLEY: I have a question.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: So for instance you said that in some of the studies you failed to document sex differences in steroid levels. How

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did you know that you would expect to see those sex differences?

DR. STEEGER: We didn't.

DR. KELLEY: So that is, in fact, then not such a useful criteria.

DR. STEEGER: We --

DR. KELLEY: If you didn't know you expected to see them and then you didn't see them, how did you know they were there anyway?

DR. STEEGER: You're talking about sex differences in the steroid level hormone concentrations.

DR. KELLEY: In steroid levels. Right.

DR. STEEGER: Well, we didn't know what to expect. Because as I indicated, this was a new area for the -- these measurement endpoints were new for the Agency to consider.

DR. KELLEY: All right.

DR. ROBERTS: Yes, Dr. Skelly.

DR. SKELLY: I just had a general question about whether in your review you considered any sort of a line below which the quality of data issues meant that you wouldn't consider the evidence from that study.

DR. STEEGER: When the Agency receives guideline studies, we have what's called the "rejection rate analysis" where there are

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certain characteristics of the study which will eliminate it from consideration. Some of the studies, one potential factor that can eliminate a study for consideration is the presence of the test chemical in control sites. Because these studies weren't conducted following guidelines, we didn't really have a criteria that would really eliminate it from consideration. But that would constitute a reason to reject a study. Does that...

DR. SKELLY: So you considered rejecting studies and decided not to.

DR. STEEGER: We considered that we would just review the studies as they existed without any consideration for what would constitute a fatal flaw in the study.

DR. KELLEY: Can I ask then --

DR. ROBERTS: Excuse me.

DR. KELLEY: -- just follow up --

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: Yeah.

DR. ROBERTS: You've got to wait for me to call on you. Dr. Kelley.

DR. KELLEY: Thank you. Just to follow up on that question. So do you use a criterion that might take into account the weight of

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the evidence, in that case just simply the number of studies? So if you had 22 studies that got an effect and 5 that didn't you would consider not having rejected any of them as weighing in on the weight of evidence in favor of the first result rather than the second?

DR. STEEGER: In this case, we are not using a -- because of the inconsistencies in all the studies, we couldn't really use a weight of evidence approach. As I indicated throughout my presentation, what seems to be a recurrent theme, a line of evidence, that there seemed to be some effects that recur over the studies. But the weight of evidence approach does not work for us in this case because there were such, in our view, glaring problems with each of the studies that it was not difficult to weight them per se.

DR. KELLEY: One last question. You had, whatever it was, 19 studies -- is that right? -- 12 and 7.

DR. STEEGER: Seventeen studies.

DR. KELLEY: Seventeen studies. After you completed the White Paper, did you become aware of any studies that were not included in the White Paper that you had missed for one reason or another?

DR. STEEGER: Our contractor provided us with -- are we talking about amphibian studies?

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DR. KELLEY: Amphibian studies.

DR. STEEGER: Not that we were aware of.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: I'm paraphrasing here. But you said something to the effect that the hypothesis wasn't accepted because no consistent reproducible effects across all species were observed. And I was wondering if that was actually a requirement for accepting the hypothesis, that consistency among all species be observed.

DR. STEEGER: No, it's not.

DR. LEBLANC: And a second question is were other species, vertebrate species, aquatic vertebrate species considered in the literature review?

DR. STEEGER: No, they were not. We do have information on other species. But our review looked at amphibians only.

DR. ROBERTS: Dr. Gibbs.

DR. GIBBS: Just quickly, in your characterization of available studies, you mentioned that data evaluation records focused primarily on methodological problems rather than statistical analyses. Could you elaborate on that?

DR. STEEGER: Because of the problems with atrazine contamination in the controls, the unresponsiveness of animals to the

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positive controls, the slow development of the animals, the high mortality rates that were exhibited on many of the studies, those were what we considered to be problematic that would have rendered the data of questionable utility. So we didn't really focus on analyzing the data per se.

Now I did mention, though, that on the aromatase and plasma steroid concentrations, there was high variability. We do analyses to verify that there was indeed high levels of variability in the measurement endpoints. But, again, because of the way that the information was collected, it would have been problematic for us to move forward with the study independent of what the analyses told us.

DR. GIBBS: Was the assumption that that peer-review process would have caught any problems or issues with the statistical analyses as reported?

DR. STEEGER: Well, the peer review process, are we talking about for open literature?

DR. GIBBS: Yeah.

DR. STEEGER: For the open literature, it's rare for journals to have access to the author's raw data. So it's unlikely they would have caught that.

DR. ROBERTS: Okay. Any other questions? Okay. I see

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none. Then we have scheduled a break now. We're a little ahead of schedule. I guess whether or not we go to break kind of depends on the length of the next presentation. So let me just ask you, Mr. Tietge, we have allotted an hour for that talk. What's your best guess?

MR. TIETGE: I think it will take about 30 minutes.

DR. ROBERTS: Then let's go ahead and move on to your presentation.

MR. TIETGE: Thank you. I'm just going to get started here.

The basis for this talk today is that the evaluation of the currently available data as previously reviewed by Dr. Steeger suggests that anuran reproductive fitness may be adversely affected by exposure to atrazine. However, the data are insufficient to conclude that atrazine adversely affects anuran reproduction. Therefore, further studies are proposed following the Guidelines for Ecological Risk Assessment to reduce the uncertainties and permit an eventual risk characterization if warranted.

These conclusions are based on the fact that there are a number of remaining uncertainties including the following: The number of affirmative studies, that is, those that seem to demonstrate an effect on gonadal development and secondary sexual characteristics, the

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sample; there's limited evidence of repeatability between laboratories; the dose-response relationship remains undefined due to the lack of sufficient dose-response data; the mechanistic plausibility of the hypothetical mode of action is currently unsupported by the available data on amphibians; and, finally, the ecological relevancy of the potential effects of atrazine exposure on amphibians remains undetermined.

Based on these observations, it is EPA's recommendation that, if the risk management process requires further reductions in these uncertainties, then additional laboratory studies need to be conducted before any additional risk assessment activities regarding the effects of atrazine on amphibian reproduction are undertaken.

The objectives of this presentation are, first, to review the concept of problem formulation as used in the Agency's ecological risk assessment process; second, to restate the environmental goals and assessment endpoints necessary to make the risk management decisions; third, propose a conceptual model for atrazine action on anuran reproduction by defining a risk hypothesis; fourth, propose an analysis plan which identifies measures of effect relevant to the assessment endpoints and risk hypothesis and includes a phased-study approach to test central components of the risk hypothesis; fifth,

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identify critical decision points in the phased approach; and, finally, to provide some conclusions.

It's not my intent here to give a detailed discussion of the ecological risk assessment paradigm, but I want to remind you or familiarize you with the basic components of an ERA. Because as already mentioned by Dr. Bradbury this morning, we are using the ERA paradigm to guide our approach on this issue.

Briefly, this process can be represented as three distinct phases: Problem formulation, analysis, and risk characterization. Problem formulation is the foundation of the ecological risk assessment process as it lays out the goals and approaches necessary for the successful completion of an assessment. Much of what I'm presenting to you today is indeed part of the problem formulation phase.

The analysis phase is the phase that implements the approach developed in the problem formulation and generates the data required to complete the final phase, which is risk characterization. I'll only touch on the analysis phase today as it relates to the approach developed and problem formulation. I will not risk characterization except to say that this is the phase that takes into account the probabilities associated with exposures and effects and results in

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some conclusion regarding risk.

In this slide, I have expanded the problem formulation box here in order to demonstrate that there are four main components to consider. The first is the integration of the available data as presented previously by Dr. Steeger. This is a critical step because it identifies the potential problems associated with a particular stressor, in this case atrazine, and serves to refine and focus the risk assessment questions and, therefore, all subsequent activities.

The remaining three components of problem formulation: selecting the assessment endpoints, developing a conceptual, and developing an analysis plan, is the focus of this talk today. Before I launch into these areas, I'd like to point out the critical connection of problem formulation to analysis.

In this slide, I've expanded the analysis phase to show its components as well. In general, in the case of a chemical, this phase typically evaluates exposure as depicted in the left side of the large gray box and effects as shown on the right. What I'd like emphasize here is that the analysis plan that I will present today, which is part of the problem formulation phase, provides guidance for how to conduct the studies on effects and is thereby a prerequisite to measuring effects.

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I'd like to return to the problem formulation phase and discuss it in terms of atrazine. First, the overall environmental goal is to ensure anuran populations are viable and self-sustaining. This goal is rather generic. But it serves to orient the assessment process by focusing on a specific objective. And although it is somewhat simplistic, it can be difficult to actually assess the impacts of atrazine, or any other chemical for that matter, on anuran populations directly in the field.

But the assessment endpoint, successful reproduction and recruitment of native anurans, is directly related to population status. And as indicated by some of the atrazine studies evaluated earlier today, some aspects of reproduction, including gonadal development, are measurable endpoints relevant to the concern surrounding atrazine exposure.

Knowing the environmental goal and the assessment endpoints, and given the fact that some studies indicate that reproductive system development and secondary sexual characteristics may be affected by atrazine, we need to construct a conceptual model. The conceptual model in the form of a risk hypothesis is an attempt to develop a model that uses existing information to form a plausible explanation of the potential effects of atrazine on the assessment endpoints.

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That model is depicted diagrammatically in this slide. The hypothesized effects of atrazine are presumed to be initiated by a molecular interaction. This interaction results in increased aromatase activity, the enzyme responsible for the conversion of testosterone to estradiol. The increased activity of aromatase results in an evaluation of endogenous estradiol which affects feminization, for example, in the male gonad.

If the effects in the male gonad are severe enough, then reduction is fertility and reproductive success could be realized. Which leads to a hypothetical reductions in recruitment thereby impairing population maintenance which is in fact the assessment endpoint.

This risk hypothesis, which is based on the information on the literature and from submitted studies, may or may not be correct. But it forms the basis of the proposed studies and can also be thought of as a working hypothesis. Because of the uncertainties associated with the risk hypothesis are relatively high, it is likely that it will be modified when data become available.

As with any hypothesis, some elements are easier and or are more important to test than others. So the questions is: At what point in the risk hypothesis should hypothesis testing be introduced to

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evaluate the specific sub-questions? Or alternatively, what is the best strategy to evaluate the train of events in the risk hypothesis to test its validity.

In the case of this specific risk hypothesis, it is our view that the most appropriate entry point is at the level of determining the effects of atrazine on gonadal development, the apical organismal level endpoint. The reasons for this are, first of all, this is the endpoint on which much of the concern hinges. But, secondly, readily available methods exist to test the sub-hypothesis with relatively inexpensive methods that permit the analysis of large sample sizes.

And perhaps most importantly, this endpoint is the linchpin in the entire train of events. That is, if atrazine is found to affect gonadal development with a greater degree of certainly than currently exists, then this result provides strong rationale to conduct studies on the proceeding and subsequent elements of the risk hypothesis.

If on the other hand, atrazine does not affect gonadal development following a systematic effort to study this potential phenomenon, then the logic train of this risk hypothesis is broken and there may be no impetus to follow up by testing the upstream and downstream elements.

So now that we have an assessment endpoint selected and a

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conceptual model in place, we can now develop an analysis plan. There are four major elements to the analysis plan: A strategy to evaluate the risk hypothesis which I've already touched on in the last slide; selection of endpoints to evaluate also referred to as measures of effects; determinization of appropriate methods; and a sequence of analysis that follows the most efficient path to accept or refute the risk hypothesis in a systematic and organized manner.

I've excerpted the first section of the risk hypothesis in the first panel that I just put on the screen. In the proposed analysis of a risk hypothesis as shown as hypothesis testing which is in the second panel. As I mentioned earlier, the entry point for testing the risk hypothesis proposed to be the effects on the gonads at the organismal level. So beginning at the organismal level, the effects of atrazine on gonadal development, particularly in the males, is the primary endpoint.

Developing data at this level is critical in that it may provide the rationale and justification for conducting relevance and or mechanistic studies. If these organismal level tests are affirmative, then measurements of sex steroids should be conducted. And if estrogen levels are shown to be elevated in the atrazine treatments, then measurements of aromatase activity could be indicated as

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previous studies have attempted. If positive, the data from these studies will be useful to establish a mechanistic basis for inter-species extrapolation, further develop the plausibility of the mechanisms involved, and develop appropriate biomarkers that could be used in future field studies.

Although sex steroid and aromatase measurements are necessary to test the mechanistic aspects of the risk hypothesis, they do not provide meaningful information on the ecological relevancy of a potential gonadal effect. Therefore, if gonadal effects are observed at the organismal level, it is possible to proceed directly to studies which evaluate fertility endpoints that are relevant to the maintenance of populations. Furthermore, if the working hypothesis is supported by organismal and suborganismal studies, then it may be possible to confirm the mode of action by conducting confirmatory studies which utilize no aromatase inhibitors. Rescue of normal morphology of the male gonad by an aromatase inhibitor co-administered with atrazine would provide substantial support of the risk hypothesis in general and more specifically the mode of action involved.

However, if any of the studies conducted as part of the hypothesis-testing phase are negative, then alternatives should be considered. If no consistent and reproducible effects are observed at

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the organismal level, then there may be no need to continue any further testing. If on the other hand, the organismal-level tests are affirmative and either the steroid or aromatase studies are negative, then an alternative hypothesis may need to be evaluated.

Since this is purely hypothetical at this time, and it is outside the scope of the current risk hypothesis, no further discussion of the alternative testing will be presented.

So now there's an analysis plan. I'd like to discuss some of the details of the proposed studies. These are labeled as phases here as they were also labeled in the White Paper.

Phase 1, the Test for Apical Gonadal Effects. The first and most important phase of hypothesis testing in this phase is to determine if atrazine exposure results in consistent and reproducible gonadal effects in males and females and determine the shape of the dose-response curve, if any.

This slide lays out the key experimental process for consideration in the Phase 1 studies. The primary species recommended for this work is Xenopus laevis. The species is recommended because it is amenable to laboratory testing and has been shown by four studies to be potentially responsive to the effects of atrazine on gonadal development and differentiation.

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Xenopus laevis, however, is not a native anuran at least from a North American perspective. It may or may not be representative of native anurans when it comes to this issue. Therefore, a secondary species is suggested such as Rana pipiens, the northern leopard frog, which can be used in corroborative studies.

There is one study that suggests that Rana pipiens is sensitive to the effects of atrazine on gonadal development. However, this species is more difficult to work with in the laboratory and most labs do not have culture methods that permit continuous breeding and are, therefore, unable to conduct studies throughout the year with Rana pipiens as can be done with Xenopus laevis. Despite the limitations associated with Rana pipiens culture, comparative studies may be useful to develop data to determine the ecological relevancy of potential atrazine effects on a native species.

The developmental stages used in these studies need to include those that are sensitive to the effects of estrogens. In a study conducted by Villalando, 1990, with Xenopus, exposure to an estrogen elicited effects on gonadal differentiation during the pre-metamorphic period. However, after entering metamorphosis, the gonads were less sensitive to estrogen exposure. The proposed studies should include the pre-metamorphic period and continue until

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the organism completes metamorphosis at which time they should be evaluated.

The test condition in the studies reviewed earlier all use static-renewal conditions. These methods did not conform to the generally accepted biological loading regs recommended by ASTM and resulted in delayed development and in some cases excessive mortality. It is likely that these problems resulted form static-renewal conditions themselves which resulted in the accumulation of nitrogenous wastes and other metabolic products and generally poor water quality. It is our recommendation that flow-through conditions be used that adhere to ASTM standards and thereby promote survival, growth, and development.

The concentrations of atrazine to be used in these proposed studies should bracket those found to be effective in perturbing gonadal differentiation in previous studies, that is, at or below .1 micrograms per liter for the low and at or above 25 micrograms per liter for the high. And, of course, these concentrations need to be verified analytically.

The use of estradiol as a positive control is recommended since the potential effects on the gonad are proposed to be mediated through this pathway.

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The sample size and replication are not detailed here, but they should be determined a priori to be sufficient to test the stated hypothesis using appropriate statistical assumptions. Sampling should include all organisms on test to avoid potential biases.

And, finally, the endpoints should include survival, growth, development, gross gonadal morphology, gonadal histopathology. From these data, male-to-female sex ratios can be derived in the shape of the dose-response curve determined for each endpoint.

Because there are issues with the existing studies that limit the usefulness of the data, we propose that quality indicators be established as a guide to evaluate validity of the proposed studies. First and foremost, the tests need to be conducted in accordance with ASTM standards for biological loading and basic water quality parameters of pH, ammonia, dissolved oxygen and need to be contained within acceptable limits and verified regularly throughout the conduct of the study.

With regard to the biological endpoints, while there is no bright line between acceptable and unacceptable survival percentages, survival of 90 percent or more is indicative of a quality study. This is a reasonable standard to adopt particularly for Xenopus laevis studies as the species is particularly hardy in the laboratory.

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Similarly, growth of Xenopus laevis should result in organisms of about one-and-a-half grams; and this will vary between laboratories. Metamorphic development should be completed within 10 weeks.

But there are no standardized methods. And some methods that are proposed here are the acceptance -- there are no acceptance criteria because of the lack of standardized methods. So these issues should be evaluated in aggregate using some professional judgement.

Measurements of sex steroids. I'll now discuss the remaining phases of the analysis plan very briefly. Since the conduct of each of these phases is dependent on the outcome of the previous phases, it is premature to discuss them in much detail. I will, however, lay out the objective and potential approaches for each phase, recognizing that these may change as more information becomes available.

The second phase of the study should be conducted if the Phase 1 studies are positive. The aim of the Phase 2 studies is to determine if concentrations of estradiol and testosterone are altered by exposure to atrazine.

The approach to this phase is based on the fact that the developmental sensitivity toward the feminizing effects of estrogen in male Xenopus laevis has been experimentally determined as depicted

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in the panel in the right. In this panel, developmental stages indicated is indicated on the X axis. The developmental period between stage 44 and 50 represent a period in which estrogen is capable of completely overriding testicular differentiation resulting in 100 percent of the test population with ovaries.

As natural gonadal differentiation proceeds, their sensitivity to this effect diminishes as is indicated by a period of incomplete feminization from androgenous estrogen in a period of apparent insensitivity coincident with the onset of metamorphosis.

This suggests that if ovotestes formation in the male is dependent on estrogen, that the elevated estrogen levels need to be present during the sensitive developmental stages. Any associated studies of this phenomenon should focus on the effects of atrazine on sex steroids during these sensitive periods.

However, it remains uncertain as to whether more developmentally advanced organisms are sensitive to the feminizing effects of estrogen on the gonad. This is an area of uncertainty that requires more investigation as well.

Moving on to Phase 3, the measure of aromatase activity, the objective of the Phase 3 studies is to determine if aromatase activity is increased by exposure to atrazine during sensitive developmental

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stages. Whether or not this phase is conducted, is dependent on whether the proceeding studies on sex steroids suggest that modulation of aromatase activity may be responsible for elevated estrogen levels for example.

Similar to the approach for measurement of the sex steroids, there's a developmental component to the approach. The developmental expression of aromatase mRNA has been determined for Xenopus laevis and is presented in the panel on the right. Expression of aromatase mRNA is apparent at approximately stage 50 and generally increases with development. This expression pattern is overlaid on the previous graphic depicting the developmental sensitivity toward estrogen-induced feminization which decreases with development.

Taken together, these studies suggest that aromatase activity must be elevated prematurely and at sufficiently high levels during the estrogen-sensitive stages to result in feminization in males. Therefore, at this point in time, the most appropriate approach may be to examine this phenomenon prior to the onset of metamorphosis which is generally considered to begin at about Stage 54.

Phase 4, Aromatase Inhibitor Study. If it is demonstrated that the previous phase of aromatase activity is increase by atrazine, then

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it may be desirable to determine if co-administration of an aromatase inhibitor with atrazine rescues the male gonad from feminization. This approach would require that, first, the effective concentration of an aromatase inhibitor be empirically determined. Then based on the dose response data in the first three phases, the organisms would be exposed to the inhibitor simultaneously will affect atrazine concentrations. Then the effects could be analyzed similar to the Phase 1 studies.

On Phase 5, Evaluating Ecological Relevancy, the objective here in this final phase is to determine if the potential effects of atrazine on gonadal differentiation results in reduced fertility.

There may be several approaches to this, but based on the premiss that feminization of males occurred in Phase 1, the approach that I've outlined here is to determine if feminization alters fertility using either in vitro or in vivo fertilization methods. Although such methods are used routinely for reproductive purposes in numerous laboratories, they are not currently used to quantify fertility as an important parameter to estimating reproductive output. If such studies are warranted, then additional research would have to be conducted to establish quantifiable methods.

So in conclusion, it is possible to reduce the major

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uncertainties associated with the potential risk of atrazine to amphibians by following a phased sequence of laboratory studies focused on the critical components of the risk hypothesis, using currently available high quality methods that are standard for aquatic toxicology, and establishing and adhering to study quality indicators.

Although the analysis plan that I have presented lays out a relatively comprehensive set of studies to evaluate the risk hypothesis, the extent to which the proposed studies are actually conducted depend on two important factors. The first is whether or not risk management decisions require reduction in the current level of uncertainties to proceed. And, second, is whether or not the outcomes of the initial phases indicate that additional studies are logical and valuable in terms of testing the components of the risk hypothesis. These issues will have to be evaluated as more data become available.

Thank you for your attention. I'd be happy to entertain questions.

DR. ROBERTS: Thank you for your presentation. I'd like to give the panel the opportunity now to ask you any questions they might have about the proposed Agency approach that you've described. Let's start with Dr. LeBlanc.

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DR. LEBLANC: Joe, do you anticipate a temporal sequence to the performance of the phases or might some of the phases be conducted at the same time?

DR. TIETGE: I think it would be up to the laboratory who's proposing to do the studies. They certainly could be conducted. Or one could, for example conduct an organismal-level study and then archive samples for further analysis that are in that tier. I wouldn't want to propose the tier as being too linear. So I think some of them could be done at the same time.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I would like to know is the hypothesis is focused on aromatase production. So what we found and up to now and what is more or less a verified is feminization or demasculinization. So this could be also, I think, obtained by anti-androgenic effects. I think for conceptional frame work, we should include all as a possibility so that we have an alternative pathway to receive feminization or demasculinization via the anti-androgenic pathways. This should be maybe from a serial point of view at least included.

DR. TIETGE: I would agree. And I tried to leave the door open in the alternative path. I think that once you get away from the organismal level effects in the risk hypothesis, you have more and

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more uncertainty especially as you go toward the mechanistic side. So I agree with you totally.

DR. ROBERTS: Dr. Kelley then Dr. LeBlanc.

DR. KELLEY: With regard to the flow-through proposal, as you know, most Xenopus colonies aren't raised in flow-through. And in the wild, of course, you don't find very many tadpoles in streams with any motion what so ever. So are you aware of data that indicate that a flow-through system as opposed to a static renewal system with large volumes of water would have differential effects on mortality?

DR. TIETGE: Well, the issue of the static renewal versus flow-through isn't -- let's see, how am I going to answer this. If one goes back to the ASTM guidelines, which I think are fairly valuable in terms of establishing guidance for biological loading, there are guidelines for static tests. However, for a typical Xenopus individual it would require probably three to four liters of solution per individual to meet those standards. So if you want to have tests that have high enough end value in order to test your hypothesis, it would require very large exposure chambers. And I think that would be very limiting.

