FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

THE POTENTIAL FOR ATRAZINE TO AFFECT AMPHIBIAN GONADAL DEVELOPMENT

U.S. ENVIRONMENTAL PROTECTION AGENCY

CONFERENCE CENTER- LOBBY LEVEL

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FIFRA SCIENTIFIC ADVISORY PANEL
OPEN MEETING
OCTOBER 11, 2007

MR. BAILEY: Welcome to the third day of the FIFRA SAP on the Potential Affects of Atrazine on Amphibian Development.

I'll be very brief, keeping it in trend with yesterday afternoon's schedule.

My name is Joe Bailey, I'm the DFO for the meeting. And for the panel you've received a few more handouts. First was a replacement slide for Doctor Van Der Kraak's presentation, page 15.

A couple of other public comments have come in, one from the New York State Attorney General's Office, one for Partners in Amphibian and Reptile Conservation and the, let's see, the final thing was Jennifer Sass' reference list, it's a complete reference list of references that she had in her opening remarks.

All the materials that have been provided so far should be in the docket today so if anybody has any interest in seeing those they should be there.

Again, just as a reminder the meeting is

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recorded so please state your name before you make any comments.

And I think that is pretty much it for me this morning. I'll turn it over to our Chair, Doctor Heeringa.

DR. HEERINGA: Good morning everyone and welcome back to the third day in our meeting on the topic of the Potential for Atrazine to Affect Amphibian Gonadal Development.

I'm Steve Heeringa of the University of Michigan, Chair for this session.

I want to ask the other members of the science advisory panel here this morning to introduce themselves and give their affiliation as well.

DR. PORTIER: Ken Portier, Director of Statistics of the American Cancer Society, National Home Office, Atlanta.

DR. CHAMBERS: Jan Chambers, College of Veterinary Medicine, Mississippi State University and I'm a member of the permanent panel.

DR. SCHLENK: Dan Schlend, Department of Environmental Sciences, University of California, Riverside and also a member of the permanent panel.

DR. BUCHER: John Bucher, Associate Director, National Toxicology Program. I'm a member of

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the permanent panel.

DR. HANDWERGER: Stuart Handwerger, Departments of Pediatrics and Cell and Cancer Biology, University of Cincinnati College of Medicine. I'm a member of the permanent panel.

DR. ISOM: Gary Isom from Purdue University, Professor of Toxicology and also a member of the permanent panel.

DR, GREEN: Sherril Green, Stanford University, Department of Comparative Medicine and I'm an ad hoc member of the SAP.

MR. PAULI: Bruce Pauli, Environment Canada, Ottawa, Ontario.

DR. SCHLENK: David Skelley, Professor of Ecology, Yale University.

DR. DENVER: Bob Denver, University of Michigan, Professor of Molecular and Cellular Developmental Biology.

DR. FURLOW: David Furlow, Section of Neurobiology, Physiology and Behavior, University of California, Davis.

DR. YEATER: Kathy Yeater, Statistician with the U.S. Department of Agriculture, Agricultural Research Service.

DR. BAILEY: Ted Bailey, Iowa State

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University, Department of Statistics.

DR. DELORME: Peter Delorme, Pest Management Regulatory Agency of Health Canada.

DR. LEBLANC: Gerry LeBlanc, Department of Environmental and Molecular Toxicology, North Caroline State University.

DR. MILLER: Debra Miller, the University of George, I'm a Veterinary Pathologist.

DR. PATINO: Reynaldo Patino, U.S. Geological Survey Texas Cooperative Fish & Wildlife Research Unit.

DR. HEERINGA: Thank you very much members of the panel.

Good morning, Doctor Steeger. I would like you to introduce your team at this point. Will you do that?

DR. STEEGER: Good morning and once again thank you for the opportunity to present to the FIFRA SAP panel.

To my right is Mary Frankenberry. She's a statistician with the Environmental Fate and Effects Division.

To my left is Doctor Sigmund Degitz, a research biologist with the Mid-Continent Ecology Division of the Office of Research and Development.

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Seated next to him is R.D. Williams who is the Acting Director of the Environmental Fate and Effects Division.

Followed by Doctor Stephanie Irene, a senior advisor in the Environmental Fate and Effect Division.

And Ms. Eda Peace who is a senior biologist in the Environmental Fate and Effects Division.

DR. HEERINGA: Thank you very much, Doctor Steeger.

At this point in the agenda for this week's meeting, we suspended our coverage of the charge questions yesterday afternoon a little early because we had made very good progress and I felt we were beginning to move ahead of the point where I think a lot of the panel members had anticipated the agenda would be and felt it would be appropriate to return this morning and pick up again.

And at this point, Doctor Steeger, I don't know if you have any additional comments to introduce things that came to you overnight that you'd like to say to the panel or should we just proceed with the charge questions?