In fact, in our laboratory, we use flow-through conditions routinely and achieve metamorphic completion in about seven weeks

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typically post fertilization and usually with 99 percent or higher survival and apparently good growth rates. Often we have organisms in the 1.8- to 2-gram range when they're right around stage 60 prior to the weight loss that occurs through metamorphosis.

I'm not sure if I answered your question. Yes, there is some basis for it. Also, I understand that even with the native Ranas, they don't necessarily live in flow-through conditions in the field; but they also don't live in a static aquarium in the field because the system is more complex.

DR. ROBERTS: If you have a follow-up questions that would be fine.

DR. KELLEY: So this does bear to the issue of ecological relevance, however. So it's not entirely clear how a continuous flow-through system would bear either on Xenopus that avoid a flow system or on Rana even if it's not totally static since much of the data, since some of the concern, at least, comes from things like drainage ditches and ponds accumulating in runoff from fertilized fields. So one should, I think, think about whether flow-through data, although well-controlled from the point of view of water quality, actually would mimic the conditions under which exposure might occur.

DR. TIETGE: I understand your point. It's a point well-taken.

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However, in aquatic toxicology, I would suggest that the more control you have over the experiment in terms of water quality, the more confidence you have that the effect that you're observing is related to the concentration of the chemical. I think it is a much different question to ask whether or not a laboratory study is directly applicable or representative of field conditions. I think that's the state of the science actually.

DR. ROBERTS: Next Dr. LeBlanc, followed by Dr. Isom, Dr. Kloas, and then Dr. Skelly.

DR. LEBLANC: In formulating your hypothesis on the mechanism by which atrazine might elicit effects on developing gonads, did you consider the work of Ralph Cooper showing in rats the effects on gonadotropins?

MR. TIETGE: The hypothalamus, hypothalamic vectors?

DR. LEBLANC: Yeah, in suppressing, glueinizing hormone.

MR. TIETGE: I'm certainly aware of it. Of course, Ralph's in the Agency so... But, no, I think what we tried to follow was the information that we thought was more specific or germane to the amphibian issue. So, no, we didn't really take it into consideration.

However, as I also answered Dr. Kloas, we did leave the door open that as you find, as the data indicates, you can go to an

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alternative, take alternative paths.

DR. LEBLANC: I think induction of aromatase may be consistent with his observations. But certainly in the young male, the profound effect that he says in terms of steroid levels was a suppression of testosterone. So I would agree with Dr. Kloas that certainly anti-androgens is something you might want to consider as a positive control.

DR. ROBERTS: Dr. Isom, Dr. Kloas, and then Dr. Skelly.

DR. ISOM: The objective of your proposed study is obviously to determine the reproductive fitness of the species. And I noted in a number of the studies that have been published that not only do we have observed or postulated effects upon gonotropic development, but also secondary sex characteristics that are important for reproduction like laryngeal muscle.

I was wondering why you aren't proposing to at least measure that in the species in your exposure studies. And then a second question is have you thought about positive controls for aromatase that is inducers exposure.

MR. TIETGE: Okay. The first question was --

DR. ISOM: The laryngeal muscle.

MR. TIETGE: Right. Laryngeal muscle. If I recall the data

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correctly, laryngeal muscle effects were observed at higher concentrations. And so the gonadal effects were more sensitive. They certainly, if you affected laryngeal muscle to a certain level undetermined at this point in time, you certainly could expect to have some potential effects on reproductive activity. And I think Dr. Steeger mentioned that in his talk as well.

I mean the door could be open to that.

DR. ISOM: It seems to me that if you're doing the study, you have the animal there. It wouldn't be that difficult to do that.

MR. TIETGE: Actually, we have -- I have no experience with that endpoint. I'm not sure how to deal with it. Would anybody.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: I do have experience with that endpoint since that's what I've studied for a good long while. And one of the things that I think would be required in a study of this kind is to enable the animals to grow until they reached reproductive maturity. If you wanted to study the endpoint of sexual differentiation functionally, both in terms of active spermatogonia in testing situations, either removing the testes or doing natural matings which would be more variable, and if you also wanted to study the endpoint of laryngeal function, which is to produce the male advertisement call among other

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calls, then you would need to actually have your animals go probably for about a year. Although, if your animals are growing fast, you can probably get them to call in six months.

So those seem like natural endpoints that relate very closely to the issue of reproductive success.

DR. ROBERTS: Okay.

MR. TIETGE: A positive aromatase inducer, that certainly could be done. We didn't include it because we were trying to stay as directly on the track as we could. But certainly it's a fine idea.

DR. ROBERTS: Next question or clarification from Dr. Kloas followed by Dr. Skelly, Dr. Denver, and then Dr. Green.

DR. KLOAS: Of course, I would like to come back to flow-through versus static renewal system. I think up to now we have no real indication of if, at least some unofficial indications, that maybe a flow-through would reduce positive control effects. For instance, for estradiol, for feminization there's one study I'm aware of they have a reduced feminization effect because it's flow-through. I'm not sure. So I think before just fixing everything, we should also maybe be aware that we need a comparative study, a comprehensive study, between flow-through and static renewal.

I know also from this flow-through experiment, the tadpole's

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grow well and also the developmental stages were reached. But I'm a little bit concerned about sexual differentiation. I'm not sure that you can say it doesn't matter.

MR. TIETGE: I'm familiar with the study, I think, that you're referring to. And I find the results to be somewhat enigmatic. However, in using the flow-through conditions in our laboratory, I don't think there's any effect on sexual differentiation based on the method itself. I think that from an efficacy of exposure point of view, the flow-through system probably ought to be more effect than in a static system because there's a lot of evidence that exists with the more hydrophobic chemicals will be depleted under static conditions. That's very well established in the aquatic toxicology literature.

DR. KLOAS: I agree from a theoretical point of view. But did you do a positive control using estradiol for inducing feminization in parallel in this system and it works?

MR. TIETGE: It works, yes.

DR. KLOAS: I would like to see it.

DR. ROBERTS: Let's go to Dr. Skelly. But before we continue with the questions, let me just remind the panel that you will have the opportunity to provide feedback on this approach as we address the questions. The purpose now is really just to get clarification on the

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Agency's approach. Dr. Skelly.

DR. SKELLY: I had a question about the designation of Xenopus laevis as a primary species and Rana pipiens as a secondary species. And I guess I'll leave it a little bit open-ended. But I'm interested in why you made that distinction and what it's going to mean in terms of the timing in your conceptual model of when things would happen and what that means in terms of how the studies will be used in terms of weight of evidence or how they'll be prioritized in your thinking.

MR. TIETGE: Well, on the first point, the speed and utility of Xenopus laevis over Rana pipiens, I think, is fairly universally accepted. I mean you can do -- I realize that some laboratories can produce Rana pipiens throughout the year for studying. However, most cannot. And most are limited to collections that occur early in the spring to conduct Rana studies.

By contrast, one could have multiple studies within one year with Xenopus laevis and make some headway without having to wait until the natural breeding season for the Rana species.

Also, I think if, based on the studies that were submitted, I think the question is: Can Xenopus be a reliable surrogate for Rana? With regard to this endpoint, taking the studies that have been done at

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face value, I guess you'd have to say yes. If you have a surrogate that's more efficient, it's probably the way to go.

However, as with any of the issues of species extrapolation, there's no substitute for a corroboration in terms of some comparative studies. And so the intent of my point that I made was that you might do the hard work, the voluminous work, with Xenopus and try to get as far into understanding the phenomenon as you can; and then, when you have a good handle on what's going on and you have a level of confidence that allows you to go forward, that you would then go back to Rana and do some confirmatory studies because I think you are limited in terms of the methods that are available with Rana. So that was my --

DR. SKELLY: I'll just follow up quickly. So does this mean with your flow chart that you might do the work on Xenopus and come up with a negative result and stop there because you don't need to corroborate a negative result versus a positive result?

MR. TIETGE: Well, that's a great question because you never know what to do with negative results. It's hard to make decisions based on negative results. But you might still have enough concern, based on the existing information, that you'd go back and do a Rana study at the organismal level even if it was negative in Xenopus.

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I think where Xenopus really has the advantage is when you get into the iterative studies, especially at the mechanistic level, where you're trying to define what's going on. Because then that would if you, for example, if the working hypothesis were demonstrated using that approach that I laid out, you might be able to very quickly then go onto Rana pipiens and verify that the same thing is going on. And that's what I was referring to as establishing a basis for inter-species extrapolation when it came to the value of the mechanistic data.

DR. ROBERTS: Dr. Denver followed by Dr. Green and then Dr. Gibbs.

DR. DENVER: Joe, some of the main concerns of the studies that were reviewed by the Agency were the variability within studies and also the variability among studies. And I'm curious if the Agency has considered ways to control for this variability in terms of the assays that have been chosen and the way to validate these assays among different labs.

I'm thinking about the two types of assays that you're proposing. One is the morphological assay where you're looking at gonadal morphology and the presence or absence of intersex individuals. And these types of scoring of intersex individuals may be influenced by subjective measures. Are there any other types of

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assays that may be applied to this, for example, by a chemical assay that may assay for testicular or ovarian antigens that may be more objective and may be even more sensitive? Has there been consideration given to developing standardized pools, say, of plasma that can be distributed to the laboratories for estimates of estrogens and androgens in blood plasma to validate those assays among laboratories, things like that?

MR. TIETGE: Well, with regard to the more mechanistic things you brought up in your last two points, no, we don't really have anything going on there.

But going back to your first comment regarding the subjectivity, I think our approach is that the histopathology has to be included. And histopathology or pathology in general is a subjective science. But I think it's often -- I should say it's often a subjective science. However, it's a science that has a lot of confidence based on the experience and the review process that's a typical, modern pathology reviews and such.

So I think that you can use a subjective endpoint effectively, I think, if you include the histopathology. But with regard to the more mechanistic-based things, I don't know of anything that's going on in that regard. And we certainly don't have anything right now.

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We are interested in developing those ideas. And I think there's movement in the Agency to develop and validate amphibian methods. But there are no validated methods currently.

DR. ROBERTS: Dr. Green then Dr. Gibbs then Dr. Richards.

DR. GREEN: Regarding some of the secondary characteristics in the male that could be studied, I wondered if you could comment on why in the literature and anywhere else that I've seen so far there hasn't been an evaluation of the nuptial pads in Xenopus laevis. They're very easy to see, quite prominent, in post-metamorphic young juveniles. And certainly if they were feminized as a result of exposure to chemicals in the wild, I would think that those would diminish and that could be followed up in field studies as well as in the laboratory.

MR. TIETGE: So your question is -- are you making a point, or are you asking a question?

DR. GREEN: No. I'm asking: Do you have plans to look at that? And it would follow up on Dr. Darcey's comment that you might have to extend the studies a little longer in order to be able to see them. But they can be detected grossly and histologically quite early, I believe.

MR. TIETGE: No. I mean our plan is just a plan, and we're

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looking forward to your input. And I think we'd accept that and move forward.

DR. GREEN: Okay. In addition to the nuptial pads, another thing would be the ventral folds around the cloaca in females are quite prominent. And you would expect, perhaps, if males were feminized, that they might become quite prominent in the laboratory as in the wild as well.

DR. ROBERTS: Dr. Gibbs.

DR. GIBBS: A quick question about endpoints. There seems to be an inconsistency insofar as in the Phase 1 studies insofar as the survival is listed as an independently varying endpoint to be measured. And yet in the recommended study protocol, survival was something to be constrained to remain above 90 percent.

MR. TIETGE: 90 percent survival in the controls is what I was referring to with regard to be an indicator the methods used in the tests were sufficient to promote survival.

DR. GIBBS: Okay. Under the various treatments.

MR. TIETGE: Under the treatments, of course, that would be given.

DR. ROBERTS: Dr. Richards then Dr. Coats.

DR. RICHARDS: Just a very general question relating to the

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two words "ecological effects." We've talked about reproduction, some aspects of that. I just wonder, in a very general sense, what has the Agency thought about that term. Is what we're talking about here so far enough to cover the realm of ecological effects? In a broader sense of --

MR. TIETGE: Broader than the ecological relevancy of, I think, Steve, you might want to jump in on this.

DR. BRADBURY: I think when you're doing a chemical risk assessment and we're starting with some observations from the field, but certainly starting from sort of the building blocks of building of a hypothesis, a working hypothesis, it's sort of building up as opposed to top down, bottom up kind of thing.

Certainly most of the ecological risk assessments for chemical stressors are across the Agency not just in pesticides, working through what are the relevant organismal responses that give you insights into population or community responses. And I think it's fair to say that we're all at sort of the edge of moving into how do you take a look at population models, how do you start thinking about meta-population models, how do you start interfacing chemical effects with habitat quality to understand the relative roles of those different stressors on a population or community structure.

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I think today, which is probably reasonably representative of other types of chemical risk assessments, we're still at that interface of how does the toxicology start to merge into population biology or landscape ecology. What are the insights from population biology information or landscape ecology information that give insights into those organismal responses that are most critical for population viability and what's a plausible toxicological mechanism to influence those endpoints.

On the surface, at least in this specific example, the discussions of measures of effects and risk assessment endpoints are at least qualitatively associated with reproductive fitness. But resolution spatial and temporal in defining that obviously would take increasing levels of information at all sorts of levels of biological organization.

DR. ROBERTS: Dr. Coats.

DR. COATS: Yes, when you're evaluating the reports, did you consider the analytical methodology used for chemistry especially in terms of the quality of the data or the selection of the methodology and how sensitive it might be or how specific it might be?

MR. TIETGE: We were aware of the level of detection that was associated with the assays that were used. We didn't have any input on the assay that were -- again, there were no guidelines for the

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registrant to conduct these studies by. So we accepted the studies at face value in terms of what the level of detection was. But it was clear that the levels of variability that were associated with the measurements themselves were high and in many cases -- in some cases, when backgrounds were subtracted from, when the assay background was subtracted from the treatment samples, they were actually negative values afterwards.

In our opinion, other methods from looking at those endpoints may be necessary. Rather than relying on ELISA assays, some other method may be more appropriate. Again, we're looking for input from the Panel to address those concerns.

DR. ROBERTS: Any other questions from the Panel?

If not, I think this would be a good time for a break. Let's take a break and reconvene at 10 minutes before the hour when Dr. Steeger will present Agency conclusions. So let's break for 15 minutes.

[Break at 10:35 a.m.; session resumed at 10:55 a.m.]

DR. ROBERTS: We're starting now. So if folks in the audience, if you could make your way to your seats promptly, please.

DR. STEEGER: The computer is in the process of re-booting.

DR. ROBERTS: All right. We'll await the computer.

DR. STEEGER: As I indicated earlier today, the criteria for

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doing a ecological risk characterization is that the characterization has to be transparent, it has to be clear, it has to be consistent, and it has to be reasonable. The Agency has reviewed a number of studies. And the process that we've used in making those reviews has been captured by Steve Bradbury, and it's discussed throughout my presentation. And critical in that process is the problem formulation. And the iterative series of processes that occur between the risk manager and the risk assessor.

Based on the studies that the Environmental Fate and Effects Division has reviewed these studies that were submitted as of February 28, it has concluded that there are lines of evidence that suggest that the exposure to atrazine may result in developmental effects in amphibians. However, there were basic inconsistencies and variability in the studies that we reviewed that prevent us from either refuting or confirming those effects.

The Agency has recommended, based on its review, that a phased process be undertaken to examine specific measurement endpoints. And in conducting this phased process that Joe Tietge outlined, the Agency would be working its risk managers to determine the level of uncertainty that would be necessary to resolve in order for some decision to be reached.

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One of the questions that was posed to Joe was what was the temporal sequence in doing the phased studies. Joe indicated studies or different phases could be done concurrently. But the critical input comes from the risk manager. The risk manager has to be able to define the level of uncertainty that they're willing to accept in order to make a risk management decision. The risk assessors collect the information and help the risk manager understand how much of the uncertainty is associated with the data that are available.

We believe that the current studies contain sufficient uncertainty that we're unable to, as I said, refute or confirm whether atrazine is indeed having effects on amphibian development. But the bottom line is, and throughout my presentation, I stressed that, given the fact that over several studies and environmental conditions and species, atrazine exposure did appear to be having some impact on gonadal development.

But because of the lack of consistency and the type of effect elicited, the lack of a dose response, the inability of the current studies to demonstrate a plausible mechanistic action, and our inability or the Agency's inability to link the measurement endpoints that have been reported with our traditional assessment endpoints of reproduction, survival, and growth currently, we're unable to make

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any statements regarding the utility of these data.

As I indicated in my presentation, though, the current studies do provide beyond the line of evidence, information on sources of variability and how future studies might be designed to better account for those sources of variability and provide reliable means of measuring the effects of atrazine on gonadal development.

Steve, would you care to add anything?

DR. BRADBURY: I think that Tom summed it up quite well. I guess as the Panel deliberates and we have some discussions, just emphasizing again using the Agency's risk assessment guidelines as a way to organize our thoughts and to think about the science at hand and the science in the context of making a regulatory decision, and sometimes that creates some different choices that one makes in terms of phrasing questions and articulating and understanding what the uncertainties are associated with different aspects of the science when the aspects of this science are in the context of making a regulatory decision.

And certainly we're looking very forward to your comments and input in terms of helping to define and understand the certainty that exists in the information today and your thoughts on those if you feel there are uncertainties that remain, the nature of those uncertainties

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in the context of how we've taken a look at it, and then some approaches to refine the knowledge base.

The ultimate decision on how much information is enough to make a decision starts to leave the realm of science, but science is important to inform that process so that the decisions that are made are reasonable and transparent and clear as Tom indicated.

So I think at that we'd be happy to turn it over to Steve and carry forward.

DR. ROBERTS: Thank you. This concludes the Agency's presentation. I'd like to ask the Panel, again, if you have any questions regarding the presentations this morning before we move on in the agenda. Yes, Dr. Kelley.

DR. KELLEY: Given that you've decided that Xenopus laevis is going to be your primary experimental target in this, I wondered to what extent you would also require before changing the current regulatory environment that effects be demonstrated in native North American species. Is that a fair question?

DR. BRADBURY: Run that by me one more time. I don't think I understand the question.

DR. KELLEY: Well, look, so you decide to use an experimental animal that's found in South Africa. There are feral

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populations here in America. But I assumed that we as Americans and EPA aren't too much worried about the survival of feral populations of Xenopus in, you know, Arizona golf courses, right, which is where they tend to live. So suppose a whole scenario was developed around Xenopus and effects of atrazine, to what extent could we then -- to what extent could you use that information to apply to our own native species here in America.

DR. BRADBURY: Now I understand your question, sorry. It's a challenging question, and I think it transcends much of ecotoxicology and ecological risk assessment where you may be able to test, get information on a handful of species and potentially have to extrapolate to hundreds of species or tens of species at least in different landscape scenarios.

I'd like to back up to the first way you phrased your question to indicate that part of the lines of evidence to determine where we are and where we may need to go, in fact, took advantage of some field studies as well as some laboratory studies to develop the lines of evidence that it's plausible to formulate a hypothesis, although we may not have the confidence right now to quantify the probabilities of risk based on the information at hand. But the epidemiology-type investigations as well as reductionist studies combined together

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increase your understanding and your ability to quantify what you know and what you don't know and the gaps that may remain.

In the context of starting with a biological model to start to get some clarity into the issue at hand for the risk assessment is sort of a fundamental question. In aquatic toxicology, typically the Agency is using fathead minnows and rainbow trout and bluegill as species to try to represent what could happen to the thousands of fish species in the country.

Now, through problem formulation and thinking about which landscapes we're talking about, one starts to narrow down the type of species that one needs to focus on. But you're still dealing with the fundamental species extrapolation challenge. What are the toxicokinetic differences between species? What are the toxicodynamic differences or similarities across species to help put some bounds on the potential variability across the species. And you have to blend that with some of the practicality of generating toxicological information that provides the ability to control some of the natural variability of the world so that one can tease out the signal the chemical may be sending in terms of a dose-response study and try to understand what that chemical is doing in light of all the other variabilities.

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So one trades off between a biological model that's well understood. What do we know about the developmental biology of a given species or the reproductive biology of a given species that helps us get insights into the potential effects a chemical may have. And it also then gives us insights into the issues we should be thinking about in terms of how do we extrapolate an effect seen in one species to other species. Do these species also contain the same receptor? How well-conserved is the receptor? How well-conserved are other biochemical pathways in terms of mechanism of action or detoxification or activation? What are the issues in terms of toxicokinetics uptake distribution? So all those things sort of come into play.

In the context of getting started on this challenge and our proposal, and, again, it's a proposal. It's a plan put before our scientific peers to gain your wisdom and insights as well. In terms of amphibian toxicology and in the context of developmental biology issues associated with a ecotoxicological risk assessment, Xenopus offers one way to get started efficiently to start to get some clarity in terms of the ability of atrazine to cause a reproducible response in terms of a developmental endpoint.

If one sees that, it gives some insights into aspects of species

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extrapolation. If that happens, what would be the observed mechanisms of endocrinology or developmental biology that would have to be present in other amphibians for an observation in Xenopus to be relevant to other amphibian species? To the extent we can extrapolate or determine a dose, be it an aqueous dose or a dose inside the organism, that gives a sense of sensitivity? What are the attributes of atrazine's physical chemical properties and the toxicokinetic properties that go on, processes that go on that help us extrapolate.

But our analysis plan doesn't rely just on -- a proposed analysis plan doesn't rely just on Xenopus and then modeled into other species. We are proposing to use at least the Northern Leopard Frog as a North American species to take a look to see if we get some consistency in an atrazine signal in a North American species.

But that's where sort of the juggling match between efficiencies and ability to get on with the question at hand. Xenopus we can use, or laboratories can use all the time. Most labs would have to wait for spring cycles before they could investigate this issue in the Northern Leopard Frog at least. So it's a balance in terms of clarity of the model we're using, what you understand about you biological model, what you understand about fundamental toxicological processes; and

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then sort of your ability to get information efficiently and effectively. And you will have extrapolation uncertainties for sure.

It also doesn't preclude, as I indicated at the beginning and as some of the questions at the end of our last session, is blending what you know from a controlled world of toxicology, the reductionist approach to sorting these things out and how does that get blended in with the landscape ecology or meta-population perspective on what this all means. And I think the ultimate sort of risk assessment, and, again, it depends on how much certainty one needs to make a decision starts to blend those sciences together to get the context of the landscape as well as the cross-species vulnerabilities.

DR. ROBERTS: Yes. A follow-up question from Dr. Kelley.

DR. KELLEY: So speaking of spring, this is a question for Joe. What Xenopus are you planning to use? Which laevis subpopulations? You know, in South Africa there are a number of different sub-populations, not subspecies just sub-populations, that have different breeding seasons.

MR. TIETGE: I haven't given much thought to that. I think there is, among the laboratories in the United States anyway, what would be a strain, I suppose, that is commonly used. But I have to -- I don't -- I haven't given much thought to that.

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If you're proposing especially going to South Africa to obtain specific strains?

DR. KELLEY: No. I think I'd be against that. But I'm just going to tell you that the groups that we have here in the States are representative of a sub-population of laevis. They were bred originally from a sub-population; and they retain to a degree, unsuspected by the unwary, an androgenous annual circannual rhythm, for instance, in hormone production. So you have to know what population you're dealing with, whether you're in their winter when they, you know, when one population will breed and another one won't or there summer vice versa.

So I think it's just worth bearing in mind where get your animals from.

DR. ROBERTS: Any other questions from the Panel?

If not, I would like to thank Dr. Bradbury, Dr. Steeger, and Dr. Tietge for your excellent presentations this morning. I think you've given us a clear picture of the Agency's analysis of the information that's available, the dilemma that lies in that analysis, and your thoughts on where to go from here. So your presentations were very helpful for the Panel and your answering our questions was very useful for our Panel so that we get a clear understanding of what the

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Agency proposes to do.

I'd like to move on now in the agenda. The next item is public comments. We've had several people request substantial blocks of time for public comments. And that includes the first public commentor. Rather than break up that public comment, I think it would be best, my preference would be, to begin public comments after lunch, to go to lunch early, come back early, get started, and begin the public comments then.

If we break now for lunch and were to come back at 12:30, that would give us more than an hour for lunchtime. But let me ask our first scheduled public commentor, which is the Eco Risk Group, if they could be ready to go at 12:30.

Is Dr. Kendall here? Not to put you on the spot. Okay. Great. Let's go ahead an adjourn now for lunch, reconvene promptly at 12:30. We will begin the public comments then.

A quick announcement from the DFO.

MR. LEWIS: Just for the members of the Panel, this room will be open during lunch. So if any valuables, please take them with you, your lap tops and other personal belongings. Thank you. See you at 12:30.

[Lunch recess taken at 11:25 a.m.;

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session resumed at 12:30 p.m.]

DR. ROBERTS: Let's reconvene the meeting. At this point in the agenda, the Panel would like to listen to public comments on these issues. And we have several individuals or groups that have requested the opportunity to address the Panel and present information.

Before we begin the public comments, I would like to remind the public commentors that the issues that we are focused on here are scientific issues. They relate to a very specific set of data and problems and issues that are of a scientific nature. There are, of course, broader issues of policy and so forth. But those are really outside the deliberations of this Panel. So I would like to request from all of the public commentors that, when they address the Panel, they really confine their comments to the scientific issues.

There are some legitimate policy issues and points to be made, but this is really not the venue to make those. There are other avenues to get that information, those viewpoints, to the EPA. So if you could in fact confine your comments to the scientific issues that the Panel is trying to wrestle with, that would be very helpful for us.

With that being said, I would like to say that the Panel welcomes public comments and different viewpoints and opinions

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regarding the scientific issues that we face. This is very helpful to us and we look forward to hearing from you.

The first group that has requested the opportunity to address the Panel is Eco Risk. And they are represented by Dr. Ron Kendall. Welcome, Dr. Kendall.

DR. KENDALL: Thank you very much, Dr. Roberts. First of all, I wanted to say thank you for the opportunity for our team to address the Panel today and we look forward to providing to the distinguished members of the SAP and you, Mr. Chairman, some perspectives we developed over a number of years now on the response of amphibians to atrazine.

We're here as an eco risk panel, but we really are university scientists, faculty members at universities across the nation and internationally. I'm going to introduce my colleagues in just a minute.

The Eco Risk organization has led a facilitative effort to bring multiple universities together and multiple members under an opportunity to coordinate, focus efforts, and to move forward in what we have felt was a very exciting opportunity to engage these cutting-edge scientific questions.

The sponsor has been Syngenta, the registrant in the case of

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atrazine. We have appreciated their support and their willingness for the Eco Risk panel to move forward in a open, forthright way and to communicate our science to the open literature as well as here at the SAP. So we appreciate that support.

In terms of the this afternoon, we appreciate the patience of the SAP in giving us the time to address you, we've been engaged in this process for a number of years now. And we've developed a core presentation which is before you. And it summarizes our efforts and tries to give you some perspective on our opinions on the subject. And, of course, we welcome your opinions as well.

Feel free to ask questions at any time. But we will go through the core presentation which will provide you a summary process as to what we've been through. And then with my colleagues, each one of them will spend a few minutes summarizing some of the highlights going on in their laboratories with their graduate students and post-docs and so on. So the SAP will have a chance to discuss with each faculty member, scientist, at the table what has been their contribution to some of the current, emerging knowledge that we have on this particular subject.

To my right as we would proceed, Dr. Glen Van Der Kraak will give the core presentation. So I'd like to proceed, Glen, to introduce

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you so if we can go to the next.

The panel members represent a variety of expertise from across the nation. We've drawn as well on consultants. But as I would go around to my right, Dr. Glen Van Der Kraak is professor and chairman of zoology at the University of Guelph and the co-editor of the document that's recently been published called "Global Assessment of the State of the Science of Endocrine Disruptors," funded through WHO. So we've asked Glen to provide the presentation and the core presentation at least.

Next to my right is Dr. John Giesy from Michigan State University. Dr. Giesy is the university distinguished professor with various appointments there and well-known in the field of environmental toxicology.

Dr. Jim Carr is in biological sciences at Texas Tech University. He has published on the subject of atrazine and amphibians and has contributed heavily to our process.

Dr. Ernest Smith is engaged with the Institute of Environmental & Human Health at Texas Tech University. He is a reproductive biologist and has engaged our subject area in environmental toxicology of atrazine from the reproductive endpoint perspective.

Dr. Louis Du Preez is from Potchefstroom University in South

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Africa, a member of the School of Environmental Sciences and is an expert on Xenopus in their native habitat. And we have been engaged in field studies in South Africa. Dr. Du Preez will report on the results of that work that has engaged the panel directive in working with him.

And then Dr. Tim Gross from the University of Florida, the Caribbean Science Research Center, and is heavily involved in amphibian ecotoxicological work in Florida on multiple species and has reported for the panel various projects over the years involving not only amphibians but fish and reptiles.

And Dr. Keith Solomon, last but not least, professor at the University of Guelph, well-known in field of environmental toxicology and risk analysis. And this is our team.

We've also had others that we have participated with over the years. Dr. Tyrone Hayes, University of California Berkeley, participated with the panel the first three years. Resigned in November of 2000 to pursue his own research. Dr. Bob Silken from Silken & Associates has served as a consultant to us and we have valued his contribution as we have engaged the statistical interpretation of a lot of these questions, both from a field standpoint as well as a laboratory standpoint.

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Again, I want to emphasize that the purpose of this scientific panel here is that we work together. We have moved forward in a way in which we design projects together. We don't send an individual faculty member out with some graduate students and they go do the work. We design work in consultation. We meet regularly. We have conference calls. We get together.

And we've designed through our standard operating procedures. Which I might add, for most of our projects they may not be totally GLP, but they're close to it. Particularly for emerging science as we are doing, no validated protocols are in place, it's kind of tough to put a GLP study together. But with the encouragement with our sponsor and the Eco Risk organization, and Ms. Katherine Vins that heads up the Eco Risk QA unit, we've been able to move in that direction. So all of our procedures do have standard operating procedures. These can be checked. All the data that we've developed to date has been turned into the Agency for full and complete scrutiny.

So never the less, we have worked as a team to combine our efforts to focus on research as we envision it to be needed; and we have designed and implemented these projects with full opportunity to freely pursue, publish, discuss, and engage our graduate students as necessary.

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So from my scientific perspective, it's been a great opportunity. And I think every one of our panel members, whether they be from North America or not, would agree with me.

So we would first like to proceed, Mr. Chairman, with the presentation of our core presentation. It might be best to let Dr. Van Der Kraak at least get through the core presentation before questions because he's worked very hard with our team to put all this together. And then let's have a question and answer period. I'm sure, based on the questions this morning, there will be lots of questions from this panel. Then we will proceed to the individual investigators if that will meet your wishes.

DR. ROBERTS: Great. That's fine. Let's go ahead and proceed with the core presentation from Dr. Van Der Kraak.

DR. KENDALL: Thank you. Dr. Van Der Kraak.

DR. VAN DER KRAAK: Thank you. Before I get into the meat of the presentation, thank you very much for the opportunity to speak on behalf of the panel to this issue.

In terms of chronology, this tends to set out some of the activities associated with the panel. In 1996, EDSTAC was formed. The atrazine endocrine panel began its work. And in '97 was the first report that came through the panel. '98 the studies began in earnest

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on fish, reptiles, and frogs. And in 2001, the panel began further studies using Xenopus as a model both in the laboratory and in the field.

Associated with this, there were other activities going on with the United States Environmental Protection Agency, formation of the Endocrine Disruptor Methods Validation Subcommittee, to try to bring together some standardized testing protocols for looking at endocrine-active substances across the spectrum of animals from humans through to invertebrates.

Our work occurred during a period of time when, as Ron had mentioned, there were no standard protocols that were available. We then shifted our focus in 2002 to begin some very detailed mechanistic and field studies in North America along with activity that was going on in South Africa.

We produced the second panel report that was available to the United States Environmental Protection Agency. We followed up with a third panel report in 2003. And this is all again occurring in the backdrop of the next generation of EDSTAC who is trying Tier 2 endocrine distruptor tests that we hope we will contribute at least in some small way to providing some of these validated methods that will be applicable for studies with amphibians.

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In terms of the panel activities, the panel activities were to establish and direct research programs in multiple laboratories to test hypotheses and to understand mechanisms. And I'll go into that in a little bit more detail. In terms of the activity that we do, we review science, we integrate and evaluate data from other laboratories, and design our own studies. And this is facilitated in part through the preparation of reports as I mentioned were made available to the U.S. Environmental Protection Agency.

The overall objective of our program is to assess the effects of environmentally relevant levels of atrazine on amphibians. We have a multi-pronged approach that you can put under two broad umbrellas. One being studies involving what we might call field studies. The other in laboratory studies. And as listed there, these studies encompass a number of different species some of which are native to North America and are environmentally relevant in our environment; others are laboratory surrogates like Xenopus, but we've gone and done the unique thing of studying this in its native habitat.

We also study some introduced species, in this case the cane toad in Southern Florida, because it's found in areas where there is overlap with potential exposures to the chemical in question.

This is just a brief summary of some of the reports that have

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been prepared by the panel. And as you can see, our focus started with a risk-based assessment of the endocrine system, moved into a more global evaluation of the endocrine system in non-mammalian vertebrates, and then specifically now has focused some of our attention looking at the questions associated with amphibians.

If you look at what were some of the highlights of these reports, these focused initially on some of the traditional endpoints associated with the ecotoxicological potential, the ecotoxicological effects that could be potentially mediated by atrazine. We looked at and identified that endocrine and reproductive effects had not been specifically addressed. Where they had been studied, they had been looked at in microcosm and in full life-cycle tests and these tended to focus on responses in fish.

In terms of reproductive and endocrine effects, test guidelines were still under development. We needed to refine and optimize assays, and we needed to establish a framework for assessing these responses given that standardized protocols were not available.

So by way of introduction, I'll get into the meat of the core presentation. And the core presentation has an overarching question of trying to understand the ecological effects of atrazine on amphibians. In order that we could accomplish that goal of increasing

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our understanding, we needed to have an approach to identify causality. And part of that came through the development and implementation of the weight of evidence approach to the question.

The other part was that we wanted to embrace the scientific method. And so we developed and tested a suite of different hypotheses that were based at different levels of biological complexity. Some of these were associated with effects on the endocrine system through modulation of various endocrine endpoints. We quickly moved to try to look at very specific activities at the tissue level and looked at tissue toxicity through studies on the effects on the gonad and the larynx. And then we also attempted in a general sense, and I will come to back to this in a few minutes, of trying to get to the tough question of what might be some of the population level impacts.

So while this approach may look like we're going from the, I guess you could say, bottom up or the top down depending on where you put these, we were trying to look at different scales of biological complexity.

In terms of the first question that came to the panel was how do we evaluate causality. And we could certainly go back into the literature and we could identify that this question has been around

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since the 1800s. There have been a very strong focus on this from a human health epidemiological perspective. But there have been very significant developments that have been made over time in terms of looking at this from an eco perspective and looking at it in the real world with wildlife species.

Some of the pivotal work by Glen Fox published in 1991. Other work by Gary Ankley who involved some of the members of our panel. But it's important to note that Gary Ankley was, in fact, one of the reviewers of the White Paper that is before the SAP today.

Now, as Dr. Kendall mentioned a few minutes ago, I was very fortunate to be involved for a period of about three years in work sponsored by the International Programme on Chemical Safety that ended up with the publication of a book that was the Global Assessment of the State of the Science Associated with Endocrine Disruptors.

Why do I bring this up here? Because the causal criteria that was developed through this document is the very criteria that we have tried to use in trying to evaluate the potential effects of atrazine on amphibian populations.

Now, I put this up as if it's my own work. In fact, it's not my own work. I was a member of a very strong team of people that

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included representatives from over 32 -- there was 32 different international experts that were associated with the construction of this document. Some of those members are in fact members of the Eco Risk atrazine panel. Others are members of the U.S. Environmental Protection Agency. But I think the key point that I wish to make here is that this was a world-wide perspective on trying to develop a criteria document for evaluating the potential effects of endocrine disrupting chemicals.

This slide talks to the fact that there are a number of mechanisms in which chemicals could be having effects on development and endocrine processes in amphibians or in other vertebrates for that matter. There were direct effects where compounds could act as hormone mimics or antagonists. Indirect effects associated with changes in the hormone titer, effects directly on tissue development such as effects on gonadal development.

And when you look back at where we were as a panel about three years ago, we were left with the starting point that there was very little in the way of responses that were evident in amphibians. The one response that was before us was a potential effect of laryngeal development in amphibians associated with exposure to atrazine. And there were discussion at the time that this possible

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mechanism may involve interference with the androgen and estrogen titer, and it was the focus of some specific hypothesis testing that we could develop that was focused not only on just androgen estrogen titers but responses associated with changes in aromatase activity.

As a panel, we developed a main hypothesis. And the main hypothesis is probably a little longer than what's written there. But what we were interested in was the question of whether exposures to environmentally relevant concentrations of atrazine caused adverse effects on endocrine function in amphibians. And by endocrine function, we mean that in the broadest sense -- changes in endocrine function, growth, reproduction, and development -- all components that are under the control of the endocrine system.

This enabled us to develop a series of sub hypotheses that I'm going to go through in some degree of detail in the next series of slides. But we're going to look at whether these effects could be mediated through estrogen-dependent mechanisms, androgen-dependent mechanisms, effect on the thyroid systems, direct effects on the gonad, and then the potential that there may be affects on the population level in exposed amphibians.

Now, I put this slide up to try to remind everyone that the endocrine system in amphibians is designed in the same manner and

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the same fashion as it is in all other vertebrates. There is a hypothalamic pituitary target organ regulation of endocrine-dependent processes. And this slide helps to illustrate some of the potential targets for which there may be effects that we could develop hypotheses around.

One of the initial hypothesis that we were interested in was whether or not there were effects on the titer estrogens and effects mediated through changes in aromatase activity. We were very interested in whether there were effects on androgen levels and effects mediated through the androgen receptor. We were, of course, interested in whether there were effects on the thyroid system because of their important developmental role in amphibians.

We were also interested, of course, on some of the apical endpoints and whether there were responses associated with secondary sex characteristics, laryngeal growth, gonadal growth as an example. And then we'll try to translate this to higher levels of biological complexity by looking ultimately at population-level responses.

So if we go into the hypotheses that we've considered, the first hypothesis we considered was whether atrazine caused adverse effects in amphibians through estrogenic or anti-estrogen-mediated

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mechanisms.

Now the next slide illustrates a number of the endpoints that we considered in doing these analyses. And for each of the hypotheses, I'm going to have a similar format. And in this case, the kinds of endpoints that we considered were binding to the estrogen receptor changes in the amount of circulating estrogens, inductions of aromatase, and whether or not the responses that were induced in studies, both in the lab and in the field, could be correlated with the responses that we saw to estrogen exposure.

I won't go through all of the details of these responses or the conclusions to the studies as these are going to be the focus of the remaining slides. But to cut to the chase, the conclusion for this section was that it's highly unlikely that atrazine could be exerting effects through mechanisms that are involved with estrogen.

An obvious question being does atrazine exert its effects through binding to the estrogen receptor. To our knowledge, this is not been explicitly tested in amphibians; but there is an extensive literature available that suggests that atrazine does not bind with any significant affinity to the mammalian estrogen receptor. Given the high degree of homology between these receptors across classes, we don't expect that this is an issue we need be concerned about.

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In terms of changes associated with atrazine and affecting plasma estrogen titer, some of the initial studies looked at both Xenopus and the green frog exposed throughout development in lab conditions, and there were no effects. Similarly, we saw no effects on Xenopus laevis adults exposed in the laboratory for periods of up to 47 days. There were some indications from field studies that there was a negative correlation between estrogen titers and triazines under field conditions. And in other studies looking at the cane toad, adults exposed to atrazine under field conditions, again, we saw no significant effects.

Just to give you an example of the kinds of data that we saw in these types of experiments, this is the result of an experiment that was conducted by a post-doc in John Giesy's lab at Michigan State University. And Dr. Hecker showed that exposure to atrazine caused no specific concentration-related response. Of the various doses that were tested, only one dose caused a reduction in estradiol concentration in the plasma.

And this finding, coupled with the other responses that we had failed to show a response in terms in changes in estradiol titer, suggested to us that this was not a particularly robust response and certainly one that was difficult to envisage from a mechanistic

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standpoint.

In this slide here on the left-hand side we slow the kinds of responses that you see when you expose animals to estradiol in the water. And not surprisingly, if you put estradiol in the water, the amounts of estrogen in the blood go up. So that's very much what one would expect.

Now we took these studies and we conducted some of these actually in the field situation in South Africa. The triangle on this slide illustrates the main corn-growing area of South Africa. The red dot here indicates the study site, and that is in the vicinity of Potchefstroom where Dr. Louis Du Preez, a member of our team, is a faculty member.

This is not Kansas. This is South Africa. And this is a picture of a corn field in the corn-growing area. And if you look at the nature of the soil type in the area, this soil type is particularly sandy. As a result of that, there is the rapid movement of any chemical that's put on fields in this area. And this raises the potential may well get into receiving environment into ponds that would be the likely home of native amphibians in South Africa.

And what we were able to do was initiate a series of studies in which we looked specifically at amphibian populations living in these

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ponds. These are two of the experimental sites in the corn-growing area E1 and E8. And this green here represents corn fields that grow essentially right up to the edge of the farm ponds.

When we've done these studies and we've gone out and measured circulating levels of hormones, in this case these are estradiol levels in males and females. And in this case, we found a significant reduction in circulating estradiol levels in corn-growing areas in both the male and the females.

I'd like to leave you, though, with a couple of points associated with this slide. The first slide or the first thought is: Is that if indeed this response of atrazine was associated with an induction of aromatase activity, this would be contrary to what one might predict. The second issue is, is that in sampling populations of frogs in this area, you have frogs at various stages of sexual maturity. And it's quite clear that if you look at the range and the variance of the data, there is considerable overlap and certainly it's very difficult to partition out responses that one might immediately attribute to exposure to atrazine.

Now, a considerable amount of attention has been paid to the question of whether or not atrazine has effects on estrogen titer through induction of aromatase activity. The first discussion of this

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point came in a publication by Hayes where he hypothesized that atrazine would be induced in frogs exposed to atrazine. In this situation, there was no data that was provided. But in subsequent studies conducted by the panel, we found no effect on atrazine activity levels in Xenopus exposed through development in the laboratory, both in juveniles and in adults. And in field studies, we showed no correlation with triazine levels in adults that were collected again under field conditions.

This illustrates some of the data that has been associated with our evaluation of aromatase activity in the gonad of Xenopus. If you look in the first instance on the right-hand side of the panel here, we see a marked sexual dimorphism in the total amounts of aromatase activity in the gonad. That's not unexpected. But we see no concentration-dependent effect of atrazine on aromatase activity levels.

The contrast to this, if you look at the data on the left-hand side of the panel, if we expose the animals to estradiol, at least in the females, we induce a significant reduction in ovarian aromatase activity in the females.

Again, back to the original hypothesis is atrazine acting like an estrogen. In this case, we're seeing no evidence that atrazine is

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having that type of a response.

We also tried to compare the effects of atrazine to those associated with estradiol. Now the first of the points that we have up there was the summary of some work that was done a number of years ago by Dr. Tyrone Hayes and his associate, looking at the possibility that atrazine would affect sexually dimorphic characters, that would be coloration in a frog species called Hyperolius. And in this case, it's my understanding that they found no response. As well as I mentioned the earlier slide, atrazine also did not mimic the effects of estradiol on sex ratio in Xenopus.

So I guess the question is where are we in terms of this overall hypothesis and using this weight of evidence criteria. In terms of temporality, we have little indication of data that we can apply in that context. But when we look in terms of the other key components of the weight of hypothesis testing framework in terms of strength of association, consistency, or biological plausibility, there's little evidence to support that type of a mechanism. And we're left with the overall summary that there's little evidence to support the concept that atrazine has affects in amphibians through either estrogenic or anti-estrogen mediated processes.

The second hypothesis that we considered, and this was one that

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was brought up in question earlier today to the Science Advisory Panel to the U.S. EPA, was whether or not atrazine may be exerting it's effects in amphibians through androgenic receptors through acting as either an androgen agonist or an androgen antagonist.

So we've considered this by both empirical studies and by looking at the literature. And we looked at a variety of endpoints including binding to the androgen receptor, changes in androgen receptor type, androgen titer, comparing the responses to DHT, and looking at whether or not atrazine mimics the effects of androgens on androgen-dependent processes.

So to summarize some of these data, in terms of does atrazine bind to the androgen receptor, to our knowledge this is not been tested in amphibians. But if we look at the extensive literature that's available for mammals, there is little evidence to suggest that atrazine binds to the mammalian androgen receptor.

In terms of androgen-dependent gene activation, this has not been specifically tested in amphibians. But again, the results are negative in mammals. And given the homology of receptors across those species, we don't anticipate that there is an issue here that we have to be concerned with in the immediate term.

In terms of effects of plasma androgen titers, in terms of

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looking at laboratory studies, we see very different types of responses. In work that has been conducted by the panel, there were no effects in Xenopus or in the green frog exposed during development in the laboratory. We also saw no effects in adults following exposure in the laboratory to atrazine. This is in contrast with a study that was produced and published by Dr. Tyrone Hayes, in which he showed a significant reduction in plasma testosterone levels in adults Xenopus laevis.

We've also looked at this under field conditions. And here we see a variety of different types of responses that are not consistent. In terms of studies with the cane toad, we saw no effects of plasma androgen levels following collection in reference and atrazine-exposed locations. In terms of studies that were conducted in South Africa, there was a correlation with lower T levels associated with the exposure to one of the metabolites, DACT, but not to atrazine or trebuthylazine under field conditions. And in the female, we did see a negative correlation between concentrations of atrazine, trebuthylazine, and their metabolites.

To illustrate some of these data, these are the responses that were observed in Xenopus laevis collected in South Africa from both reference and corn-growing locations in males. We did not see a

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significant change in the median androgen titers in the blood. In contrast, we did see a significant reduction in females. But, again, the levels of androgens in the plasma of these frogs are highly variable and it makes interpretation of these data somewhat difficult.

Now, there was an initial report that suggested that one of the androgen-dependent processes that occurs in frogs, that being the size of the laryngeal dilator muscle, may well be affected by atrazine. And this was a study that was produced Dr. Tyrone Hayes. And he reported a decrease in laryngeal dilator muscle using cross-sectional area as the indicator.

In three other studies involving Xenopus, members of the panel have failed to show an effect of atrazine on laryngeal dilator muscle size. And if we test the original hypothesis that atrazine may well be functioning as an androgen-receptor agonist, we would anticipate that atrazine would mimic the effects of DHT. And, in fact, in our studies, we have consistently failed to show that atrazine mimics the effects of DHT on the size of the laryngeal dilator muscle.