DR. STEEGER: Actually a couple of things

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came to me overnight. Only a couple of them though were relative to this SAP.

With respect to question number 1, yesterday's discussion sounded as though the panel concurred with the Agency's evaluation criteria for open literature.

These same criteria were applied to the registrant's submitted studies as well.

The panel also seemed to agree that the open literature consisting of both laboratory and field studies did not across multiple evaluation criteria, meet the standards of acceptability.

It was unclear after yesterday's discussion whether the panel believes that the open literature continues to have some utility in refuting or confirming my hypothesis that Atrazine exposure causes amphibian gonadal developmental affects.

Yesterday Doctor Jim Carr from Texas Tech University and Doctor Jim Wolfe from the Experimental Jeff Wolfe, I'm sorry, from the Experimental Pathology Laboratories provided a brief overview of their re-analysis of tissues which were initially reported as inter-sex tissues in the open literature.

This re-analysis concluded that none of

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the animals originally reported as inter-sex were indeed inter-sex. Therefore to our knowledge the only literature reviewed to date claiming to result in inter-sex is that of Doctor Hayes.

If the panel believes that open literature has some utility relative to the DCI studies, do they believe that multiple lines of evidence are consistent with the outcome of the DCI studies indicating that Atrazine is not affecting amphibian gonadal development?

With respect to question number 8, it's unclear whether the panel's final recommendation is for the Agency to require a review of sub-samples of slides from the DCI studies by a pathology review board or whether the panel is simply noting that such a review board would be of an added benefit.

If the latter, is the panel concerned regarding the identification of the apical end points or mixed sexed or are they concerned regarding the secondary measurement end points such as aplasia and mineralization?

If it is the latter, has the panel determined the biological relevance of these secondary measurement end points?

How much would these secondary

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measurement end points have to change before the conclusions regarding the apical end points would be affected?

And finally, with respect to the discussions regarding Atrazine's degradate exposure potential in the flow through study, data from the Health Effects Division indicates that in vivo metabolism results in the formation of diammino chloroatrazine, DACT, deisopropylatrazine, DIA and deethylatrazine, DES, those are the principal degratives of Atrazine that are also found in the field.

So if that is indeed the case, then exposure in a flow through system, those animals would be expected to be producing those metabolites in vivo.

And the question would be, what additional benefit would be gleaned from having a static renewal study to examine those, rather than having formal flow through studies that would indeed test whether each independent degradate was causing an affect?

DR. HEERINGA: Doctor Steeger, with your permission I think that what I would like to do is if a member of your staff could maybe put those up so that we could get those on the screen and we will return to

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those during the questions this morning.
DR. STEEGER: Okay.
DR. HEERINGA: I'd like to do it systematically rather than sort of right off the bat here.
DR. STEEGER: That's fine, that's just
DR. HEERINGA: But definitely we will systematically review each of those and I think certainly in some of the points I recognize the issues that you're raising here.
So what I'd prefer to do is maybe return to our question 9, if we could have those put up so we could see them and consider them systematically, because I think it's taking us back to a few issues, but those are excellent points of clarification and I think getting us a good discussion of each of those points would also clarify our report on those matters too.
What I would like to do at this point Doctor Irene, I'd like to return to charge question 9, and would you read 9a into the record again please?
DR. IRENE: That's great. After an evaluation of the laboratory based studies submitted in response to the DCI, the Agency has concluded that these sutides do not provide sufficient evidence to

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support the hypothesis that Atrazine causes adverse gonadal developmental affects in amphibians.
A, in light of the responses to questions 3 to 8, please comment on whether the results from the study in response to the DCI are sufficiently robust to address the hypothesis that Atrazine exposure causes gonadal abnormalities in Xenopus laevis. If the SAP concludes that these results are not sufficiently robust, what recommendations can the SAP provide to efficiently and reasonable address remaining uncertainties?
For example, if the SAP does not believe the DCI study is sufficiently robust to assess the hypothesis, does the SAP believe either of the two experiments or a specific component of the two experiments should be re-analyzed or repeated?
Please provide the rationale for recommending any additional analyses and/or experiments.
DR. HEERINGA: I'd like to return to Doctor Delorme again to review his comments oR add additional comments.
DR. DELORME: I think we're a little bit more on the ball this morning having been able to put our thoughts in a little bit better order.

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I'm just going to go over some of what was talked about yesterday and then go back to the corespondents to see if they have anything to add.
Just start it off by saying that the panel noted the question was somewhat confusing in that it presents two hypotheses. In the original question is refers to adverse gonadal development affects in amphibians. Well within sub-bullet A question, it refers to the DCI studies and uses the words, causing gonadal, the hypothesis refers to, causing gonadal abnormalities in Xenopus laevis.
In order to provide a clear response the panel has restated the hypothesis being considered in this question to better reflect the result of the DCI study as follows: Exposure to the parent compound Atrazine causes adverse gonadal development in Xenopus laevis within the range of concentrations tested, i.e., 0.01 to 100 micrograms per liter.
Responses to the more general hypothesis concerning adverse gonadal development in amphibians are addressed in questions 12 and 13.
In general the panel believes results are sufficiently robust to test or address the restated hypothesis based on the discussions and considerations identified in responses to questions 3 to 8.

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In brief the panel concluded that the study design was appropriate for testing the parent, testing parent Atrazine and that the study design addressed many of the concerns regarding water quality, loading rates, et cetera that were identified by the '03 panel.
Panel members agreed that the use of a flow through exposure system and lack of measurement that did not allow for testing of the hypothesis related to effects of transformation products on adverse gonadal development. And we're probably going to talk to one of the points Doctor Steeger just brought up.
Parent Atrazine exposure concentration profiles are well characterized and sufficient for documenting the potential affect of Atrazine over a broad range of exposure concentrations.
Actual concentrations were generally stable, although the panel had concerns about low concentration values at the two lower doses for the IGB study compared to the target exposure concentrations. These concerns are balanced by the robustness of the measured concentration data which allows analysis.
It was suggested that results could be stated in terms of measured rather than nominal concentrations.
The panel generally agreed that primary

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apical end points were well characterized, both technically and statistically for negative control, positive control and Atrazine exposed groups.

There remains uncertainty with respect to the biological ecological relevance of secondary end points.

With respect to the histological analyses the panel recommended a verification of results by independent pathologists.

In addition, some other comments or some other points. The strength of the concentration response relationship, studies provided no evidence, and these are more related to the results in general, studies provided no evidence for a concentration response relationship between Atrazine and primary end points such as sex ratios and inter-sex testes. One study provided significant evidence for concentration response relationships with several secondary end points, and they're listed here.

Strength of cause/effect relationship, the effects observed with Atrazine were modest despite robust responses in the positive control. Furthermore the noted concentration response relationships were not reproducible between two studies performed in the same study protocol.

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Mechanistic plausibility, no mechanistic plausibility. The predominant hypothesis for the purported action of Atrazine is the induction of aromatase but while the aromatase gene is inducible in some cell lines by exposure to high concentrations, we are aware of no precedence for the induction of aromatase by Atrazine, in Atrazine exposed frogs.

Failed attempts to induce aromatase in frogs by Atrazine have been reported.

The ecological relevance of effect, end points for which there exists weak evidence for an effect of Atrazine are not recognized as relevant to reproductive fitness.

Conversely, end points that are more likely to impact reproductive fitness, sex ratios, intercepts were unaffected by 100 micrograms per liter of Atrazine.

Now, despite the robustness of the DCI studies for addressing the hypothesis, several other concerns have been identified by panel members.

Some of the members were concerned by the total rejection of the hypothesis based on negative data, i.e., no affect and only two studies effectively. In part their concern was based on the uncertainty caused by the relevance of the exposure system to the

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natural life history of Xenopus laevis, pointing out that flow through, the flow through paradigm is likely father away from what a Xenopus tadpole experienced in the wild than is static renewal.

Another concern expressed by some panel members was based on information presented by Syngenta related to the specific strain used and the apparent differences in the presence of testicular ovarian follicles in different strains of Xenopus laevis. It was not clear if the differences in the presence of TOF's are the result of differential sensitivity or differential presence of other factors which could cause them.

The Xenopus laevis used in the DCI studies were derived from strains with no reported TOF's apparently. While the DCI studies included positive control the possibility of differential sensitivity introduces added uncertainty to the interpretation of the results.

And yesterday, Doctor Steeger, you mentioned that you wanted, you would like some input on this and you noted that there was a positive control. I'm not sure whether or not sex reversal and TOF are the same thing. So whether or not the positive control is relevant for this question or not. Perhaps other

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members of the panel can comment on that.

But I would suggest that perhaps a comparison of the responses to a positive control by the different strains could help reduce the uncertainty.

As noted earlier the panel concluded the current study did not address potential affects caused by exposure to transformation products. The panel recommended that the Agency could use existing monitoring data which includes information on environmental concentrations of transformation products to determine the extent to which they might want to consider transformation products in the future.

In other words, look at the monitoring data. If the exposure in the environment is reasonably high, that you might want to pursue that or not. That's a call that EPA would have to make.

In addition a literature search could be conducted to determine if information exists on the potential for transformation products that interact with the endocrine system, for example, receptor binding assays and such.

And I'll go back to my fellow inputs to see if they have anything to add.

DR. HEERINGA: Let's go back to Doctor Denver and see if he has anything to add?