Now, here I have redrawn some work that came from Dr. Tyrone Hayes. And he reported in the Proceedings of the National Academy of Science a response in males such that exposure to concentrations of atrazine at the highest doses caused a significant depression of the

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size of the laryngeal muscle in terms of cross-sectional area.

Interestingly, he showed a similar kind of trend, although not a statistically significant response in females. And this was an interesting observation and one which the panel was very interested in trying to see whether it would hold up under other studies. But this reduction is not synonymous in our minds with an effect that would likely be mediated by an induction of aromatase.

In the kinds of studies that we've conducted as a panel, these are some work from Jim Carr, published in 2003 in Environmental Toxicology and Chemistry, we see in the course of our study at Stage 66 that the sexual dimorphic response is quite evident, suggesting that they are responding to androgenous hormones. But we see no dose-related affects of atrazine in males or in females. By comparison, if we do treat these animals with DHT, we see the anticipated and expected rise in the size of the laryngeal dilator muscle.

In other studies conducted at Michigan State University looking at this endpoint in terms of responses looking at atrazine, we saw no significant differences associated with the size of the laryngeal muscle in males or in females. But once again, the positive control of DHT had a clear stimulatory effect on the size of the muscle.

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When we looked at Xenopus from the field situation in South Africa, we did these data a little bit differently. We tried to co-vary this with the weight of the frog. And so we calculated a larynx-somatic index, the weight of the dilator muscle and the associated cartilage versus the body weight of the frog. Again, we saw a clear sex-related difference in both the references areas and the corn-growing areas. But there was no association with whether the animals were collected in reference or corn-growing locations.

So in terms of this second hypothesis, if we looked at whether or not there was evidence to support the conclusion that atrazine effects or exerts effects through androgen-mediated processes, the evidence was either there was no evidence available; or where there was, the evidence was scant and certainly not indicative of a robust type of response.

The third hypothesis that we considered was one of whether or not atrazine would exert it's effects through influences on the thyroid hormone system. And this was an obvious hypothesis to us given the importance of the thyroid in mediating both metamorphosis and what is known across vertebrates in terms of the permissive effects of thyroid hormones on other aspects of development such as gonadal development.

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So we were interested in whether or not there were changes in thyroid hormone-mediated responses. And the bottom line here is that atrazine does not appear to affect metamorphosis. In terms of an obvious other place to look, would be whether or not atrazine had effects on plasma thyroid hormone titer. There is no information available at this time.

So binding to the thyroid hormone receptor has not been tested in amphibians. And in terms of effects of thyroid hormone-dependent gene activation, there was no effect on metamorphosis in a suite of different studies using a range of species including Xenopus, the green frog, and the Leopard Frog.

So in terms of the bottom-line conclusion for thyroid-mediated response, we see no evidence that atrazine affects thyroid-mediated processes in amphibians. And this conclusion falls well in line with the conclusions that are coming out in terms of the mammalian literature. Again, in mammals, there is no indication that atrazine is affecting thyroid-dependent processes.

The fourth hypothesis and one that has been the focus of much of the attention of the panel, but also of the discussions today, was whether or not atrazine causes adverse effects on gonadal development in amphibians.

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And in this regard, we focused our evaluations on both testicular morphology and ovarian morphology and development. The bottom line in these studies is, that if you look across the literature, if there is a sex that has the potential to be affected, it is more likely the males. But in this situation, it is clearly a variable type response. And I'll try to highlight some of those differences in various studies that have been evaluated.

In terms of the effects on testicular development, the kinds of endpoints have included -- well, first of all, there's been a variety of endpoints that have been evaluated. In terms of the ones that I'm going to highlight on this slide was in terms of effects both hermaphrodism and on the presence of discontinuous testes or breaks of the structure of the testes.

Dr. Hayes reported in the PNAS paper that there was an induction of both of these events at doses greater than or equal to 0.1 microgram per liter. The work done by the panel showed aspects of similar responses, but at doses that were about 250-fold higher in concentration. And in other studies using Xenopus, there were no effects in field and microcosm-exposed populations in South Africa. And there was no effects in the laboratory study conducted at Michigan State University.

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There was one other study. And that was the study by Tavera-Mendoza and colleagues who reported a decrease in testicular volume in Xenopus. They showed this response at 21 micrograms per liter. But we have serious questions and reservations about that study as there is inconsistency between the published work and the replicate experiments that are reported in the thesis describing the entirety of the work conducted in that laboratory.

In other studies, there was no effect at doses less than or equal to 25 micrograms per liter in the green frog in work by Hecker. And then there was study by Hayes showing that there was in increase in hermaphrodism in Rana pipiens, the Leopard Frogs; but it was an inverse concentration response that was somewhat difficult to interpret.

There were other studies that have looked at hermaphrodism in frogs. And this turns out that this has been a response that has been observed for decades. There was response indicating that hermaphrodism does occur in other frogs well prior to the use of atrazine. And in the cricket frogs, there was a clear indication of intersex in a retrospective study that evaluated museum specimens.

In terms of the types of responses that have been seen, these are some work from Jim Carr's studies that were published in

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Environmental Toxicology and Chemistry. In terms of discontinuous testes, there was an increase associated with atrazine exposure but only at the highest dose both in terms of discontinuous testes and intersex. If we treated with DHT or estradiol, we saw no effect in terms of discontinuous testes but an induction by estradiol of an increase in the proportion of intersex.

In other studies that were conducted by Hecker and associates at Michigan State University, we saw no significant differences associated with exposure to various doses of atrazine in terms of looking at discontinuous gonads, mixed-sex gonads, size irregularities, intersex, or other anomalies. So, clearly, there is a discordance between different laboratories in terms of types of responses that are seen in terms of testicular development.

In a field study, this was work conducted in Iowa in which there was an evaluation of various -- pardon me. This is work from South Africa. And this was, again, looking at Xenopus from areas which were references sites and corn-growing sites. And here a serological evaluation was done to look at the distributional volume of different cell types within a microscopic field, looking at spermatogonia, spermatocytes, sperm, blood vessels, and other cell types. And we could differentiate no difference in this distribution of cell types from

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both corn-growing and reference locations.

If we switch gears a little bit and we look at what happens in terms of some of the types of responses that we see in amphibians, this is an interesting observation that we and now others have clearly made. And that's the presence of an oocyte that is found growing, not necessarily growing, found present in the vicinity of what appears to be normal testicular tissue. In this case, we have an oocyte with multiple nucleoli. We have a development of epithelial cell layer. And this testicular oocytes are turning out to be almost a ubiquitous feature of the development of amphibians.

If you look across a range of studies, various authors, Hayes, Smith, Du Preez, Hecker, and others going back to Witschi in the 1920s, have identified that there are testicular oocytes that are present in amphibians.

In terms of whether this response is associated with atrazine, there's one paper suggesting that these occur in association with exposure to atrazine. That being the Hayes work. But the other studies show that these are present at all doses associated with both reference and doses lower than 30 or 25 micrograms per liter.

Just to give you an example of the kinds of responses that people have seen, this is some work on testicular oocytes in Xenopus

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that were exposed as juveniles until Stage 66 and then allowed to grow up or grow out from there. When we looked at these data in terms of the incidence of testicular oocytes or of intersex, there was no significant difference in that distribution associated with exposure to atrazine.

Now, I mentioned earlier that if we switch and look at the opposite sex and we look at responses in females, the general scheme of things is that there's no evidence that there are affects on ovarian morphology in Xenopus associated with exposure to atrazine. Hayes in his work showed no effect at doses up to 200 micrograms per liter. Others at the highest doses that they looked at 25, approximately 30, in the field studies, or 25 in the Michigan State study by Hecker, showed no effect.

The one study that seems to be contrary to this is the Tavera-Mendoza in a second paper. And this group reported that associated with exposure to atrazine, there was a reduction in the number of primary oocytes but actually an increase in the number of secondary oocytes. An interpretation of this would be that atrazine is actually promoting ovarian development.

Again, we have some concerns about this, that the replication between the published study and what's reported in the thesis is

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certainly not there and it's not consistent.

In other studies where investigators have looked in a number of ranids, again, there was no effect in terms ovarian development.

So in terms of evaluating whether or not atrazine exposure is associated with effects on gonadal development, in terms of the temporality, there seems to be a very serious question associated with causality in that these responses were present well before the introduction of atrazine to the marketplace. In terms of the strength of association, there is some evidence of responses. But it's an inconsistent concentration response.

In terms of the consistency where there are concentration responses, these are typically not dose-related. There's clearly some indication that there may be some effects that are occurring. There's little evidence to indicate that those are severe effects. But at this point in time, we have little or no evidence in terms of the mechanism that may be contributing to these types of responses.

So our overall assessment here is that there's little evidence the atrazine affect gonadal development in male frogs.

The last hypothesis is one that we very much wished to get to. And that is to address the question -- some of you may call it the select question. And that was whether or not atrazine causes adverse

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effects at the population level in exposed amphibians. And so the kinds of endpoints can be and have been considered in this regard are looking at the abundance of species, age-size class distributions as examples.

And the conclusion to these studies is relative to causality, there's little evidence of effects linked to atrazine exposure.

Now, I'll go through this slide, but I'd to remind those ecologists in the group that there is a caveat coming on the next slide.

When we've looked at population responses, the kinds of responses that we've measured are there are robust populations and there are no differences in age-size class distributions of Xenopus in corn-growing and reference sites in South Africa. When others, Hayes, has looked at the Leopard Frog across a range of atrazine exposures, he found robust populations. When we looked in South Florida, we found much higher populations of the cane toad in areas that were associated with sugar cane production which would have higher exposure to atrazine than our reference locations.

And when we looked at the bullfrog across a range of atrazine exposures in Southern Iowa, there were numerous individuals in what appeared to be robust populations. But my caveat for the ecologists in the group was that few studies have been undertaken to explicitly

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address the question of whether there were adverse population level impacts associated with atrazine exposure in amphibians, at least in native amphibians.

Now, just a couple of slides here so we don't think all of the work occurs in the lab. This is Louis Du Preez taking an oxygen meter sample from a study site in South Africa. These are the traps that we used to collect Xenopus. These are weighed down and put underwater because Xenopus is obviously an aquatic species. We then collect the frogs in the traps. We then can do mark, recapture, and release studies.

And when we do these kinds of things, these are types of data that we have seen. And these are looking at both reference and corn-growing locations in South Africa. And the various colors on there represent various age classes. In terms of statistical evaluation, there was no difference in the proportion of different age classes across the reference and corn-growing sites. And if you look at those population structures, you've got a blend of young and old frogs in all of the locations.

So in terms of our overall evaluation here in terms of whether or not there are responses that are manifest at the populations, to our knowledge, there's no evidence at this point linking atrazine exposure

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and adverse effects at the population level.

Now, where are we in terms of the summary? In terms of the overall strength of association, if we look at the global characteristics of temporality, we see no evidence of a correlation between the occurrence of gonadal effects and the introduction and use of atrazine.

In terms of strength of association and the various kinds of parameters that we've looked at, on general or in general, there's little evidence to point to a concentration-dependent response with atrazine and the various endpoints that we've looked at. No one has evoked cautious postulates to remove the stressor to try to establish causality. That's something that with a robust responses, we certainly would be willing to and would like to consider.

In terms of incidence rates in the population, these, for the variety of parameters that we've looked at, are clearly inconsistent. And more often than not, the various types of confounders that could have influenced the types of responses that we've seen, particularly in the field situation, have not been specifically addressed.

In terms of consistency, generally, there is not particularly good consistency where there have been responses measured. In terms of biological plausibility, in terms of the kinds of mechanisms

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that are evoked in the literature, in terms of effects through estrogenic, androgen, or thyroid-mediated mechanisms, we see little evidence to suggest that atrazine is exerting effects in that way.

And we can't specifically address the question of recovery given that there's a lack of consistent and robust response in the types of endpoints that we've looked at.

Now, one of the things that you may have picked up is that the group that we work with is an atrazine ecological risk analysis panel. So what we should be doing is conducting a risk analysis. Well, we're hamstrung and we're unable to do a risk analysis in the sense that we're not seeing consistent effects, there's no consistent concentration-dependent responses. And at this point in time, a risk analysis is not feasible or possible.

Thank you very much for your time and attention.

DR. ROBERTS: Thank you for your presentation. I would like now to ask the Panel if they had any questions for you. Dr. Kelley.

DR. KELLEY: Yeah, I have some questions about the Carr study that was published in 2003. Is that okay to ask you about? One of the puzzling aspects of the Carr study were the results with the positive control which was raising the tadpoles in estradiol. And as I understand it, they were raised beginning 48 hours after fertilization

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all the way up until they got to Stage 66, and you only looked at Stage 66 animals. And there are a number of studies in the literature that indicated that these animals in other studies would have a hundred percent female at that dose and nobody had ever reported intersex at that dose; although it is reported within a smaller time window.

And I wondered if you had a feeling for why you had relative insensitivity in this paradigm to the positive control?

DR. CARR: I don't know if it was a lack of sensitivity. If you actually look at the estradiol levels in the tanks, which is in the technical report, they are a lot lower than they should be. So it may have been a dose response effect.

DR. KELLEY: Oh, so you think it was actually sticking to the glass.

DR. CARR: No. I think it might have been a fact of the tank change paradigm. We didn't do complete tank changes. We didn't think that would affect atrazine. In fact, it didn't affect atrazine levels. And that was the purpose of the experiment.

We've done other studies to show that that concentration, if you maintain target concentrations at 100 parts per billion estradiol, you will get 100 percent females.

DR. KELLEY: Well, so what I'm disturbed about is in Figure 2.

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So the summary statement from Figure 2 is that, in fact, the effect of atrazine did not resemble the effect of estradiol. But if you actually look at Figure 2, in fact, they look quite similar; although clearly the effect of atrazine is not as significant as the effect of estradiol. So I'm just worrying that the paradigm itself diluted the delivery to such an extent that an effect present could not have been picked up.

DR. CARR: Diluted the delivery of --

DR. KELLEY: Well, clearly, you've just told me it diluted the delivery of estradiol so that you didn't have an effective concentration.

DR. ROBERTS: Dr. Kelley, I'm sorry to interrupt. But can you make it clear which figure you're looking so the rest of the panel can see what you're referring to.

DR. KELLEY: Yes. This is a figure -- there is a published paper this year on whom the first author is Dr. Carr who's down at the end of the table.

DR. CARR: So this is Figure 2 in our paper. Okay.

DR. KELLEY: Yeah, this is Figure 2 in the paper. I've also read the technical report which I'm looking at here. But, you know, the paper is a lot shorter. It's easier to get through.

Anyway, so I guess I'm concerned about the fact that, although,

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of course, applying statistics to it -- in one case, you have a statistical effect and in the other you don't. But, in fact, the graphs are actually rather similar.

DR. CARR: You're right. There was a reduction in the percentage of males in the highest atrazine concentration. It was not statistically significant. There was no alteration in the percentage of females.

DR. KELLEY: Okay.

DR. CARR: And that led us to our other conclusion in that paper that atrazine was principally affecting male gonadal differentiation.

DR. KELLEY: Okay. So you're attributing your lack of the positive control to the fact that you didn't have an effective enough dose of estradiol.

DR. CARR: Correct.

DR. KELLEY: But you don't think that that applied to the atrazine in the study --

DR. CARR: Well, we know it didn't because --

DR. KELLEY: -- because you measured it.

DR. CARR: -- we measured it.

DR. KELLEY: That's my first question. I will cede the stage to

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somebody else.

DR. ROBERTS: All right. Dr. Skelly and then Dr. Green.

DR. SKELLY: During your presentation, you suggested that the work of one group, Tavera-Mendoza. You suggested that this study design was flawed and that you sort of implied that that should influence how we think about the evidence that came out of that. I wanted to ask you a general question and a specific question. And that is, in general, do you think study design flaws should influence how this panel views the evidence that we're being asked to look at? And specifically, if atrazine is being detected at control sites, should that influence how we think about study outcome?

DR. VAN DER KRAAK: In terms of the Tavera-Mendoza paper, we were taken back by the lack of reproducibility of the data across what was reported in the thesis and what was reported in the published literature. So in terms of our evaluation, we felt that it was appropriate that we identify that there was that inconsistency. And so rather than in our weight of evidence providing a very resounding positive response, as an example, we felt that it was inappropriate to do that; given that within their own hands, that wasn't a reproducible effect. And so we've tended to diminish the value of that in our scheme. We didn't exclude it to completion in that we reported the

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data and said what they found. So 50 percent of the time, they get a response.

In terms of the specifics of their experimental design, I think there's a number of issues associated with the type of experiments they did in terms of a very short duration response, a short duration exposure paradigm, looking at a response without subsequent follow up to find out whether this was a long-term advancement in ovarian development as the case was in females or a significant change in testicular development as they seem to report in males. So it was difficult to try to address that from the robustness perspective.

Should you exclude that in your evaluations? No. I think you should include it in your evaluations. But you should look at all of the available data in arriving at your individual conclusions as to how you placed weight on individual studies.

The second questions I'll give my response to it, and then I'll ask others on the panel if they wish to add something additional.

Your questions was whether or not the presence of atrazine in some of the experiments in the controls would be something that would cause me to throw out that data. And the answer to that is that in my mind, that's not the issue that would throw it out in my perspective would be because we would be looking at that in a

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dose-response-related paradigm and seeing no evidence of dose response. I wouldn't be -- I would feel that that negated the nature of the experiment.

Does anybody wish to add to that?

DR. GIESY: I think that's an excellent question. And the way I would answer it is I think that would preclude being able to ask certain kinds of questions. But it wouldn't negate the ability to ask other kinds of questions.

The way we've approached it in the field where it is difficult to find situations where there is no atrazine but very low concentrations, is to take a Type 2 statistical approach or regression-type approach to look at that data because it is difficult to ask the question completely without and with atrazine.

So I think you have to look at each study specifically. And I would reiterate what Dr. Van Der Kraak said, I don't think you throw all the data out. But I think it does preclude the ability to ask certain questions.

DR. ROBERTS: Moving on then. Dr. Green.

DR. GREEN: This question is along the same lines. There were two studies that you referred to quite frequently, the one by Dr. Carr in 2003 and the one published Dr. Giesy in 2003, in which you

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said that data was interpreted as atrazine had no effect on the various parameters you were looking at. A very nagging concern I have about those studies has to do with stocking densities, loading densities, and the water quality in those tanks.

Ammonia levels as high as 27 milligrams per liter are quite toxic. And that alone could affect the outcome of that study. It could inhibit the growth of the animals, make them susceptible to infectious diseases. And given that you have such variability in stocking density, the tadpoles were stocked quite heavily, as well as variability in water quality, how can you support the conclusion that atrazine had no effect in the face of such background levels of other toxic substances.

And I have a follow-up question to that, too, if that's okay.

DR. ROBERTS: That's fine.

DR. GIESY: Yeah, that's a good question. The studies with my name on them were field studies from South Africa. They weren't the lab studies. I think the ones you refer to are the ones by Hecker, Environmental Toxicology.

DR. GREEN: Yes.

DR. GIESY: Yeah, and you're right. All those issues are ones we identified in our report that are limitations of the studies. When

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we went into the study, we tried to do a power analysis to look at what we needed in the way of sample sizes to be able to make some conclusions. And that included stocking numbers and numbers of tanks to look for tank effects and number of doses.

So when we did all that, in the end, everything was a compromise. And in hindsight, certainly, if we had the space and ability to do it, we would have chosen to use lower density stocking for sure. So all those criticisms that the EPA has pointed out, and I'm sure the Panel will pick up on, are valid and we certainly would recognize those.

Whether it completely negates the utility of the data, I personally don't think so. I think it would be nice to be able to do it again. That's why personally I think the EPA's conclusions are sound. And their proposal to move forward is a good one, to try to remove of those uncertainties that we readily admit are there.

DR. ROBERTS: Follow-up by Dr. Green.

DR. GREEN: Yes. I'd like to know just in general by members of the Panel who have labs where they are conducting these experiments. What test kits do you use, and how frequently do you monitor water quality analysis in these studies? Are they color metrics, that sort of thing?

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DR. CARR: The standard operating procedures, I think, were made available as part of the GLP conditions for the study and there should be water quality operating procedures in there. We measure temperature, dissolved oxygen, conductivity, ammonia, using the Hawk photometric method on a weekly basis. In many cases, dissolved oxygen on an every-other-day basis or every three days when we do tank changes.

DR. ROBERTS: Dr. LeBlanc then Dr. Kloas and then Dr. Thrall.

DR. KENDALL: Mr. Chairman, I think Dr. Carr wants to add to the first question.

DR. ROBERTS: Okay. That's fine.

DR. CARR: Right. Dr. Green was asking about water quality. There are concerns about water quality in a static exchange design. The 27 ppm levels that came up -- towards the end of the study when the animals are larger, completing metamorphosis, the unionized ammonia levels were about .2 ppm. We didn't see high mortality. And we don't think that ammonia was toxic to the animals.

Did it effect growth? Well, the animals did develop slowly. They were at a lower temperature. But we also saw 99 percent of the animals sexually differentiated. So we don't think it impacted the

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degree of sexual differentiation and that the critical aim of the study was to examine atrazine effects on gonadal development.

DR. ROBERTS: Okay. Were there other members of your group that wanted to respond to Dr. Green's question? Dr. LeBlanc.

DR. LEBLANC: Thank you. When I think about androgens and estrogens, I tend to think about them having different roles in adults versus juveniles. That is, in the adult, I think about them having roles in reproduction. And in the juvenile, the larvae, I think about them having roles in development. And I think what we're concerned about today is a role that atrazine might have in perturbing development of these larvae.

But it seems like a lot of the negative data that was just presented discounting or at least not being able to demonstrate any effect of atrazine on androgens or estrogens, were in the adult. Correct me if I'm wrong if that's not the case. But in terms of steroid hormone levels, aromatase activity, I just got the feeling like you were aiming at the wrong target when generating this information. Could you comment on that anyone?

DR. ROBERTS: Dr. Giesy.

DR. GIESY: I wanted to make sure you introduced me.

Yeah, that's a good question. Some of the studies were with

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adults. The Xenopus studies, there was one that was conducted through Stage 66 where there were samples taken for analysis at that point. And there was a subpopulation that was grown out for about two-and-a-half months beyond that.

A similar study was done with the ramaclamatns. So those were exposed throughout their entire development. The Carr study was terminated at Stage 66. And those were exposed throughout the entire developmental period. There were other studies that were the field studies where those were adults. So they were collected as adults, but presumably, they were exposed to the environmental concentrations of atrazine in those situations throughout development.

And then there were some studies that were done only as adults to look at potential mechanisms of action at a fairly crude high level to see if we could get induction in the gonad because that had been reported in the literature previously. So in the adults, we did want to see if we could reproduce that.

So it was a combination of adults. But mostly it was throughout development.

DR. LEBLANC: Can I follow up?

DR. ROBERTS: Yes, please.

DR. LEBLANC: As related to androgenic or anti-androgenic

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effects of atrazine, one of the conclusions that was reached by the group was that atrazine did not mimic THT. And I think the data demonstrated that rather clearly, though I suspect that never really was a hypothesis. So I don't think any of the data that's been reported suggests that it is acting as an androgen. And if anything, perhaps it's acting as an anti-androgen.

And I was wondering if the Eco Risk group has ever evaluated it, an anti-androgen, to see if the effects are consistent with atrazine.

DR. VAN DER KRAAK: The short answer is no. Have we considered it? Yes. But it has been considered in relation to a whole host of various hypotheses that in the goodness of time will get tested.

DR. ROBERTS: Dr. LeBlanc, I thought your first question might have encompassed not only the time of exposure but the time of assessment, developmental stage at assessment. And I wasn't sure whether the response -- Dr. Giesy, I think, focused on the duration of exposure and the developmental stages of exposure but not necessarily at the times of assessment.

So if I might jump in and follow up. Dr. Giesy, can you touch on that in terms of stages of development at which assessment was conducted and how that might factor into the interpretation.

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DR. GIESY: Yeah. We, as a panel, have talked about that a lot and think that's really critical to doing experiments and also interpreting the data. In our studies, we developed protocol. If we were to do additional studies, certainly, we would want to design, I think like the EPA is proposing to design, a system where we could look at some of the critical windows. We think that's very important. And I'm going to let Jim Carr mention things in a minute.

But we think, also, that it may lead to some of the difficulties in interpretation and comparison among data sets, among laboratories, how animals are exposed and when they are collected, and whether or not they're grown out. We agree with the EPA that to do that grow-out study is important. And I think Dr. Kelley mentioned that this morning. I couldn't agree more. It's very, very appropriate to do that.

So interpreting the data, the timing of exposure, and in a minute, I'll make some comments relative to aromatase when the time is appropriate that also would impinge on when you collected it in the developmental cycle.

DR. CARR: One of the technical issues with looking at hormone levels in the tadpoles, of course, is that you're restricted by the amount of blood that's available to look at blood hormone levels.

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And what's often done is to look at whole-body hormone levels. So that's a technical issue that would need to be addressed in the interpretation of whole-body hormone levels relative to the onset of gonadal steroid secretion.

The other issue is the transfer of maternal steroids into the egg and the contribution of those steroids and separating those contributions out from the steroids that are produced andogenously from the animal's gonads.

So I think there are some technical issues that would need to be addressed, too. And we have discussed those several times. And it would be important to look at those, I think.

DR. ROBERTS: Dr. Kloas and then Dr. Thrall.

DR. KLOAS: I would like to continue in this field. I would like to know something about why did you use this experimental design for measuring aromatase steroid levels. So as you are aware, the endocrine system you have some counter-regulation. So you assessed estradiol and testosterone after at least 26 days. And why didn't you use the short-term exposure. For instance, let's say half a day, one day, three days, seven days? And then if there is any change in aromatase activity and also in estradiol and androgen levels, because after 26 days or 43 days, there might be a readjustment by

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endocrine counter-regulation by hypothalamic pituitary levels. So I think you cannot really exclude any aromatase effect.

And, furthermore, my second question is methodology by Miyita, environmental toxicology al., would also allow to assess aromatase activity in tadpoles. Just a couple of seconds ago, you were talking about sensitive windows. Why not to do short-term exposures in tadpoles and measuring aromatase activity by using semi-quantitative auto PCR?

DR. GIESY: All great suggestions. I'd love to do it all.

DR. ROBERTS: Dr. Giesy's responding.

DR. GIESY: Those are all great suggestions. In fact, in our laboratory now, we've developed molecular beacons for CYP19. We can do that. So these were initial studies. We wanted to start at a sort of a high level, gross look and see if we could reproduce what was reported in the literature. But I would agree it does not allow us to preclude the potential effects through an aromatase mechanism of action in specific localized tissues. So I think timing is important to do that and look at it. To do that in small tissue amounts we would have to use PCR. And like I said, later, whenever it's appropriate, I'll talk more about the aromatase hypothesis and what I think about it and its future.

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But I think all of those are great suggestions. I would not disagree with any of them.

DR. ROBERTS: Dr. Thrall then Dr. Kelley.

DR. THRALL: Maybe I missed it. But I was still just a little confused about what the inconsistency was on the Tavera-Mendoza study. You said there was an inconsistency between the thesis and the published paper. And I wondered if you could be more specific about that. This was in relation to testicular volume.

DR. SOLOMON: We originally saw these papers only in publication, and, subsequently, obtained a copy of the thesis. I don't know. Has the Panel seen the thesis?

DR. ROBERTS: I do not believe that that's been entered into the docket.

DR. SOLOMON: One of the issues in the published paper was that they exposed the animals for a relatively short period of time. And then they reported a decrease in the size of the testes, in the volume of the testis, up to 70 percent as I recall. However, they didn't actually measure the size of the testes in the animals when they started. It was just comparing controls to the treated or the exposed animals which raised concerns.

Some other concerns were differences in the numbers of

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animals reported between the figures and the text of the papers that were not consistent. We, subsequently, obtained a copy of the thesis and found that a second experiment had been conducted with greater range of concentrations; not just 21, which was actually measured at 18, three different concentrations. And they had not seen a concentration response and no statistically significant differences.

So on that basis, we felt that there was obviously some design flaws in addition to the small numbers of animals used, the small number of tanks. There were only two tanks used. So they couldn't look at inter-tank variation. So that to our mind, diminished the value of that data in interpreting these responses.

DR. ROBERTS: Dr. Kelley and then Dr. Skelly.

DR. KELLEY: So I have two sets of questions. The first really to the field data in South Africa since I have to report on that.

So the animals were sampled in April and May which is just at the end of the rains. Could I have some information on the relation between the data sampling of the adults and the onset of the breeding season?

DR. DU PREEZ: In the Potchefstroom area, Xenopus breed from September, September, October, November. That's the onset of the rainy season. But Xenopus has got a prolonged breeding season

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from September right until April, end of April. So --

DR. KELLEY: So you were sampling at the end of the breeding season then.

DR. DU PREEZ: Yes. We sampled the great majority of the specimens at the end of the breeding season. In a few of the ponds, we had difficulty collecting the targeted number of specimens; and we collected those during subsequent months after that.

DR. KELLEY: Did you see any difference in your measurements of plasma steroid levels depending on the time of year? So you had most of your animals in April and May. But you had this one group where you collected at four different times. Were they pooled? Or were you able to look at those data separately?

DR. GIESY: That's an excellent point one we've discussed at length within the panel. Let me cut to my conclusion. Then I'll go back and try to backfill with some details.

From where we are now, I would have two conclusions. One, I don't think it's very useful unless we understand the seasonal cycles and are able to stratify our sampling to use measurements of estradiol and testosterone as functional endpoints. EPA has come to that conclusion in their White Paper, and I agree.

Now, the reason for that is the sample sizes required to have

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any power to show effects would be pretty large. So we recorded when we collected them. We also determined gonadal stage development with a scoring system, and we also determined age. But when we went to go to try to stratify the organisms by time of collection in the season, gonadal stage, and age, things got pretty thin in the sample sizes. So that's a trade-off. So I think any field work that people want to do is going to be very limited because of that problem.

Now, in South Africa, as you well know, the Xenopus are not synchronous spawners. They spawn continuously throughout the season. Some may not spawn at all. Some may spawn once, and some may spawn several times. So I think that's what leads to the great amount of variation.

So at the same time, I then think, well, with the effects we saw, we do see effects in the corn-growing region. They are fairly small relative to the variation that we see. And one question is what ecological relevance does that have.

But to answer your question, I think it's absolutely critical that we be able to stratify our sampling by season, by age, and by reproductive condition to be able to interpret any potential effects of compounds like atrazine on hormones. It's a difficult problem as you

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well know.

DR. KELLEY: So if I could just bring the attention of your group to some available data on hormone levels. So we measured hormone levels both in serum and in mid-sections of bodies at various stages and development which is in the Kang, environmental toxicology al., 1995 paper from General and Comparative Endocrinology. And where we could compare the mid-section level to the serum level because we had enough tissue, they were very close. So it may be, in fact, that that's an adequate way to do that study.

And this is also the way that a more recent study by Bogge, environmental toxicology al., in Comparative Biochemistry and Physiology, Part B in 2002, measured both 17-beta estradiol and androgen, both T and DHT, throughout development, were able to document the contribution of maternal hormones very early in development, and then the later contribution of hormones.

And in their paper, although we did not see this in ours, the levels are comparable. But it looks like their variability is lower. They actually have a sex difference in androgen level and also in estrogen level at the time of sexual differentiation.

So there clearly are some data available now that would enable or approaches that would enable you to look at that.

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That was a comment.

DR. GIESY: One I didn't fully appreciate. Can you tell me about the method? Could you resample the same individual? Is that what?

DR. KELLEY: No. What we did was, you know, you can't get enough blood out of a tadpole to run a reliable radioimmunoassay. They're pretty small. And we actually did these assays down to Stage 56, which is just towards the beginning of metamorphosis. But at Stage 66, the end of metamorphosis, we were able both to get tissue samples, not from the -- actually, did we do them? I think actually we did do them from the same individual come to think of it. But it didn't make any difference. The variability was quite low. And in that case, the serum levels agreed quite well with -- this is a mid-body segment that includes both the liver, which would be the major clearance organ, and the gonads. So I think it is possible to do.

And the comment which we got from the reviewers, which I will forward to you, was that they were worried about contamination from lipids. But we were able to extract lipids and come up with exactly the same numbers.

DR. ROBERTS: Dr. Skelly followed by Dr. Green and then Dr. LeBlanc.

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DR. SKELLY: In summing up what you had concluded from concerning Hypothesis V, which is atrazine causes adverse effects at the population level in exposed amphibians, you mention that a number of studies, which you had done and which are part of the open literature, had sampled robust populations. And wondered if you could tell me what your group defines as a robust population and what sort of demography, breeding behavior and breeding success sorts of parameters you've measured and you plan to measure.

DR. DU PREEZ: As part of this study, we did a mark and recapture study to determine populations in both corn-growing and cattle-farming areas. And in all of these sites, we found large numbers of Xenopus. Male female ratio were the same. No statistical difference. Xenopus populations do fluctuate sometimes due to introduction of catfish. And as we've seen this past year, catfish can wipe out a Xenopus in one specific pond in a relatively short time. So you have this constant fluctuation.

But if you set the traps, you get a feeling for the population. If you have difficulty getting the specimens during a certain part of the year, it's easier to trap Xenopus in spring. You get larger numbers in the traps. But in all, those populations appear to do very well.

DR. SKELLY: I spend a lot of my time wearing rubber pants as

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I'm sure some of you do. I guess what I was asking specifically is, based on what you have done which is look at literature, published literature studies, and your own studies which seem to be, maybe with some exceptions, going out and sampling either over a short interval or just looking at a study that might have gone in just once, you're declaring something to be a robust population.

I'm a population demographer. That raises antennae. So what is a robust population. And if that's just sort of a vague descripter, I'd like to know that.

DR. KENDALL: Mr. Chairman?

DR. ROBERTS: Dr. Kendall.

DR. KENDALL: I'd like for you to tell us what you think is a robust population. No disrespect. I would like for you to address that. And then we will respond.

DR. KELLEY: Okay. Well, I mean, I guess going back to the conceptual model that's been forwarded here, we're ultimately trying to get at viable populations. And I guess you could also define it comparatively. You've gone out and measured atrazine-exposed sites and control sites and you could do comparisons as well.

What I was asking, none of that information was presented when you mentioned robust populations. I didn't know whether you

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had collected information that you weren't showing us.

DR. KENDALL: I think that point's well-taken. Dr. Ernest Smith would like to respond.

DR. SMITH: We are responsible for the Iowa study. We have not done mark and recapture. But based on the profile over our first year, which was really a pilot study, we observed a significant change in the number of juveniles as we sampled during the late spring, early-late summer, and early fall. And as a result of that, I think from that standpoint, I would say there is an evidence of reproduction, evidence, indication of juvenile metamorphosis, differences in that increases as you sample.

So we're back into the same site for a second year. And I think we'll have a better feel for what is a robust population relative to last year's. But at this point, I think there is enough indication there from the numbers that we have captured and released back into those sites.

DR. KENDALL: Dr. Gross.

DR. GROSS: We've been coordinating and looking at the cane toad which was summarized in the previous presentation in South Florida. And at least in our studies on the sugar cane sites, we see populations we consider robust, to answer you question in part, due to the fact that we see all age classes present within the group that we're

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looking at. We're able to collect several hundred animals, actually, on those sites within a couple of hours time frame representing all these age classes as compared to control or reference sites where it would usually take us weeks to collect similar numbers if even possible to collect those numbers.

We've also been able in data you haven't seen demonstrate that there are tadpoles on those particular sites, egg masses, and so on. So reproduction is obviously occurring on those particular sites. And we consider them to be fairly robust for those reasons.

DR. ROBERTS: Did you have a follow-up, Dr. Skelly?

DR. SKELLY: I'm done.

DR. SOLOMON: Just an additional comment on the South Africa studies. We obtained estimates of the total population size based on the mark and recapture. And these were not inconsistent with the sizes of the sites. The smaller sites had smaller populations. The larger sites had... So if one thinks that a site may have a certain carrying capacity, it was consistent with what we saw there except for the cases of introduced predators, which would obviously affect numbers for different reasons.

DR. ROBERTS: Dr. Green then Dr. LeBlanc, Dr. Richards, Dr. Gibbs, Dr. Kloas, and Dr. Kelley.

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DR. GREEN: I think you just answered part of my question about the viability of the eggs produced by these females on sites where atrazine contamination is known and the viability of the sperm from the male, and are the eggs able to be fertilized. Apparently so if you say there are healthy populations.

It came to my attention here when you were presenting some of your data in the core presentation, the Hayes and Hecker studies, where you cited ovarian morphology in frogs and other species was normal. And these were laboratory frogs; correct? So I was wondering if anyone from the panel had extended those studies to actually if those eggs were fertilizable because, in my experience in the laboratory, a good healthy looking egg may not yield the kind of data you're looking for. It's not viable even though it appears to be so by physical characteristics.

DR. GIESY: I knew you had assembled a super panel, Steve; but these questions are great.

Once again, we couldn't agree more. We've thought about that. We've even gotten to the point of designing some studies, both ex vivo type studies and I think that it's very appropriate to do that kind of a grow-out study. So both ways that I heard suggested earlier today in discussion, I think, have some merit. I think the EPA

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suggested, and I think Professor Kelley might have mentioned, maybe ex vivo approaches to try to get at that in a staged way. I think that's a good thing to do. But the definitive study is that grow-out study. Absolutely. And I think the way the EPA has proposed to stage that and work through it has some merit.

But I would add the caveat that I'm not completely comfortable with the decision tree of saying if we don't see testicular oocytes, for instance, or some other histological response, that the decision would be to not do that study. I personally am not particularly comfortable with that. I think it is a very important thing to do.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: When discussing testicular oocytes, Dr. Van Der Kraak commented that the phenomenon seems to be relatively common at least in the experiments unrelated to atrazine. None the less, if we look at the figure, and I'm referring to the figure titled, Testicular Oocytes in Grow-Out Xenopus by Hecker, it certainly at face value it appears that testicular oocytes are regulated by hormones that negatively regulated by DHT and positively regulated by estradiol perhaps.

What I was questioning is as related to atrazine we see what appears to be a greater than two-fold increase in testicular oocytes

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with .1 microgram per liter atrazine. There's no error there to indicate the level of variability and there's an indication that it's not a significant increase.

But my question is, and I recognize that we as toxicologists would be very uncomfortable looking at dose-response curves that don't conform to a monatonic response. But was any consideration given to the fact that this might be real increase in that the inability to detect a significant increase reflects the statistical design or design of the experiment?

DR. ROBERTS: Before you respond, can we get the particular figure? Is this the one? Okay.

DR. GIESY: I'm not quite with you. Can you repeat it again, please? I've got the picture now.

DR. LEBLANC: Okay. So now we're looking at -- well, first if we look at the bottom, would you concur that testicular oocytes are regulated by hormones? It's hard to make judgements here because we don't see what the error is associated with these values. But --

DR. GIESY: This is one of our studies. In the report that you have, the means, medians, ranges, and all of the statistics for this data. So that's maybe what you want to look at, Gerry, to get the specifics.

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The one problem is sample sizes are fairly small relative to the incidences that we're seeing. So I'm not sure we can draw much conclusion. What I can say is based on this, at least with atrazine, it doesn't look like a very robust response. It's not a huge response. Whether estradiol affects this, I'm still open on that. I think it potentially can. That's why I'm concerned about some of the circulating plasma concentrations and some of the regression relationships we did see from the South Africa study. So that's why I wouldn't necessarily focus only on the testicular oocytes.

But the problem here is I think the sample sizes are fairly low and the incidences are low. And that's what leads to not being able to show a statistical difference. So in planning, if the EPA moves ahead and has this study repeated, they can look at this data as a way to do their power analysis to figure out exactly what size they need. But they're going to be very, very substantial sample sizes you're going to need to show a difference.

DR. ROBERTS: Dr. Richards, then Drs. Gibbs, Kloas, and Kelley.

DR. RICHARDS: I'm interested in the South Africa field studies. And it probably relates to other field studies also.

DR. ROBERTS: Dr. Richards, can you get the mike?

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DR. RICHARDS: I'm sorry. I'm interested in the South Africa field studies. But this relates to other field studies in general.

With essentially eight data points, I wonder, it's certainly critical to have some confidence in the degree of exposure that we think the animals were exposed to. And my question is sort of general in that how well do you think you've characterized it in the way you portray the data, four-week-mean concentration. I can't remember exactly your procedure or how frequently you measured. Certainly, with hydrologic events very dramatically influence concentrations of atrazine. I just wonder how well do you think this portrays what they were exposed to?

DR. DU PREEZ: What you have to take in mind is over one field use season we measured atrazine and other triazine concentrations. But that is not the concentration that that specific animal was exposed to during its development. That specimen might be four, five, six years old. And we don't know what those atrazine or triazine concentrations were five, six years ago.

But from what we've seen in the specific season, it was a season with a very high rainfall. And we would hypothesize that those animals were exposed to probably much higher atrazine concentrations than was recorded in the specific season. The answer

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is we don't know what those animals were exposed to.

DR. SOLOMON: Could I just add a comment to that?

In designing the experiment, we realized we couldn't go back in time and measure prior atrazine concentrations. We did have historical data from theses and other sources that showed the presence of atrazine at relatively high concentrations in surface waters in that area. So we realized that they could be quite large.

The season, as I said to somebody earlier today, if I could have predicted that rainfall, I would have sold my shares in Enron. But I'm not that good.

It was interesting, though, that in high rainfall, it's dilute. But we did measure concentrations every week during the application season because not all the fields are treated on exactly the same day. And then every two weeks after that. So we have a fairly good temporal exposure regimen. And for the purposes of the assessing the effects on hormones and aromatase, we decided to use the concentrations in the four-week period prior to the capture and sampling of the animals because we suspected -- or expected rather -- that these kinds of responses would be related more to recent exposure than previous exposure.

And, of course, the metamorphose that we collected in the study

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year, were exposed to the concentrations that we measured. So we know what they were exposed to. And we also know what their responses were in terms of gonadal responses, environmental toxicology cetera. So I think we have a good handle on both temporal exposures and for the site exposures as well.

But then as Dr. Du Preez pointed out, we know that they were probably exposed earlier; but we don't know to what concentration or that we theorize that it may be greater.

DR. DU PREEZ: If I might add another comment. What we've observed is a definite peak in atrazine directly after the application. That would be from December, January, February we observed a peak. But the Xenopus started breeding end of September. So those first couple of months, Xenopus would breed in fairly low atrazine concentrations. And then those that spawned after December, would be exposed to higher concentrations. So that is making this whole interpretation of the field use of the field data even more complicated.

DR. KENDALL: Dr. Bob Silken.

DR. SILKEN: Bob Silken, statistician-consultant to the Panel.

Given that you didn't have exposure concentration information over the entire profile of the animals life, although you did have a pretty good handle on what it was in the recent past, and also in

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keeping with Dr. Giesy's comments earlier that even though you may have some concentration of atrazine in the control sites or the reference sites, that doesn't eliminate making some use of the data. We not only did comparisons between non-corn-growing sites and corn-growing sites, but we also took what concentration information was there and compared the sites with the four lowest atrazine concentrations with the four highest. And they were fairly divided.

We also did the separation for anything below one and anything above two and compared the five sites that were below one with the three sites that were above two. So that even though you didn't have exact, precise exposure concentration, you could still do comparisons at different levels of exposures. And the comparisons were reasonably consistent across. No matter how you grouped them, reference, low, low three, low five, low four, the analyses came out pretty much the same.

DR. RICHARDS: One follow-up?

DR. ROBERTS: Sure.

DR. RICHARDS: I'm just curious about the biology of this creature. I know in the mid-west, sometimes when I'm sampling Rana species, when I get a heavy rainfall, there's water everywhere and there are Rana everywhere, moving around between ponds. That's

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probably part of their metapopulation dynamics and so forth.

Does this occur during high flows with this species in South Africa?

DR. DU PREEZ: Yes. Xenopus has been well-documented to migrate and sometimes en mass. Sometimes you find mass migrations. What we did in this study is we tried to determine, was there any migrations. And what I did was to brand specimens from a specific pond with a digital number corresponding to the site number. And we did not observe any migrations during our study. But, yes, they do migrate.

DR. ROBERTS: Dr. Gibbs.

DR. GIBBS: Yes. In your presentations, there were repeated presentations of negative results. I had anticipated more of a consideration of a power of tests. And I'm just curious particularly with the field studies. And I'm just curious how widespread power analyses were in your analyses both perhaps post hoc or a prior.

DR. KENDALL: Good question. Bob Silken.

DR. SILKEN: I guess I'm not going to get to sit over there. Yes, we did try and do power analyses before we went into the tests. And, of course, the power analyses varied in requirements depending upon whether it was a lab study, a field study, or what endpoint for

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the obvious reasons about the difference in variability.

For example, in Dr. Carr's lab study, we found that we definitely at least eight tanks because we felt the tank effects were going to be a strong factor. We went with 11 tanks in order to maximize the power, given that 11 tanks times the number of treatments was a many tanks as they could work with, that they had room. So power was in there.

We also did power calculations as far as the numbers per tank which may have led to some overcrowding. Blame it on the statistician who wanted bigger numbers. Although we found the number of tanks were much more important than number of animals per tank.

When we did the power analyses, for example, for Dr. Carr's study, we did look at the high degree of correlation within a tank. And we looked at what the power would be if we effectively had one animal per tank. Even though we put 30 in there, if they effectively all behaved the same, what would the power be if we had one animal per tank. And it ranged in his study from something like 70 power to detect the types of departures that were being talked about. A power in the range of 70 to 98 percent.

If we had four animals per tank, for all the types of changes, the

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powers went up to 99 percent. So we did look at power.

We looked at powers also in the field study. And, again, the variability there makes it such that the powers would be less. We would strongly encourage that if you're going to do field studies, that the site-to-site variability probably dominates everything else. And that that means that you need a large number of sites, both control sites and treatment sites because the variability in the controls is huge as well. And two or three control sites is not enough.

So we were looking heavily at power. What else do you want to know?

DR. GIBBS: You've considered it. Did you do any post hoc analyses? Or perhaps you're opposed to those of what sorts of effects you could have detected given your final sampling design.

DR. SILKEN: They ranged from kind of a post hoc analysis gives you better estimates of variation. You can go back and ask the question of, given those variations, what could you have detected. And we found, for example, for laryngeal muscle that we had plenty of power. That was not an issue.