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DR. DENVER: No, I don't have anything to add. I think you've summarized our comments.
DR. HEERINGA: Doctor LeBlanc?
DR. LEBLANC: I have nothing to add.
DR. HEERINGA: Doctor Furlow?
DR. FURLOW: I have nothing to add. I look forward to a continued discussion about strain usage and things like this that I think will continue throughout the rest of the questions.
DR. HEERINGA: Agreed, thank you. Yes, Doctor Patino?
DR. PATINO: Reynaldo Patino. I agree with everything generally with what's been said.
One additional comment I would make and this discussion came up I think when we were talking about questions 3 and 6, that, you know, the way the question is posed seemed very limited in the sense that it said in part A, that it talks about gonadal abnormalities and somebody suggested that additional information to that question should be added to response to that question and add, within the range of concentrations tested.
I would add to that too that probably we should also say that we had no actual terminated Stage 66 frogs because on the questions 3 and 6 we talked

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about the long terms affects, functional affects that are known.
The question does not address that so I just, within the, if you add that parameter to the question I would agree that it would clearly show, or answer that question.
DR. HEERINGA: Yes, Doctor Miller please.
DR. MILLER: Debra Miller. I agree with that and I would probably even make it more specific in saying Xenopus laevis and I know we'll get into this later but amphibians are a class and there are many species and there are many orders. And this is a great lab frog but just like the lab mouse is to mammals you don't say in mammals.
And I just don't think that you can make such a broad statement and say that it's the same in all amphibians.
DR. HEERINGA: Doctor Delorme?
DR. DELORME: Yeah, that was why we wanted to restate the hypothesis, it was ambiguous.
DR. HEERINGA: Doctor Delorme again.
DR. DELORME: I was just wondering if anybody could help us out on Doctor Steeger yesterday asked the question, with respect to the strains what our concern was given that there was a positive

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control?
Now the positive control was E2 estradiol and that causes sex reversal. The differences in the strains was with respect to the presence of testicular ovarian follicles. And I was just wondering if somebody could help us out on what, if that's a valid concern or not?
DR. HEERINGA: Members of the panel?
DR. PATINO: Could you restate the question, I didn't this is Reynaldo.
DR. DELORME: In the presentation by Syngenta on Tuesday they indicated that there were differences in certain genetic strains of Xenopus with respect to the presence of testicular ovarian follicles with some populations having them in areas that are unexposed to Atrazine and other populations in unexposed areas not having them.
The strain that was used for the test was derived from the populations that do not have them in their background.
Is that a concern, should that be a concern? And it goes to state, is there a differential sensitivity to some factor in the environment and could that impact the results of the test?
If we don't think it does then it's a

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nonissue and we can take it out.
DR. HEERINGA: Doctor Green, or Doctor Patino.
DR. PATINO: Reynaldo Patino. I would say my experience with fish and reading of the literature and I don't have a specific paper in mind, but just general knowledge of both sex reversal and presence of testicular oocytes are often the cause of feminizing, when animals are exposed to feminizing agents. You can see both.
So I would say, you know, perhaps that if you find testicular oocytes or inter-sex or mixed sex they generally indicate the same or their symptoms are the same phenomena which is feminization.
DR. HEERINGA: Doctor Green.
DR. GREEN: There is a textbook called, The Biology of Xenopus by Tinsley and Kobel which is published I think, the last edition came out in 1996 and in that textbook they do describe multiple sub-strains of Xenopus laevis that are geographically distributed around the world in different places.
And I don't think there is any data regarding those specific sub-strains about sensitivity differences to stressors. However there are very subtle differences on the phenotype, some of which are

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secondary sex characteristics which I think, given the fine nuances by which we are interpreting differences in gonadal development and the low incidence and the negative results, that could come into play if you are using one of these different sub-strains that has very subtle difference like a different snout to vent length, a different from metamorphosis to sexual maturity.

And some of those sub-strains are characterized and in the last 10 to 15 years I believe there have been even more reported and described.

So that would be something to consider in terms of using a different sub-strain of Xenopus laevis in studies such as this.

DR. HEERINGA: Any additional input on that particular. I appreciate those two contributions yes, Doctor Furlow.

DR. FURLOW: I just guess I can add to this, just one point. We all consider Tamoxifen to be an antagonist for the exteroreceptor, everybody believes that in terms of subculture experiments, binding to the receptor, et cetera and yet it has different affects on different tissues. It can be an agonist in bones and it can be an antagonist on the uterus.

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In addition, if you compare rats and mice, if you give Tamoxifen to a rat it acts as a pure antagonist in the uterus. You give Tamoxifen to a mouse and it's an agonist. It has a completely opposite affect.