We found that for aromatase and some of the hormones, you're going to need an awfully big study unless you really want -- unless you only want to detect really big differences. Kind of looking at the

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slide that you all were looking at up there earlier about the different effects of atrazine at .1, 1, 10 and 25, you had power to detect big differences relatively easily. And if big differences were all that were ecologically relevant, then you have plenty of power. If you wanted to get down and say I really wanted to fine tune this and be able to differentiate between a 3 percent response and 4 percent response, we didn't have that much power.

But you sort of want a trade off between what's ecologically relevant and how-big-can-you-make-it type thing.

DR. ROBERTS: Dr. Kloas and then Dr. Kelley.

DR. SOLOMON: Could I just add something to the comment?

In designing the South Africa studies, we started those in 2001. And at that time, our initial interest was laryngeal dilator muscle. And there was an ongoing study in the lab at the time, and that was the effect we were interested in. With that in mind, we chose the best possible situations. We wanted to find reference ponds where we could detect no atrazine at the time of the study which was the season before we started. And we also wanted to have a reasonable number of ponds within reasonable operating distance of the university as we didn't want frogs dying in the field because we couldn't collect and empty the traps fast enough. So a lot of logistical problems

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associated with getting really large sample sizes. We couldn't go very far a field for practical, logistical reasons.

DR. SILKEN: Let me just add one other comment. One thing that we discovered in looking at this data relatively extensively is that the tank effects, both in the field studies and in the lab studies, were such that if you were to pool the data and ignore tank effects, you get yourself in some very unexpected trouble.

And, in fact, and I know experimenters do this all the time. They'll do a test to see whether there's homogeneity among the tanks. And if it passes an F test or another test for homogeneity, then it passes the test that apparently there are no tank effects. So you pool all the data together, and then you do a test for, say, treatment differences.

We found that when you do that, instead of having a 5 percent error rate, you have between a 30 and 90 percent error rate when you follow an F test for homogeneity with then pooling the data and checking.

So as far as your power is concerned, there's another aspect, too. And that is the tank effects and whether or not you pool animals within tanks. A very dangerous thing to do. So we encourage the Panel not to do that and to do almost all of their analyses on a tank

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basis.

DR. ROBERTS: Dr. Kloas, then Dr. Kelley, Dr. Denver.

DR. KLOAS: I appreciate very much that you mentioned the hypothesis of possibility of anti-androgenic effects of atrazine. I think why didn't you go forward to test this hypothesis? You stated just something about androgen effects but not about anti-androgenic effects.

So for instance, I would have liked to know something about effects on 5-alpha reductase or the relationship between testosterone to dehydrotestosterone because I think we all are aware that dehydrotestosterone is much more power full, it's an androgen, which is leading to masculinization. And we have a demasculinization effect. I think this could be also another key enzyme or another key target for having adverse effects on demasculinization.

DR. ROBERTS: Did you want to respond?

DR. VAN DER KRAAK: I mean I think the answer is simple. I agree. I mean it's just a question of -- we've talked about a number of potential experiments. We've not got there yet. And we'll certainly continue to consider that. And if that wishes to be recommendation that goes forward by the SAP, we and I'm sure others would consider that very seriously.

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DR. GIESY: If I could respond. Once again, I have to completely agree. We've thought about it. We've talked about it. I have a proposal written to do it. So I, obviously, agree with you that it's a thing to do. But it's just a matter of time, how much time there was. And also it was driven by trying to look at what was being published in the literature and get a handle on, could we reproduce that, were those mechanisms of action that were being proposed plausible.

So in part, if we could just design experiments ourselves and not be looking at the literature, certainly, we would have agreed with you and gone straight ahead to do that.

DR. ROBERTS: Dr. Kelley then Dr. Denver and Dr. Herringa.

DR. KELLEY: So these are, once again, questions about the field data. From the mark and recapture data and from, of course, you know the size of the ponds, can you give me an estimate of how big the ponds were and how many frogs there were in each pond? Were these high-density ponds? Low density ponds?

DR. DU PREEZ: I would say medium to high density. In the report that's been submitted, we gave the estimate surface area of the ponds. And they varied from small to really big ponds. And the estimated populations that we've calculated corresponds very well

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with the size of the pond.

From my personal experience, what I would regard as a good Xenopus pond would be a pond with muddy water because predation is that much lower. So some of our ponds were muddy, muddy water. Some of the other ponds were clear.

DR. KELLEY: So in your smallest pond, how many frogs do you think you had?

DR. DU PREEZ: The population estimates that we did were very conservative. But they were in excess of 300 in the smallest pond. And then a couple of thousands would be in the largest.

DR. KELLEY: Okay. In the document here, the laboratory number SA01A, Table 3, you give the ages of male and female frogs collected. And yet having only started in 2001, this can't be actual yearly observations because it goes up to an age of eight unless that's eight months.

DR. DU PREEZ: Those age determinations were done through scoliotic chronology. So we did histology, sectioning through a toe and then by counting the growth rings to determine the age of the frogs.

DR. KELLEY: So the eight on this is years?

DR. DU PREEZ: Eight years.

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DR. KELLEY: Is eight years. Okay.

Now my final question has to do with your measurement of the estradiol levels in the two sets of ponds. There are published estradiol levels for laboratory reared animals. And I guess my question is as follows: The estradiol levels that you have for males in this study -- I'm now looking at Figure 5, page 29 of 138, on the Eco Risk No. MSU07 -- in which your measuring plasma estradiol levels picagrams per mil in male and female from both your control ponds and your atrazine-sampling ponds. My concern here is I don't think I've actually seen such high estradiol levels in males. Females, yes. But males, no. And I'm wondering if there might not be some other contaminant in the ponds that don't have atrazine that could be accounting for this.

Both my studies and Shapiro's and almost anybody who looks at vitellogenin induction fails to see very much, if any, estradiol in adult males. And yet you have levels that are 1,200 picagrams per mil which is way in excess of the published values.

DR. GIESY: We did those measurements in our laboratory using an ELISA technique. They are what they are. We've provided to the panel our QAQC. But we would be glad to have people look at it. And if there's a problem, let us know.

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Relative to other contaminants, I don't know other than what's been measured. And I would defer to Dr. Solomon to talk about the other measurements that were made. But I don't know of anything that might cause that interference.

DR. KELLEY: But the literature, people looked at this quite carefully because they were interested in using induction of the vitellogenin gene as an assay for steroid hormone control of development. And Shapiro, a number of years ago, pointed out that there's this very interesting memory phenomenon. And he used male Xenopus because they had no induction of vitellogenin gene. And he showed that if they'd even once been exposed to estrogen, the second time they saw estrogen he got a very rapid, very large response. And he went back and measured estrogen levels during development, and we've measured them as well. Both of us felt that the data were consistent with males having almost having no estrogen available. And so to see an animal with circulating levels that aren't any different from females, they're identical, even at the end of the breeding season and that are so high for estradiol, I mean, this is a huge level for estradiol, makes one wonder what's going on in both sets of ponds.

DR. GIESY: That's a very good point. We'd love to be able to

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split samples with people and make sure the results are accurate.

DR. ROBERTS: Dr. Denver. Dr. Solomon.

DR. SOLOMON: Could I just address the other contaminants?

The reference ponds were located in a totally different geological area where corn production was not possible just because of the type of soils. There were, however, cattle present in the system. And of course, cattle do come and drink at the ponds. So there is a possibility of contamination with both urine and feces in cattle which may result in presence of animal estrogens in the system. But whether this was causing any response, we don't know.

The populations in those ponds in terms of age, size, class, sex ratios, et cetera, were what we would expect them to be. So whatever those numbers are, they at least appeared to us to not be affecting the populations.

DR. ROBERTS: Dr. Denver.

DR. DENVER: Yes. My question has to do with the laboratory studies. And it goes to potential vehicle effects. And I was wondering if the group could comment on that. I've noticed that there are a number of instances of ethanol having an effect. And I think tht I recall from reading the literature that you published, the Carr study, that the atrazine was actually not dissolved in ethanol; whereas in the

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Hayes study, it was. And I wonder if you've considered possibly there being an interaction effect between atrazine and ethanol that might be responsible for the differences between the laboratory studies that we've seen today.

DR. CARR: We chose not to use ethanol as solvent because atrazine soluble in water up to 30 milligrams per liter. And there was no reason to use ethanol as a solvent for our study because the concentrations were low enough that they could be dissolved in an aqueous medium.

In terms of addressing the differences in the study and hindsight, which we didn't know before going into the study, it might be important to look at that. And I think there is in some of the data evaluation records concerns about using ethanol as a potential solvent when it's not necessary.

And that's something that would need to be done. We haven't done that.

DR. DENVER: On the graph that's there, there's clearly the potential for effects of ethanol on some of the parameters that you're looking at.

DR. GIESY: I would agree. In the report that we provided to you, when we did our statistical analyses, we compared to the

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appropriate control. So for atrazine, we controlled to water-only control. And for the positive controls, the DHT and estradiol, we compared to the ethanol control. We can't get enough estradiol or DHT into solution readily to do the studies without using the carrier solvent. So that's a real limitation of the study.

So we chose not to put estradiol across everything for the reasons that Dr. Carr pointed out. So could that lead to a difference in results in the designs? It certainly could. And I think that's the kind of thing that might led to some of the differences that you see among studies.

DR. SOLOMON: If I could just add, I have a comment. We've done some studies with fish exposed to estradiol and effulents containing substances that are supposed to mimic estradiol. And we've had a lot of problems with ethanol as a carrier solvent in terms of mortality and lack of growth in the controls in full life-cycle studies. So we try to avoid it when we can. But, obviously, in this situation, it's the only way to get it in so you minimize it.

But I did at a meeting a couple of weeks ago find out that ethanol is a good inducer of mixed-function oxidizes in some organisms. So it may be something worth following up there.

DR. ROBERTS: Dr. Herringa then Dr. Green.

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DR. HERRINGA: Bob Silken's comments on these inter-tank variability in some of the lab experiments, it prompts another thought that I had as I was reading these papers. And that relates to potential genetic isolation, genetic effects. I presume that in the laboratory setting, it's not possibly clearly in the pond-field studies, that genetic isolation is probably a very real phenomenon in fact. But in the lab studies, is it standard practice to split -- I want to say litters. It's not litters here -- but whatever the egg pool is. And sometimes it's not even just sort of a biological strain you're using, but you're actually field-capturing animals. Do you have fertilized and then randomize them between the control and the dose levels?

DR. CARR: In our studies, yes, we mixed eggs between breeding pairs. In our studies, we used five or seven breeding pairs. The eggs were randomly selected and assigned to treatment tanks.

DR. HERRINGA: If I may follow up. Dr. Carr, is it your impression that in these studies that that's standard practice or would be standard practice for these types of studies?

DR. CARR: It is in our standard operating procedure. Again, there are no standardized and validated tests for these chronic exposures. I'm trying to recall the ASTM guidelines for FTEC studies. I don't know if anybody remembers off the top of their head

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the numbers of animals to be used in those.

But it's our sense that for statistical purposes, it's better to use multiple breeding pairs than one breeding or two breeding pairs.

DR. ROBERTS: Dr. Silken.

DR. SILKEN: To follow up, Steve, the eggs were randomly split up among treatment groups in the Michigan's MSUO3 study as well as well as they were in Dr. Carr's study.

DR. ROBERTS: Dr. Green.

DR. GREEN: I just want to get a feel for the kind of atrazine exposure the frogs in the wild might have had. And one way, I think it would help me to do that would be if you could tell me, over the time period preceding your collection of the new metamorphs and the young juveniles, could you tell me things like what was the average daily temperature, what was the average daily rainfall.

And, of course, I'd be interesting in knowing the water quality parameters of the pond water, for example, do you know what the ammonia levels got to at the worst and the best? Then I could gauge, you know, did they experience a period of drought where atrazine might be at its highest versus rainfall where you're going to have a dilute run-off?

DR. DU PREEZ: In this study, we measured those couple of

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parameters on a weekly basis and later on bi-weekly. As we've collected water samples, we measure temperature. We measure dissolved oxygen. We measured conductivity, pH. And that's all been documented in our reports.

DR. SOLOMON: There was some additional water quality analyses conducted and, actually, sediment as well just for routine water chemistry parameters. This was done less frequently than the sampling measurements. But we've characterized and other components of the system. And that's also in the reports.

There were differences between the reference and the control -- I'm sorry -- the reference and the exposed sites in terms of some of the major ions, calcium, sodium and some of those. But in general, there didn't seem to be any difficulties except some of the elements were relatively high in concentration. But the analysis was conducted on unfiltered water samples. So we don't know the speciation of some of the metals in the water because it was total element analysis rather than soluble. Doing it again, one might look at filtered water or look for soluble form rather than suspended forms of some of the elements.

DR. GREEN: If I could follow up. I haven't gone back to pull this information out quickly. If you could summarize for me, was there a period of heavy rainfall at some point that might explain low

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levels of atrazine and no effect. That's really what I'm looking for. Versus a period of drought which...

DR. DU PREEZ: During this study, as I've mentioned early on, we've had more than double then annual rainfall. So it was a very wet season. That's one point.

Average, minimum, maximum temperatures was spot-on with a 10-year mean. So there was no really very cold or very hot periods during this study. But the rainfall was double.

DR. ROBERTS: Okay. I have a very quick question. Dr. Silken, I saw him leap to the table. Did you have something you wanted to throw in on this.

DR. SILKEN: I just wanted to follow up with Louis' response that the rainfall, did that increase the concentrations or decrease them?

DR. DU PREEZ: Increased the concentration?

DR. SILKEN: Of atrazine.

DR. DU PREEZ: No. We had a definite dilution of atrazine with this excessive rainfalls. But all these crafts are in the reports, the temperature, the rainfall, everything has been reported.

DR. SOLOMON: I have a comment to that. Work done in relation to another component of the atrazine risk assessment

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extensive modeling done by Marty Williams has looked at this issue of rainfall and dilution in ponds, specifically to try and estimate concentrations that might occur in ponds. And he sees in his models basically the same thing as if you have an out-flow to the pond, which many models interestingly enough don't have, with a lot of rainfall, you actually end up with lower concentrations than if you have moderate rainfall. If you have no rainfall, you get not runoffs and no contamination. So highest concentrations would be in moderate rainfall years, which preceded our year and have followed our year of study in South Africa.

DR. ROBERTS: My quick question, it was for Dr. Van Der Kraak. It's just a clarification. When you discussed your causation criteria, one of them was temporality. And you made the statement that you didn't think temporality was met because of the prior existence of some of these phenomenon like testicular oocytes. Were you really viewing that in a qualitative sense or in a quantitative sense? In other words...

DR. VAN DER KRAAK: That was more in a qualitative sense.

DR. ROBERTS: That's what I thought. I just wanted to be clear on that point.

DR. VAN DER KRAAK: Yes.

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DR. ROBERTS: Dr. Kelley.

DR. KELLEY: So let me follow up on the testicular oocytes. So in other published work, my understanding is that you can actually detect the testicular oocytes only when you serially section through the testes and then you see the occasional oocyte; right? So maybe that's just normal.

But in the 25 picagrams per liter treated atrazine group in the Carr, et al., study, were there frank -- were there testes that upon visual inspection had testicular parts and ovarian parts? We're not talking about the stray oocyte but were frankly hermaphroditic comparable to other reports in the literature.

How did those testes look really?

DR. CARR: The animals that we looked at in our study, were Stage 66. 99 percent of the controls were sexually differentiated. And in our study, it was very clear to see animals that shared both male and female gonadal characteristics.

The most common finding when we actually did the histology on the ones that were intersex at the gross morph level was that we could detect an ovarian cavity, for example, in the females and call it a female-like gonad. But most cases in the males, the gonads were smaller. And so it was more a difference in the shape and

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organization at the gross level that we were reporting.

Now, one of the features we did see even in the estradiol exposures were rostral deformities that resemble some of the things that Chang and Witschi had reported back in the '50s. We saw that in both the estradiol and 25 part per billion atrazine group.

Those weren't entirely that common. In fact, the intersex -- and this was another point I was going to bring up -- was fairly rare. When we looked at 300 animals in the 23 part per billion atrazine group, we saw it in 12. So in our study, it was a relative low incidence. But they stood out fairly clearly. It was fairly easy to detect.

DR. KELLEY: So if I could follow up. I think the point I want to make is that the testicular, having a couple oocytes in your testes, you know -- I mean, this just may be a normal thing, nothing to get excited about. But having a gonad that has frank ovarian parts, I think might be a qualitatively different phenomenon.

So the fact that there might have been oocytes throughout all of the literature and the occasional testes wouldn't, actually, I don't think, bear on the question of intersex. And the Panel has had informal discussions about how many Xenopus they've opened up and how many intersex they've ever seen in their entire life. And I have

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to tell you, I have never seen an intersex. And I'm sorry to report, I've opened up thousands upon thousands of Xenopus.

Yes, testicular oddities. Sometimes only one testis. But a gonad that's hermaphroditic? Un-huh. At least not in normal adult lab populations.

DR. CARR: We've never seen anything that was grossly hermaphroditic in our atrazine animals. The intersex we used as, in terms of the terminology, was to mean that we couldn't identify it as male or female at the gross level. We did not find testicular oocytes in our intersex animals.

DR. KELLEY: Again, I guess I suggest we're going to have to go forward and grow these animals up.

DR. CARR: Absolutely.

DR. KELLEY: Because it may be a phenomenon that becomes more obvious as the animals get older.

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

DR. MATSUMURA: I was just wondering what the mechanisms which can create these kinds of effects if there is effect. I think you went pretty describing the major hypothesis. And you didn't mention anything about the LH or prolactin or gondaltropins. Did you do any experiment, or were you just guessing?

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DR. VAN DER KRAAK: I apologize. We were talking between us at the beginning of your question.

DR. MATSUMURA: Well, of course, we must get some mechanistic information. And you mentioned about the hypothalamus, pituitary, LH, FSH; and you didn't say anything about data.

DR. VAN DER KRAAK: Correct. And I think there are a couple of responses to your question. The first one is, in formulating the questions that were raised in the White Paper, we did go into a discussion about the possibility of looking at aspects of various hypothalamic hormones, LH, FSH, and the like. The question there becomes one of what hypothesis is one expecting to be testing. And then we've got some specific questions that we need to do some additional biological detective work in terms of if the question is related sexual differentiation, is there, in fact, significant expression of LH and FSH genes at that time in development and whether it's a viable hypothesis to test.

We've identified, again, as a priority -- pardon me. We've identified it as a potential question, but we've certainly not gone there. I know Dr. Giesy has talked about it extensively in our panel meetings, and we all agree. It's a question, again, of time and effort.

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In terms of are there other hypotheses that are out there that one could test? Sure. I think the number of hypotheses that one could generate are not endless, but there are large numbers of those that it could be. I mean there a number of genes that are turned on during sex differentiation. We could go systematically and look at the expression of every one of those genes. Or we could take a molecular approach to try to evaluate those. But I think the approach that we've attempted to adopt was, on the one hand, let's look at some of the obvious candidates. And then number two, if we have evidence of a frank effect that's reproducible and robust, then let's go back in and do those directed, mechanistic studies at that point in time. Otherwise, it tends to be a little bit of a fishing expedition.

DR. MATSUMURA: I understand. It's priority. This is one mammalian people have really found an effect. Sos there's no question that the Long-Evans rats, this is a major finding. So that's why.

DR. ROBERTS: Dr. Giesy.

DR. GIESY: Well, as usual, Professor Matsumura, you're very perceptive. We've certainly thought about that. We've looked at the mammalian literature and actually think that is an area we need to look at.

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Now, what I have to do is put things into perspective. We have really two ends. One, if we know a mechanism of action, then we can look at the response that we would expect to find and use that as our measurement endpoint and we can put that into perspective relative to environmental risk assessment. Or we can choose the endpoint that we think is important and try to work back and see if there's a plausible mechanism.

Where I see us now is we're in the middle. We don't know what endpoint we need to look at because we don't know the critical mechanism of action. And if we did, then that would be better. But what EPA is saying in their White Paper is that we're not going to look into those mechanisms of action until we have looked at a couple particular endpoints.

I think I speak for the panel when we would say, well, there may be other critical mechanisms of action. We've looked at aromatase a lot. A lot of that proposed mechanism of action is based on work that was done in my laboratory. We've looked at that. And at this point in time, we could give you some suggestions on how you could further test that specific hypothesis. But at this point in time, my feeling is it's more efficient to look other places. And so we've identified the hypothalamus and pituitary. Hypothalamic pituitary

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axis is a key place to look.

Now how do you look at it? Well, you have two choices. One, you could look for specific responses. And we've developed a preliminary proposal to do that. Or as Professor Van Der Kraak indicated, you could look for more general responses. And some work he's done in his laboratory using things like differential display, are useful techniques that we could ask the question, is anything change in that axis and work from that position. So that's another idea you might think about.

But we couldn't agree more. I don't think at this point in time we can key in on any one specific mode of action. Absolutely.

DR. ROBERTS: Dr. Coats. Oh, Dr. Solomon, did you want to respond also?

DR. SOLOMON: Just to follow up. And, Fumio, thank you. The effects in the Sprague-Dawley rat were reproducible and consistent at different times, different labs, et cetera. Once the mechanism was understood in the fact that it was specific to Sprague-Dawleys that, I think, was very helpful. I was at the SAP here in Crystal City a few years ago to listen to that discussion, so it was very interesting to see it being used in a risk assessment context.

But we haven't yet, at least in our own hands, been able to get a

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reproducible robust response that we can use to track back to a potential mechanism. Given that, obviously, we would love to do it.

DR. ROBERTS: Dr. Coats and then Dr. Isom.

DR. COATS: In the field studies, I guess it was particularly in the South African one, there are quite a few other triazine or metabolites note there. I was curious about the water levels that are reported. Are they filtered samples or unfiltered? And are there sediment values, and do you think those would be important or not?

DR. DU PREEZ: If you're referring to if they're filtered or not, you're referring to the weekly sampling of the water?

DR. COATS: The water, yes.

DR. DU PREEZ: No. Well, I'm not -- I wasn't involved in those analyses. But to the best of my knowledge, they were not filtered.

DR. SOLOMON: The analyses were done by Piet Johnson from Rensburg at the Potchefstroom University using GC mass spec. And he used SPE cartridges and liquid-liquid extraction using both methods to confirm. And then he used unfiltered water. But it was filtered through the SPE cartridges which were then eluted. So anything that was trapped on a solid, would have been extracted. And then the liquid-liquid were extracted from the direct water samples,

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no filtration.

Sediments samples were looked at and no pesticides were detected in the sediment samples. Given the KD-binding constance of atrazine which is relatively water soluble, that's consistent with what we would expect, that nothing was present in the sediments.

DR. COATS: Thanks.

DR. ROBERTS: Dr. Isom.

DR. ISOM: Dr. Giesy, I'd like to follow up on your comment you just made a few moments ago about aromatase. The EPA is proposing that that will be the second tier, that is, if we can pass the first tier, to start to look at that. And you made the comment that perhaps -- I'll paraphrase you that perhaps we're looking in the wrong direction.

Yet if you look at the literature Sanderson's group has shown in human cells, tumor lines, that it atrazine induces aromatase. And I think at higher concentrations perhaps even inhibits so that you can get that inverted U-shaped dose response curve.

I'd like to have you follow up on that comment then and any other studies that you have done, could you describe those with that enzyme?

DR. GIESY: Absolutely. Well, yeah, I'm an author on those

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papers; so I do know about them. I think it's important maybe to explain a couple things about how those studies were done and why they were done and I'll do that.

But let me answer your question very specifically. I think what I meant was, if we look at aromatase, if there are open issues there, I think we need to take a fundamentally different approach than we've taken previously. Not to dismiss it entirely. But if we do go ahead with that, and I do agree with EPA that it shouldn't be first tier. But if we go ahead with it, I think we have to do it in a way that we look in specific tissues. And we can do that by QRTPCR.

In our laboratory, we've now developed a molecular beacons to do that. So we can do the quantitative PCR. I think it would be important if we follow that mechanism up that we do it through the developmental stages where we know there are changes in aromatase expression and we do it in a tissue-specific way.

First of all, my comments were not to just look in a gross way because I don't think we'll see. So I think it is stage-dependent and it's tissue specific. So if we do go that way, that's what we need to do.

But at the same time, based on the literature we do know, it would be appropriate at the same time to look at other plausible

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mechanisms. And one of the other plausible mechanisms is through the hypothalamic pituitary gonadal axis. I think we've got enough evidence as Professor Matsumura point out to not completely dismiss that.

Why do I say that? Because, in fact, I think some of the effects that we do observe are more consistent relative to what can be caused through that mechanism of action relative to testosterone depression for instance.

Now, beyond that, the studies that Thomas did when he was in my laboratory were designed specifically to try to understand why we observing what we considered to be anomalous results. And that is we knew that atrazine didn't bind, at least in our hands, to the estrogen receptor. But in some cell lines, we did see what looked like estrogenic responses. The question was why.

Now the first experiment I had Thomas do was just to dose them with perpronobutoxide because I though maybe what we were looking at were metabolites that were being formed, hydroxy metabolites which, in fact, might be estrogenic. Subsequently, we tested all those. They basically weren't estrogenic. But in doing that, we thought we were knocking out the MFO activity that might form the metabolites; when, in fact, what we were doing most likely was

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knocking out the aromatase.

So we did those studies in part to understand mechanistically why we were observing this effect in vitro. They were done at pretty high concentrations. They were done at 30 micromolar, which is about 6 parts per million in the tissue. And they were done -- and the result, then, of that, too, was -- and we looked at message. We looked at expression, and we look at the activity. Depending on which one you looked at, the range of response that we got was somewhere between two- to four-fold. Not a huge response.

So I don't dismiss it entirely because I think we haven't investigated at these time-specific and tissue-specific responses. So if we do go ahead, that's where we need to look. But I really think based on the literature that's out there, we shouldn't dismiss the other potential pathway at the same time. That was my point. Not that you shouldn't consider it at all. But how you do it if you do it and not forget about this other pathway which I think is also consistent with the results that were observed.

DR. ROBERTS: Dr. Kendall, I think that before your panel continues with the next aspect of your public comments, my panel needs a break.

DR. KENDALL: Mr. Chairman, our panel yields to your panel.

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Let's take a break.

DR. ROBERTS: Let's take a 15-minute break.

(Break; session resumed at 3:45 p.m.)

DR. ROBERTS: Before the next round of presentations, Dr. Green had a question as a follow up from our discussion right before the break.

DR. KENDALL: Very good.

DR. ROBERTS: Dr. Green.

DR. GREEN: Yes. I just have a quick question for clarification to Dr. Du Preez. You mentioned that some of the answers to the questions I posed earlier were available in a document that was submitted. Can you clarify which document that was so I can go back and make sure we all have the same details?

DR. DU PREEZ: The documents that we've submitted to the Panel, that would be SA01B and SA01C.

DR. GREEN: And in those documents, there are the details regarding water quality analysis on the ponds, frequency --

DR. DU PREEZ: Everything is in there.

DR. GREEN: -- changing and rainfall and that sort of thing. Okay.

DR. DU PREEZ: Including the raw data.

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DR. GREEN: Thank you.

DR. ROBERTS: Let's then proceed with the next round of presentations. The Panel will have opportunity to ask more questions after those.

DR. KENDALL: Thank you, Mr. Chairman. As we earlier indicated, we would lead off with the core presentation by Dr. Van Der Kraak. And we, as a panel, are very impressed with your panel. You came very well-prepared. And we do accept the criticism and welcome the comments and have gained a great deal of future insight related to how we would proceed with our research.

I think the panel can relate to particularly the last couple of years, spent a lot of effort and we've amassed now a great deal of information and a lot of manuscripts are stacking up that are going out for review as we speak. So this discussion, interaction is welcomed.

And from our core presentation, our panel is continuing, as I speak, to evolve data. And I wanted each of them to have a brief opportunity to -- I didn't realize we would have as many questions as we did. But we welcome them. But I wanted each panel member to have a chance to briefly address this SAP to give you an opportunity to further understand what their contribution was to our overall

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effort. And I emphasize our efforts have been collective. And our publication, we have discussions on our publications and they are collective.

And I also wanted to make a comment about Dr. Bob Silken. We probably ought to make him an honorary panel member. He's made himself so available. He sits in on all panel meetings now, conference calls and so on. And he's very gracious with his contribution, although he drives us crazy sometimes related to all of his questions. But I think it's made our science better.

But anyway, I'd like to begin. Dr. Van Der Kraak gave our core presentation. Dr. Giesy, I'd like for him to comment as he feels appropriate. And the Panel is welcome to ask any questions of our scientists as we proceed. And then we will close our comments today after this period by offering you some responses to the White Paper questions from our panel that can be shared with you. So Dr. Giesy.

DR. GIESY: First of all, I'd just like to say I think this is an excellent panel. I'm very impressed by how well-prepared everyone is, but you're all experts. And, two, to reinforce that I think the EPA White Paper was an excellent document. They have a really tough task to do, to balance all this and try to find a way forward. So I thought they did an excellent job.

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We've been involved in this for a couple of years now. I guess what I've learned from that is that we started from not much and have made a lot of progress. But it's certainly a work in progress. Certainly things aren't perfect and can be developed much more in the future.

My main interest in really in the mechanism of action, what is the plausible mechanism for how atrazine may cause effects so we can put into context what are the right endpoints to measure, what we refer to in risk assessment as measurement endpoints, what would be the most sensitive and relevant endpoint to measure that then could go into an assessment endpoint; which is ultimately what the EPA has to deal with. And so I feel strongly that we do need to know what the critical mode of action is. And that's my interest. So anything relative to where we are, where we go relative to that is something I'm interested in.

So we've set up some studies. When we started, we wanted to work with some native species. The protocols aren't all completely worked out for that. One of the grad students in my lab, Katie Cody, did a lot of work just to be able to figure out how to do a study with green frogs. Having done that, if the EPA asks me should we use green frogs, I'd say no because there are some real issues with time to

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metamorphosis for green frogs.

But I think we have learned a lot as a panel. And I think as a scientific community, things are unfolding in this whole endocrine disruptor area.

With that, you know, I think this panel can have a huge impact on the future, where we go, where EPA goes, where the science goes. And I think that would just be great. But I don't have any other specific comments.

We do have one ongoing field study. What Dr. Kendall wanted us to do was get you up to speed on anything that's been done since the reports that we've provided to you. From my laboratory, there isn't anything else really. But we do have an ongoing field study. We have students in the field right now that will continue with all the warts and imperfections of trying to do field work.

But other than that, you have everything that we've done at this time. And if you have any further questions, I'd be glad to try to field them.

DR. ROBERTS: Any question? Dr. Kelley.

DR. KELLEY: So I'd like to comment and I think this also applies to Dr. Hayes about the use of this laryngeal dilator muscle cross-sectional areas and assay.

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So we studies the development of the larynx in Xenopus. And there are two features that I think are important for your use of it as an assay for masculinization. First of all, let me point out, it's a very good assay for masculinization as you show yourselves. It's a very androgen-sensitive organ. And it's certainly extremely sexually dimorphic in adulthood.

So the cross-sectional area that you guys measure represents two things, the size of muscle fibers and the number of muscle fibers. And we actually never measured cross-sectional area because it confounds the two. We looked at number of muscle fibers. And we also showed that the ability of androgen to cause growth of the larynx, both hypertrophy and hyperplasia, was dependent on prior exposure to thyroid hormone. So in an assay system where your animals are taking a long time to metamorphose -- all right. So let's say normal is two weeks. These guys are taking a month and a half or longer, we've also shown that the animals are exposed to androgen during that period. So it's possible that variability in time to metamorphosis can contributed substantively to variability in whether you see a sexually differentiated laryngeal cross-sectional area.

Now, that all washes out by the time the animal is three months old. So you don't have to worry about that any more. So I make a

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plea for you guys to stop looking at laryngeal cross-sectional area at Stage 66. I don't think it's very appropriate time. And just give it a couple months more.

We divided post-metamorphic development into six stages that are very well-characterized by laryngeal weight. You can standardize all of your animals no matter how long it's taking them by body weight and laryngeal weight to those stages. And it should be possible to come up with some well-characterized, low variability assay for whether atrazine has an effect on masculinization by using that assay at a slightly later time period.

Anyway, that's my input on the laryngeal bioassay which has, in fact, been variable. But I think it's been variable for reasons of rearing variability in terms of length to metamorphosis in the studies that pretty much every has done. And that makes concordance between the studies very difficult.

DR. KENDALL: Good points.

DR. GIESY: Excellent point.

DR. ROBERTS: Any other questions for Dr. Giesy? Okay. Let's move on.

DR. KENDALL: I did want to make sure of one thing. Dr. Kelley, you mentioned two references, two papers on the hormonal

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measurements. And we need to make sure we get those for Dr. Giesy.

DR. KELLEY: I have the papers. I'd be happy to give them to you.

DR. KENDALL: Very good.

DR. CARR: I really don't have any new data to present. And I don't have any additional specific comments that weren't addressed in the previous session.

I did have a general comment. I think there was discussion earlier this morning regarding the subjectivity of some of the gonadal assessment. And you can only come to that conclusion when you look at all the different terminology that's used to assess intersex, hermaphrodite, et cetera.

But one thing to remember in our study and most, if not all, of the other studies, is that these are analyses and the treatments are conducted with no knowledge of what the treatments actually are. These are blind tests in essence. And so I feel very confident about our data in terms of when we see something that is unusual and this is an intersex, it's very different from what we would expect to see in a normal male or female. So I think that reduces some of the subjectivity at least in Xenopus.

We have finished an experiment in Rana pipiens that the things

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were a little bit different there. We don't have data to share on that yet.

But that's really all I had to say. And I'll be happy to answer any additional questions.

DR. ROBERTS: Any questions for Dr. Carr? Great. Thanks. Dr. Smith, did you want to proffer something?

DR. SMITH: We have continued the work in Iowa, the field study in Iowa, primarily, the laboratory component of it. From the histology standpoint, we have found so far one testicular, one animal with testicular oocyte which turns out to be about .6 percent of the total number of animals that we have analyzed.

We have also taken the plasma for testosterone analysis and gone over the period of collection because, as I said earlier, the representative time representing Period 1, 2, and 3 which would be late spring, early summer, later summer and early fall. And the indication there is that there is time-dependent change in plasma testosterone concentration. So time becomes a variable and a factor that has to be taken into consideration. However, over the period, there is no significant difference for plasma testosterone when you compare the adult animals during the specific period.

The other observation is that during that period, there is

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significant difference between the adult and the juvenile plasma testosterone concentration using a 60 gram cut-off point as the difference between the adult versus the juvenile for that comparison.

We are presently in the process of utilizing the steriological approach that we use for the South African Xenopus laevis adult testicular histological analysis to evaluate the fractional volume sperm, spermatocytes, spermatogonia and what we consider as other cells, which includes blood vessels, connective tissue. And that data will be made available pretty soon as to the contribution from that standpoint.

And that's where we are presently. I'll entertain any questions from you.

DR. ROBERTS: Questions for Dr. Smith? Great. Thanks for the update. Dr. Du Preez.

DR. DU PREEZ: Last night I prepared a quick PowerPoint presentation, so I'm going to walk you through a couple of slides.

This a picture that I've quickly inserted here to give you an image. This is one of the larger ponds that we've used. This specific pond was referred to as Site E6.

We did four studies in South Africa. SA01A was the evaluation of the sites where we characterized the different sites, the

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populations, the mark and recapture studies. And based on this study, we identified five experimental sites and three reference sites.

Phase B, SA01B, was the study of a one-field use season. We on a weekly basis at first and later bi-weekly collected water samples that were analyzed. Elemental scans were performed on water samples as well as on sediment samples. And then SA01C, where we've collected the specimens. The blood samples were collected, shipped over to Dr. Giesy's lab where they've conducted the hormone aromatase analyses. Gonads were shipped over to Dr. Smith's lab. And he just mentioned the histology.

Then SA01D is the study on the microcosm that I'm going to expand a little bit more on. And then just for interest, I've been busy with the first study at this stage where we're going to look at the reproductive cycle of Xenopus. Our period of months, we're going to quantify the nuptial pads, the cloacal folds, the hormone levels, and so forth.

If we now focus on the microcosm study, this formed part of a thesis of Alaric Uester. We had a microcosm. We had 12 ponds, and this was the layout. Three reference ponds, three ponds with one microgram per liter, three with 10, and three with 25.

These were initially earthworm pits that we referred to. We

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emptied them, lined them with a membrane, filled them with water, add some microphytes, leave them for six months to stabilize, and then started the experiment.

Individual microcosms were covered with that frame with hail netting. And that would be to keep predators out, primarily dragonfly, because the dragonfly larvae is the one animal that you don't want in a microcosm, and also birds.

Just a couple of tadpoles there to show that the tadpoles schooled in my opinion in a natural fashion. And at Stage 66 animal, they are in the water. The water quality from measured concentrations and from a visual inspection was very good.

And there is a set of microcosms. What I want to point out in this slide is, from a logistical point, we had a problem that not all the ponds were exposed to the same amount of sunlight. You can see that these first four ponds are shaded here by a tree. Then there are a couple of ponds in the middle that received more sunlight. And then on the other side, again, two ponds that were semi-shaded.

The water temperature was much lower than you would expect in a natural pond. And this had an effect on the development. And we, indeed, experienced a delayed development. But that's, in my opinion, not a concern in this study.

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This is the actual recorded atrazine levels. At one instance, in one of the reference ponds, we did detect 0.1 microgram per liter atrazine. Again, I was not worried about this because two weeks later it was gone. I think it was either an error or contamination. But to make sure, I went back and I split the reference pond samples in two. I compared the specimens collected from that specific Pond No. 3 with the other reference samples, and there was no difference.

Then on cross morphology, that would be a normal male, the female. And this is the type of deformities that we've observed. And the only deformity that we found was discontinued testes.

Now, I'm actually a parasitologist working on parasites of amphibians. And for over 15 years or more, I've literally opened up thousands of frogs. And this is what you see from time to time, a single testis. Usually, when there's a single testis, it would be larger. You do find discontinued testis, but I've never come across a true hermaphrodite in frogs that I've collected. And I've worked in both corn-growing areas where atrazine would be applied and areas more pristine reserves and so forth.

Based on the gross morphology, we've observed a 4 percent in the reference, 1.3 in the 1 microgram per liter, .6 in the 10, and 3.7 in the 25 microgram per liter. So, again, no statistical difference here.

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And this is up to the point where we've reported in the study SA01D. In the recent weeks, we've conducted the histology. And I'm quickly going to report on our findings there.

This is my slave, Alaric Uester. We selected 54 specimens per concentration, randomly selected. That adds up to 216 of the 600 specimens selected for the gross morphology. Six specimens were lost in the preparation for the histology. 120 to 200 sections per specimen, four to six slides per specimen. That all adds up to more than a thousand slides. And 31,000 histological sections that were examined individually. So that's why I'm not very popular with my students at this stage.

What we've observed and what I want to point, testicular oocyte would appear and disappear in seven slides sectioned at 6 micrometer. So what I wanted to stress with this is it's not good to check every 20th section. You have to check every one if you want to detect the testicular oocytes.

A testis with a testicular oocyte there, a cross-section, longitudinally section, and the ovary. And you can clearly the lumen in the middle.

This is what the histology revealed. No less than 56 percent of the animals from the reference ponds had testicular oocytes. Again,

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56 in the 1 microgram, 58 in 10, and 38 in the 25.

Just this past week, we did another study where we looked at grow-outs. Now the rate columns represent Stage 66 and the blue, 10-month grow-outs. And there is a reduction in the number of oocytes. Now I must point out that this blue set, the 10-month grow-outs is only 10 specimens per concentration.

Then we calculated the number of specimens with testicular oocytes where we found the oocytes in the single testis or both testes and no statistical significant difference there.

If we now look at the mean number of oocytes per specimen with testicular oocytes, we find that there is an average of about 10 oocytes per specimen. But there is a significant reduction if we look at the 10-month-old grow-outs. And then if we split up the sample and divide the number of oocytes in categories, zero, 1, 2 to 10, 11 to 12 and so on, 51 to 60, we find the following. And, again, not the significant reduction in the number of oocytes.

The maximum number of oocytes in the Stage 66 samples was in this 25 microgram per liter. And that was 58 oocytes in a Stage 66 Xenopus. The maximum for the 10-month grow-out was 5. Only one specimen had 5, three had 2, and the rest had a single oocyte.

So from this, tadpoles developed slow as a result of cold water.

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I've already addressed that. Ponds were partially shaded, covered with hail netting and did not have a shallower part as you would find in a natural pond. Often these Xenopus in a natural pond would school in the shallower water.

I've already addressed this point of the atrazine that was detected in this one reference pond. Again, no reason for concern there.

No gonadal abnormalities were observed in the females. No hermaphrodites were observed. Males showed a low percentage of deformities at a cross-morphology level. A high percentage of frogs had one or more testicular oocytes at all concentrations, but there was no dose response. And then a significant reduction in the number of testicular oocytes as metamorphs grow older.

And from the South African studies, for me the take-home message would that atrazine does not appear to affect the gonadal development of Xenopus laevis at environmentally relevant concentrations. The second point would be that, in my opinion, one would expect that if atrazine had a negative or adverse effect on Xenopus laevis, it would reflect in the population dynamics after four decades of intensive use of atrazine. And in previous decades, it's been documented that in South Africa they used atrazine by the tons.

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I mean they really applied it.

Now days, they use more conservative, recommended amounts. But surely, if there was something to be worried about, you would see it in the Xenopus populations out there.

And then in my opinion, field studies and microcosm studies does have its limitations as greatly pointed out by the EPA's White Paper. But I don't think we should underestimate the value of field or microcosm studies. Because in the end, the question is what's happening out there.

Thank you.

DR. ROBERTS: All right. Any questions from panel members. Dr. Green.

DR. GREEN: I think you could probably anticipate this question from me. How cold was the water? And you didn't mention anywhere in this document the conditions of the water quality, pH conductivity. If someone were to try and reproduce this experiment, it would be very important to have that information if you're going to reproduce this microcosm in a lab.

DR. DU PREEZ: That's correct. That data is available. What we've handed out is a handout to summarize this presentation. That's not the full report. In the full report, we have all the other data.

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DR. GREEN: But in my opinion, I think the water quality and the parameters that define this microcosm are very important, especially to a laboratory Xenopus person. So what was the temperature on this?

DR. DU PREEZ: Too cold to swim in. We did not measure water temperature on an hourly basis for example. We measured in this study at 10 o'clock in the morning when we took the water sample to check for atrazine. And that was in the low teens, around 10 to 14, which is lower than you find in the natural population.

DR. ROBERTS: Any other questions. Yes, Dr. Kelley.

DR. KELLEY: If you wouldn't mind my asking you a question about the previous field study. I'm looking at the mass of the frogs on page 59 in the study where you collected them from the three reference ponds and the five experimental sites. And I just want to make sure I'm reading this right.

So I think the goal was to get 20 of each sex from each pond. But it looks to me like that was difficult to achieve at the experimental sites. Was there another reason for that that you could think of?

DR. DU PREEZ: I was really upset when I wanted to collect the specimens, especially from Site E1. Because with this excessive

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rains, some catfish were washed into that pond and literally wiped out the Xenopus population in that pond to such an extent that we had difficulty to collect the target number of specimens.

DR. KELLEY: So your feeling that the difficulty in collecting at this sites where you have full representation in your reference sites from the cow pastures, but a sparser representation for the experimental site was due to predation.

DR. DU PREEZ: That's my opinion.

DR. KELLEY: Thank you.

DR. ROBERTS: Any other questions? If not, let's move on to Dr. Gross.

DR. GROSS: My comments will also be relatively brief. We have been coordinating now for the past year and a half the studies looking at another one of the mini-field studies, looking at the cane toad in sugar cane agricultural areas in South Florida. Those studies are currently continuing and ongoing, so we have little to add at this point that's new that the Panel has not already seen the documents provided.

I think several things can be noted, though, from the materials that have been provided. First, I think as indicated in EPA's review which we fully agree with, these experiments were simply preliminary

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examinations, mostly fact-finding, range finding, site identification, species identifications kind of studies. And, therefore, it's very difficult from these studies if not impossible to draw any conclusions relative to causation of single chemicals or even mixtures of chemicals or other factors.

None the less, the studies that we've seen do demonstrated some effects. We think that these effects, though, are vastly different in this species compared to what else has been reported for Xenopus or the various ranid species that had been examined because we're looking at a toad species, the cane toad, which has a bitters organ and rudimentary ovarian structure, which basically, I think, allows a differential kind of response. We believe these responses currently to be mostly in the adult or sub-adult rather than during the metamorphic phases that have been described previously for the other species.

None the less as I indicated, we are continuing with these studies. Actually, we've been continuing since February, have little data yet to add because these studies are ongoing. I think the comments of the panel today have been mostly addressed as we designed this second phase of study, the second tier; and, hopefully, we will have better answers in the next year.

But I'll be happy to entertain any questions you might have

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relative to the studies we've conducted on cane toads.

DR. ROBERTS: Any questions for Dr. Gross? All right. Dr. Solomon.

DR. SOLOMON: I'm sort of wrapping up this with a few brief comments. And my role in the panel, apart from helping formulate the reports, was to bring the concept of risk assessment to the data that we were developing. And as we heard earlier from Dr. Bradbury and others, risk assessment involves a component of integration of exposure and effects data. And as you probably very well know, there is an excellent data base for atrazine concentrations from the ecosystem through the efforts of the U.S. Geological Survey and other labs. We have very large data sets in the hundreds of thousands of data points. Maybe not hundreds of thousands, but certainly tens of thousands of data points. So there is a good data base to go out there to work on a measured concentrations to compare to effect concentrations.

We also have good modeling data that allows us to estimate exposures in areas where we don't have good measured values and helps to get a tighter definition of temporal variation that we don't get from sampling once every week or two weeks.

I've been somewhat frustrated in this because the other side of

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the equation, the effect side, has not materialized to the point where I can use this in the risk assessment process. And I think Dr. Van Der Kraak pointed out in his slide that we need robust and consistent concentration responses that we can feed into the risk assessment process to determine probablistically or deterministically what types of responses we might expect to see in the environment. So that's been my frustration. And, hopefully, it's a frustration on the Panel as well. I certainly heard that when I read the White Paper as well, that there was insufficient data at this point to do a risk assessment.

Thank you very much.

DR. ROBERTS: Any questions for Dr. Solomon? Yes, Dr. Van Der Kraak.

DR. VAN DER KRAAK: They overlooked me.

Just to comment in terms of some ongoing studies that are occurring in my laboratory. Some of the experiments are being done in relation to some of the questions that Dr. Kloas addressed in that we've been looking at more of the responses of Xenopus to atrazine in terms of effects on steroidogenesis. And so we're looking and doing some mechanistic studies, trying to tease apart places in the pathway that are responsive and develop the methodology in order to do in vivo exposures and to take gonadal tissue outside of the animal and do

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ex vivo incubations to test for specific lesions in the steroidogenic pathway. So those studies are just ongoing.