Xenopus is a very ancient species. You know, you could say, okay, come on, you know, Xenopus from different areas of South Africa can be as different as rats and mice. But I don't know that we know that in terms of their ability to handle these different compounds.

The use of the positive control also implies to some degree, even though Doctor Steeger was careful to mention this previously, but there is some underlying implication that the mechanism between Atrazine versus the positive control would be common and that's given the aromatase, the lack of aromatase data I think that's pretty far from clear.

It's entirely possible that differences in strain may result in differences in metabolism binding to receptors, et cetera. It's unclear what those strain differences might be. And so I think there is uncertainty underlying these results because of the strains.

DR. HEERINGA: Thank you, Doctor Furlow.

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At this point I'll ask Doctor Irene to read the second part of question 9, or Doctor Steeger, sorry.

DR. STEEGER: Just as a point of clarification, each one of our risk assessments that the Agency produces makes a, has a boilerplate language in it to indicate to the public that we rely on surrogate species in order to conduct risk assessments.

The, traditionally we don't even look at amphibians, we use fish to estimate the risks to aquatic phase amphibians. We use birds to estimate the risk to reptiles and terrestrial phase amphibians.

We acknowledge that there are thousands and thousands of species out there. But the reality is that we get two birds, two bird species to represent risks to all bird species, all reptiles, all terrestrial phase amphibians. We get two fish to represent the risks to aquatic phase amphibians and most of the vertebrates that are in water.

That's the reality that we face. We don't really have the luxury of testing every one because no pesticide could be rather strict. So the reality that we face is we were lucky to get amphibian data at all. And yes, there are uncertainties, there are incredible uncertainties in terms of whether the strain of the animal that's used is more or less

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sensitive than what's out there.

But the data that we have, we're just making the best with what we can work with, suggesting this was data that was presented yesterday representing the work of Doctor Hayes shows that Xenopus laevis is a sensitive indicator to estrogenic compounds. It's one of the most sensitive.

Does the strain difference play a role in our assessment? It's a consideration but we have a positive control that suggests that the test system was sensitive to an estrogenic compound and that it could demonstrate that a chemical could impact amphibian gonadal development.

In that test system, did Atrazine show an affect or didn't it? That is the question that as a risk assessor I have to be evaluating.

In the context of how we do regulatory science here at EPA and the limitations that we have that we do not have the luxury of testing every species out there and addressing every uncertainty, we can caveat those uncertainties, but the likelihood of our getting data is becoming increasingly difficult.

I'll just let it go at that.

DR. HEERINGA: Thank you, Doctor Steeger. At this point I would like to move on to question 9b.

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DR. IRENE: Stephanie Irene, 9b. Please comment and provide recommendations on alternate statistical analyses if any to evaluate the data derived from the study. If alternative approaches are suggested, please comment to the extent possible on the rationale for these approaches and how they represent improvements in the existing statistical interpretations.

DR. HEERINGA: Doctor Bailey is our lead discussant on this subpart.

DR. BAILEY: We recommend that a combined analysis of the data from the two studies be completed.

The usual procedure for analyses when an experiment is repeated two or more times, the study usually involved two phases.

First, analyze and interpret each study separately. This was done for the most part very well in this study.

Secondly, carry out an analysis of the combined data from both studies. Important advantages in the combined analysis include stronger, more powerful tests of hypotheses can be made than in each experiment.

For example the dose relationship of Atrazine.

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Secondly, more importantly the combined analysis allows us to ask the questions like, do differences between controls and levels of Atrazine differ in the two studies? Does the dose response relationship differ in the two studies? Does the unexplained variability, the experimental error in the two studies differ? If so, why?

Answers to these questions provide information on the repeatability of affects of interest in the study.

Second item for comment, we recommend the use of blocking be considered in the design of lab studies like this one. There are many reasons to introduce blocking in the design of experiments, including the control of experimental error.

It is surprising to us that none of the lab studies reviewed in preparation for this panel took advantage of the benefits of blocking.

We strongly encourage more and better interpretation of statistical tests. To state that a test is statistically significant or that it is not significant is seldom sufficient. One wants to learn what the magnitude of the differences between the treatments are or we want to learn the magnitude and the strength of the dose response relationship.

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Making a test of hypothesis is not the same as interpreting the result of a test.

The use of competence intervals are especially recommended for presenting and interpreting results from the studies.

We recommend that in designing experiments the essential choices of experimental unit, treatment design, experimental design and the method of randomization of treatments to experimental units be clearly specified.

Application of these principles in design not only leads to efficient experiments, they also ensure unbiased estimates of treatment effects and estimates of experimental error.

The information with respect to the design of the experiment should be shared with all relevant individuals.

DR. HEERINGA: Thank you, Doctor Bailey. Doctor Yeater.

DR. YEATER: Kathy Yeater. In addition to those comments with which I concur, I would also like to add in that I really feel that there is a high quality of data collected in these two studies.

And there was a previous mention during this SAP meeting was that the idea of perhaps

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developing a standard protocol from this, and so that leads me to suggest and recommend that more sophisticated statistical methods be considered.

And I sometimes think this is a failure of statistical education in the applied sense is that we don't get beyond a standard ANOVA t-test. And I want to suggest that well, let me get into this here.

Specifically the data analysis presented for the DCI study reveals no information on any associations that may or may not be present between the measured variables.

Also there should be more consideration given towards the male/female ratio in the tank. From the data reported in the DCI study it is observed the differences between male and female means are significant in several of the end points. However the overall tank means are not weighted or standardized for this differential which could be influenced by having a skewed male/female ratio.

These two methods can be approached by transposing the data set into a multi varied data sets where each larva and its corresponding measurements are the units of analysis. This is not such a stretch considering we have already accepted that the individual tanks are the unit of analysis.

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By using each larva as an observation we can incorporate all measurements to that sample from which it was measured as well as incorporating the observed sex of each sample at metamorphosis.

This would enable a multi varied approach such as an ANOVA which is a multi varied analysis of variance of even a canonical analysis and having some better understanding of possible associations and correlations between the measurements and observations within and across treatment affects. It would also enable the easy inclusion of the male/female as an appropriate measure variable.

DR. HEERINGA: Thank you very much, Doctor Yeater. Doctor Portier.

DR. PORTIER: I don't have much to add. We talked about this among ourselves and since I'm third I didn't have to add a lot.

The one thing I will point out is that even with a more sophisticated analysis that we're recommending, a multi variate look at the data, and there's a lot of benefits to doing the multi variate because I feel a lot of the responses that were observed as significant are probably significant together, so there's some underlying mechanisms that are causing those events to be significant.

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And we haven't really looked at those. We've talked about them.

But I don't think it's going to change and increase the number of significant findings that we have in the data. So we're not really criticizing the analysis, we don't think you missed anything. The only opportunity you've missed with a high quality data set like this is just doing a better statistical analysis that uses more powerful tools to look at it.

But I think my colleagues would agree, I don't think you're going to find some things, magically find some additional affects here that were significant. And in fact my own personal feeling is that some of these are going to go away, some of the things that are not consistent are going to be lost in the even though we're increasing the power of the test by combining the two results, some of these findings are just not going to hold up.

DR. HEERINGA: Thank you to each of you for those contributions. Other members of the panel, do you want to contribute on the statistical design and analysis, they go hand in hand obviously?

Yes, Director Williams.

MS. WILLIAMS: R.D. Williams, thank you. I just need to try and get something clarified in my

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mind if I may.

One of the things that was mentioned is that by combining the analyses it gives you a better indication of whether the results are repeatable. Is that did I hear that correctly?

DR. BAILEY: Ted Bailey. It does because with the combined analysis you are able to see if the same result is obtained in two different labs. If so, that reinforces the results.

And also you're able to look at interactions if you combine both, interactions of the factors that are in the study that you cannot do without a combined analysis.

MS. WILLIAMS: And I do not, I'm not a statistician either, or on t.v. so I guess I'm a little confused, because I know one of the things that we always look for when we're using data, is whether the study has been repeated and the results are repeatable. And one of the reasons that this study was done at two separate labs was so we could, at the same time have the repeated study.

So I guess I'm confused about why we now would combine everything and make it like one study.

DR. BAILEY: Yeah. During this panel what we've seen is a thorough study and interpretation

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of each of the studies separately.

But you can gain some things by doing the combined analysis that you cannot do by looking at the individual studies separately. And those, that is what is to be gained from the it's a very standard procedure when studies are repeated in different locations or in different labs in many situations.

It's a very standard thing to do to come back and look at be able to make these comparisons when you have the data combined.

DR. PORTIER: This is Ken Portier. I think Doctor Bailey had a good example.

In both studies you talk about a dose response and you can see that it's not significant in either one, but when you put it together you have more power. So one, you have a more powerful test of whether there is a dose response. But one of the things you could test is whether that dose response was the same in both studies.

Now if you knew it were the same that's a more powerful finding, right? You could say, well, we've repeated not just the finding of a dose response, but we've repeated a finding of the same dose response.