DR. ROBERTS: Any questions for Dr. Van Der Kraak. Dr. Kendall, I think there's another phase of your public comments.

DR. KENDALL: Yes. Thank you, Mr. Chairman. We were encouraged to as a panel, and we did, respond to the White Paper in terms of the questions being posed to the SAP and would offer, Mr. Chairman, that we discuss that or summarize it or provide this for the record. But each member of the SAP has been given our panel's response and literature backup as to some of the scientific issues we see. I would yield to how you recommend that we proceed.

DR. ROBERTS: I think that if you could summarize it for us, your opinions and responses on these, that would probably be the most useful. Let me be sure that I've got it.

DR. KENDALL: This document is entitled, "The atrazine Endocrine Ecological Risk Assessment Panel's Response to The United States Environmental Protection Agency's Questions in the `White Paper on Potential Developmental Effects of atrazine on amphibians.'"

DR. ROBERTS: All right.

DR. KENDALL: The date is June 17, 2003.

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ATTY2: Got it. Thanks. If you can maybe just proceed and summarize it for each question, that would be very useful.

DR. KENDALL: The members of the SAP, of course, will receive your charge and the questions probably Thursday morning. Mr. Chairman, I really don't want to read each question, not unless you want me to. But we thought we'd at least project them on the board. And I wanted to just very quickly touch on some of the responses that we had as a panel.

Initially, we as a panel would like to compliment the Agency on their effort in bringing this White Paper together. Again, this is an emerging and one in which there's not a lot of standardization both in the science to do the research as well as the terminology. And would encourage the SAP to engage, not just the science, but the terminology so we call all begin to speak the same language.

Never the less, in terms of Question 1, in particular 1.a., "Does the SAP have any comments and recommendations..." In general, our panel supports the Agency's evaluation of the existing body of data and generally agrees with the conclusions; although there were two point from our perspective that needed to be considered.

We as a panel felt that the Agency focused more on the limitations of the data versus what the data could offer if it were

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looked at in a robust, comprehensive manner. For instance, the limitations of low concentrations of atrazine in the reference site, relatively great inherent variability in hormone concentrations, and other issues such as the time to metamorphosis.

Again, we accept the criticisms. We welcome the opportunity to address these criticism in the future. But let us not lose sight of the forrest for looking too hard at the individual trees.

The second point that we think merits attention is the Agency's statistical analysis of the data. Whenever the Agency reanalyzed our data, I want to emphasize for any Panel member, we provided all raw data for all studies. So from that perspective, one could take this and analyze this as one deemed appropriate. Never the less, the Agency's analysis of our data, if they found a statistically significant difference that our panel did not find, it was not always because EPA's analysis in terms of how they approached it in many cases EPA's analysis pooled animals together over sites, tanks, replicates rather than approach it as we did in preserving the structure in the data and including the animal site, tank, and replicate in the analysis.

And Dr. Silken was adamant as to how we approached these analyses in the context of statistical background. And we would off that it just be considered as one looks at all of these data together.

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In terms the b. part of Question No. 1, from a statistical perspective, the Agency's overall characterizations of the currently available studies tend to treat differences that are found to be statistically significant by one method analysis as true differences that are biologically significant and beyond question but tends to treat results, in terms of no statistically differences, as questionable.

So as we approached it, and I heard the comments today as my colleagues did. We approached it with the best science and the best statistics that we could apply. We are not seeing robust, repeatable statistical differences. Never the less, we would welcome the SAP to look carefully at this as well as assist the Agency in looking at their statistical approaches.

In 1.c., a number of studies have been done that address the effects of atrazine on development of anurans and also on the occurrence of testicular oocytes have been published in the literature. Others that should have been included include Allran and Karasov 2000 and 2001, and Brown-Sullivan and Spence of 2003. And we go into more detail on that particular area.

In Question No. 2, we agree that field studies have limitations as discussed by EPA. While these limitations are acknowledged, field studies we believe are extremely useful in a weight of evidence

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approach and the results are still relevant.

This is particularly important for emerging areas of science where we need to make decisions. Data are coming in. And we believe that a comprehensive field and laboratory integration are critical.

The studies conducted in the field we believe were designed to test the presumption of risk. That is, they were designed such that, if there were no differences locations or correlations between exposure to atrazine and responses, it could be concluded that atrazine did not cause these effects under relevant environmental exposures. And we would welcome further deliberation by the SAP on this particular approach.

Never the less, Dr. Du Preez comments and I think his elegant summary of some of the works going on in South Africa, we challenge the concept that if atrazine had caused robust, sustained, comprehensive population-level effects on native Xenopus laevis in South Africa as one may have suspected from preliminary studies, then it would have been reflected in disturbances of population structure, particularly after 40 years of application in that area.

Moving on to Question 3, in terms of 3.a., we believe that laboratory studies provide a plausible basis to establish a hypothesis

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concerning the potential for atrazine to cause developmental effects provided that sample size is adequate the small incidence of abnormalities that have been reported in some studies, and these have been relatively small in some studies, and provided that the exposures overlap with critical windows of gonadal differentiation, and provided that sampling design takes into account the variability of timing in gonadal differentiation in different anuran species.

Many of these issues were raised today. I think this SAP panel is highly alert to these concerns and I think will address this nicely and assist the Agency with their recommendations. We believe that the overall body of data clearly indicates that the response of Xenopus laevis to atrazine varies under the conditions described in the available studies. And there are a lot of reasons for this. And, of course, this SAP will address many of those reasons.

Although the degree to which differences in experimental design and husbandry influence the contradictory findings remains a matter of debate, the fact that a relationship between atrazine exposure and development of gonadal abnormalities is not consistently found raises the question of the ecological significant and the relevance of observed effects of atrazine on gonadal differentiation. We think that's at the essence of the questions that

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you will be addressing, Mr. Chairman.

In Subsection b. of that question, to date, the laboratory and microcosm studies have followed logical assumptions inherent in the scientific method. And we believe that given the inconsistent responses observed, it is not possible at this time to predict how Xenopus laevis will respond to atrazine under yet a different set of environmental conditions.

Although it is relatively clear that atrazine does not dramatically affect thyroid function and does not influence estrogen receptor activity at environmentally relevant concentrations, the ability of atrazine to influence gonadal differentiation is an as of yet unelucidated pathway cannot be predicted for available studies. And we are still searching, as Dr. Giesy and other members of our panel related to you, for these underlying mechanisms of action for potential effects.

Give the lack of a repeatable effect and the absolute lack of evidence for a cellular mechanism underlying the reported effects of atrazine on gonadal differentiation, it is not possible at this time to predict the dose response relationship or the rank order potency of atrazine metabolites relative to the parent compound.

In Question No. 4, we concur with the conclusions reached by

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the EPA that there is no evidence to conclude that atrazine causes induction of aromatase in frogs, at least at this time.

In Subsection b. of that question, there is not evidence to suggest that analytical issues are a major factor contributing to variability in plasma sex steroid hormone levels or aromatase activity measures in frogs. I might add that, again, I emphasize, our projects were implemented on GLP-like performance standards under SOPs where standards of performance were measured. The Eco Risk QA unit went to every laboratory, check and recheck process. So we did take into account analytical and measurement performance.

At least for the research associated with the Eco Risk panel here, our quality measures are in place. And we believe they are within acceptable bounds. And we do believe that perhaps variations in analytical results, particularly in the area of sex steroids, may have some biological underpinning moreso than analytical underpinning.

In Subsection c., we believe that in anurans, sex differentiation is sensitive to sex steroids during critical periods of development. And I think this SAP will help elucidate for the Agency how one might approach a better measure of that for repeatable type data acquisition. There is little evidence to suggest that anurans are particularly sensitive to estrogens in terms of the induction of

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testicular oocytes following sex differentiation. And our panel has discussed this issue. So this area, we would welcome the SAP to fully explore and offer some perspectives to the Agency on the relevance in either the reproductive or ecological relevance of these testicular oocytes.

Compounds acting as estrogens or anti-androgens would be predicted to cause feminization of males. There are no data available to suggest that atrazine functions as an estrogen receptor agonist through binding to the estrogen receptor or induction of estrogen-dependent responses. Similarly, there is no evidence that atrazine binds to the androgen receptor and functions as an anti-androgen.

There is some real experts on this SAP related to this particular subject area. This is what we believe to date as the state of the science. We welcome your critical review.

Moving to the next question, just a few more minutes, Mr. Chairman. I'm trying to roll through this.

Question 5, Subpart a., our understanding of spermatogenesis in anurans lags far behind that for mammals, especially for amphibians. It is not known if accelerated growth precedes histological differentiation of gonads which would influence the developmental

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rate at early stages as well as gonadal morphology. It appears that sex determination exhibits considerable plasticity and the ability of gonadal differentiation to respond to environmental factors may be highly adaptive. Therefore, it may be resulting in some of the differences in data that we are seeing currently.

Gonads in Rana curtipes initially differentiate into ovaries, and later, in the prospective males, the ovaries degenerate and transform into testes. This represents a semi-differentiating type of gonad. Thus, interpretation of background rates of ovotestes and/or testicular oocytes occurrences in amphibian species, as well as interpretation in the context of environmental exposure and risk assessment, requires significant experimental evaluation under controlled laboratory conditions in addition to the evaluation of populations in the natural habitats.

And so we believe this SAP can contribute much thought and idea in the proposed future laboratory work necessary with atrazine and frogs. But let us not discount the importance, relevance, and contribution our field work can contribute to this whole subject area.

In subpart b. of this question, there is no information currently available that explicitly tests whether the presence of a few testicular oocytes would result in any impairment of reproduction. Therefore,

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one would wonder is this an aberration or not.

The presence of robust populations of frogs, and this is debatable, Dr. Skelly, and we respect your opinion and welcome your seat on the SAP in contributing to the population understanding of this subject area. But there were presence of robust populations of frogs where there is a relatively great incidence of testicular oocytes further argues against such an adverse effect of this condition. This is point we want to make.

Recently, the research accomplished by our research team in male amphibians does not suggest that the presence of testicular oocytes and ovotestes results in the reproductive impairment via reduced fertility.

In Subsection c. of this question, the major point here -- we addressed other points. But the laryngeal development in frogs, as Dr. Kelley has mentioned, is a sexually dimorphic process; and the formation of a larynx capable of a male calling behavior is androgen-dependent. Under normal conditions the laryngeal dilator muscle of the male Xenopus laevis is larger than that of females. It's been hypothesized that atrazine could decrease plasma concentrations of testosterone in Xenopus laevis by up-regulating the expression of aromatase, the enzyme that converts testosterone to estradiol. Yet

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this remains to be proven.

And, again, this whole area, I think, would welcome further input from the SAP. It is probably inappropriate, we believe at least at this point with the laryngeal dilator muscle, to be using this effect as an assessment endpoint, perhaps a measurement endpoint. But this point may be moot, since at least at this point in time, we do not seem to have reproducible, sensitive measures of potential estrogenic or anti-androgenic effects of atrazine.

In Question No. 6, our Eco Risk panel supports EPA's conclusion that the data currently available from both laboratory and field studies involving a wide range of amphibian species does not support the hypothesis that atrazine causes development effects in amphibians. That is yet to be proven. Given the low background incidence of gonadal effects reported in most studies, particular attention should be devoted to sufficient sample size so that the statistical power of the study is sufficient to properly test the null hypothesis. This has already been described by Dr. Gibbs.

The use of concentrations greater than those expected in the environment would allow evaluation of a threshold for gonad-specific effects to be determined. Therefore, levels of exposure greater than 25 ppb would be necessary. The study should also consider the

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possibility that effects could be caused by metabolites and/or environmental break-down products of atrazine and related triazines.

Hazard and risk assessments for pesticides and other substances have always been based on the principle of dose response and/or concentration response. We believe there is neither a framework nor a general precedent for risk assessment based on U-shaped, dose, or concentration responses. We will welcome SAP deliberation on this subject.

In Question 8, while some aspects of the study plan proposed by the U.S. EPA are reasonable; again, we compliment highly EPA's White Paper and their effort to engage the best science possible on this whole area of atrazine exposure in amphibians. It may be unwise to base the decision tree solely on histological effects in the gonad when there is currently no reason to believe that this the most sensitive endocrine response. And there has been no plausible mechanism of action that has been suggested by studies done to date.

Dr. Kloas's eloquently argued on other enzyme approaches and other endpoints that may be more elegantly sensitive to such effects is being considered by the SAP.

In the b. part of that question, it appears that the major set of endpoints is covered under the present approach that EPA has offered

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except some of the ones I just mentioned. Work so far has revealed that the degree of gonadal differentiation at completion of metamorphosis is highly variable within species and especially between species. In fact, there is no a priori reason to suspect that gonadal differentiation would be timed with completion of metamorphosis. So it is unclear why the Agency is using this same sampling time, end of metamorphosis, to gauge the degree of sexual differentiation.

And I'm sure the SAP will deliberate and offer the approaches in particularly the lab component of these future proposed studies that will address these concerns that we have.

In Subsection c., it is extremely important to properly design for possible tank and site effects. It is also important to include possible tank or site effects in the analyses of the study data.

Not only is it important to design studies with multiple tanks or sites per treatment level, but it also important to include multiple tanks or sites at the control level. Even two or three tanks or sites at the control level may be insufficient to capture the true tank-to-tank variability. And this has already been discussed by Dr. Silken previously at the table today.

Based on initial power analysis performed by our Eco Risk

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panel prior to conducting its laboratory studies, a minimum of eight tanks per treatment are necessary to account for inter-tank variation.

In terms of the last few comments in Subsection e., within ranid species there are differences in the expression of androgen hormone-dependent secondary sexual characteristics. This will have to be take into account. There are also differences at the hormonal and potential differences at the developmental level that are not clearly understood in ranids which would point to caution in assuming that the developmental processes and the mechanism of gonadal differentiation can be appropriately tested in Xenopus laevis alone which argues that Xenopus laevis may in fact be an initial good model species to test. But we should not count other native frog species that may be differing in their sensitivity, gonadal differentiation, et cetera.

Many ranids, for example Rana catesbeiana, are difficult if not impossible to breed under laboratory conditions, meaning that eggs would have to be collected from natural ponds with unknown exposure histories. Rana pipiens can easily be breed in the lab. So we have differences of how these various species will reproduce in the lab and/or have to be collected from the environment.

In Subsection g. of the last question, we believe and we hope

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that you will talk about this. But a toxicokinetic study of the uptake, organ distribution, and depuration of atrazine in Xenopus laevis, rana species, as well as other frogs will be useful in determining whether extrapolation is possible between frogs. In other words, we offer caution that to take a non-native laboratory model and try to extrapolate this to robustly to our native frog populations.

Thank you, Mr. Chairman. That concludes all we have to say in a summary nature of our document.

DR. ROBERTS: Thank you, Dr. Kendall. Reminding the panel that our opportunity to respond to these questions will come later, are there any questions to Dr. Kendall or other members of this panel regarding their rationale or basis for their responses to these questions. Dr. Kelley.

DR. KELLEY: I wanted to make sure I understand your summary. As I understand it, you felt that there was no robust and reproducible effect of atrazine in terms of development of intersex. And yet in the Carr, et al., study, you say, and I quote, "Exposure to either estradiol or 25 micrograms atrazine per liter increased the incidence of intersex animal based on an assessment of gonadal morphology."

So do you mean that that was not a robust effect? I mean, there

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were 11 replicates of that particular treatment group.

DR. CARR: Again, we found 4 percent intersex in that particular treatment. In terms of the percentage of animals that were affected, I wouldn't consider that a necessarily robust response. Neither was the qualitative assessment of the intersex. Again, we had initially used the term hermaphrodites to refer to these gonadal abnormalities. But they weren't true hermaphrodites in the sense that there were testes and eggs sticking out. In that sense, they were fairly subtle.

And, again, when we looked at the histological level, they were identifiable as male or female at the histo level. But there were differences in the shape and structure of the gonad that popped out at the gross level.

DR. KELLEY: But it was statistically significant.

DR. CARR: Absolutely.

DR. KELLEY: It was reliable within your groups.

DR. CARR: Right.

DR. KELLEY: So you would conclude that this particular dose of atrazine did have an effect even if you felt that it was a high dose and it was a small effect.

DR. CARR: Absolutely.

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DR. KELLEY: But still you would stand behind the effect.

DR. CARR: Yes.

DR. KELLEY: Okay. Thank you.

DR. KENDALL: That's a good question. And, again, in the presentation what we emphasized, we went through enormous planning on the statistical power of that experiment. And had not that statistical power been so strong, we probably would not have detected that effect. But we stand by it, although it was small and we do not believe robust.

DR. VAN DER KRAAK: I think there's an additional point to add to that. There's another component to your question, though. And the question was: Whether the response was reproducible across multiple studies. And in fact --

DR. KELLEY: Oh, yeah, within your study. That's all I asked. You did 11 replicates. I wanted to make sure it was reproducible within your study.

DR. VAN DER KRAAK: Correct.

DR. KELLEY: Right. Good.

DR. VAN DER KRAAK: The question, the additional point was whether it was reproducible across studies.

DR. KELLEY: 25 micrograms at that dose.

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DR. CARR: Right. And --

DR. KELLEY: I really want to know about your study, whether you stood behind that conclusion.

DR. CARR: Right. I do. And the thing that might add some clarity is that those abnormalities were distributed across the replicates. They weren't from one tank. That's the point I wanted to make.

DR. KELLEY: Great. Thank you.

DR. KENDALL: That's a fair question.

DR. ROBERTS: Any other questions? Dr. Green.

DR. GREEN: One last question. I was wondering how the panel felt about the possibility that in order to get a handle on the significance or the number of frogs in the wild population in different species that live with ovotestes. How does the panel feel about the possibility that field studies would involve going out and capturing healthy frogs from healthy frog populations and literally having to kill hundreds of them, maybe thousands depending what the statistician tells us, just to verify that it is or isn't a problem in the absence or presence of atrazine?

DR. KENDALL: We may ought to wait to let the SAP answer that question. I think that's a great question.

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DR. KELLEY: They're not our frogs. They're SA's frogs.

DR. GREEN: I assume there would be some in the United States. You can see I find it's a bit troubling. Do you think the data right now supports, for the edification of the panel, that we consider having to go out and capture healthy frogs to determine how significant this is in a wild population?

DR. KENDALL: I think initially what we believe is going to take a lot of frogs to have the power to detect the effects because we don't believe the effects are that robust. That's a very good point. Never the less, if one is going to get the correct kinds of data for future purposes to regulate this chemical relative to frogs, we will need to take the lab work to the field to see if, in fact, these effects are occurring and that they have any reproductive and/or ecologic consequence.

DR. GIESY: That's an excellent question and one, certainly, within my group we consider all the time. We have ongoing field studies that we're doing where we are collecting a lot of frogs. And, of course, we do that under our animal use permits. But it's something we don't do lightly. And I don't think we should do lightly. And I would encourage the Panel to consider that in whatever design you come up with for experiments. We would not want to do that

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wantonly.

DR. DU PREEZ: One can also look at museum specimens. There are literally thousands of Xenopus sitting in bottles throughout the worlds. But it's difficult to persuade the curators of those collections that you want to open up some specimens. They are sometimes very difficult. But that is a possible option. And now we know why frogs are declining.

DR. ROBERTS: Other questions from the Panel? Sorry. Dr. Skelly.

DR. SKELLY: Perhaps I should have asked this before. It dawned on me, thinking about this, that one of the questions I wanted to ask your group was whether you have looked or whether you plan to look for any evolved tolerance to atrazine.

DR. SOLOMON: Perhaps I can answer on behalf of the group. We certainly thought about this. Two aspects. One is has there been a genetic bottleneck that these frogs have gone through. And, in fact, in frozen at minus 80 degrees celsius we have blood waiting for DNA analysis if anybody's interested in it. We'd welcome contributions from the Panel and the audience as well. Not contributions financially, but suggestions as to how we do that in case you get me wrong.

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The other issue is -- I mean there have certainly been some suggestions of resistance. And I think the White Paper mentioned and we know that there's resistance in plants. Plants have a mechanism of action, receptor mechanism, that is susceptible to selection, change in the protein sequence. Of course, plants are under strong selective pressure, so they have evolved being selected for this resistance.

For this to have happened in frogs, I suspect we'd have to have a fairly strong response that either causes death or change in reproduction. And for my part, I haven't seen that yet. In my own experience with resistance, I think you need one of those or both of them to actually have selection take place.

DR. SKELLY: Just as a follow-up. I guess that's my point is that there are two ways to document a demographic effect. One of them is to watch a population for a really long time. The other ways is, if you find an evolved response, that implies a past demographic effect.

DR. SOLOMON: Yes.

DR. KENDALL: That's a good point.

DR. ROBERTS: Any other questions? Or more response from Dr. Giesy.

DR. GIESY: The issue of resistance, I think, is a very

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interesting one. And one that I'd like to hear some responses from the Panel. I personally have some ideas from a molecular biological techniques how you could approach that. I think it is a question you can pose. I think we do have tools that we can look at that. And I think it's probably worth looking at.

DR. ROBERTS: Any other respondents? Dr. Kendall.

DR. KENDALL: I just want to say, Mr. Chairman and distinguished members of the SAP, thank you for the opportunity. We're very grateful to have had this time frame to discuss these issues with you.

I also appreciate EPA for setting up the opportunity to have such a scientific discussion and to give us the opportunity to look towards the future. We also appreciate Eco Risk for facilitating our efforts between our universities. And we appreciate our sponsor for giving us the support necessary to engage what we believe will be some important discussion in the next few days. And we welcome hearing from you. And thank you for your criticism, you input, and particularly your patience this afternoon.

DR. ROBERTS: On behalf of the Panel, I'd like to express our appreciation for you and your colleagues willingness to come here and discuss your research in very open fashion with the Panel and

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answer all their questions regarding it. I think that's been very helpful in us understanding these issues.

DR. KENDALL: Thank you.

DR. ROBERTS: All right. Well, that concludes this public comment. There are plenty more to come. But at this point, I want to assess, do a stamina check on the Panel. The next public commentor, we have two public comments from the registrant. They will take a total of two hours, but they could be divided. Potentially, we could do one of them. Then next one will take about an hour.

Okay. I can see by the looks on your faces. That answers my question. I just thought I would bring it up, but I got a pretty clear non-verbal response on where we stand today.

In view of that, let us go ahead and adjourn the meeting today. We will reconvene at 8:30 tomorrow morning. We will continue with public comments, the first of which will be two public commentors from the registrant, Sygenta. And then we will proceed with other public commentors beyond that.

Paul, do you have any announcements before we close for today?

MR. LEWIS: Nothing to add, Dr. Roberts. I'm looking forward to continuing our discussion tomorrow morning. Thank you.

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DR. ROBERTS: Today's session is closed. We'll reconvene tomorrow morning at 8:30.

[Meeting convened at 4:35 p.m.]

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

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TODAY'S DATE: 6/26/03

DATE TAKEN: 6/17/03

CASE NAME: FIFRA SAP/Potential Developmental Effects of Atrazine on Amphibians

TOTAL: -- PAGES: 363

LOCATION OF DEPO: Crystal City, VA

DELIVERY: 7-day --

SPECIAL INSTRUCTIONS: Tapes to be included

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