And that's something you cannot get by looking at them separately, right? You have to put

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them together.
We found in one study that there were some differences in some of the treatments for one of the responses, I forget which one it is, and we didn't find that in the other one. When you put them together you have more degrees of freedom for residual variability, you have a more powerful test.
Now we can say, you know, when you combine the data, are those things that we saw in one study, is it still important? If it's important that's a study by affect interaction that we can actually test and put a P value to and say that we had differences in this responses by the studies with this level of significance.
DR. HEERINGA: Yes, Doctor Steeger.
DR. STEEGER: I have a couple of questions. With regard to the idea of combining these studies, keep in mind that they were intended to be an effort to duplicate or reproduce the study in two labs so we could get at the issue, that we wouldn't be dealing with a single study but we would actually have more than one study.
In suggesting that they be combined, does that compromise in any way the interpretation of the studies as being independent and as being intended

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to be reproduced?
DR. BAILEY: Ted Bailey. No.
DR. STEEGER: Okay.
DR. BAILEY: The integrity of the studies remains. I think it's good to analyze each study separately first to be sure we understand what's happened in each study. But then we have the advantages that have been mentioned that you can do other tests about the repeatability of effects and so forth, which would be very important.
You could confirm that you're getting similar results in the different labs which would be important to know, but you may also find out that the effects, the dose response relationship may not be exactly, may not be the same in the first lab as the second lab. It's entirely possible.
DR. STEEGER: This is Tom Steeger again. I think one of the difficulties that we had in originally combining or deciding whether to combine the studies was that they are using different animals, they are not from the same 10 breeding pairs and completely different set of breeding pairs that were used in the IGB study compared to the Wildlife studies.
My concern would be, and I can appreciate the fact that the power of the test to

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discriminate statistical differences would be increased by combining them, I will presume it would be increased, and then there would be the concern whether statistical differences that might appear were driven by sample size as opposed to what might have been a biologically significant affect.
The white paper does go into an analysis where there was a significant effect, particularly related to body weight and body size at IGB in female animals, did look at the percent of the affect and did discuss whether that is of biological significance.
In doing so we noted that first of all there wasn't a dose response, the affect was skipping every other dose. And for weight, the weight did not change, the animals were .52 grams in all three significant affects. It was a decrease of 7 percent. It wasn't, because it wasn't a dose dependent increase the weight was constant across all three concentrations that were significant and it skipped every other dose. It did not appear to be a biologically relevant measurement end point.
It is true that if you were to combine the studies that might become, what might have been even more subtle affects in the other concentrations might have shown a concentration dependence.

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But the fact that the weights weren't changing at all, that would be of concern to us.
Let me mention one other thing. It's not in the white paper. When Mary Frankenberry was doing the analysis, and I would add that Lisa Eisenhower was also a statistician with the Environmental Fate and Effects Division, contributed greatly to the statistical analysis, I did request that they take many of the apical end points and secondary end points and do a correlation analysis to determine whether any of the changes that were occurring across the different treatment groups were correlated with other end points.
So those analyses all proved to be negative. But they were conducted although they are not included in the white paper.
DR. PORTIER: You know, I just wanted to address the issue of statistical significance versus biological significance, we're always aware of that.
You know, the statistics can only point you to where we think something might be happening, but you also have to look as Doctor Bailey pointed out, you have to look at the magnitude of the affect through your biologist's eyes, not your statistical eyes, to say, yeah, that might be a statistical difference of .1

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but who the heck cares, right? Because that's not a biologically important thing.

But if you look at the power analysis you saw for some of the outcomes you needed to observe like a 10 percent affect size, right, for any one of these experiments. You put it together and it might be more like a 7 percent affect size.

Well 10 percent sounds like a big affect to me and that's kind of a biologically big difference to be shooting for. I'd be more interested, there might be some findings, unlikely, it might be some findings that we'd see statistically significant that were still in the biological affect area.

The second thing is, you know, in agriculture we've been doing studies like this for at least 60 years where, you know, you raise a new variety of corn, you don't just depend on one field trial in one location to establish that that's a better variety, it's got to be planted in three or four or five different varieties that have different soil types, different climates to establish that you actually have an affect here.

And nobody ever challenges that these things are independent field trials even though they may be done by the same ag experiment station or a set

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of scientists that are working together.

And the same thing in clinical research, we do clinical trials for new drug therapies, not in one hospital or one clinic, but in multiple clinics with multiple researchers with multiple sets of patients and then we have study centers and data centers that combine these to look and establish the true affect.

And all we're saying is you've done the same thing here. You've done two different studies in two different locations with different scientists but the same protocol. Different feed stock, high quality data, you have an opportunity to actually address with more power what affects are there.

You still have to bring a panel together to look at the affects and say whether they are important or not. But that's the question here is how to improve the statistics and I think combining it does improve the statistics.

DR. HEERINGA: Doctor Bailey.

DR. BAILEY: Ted Bailey. You just mentioned a correlation analysis and we didn't discuss that here. But I'd have to be sure that you to say to you that when you, if you use correlations when you've got structure in your data, structure like the

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different treatments, then you have to be very careful because those effects can effect, can influence what the magnitude of the correlation is.

And I'm sure you know that.

DR. FRANKENBERRY: This is Mary Frankenberry. Just two thoughts and we thank you for your suggestions definitely and frankly did think about a lot of other things that we might have done with more time and we're grateful for the ideas.

But I think also the idea with reproducing something in two labs was one of reproducibility and not replication originally. And I think we've heard a lot of talk about we didn't see the affect in both labs and my personal opinion was simply that if we saw it in one lab, that was good, if we saw it in two, that was better. But it did not detract from seeing it in one, no.

I think there's more to gain if we wanted to look at them together but my concern was I think with Doctor Portier I was afraid that the increased variability we may have between the two labs would do away with any increase in power that we might have.

But again we would have an increase in information so we can look at that.

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With regard to anything dose response related I think part of that was a time element, but also we never saw anything very strongly in either lab along those lines to inspire us to go much further.

But, you know, any other ideas you have we're happy to listen to.

DR. BAILEY: Ted Bailey. The, this is the general process when you've got data collected in two or more different locations or whatever. This is just the regular procedure recommended is to go ahead and analyze it individually and then come back and do a combined analysis.

And Doctor Steeger gave a really great example of where that could be beneficial. You had the different strains in the two labs and if you did the combined analysis and then you found an interaction between the sex and the treatments, one explanation for that interaction would be that there were different strains.

You wouldn't have been able to get a test on that unless you did the combined analysis, that's the reason for doing it.

DR. STEEGER: Just as a clarification, they had different parentage, the were the same strain.

DR. HEERINGA: Thank you very much. I

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think certainly in terms of the design aspect the two independent laboratories repeating the experimental process, I think the panel is in complete agreement with that.

We'd be at a very different point in this discussion if it hadn't been done that way I believe. And so I think these are all additional analyses that are proposed as Doctor Bailey suggests that in a typical sequence of independent analyses and then a combined analyses and with the potential benefits as he just discussed.

But I think that certainly there is, I don't see, or haven't heard any critique on the two laboratory design from any members of the panel.

Additional input on the statistical analyses that could be added to what has already been done in preparation of other reports or the white paper?

Again we'll have a chance to revisit these things if new ideas do arise, but at this point I'd like to move on then to charge question number 10.

DR. IRENE: This is the first of the concluding questions. Is the SAP aware of any other laboratory based or field based studies not included in this white paper that contradict the Agency's

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conclusions that one, the design associated with current studies available in the open literature are not appropriate for evaluating the hypothesis that Atrazine affects amphibian gonadal development?

And two, the available data in the open literature combined with the results of the DCI study indicate that Atrazine does not cause adverse affects on gonadal development in Xenopus laevis when investigated under conditions consistent with those recommended by the SAP in its previous report in 2003.

If so, please identify the studies and briefly outline how the results from these studies would contradict the conclusion that Atrazine in concentrations up to 100 micrograms per liter does not cause adverse affects on amphibian gonadal development.

DR. HEERINGA: Doctor LeBlanc is our lead discussant on this response.

DR. LEBLANC: Gerry LeBlanc. This charge question is divided into two sub-questions and I'll address the first and then proceed into the second.

And to paraphrase the first question, are there studies available in the open literature that challenge the design of the studies that were submitted in response to the DCI request?

Such studies for example might indicate

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that Xenopus does not perform well under flow through conditions. Alternatively there may be studies available in the literature that would indicate that initiating these studies at Stage 46 was inappropriate.

This is the way at least that I interpreted this part of the question.

And in response I'm aware of no such studies that would call into question the design of the DCI studies.

The second part of the question gets more to the meat of the issue, that is, are there any other published studies available in the literature that would contradict the conclusions reached by the Agency with respect to the DCI studies? That is, are there other studies that were conducted according to the recommended guidelines provided from the 2003 SAP that come up with conflicting results?

And again I am aware of no such studies.

DR. HEERINGA: Doctor Delorme.

DR. DELORME: I don't have anything further to add.

DR. HEERINGA: Doctor Skelley.

DR. SKELLEY: Doctor LeBlanc and I discussed the response to this question before the meeting today and I have nothing to add.

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DR. HEERINGA: Any other comments from panel members in response to the two parts of this question? We've had some conversation on this earlier too.

Doctor Steeger, are you, do you feel that you understand the response of this panel and if so, do you have any comments or requests for clarification?

DR. STEEGER: No, I understand the comments clearly, thank you.

DR. HEERINGA: Okay. Doctor Irene, would you read question 11 into the record please?

DR. IRENE: Yes. The Agency is not aware of data that establish a mechanistic basis for how Atrazine could affect amphibian gonadal development. Please identify and comment on any studies that demonstrate the mechanistic steps by which amphibian gonadal development could be affected by Atrazine and thereby contradict the Agency's overall conclusions based on the studies evaluated for this SAP.

If the SAP is aware of any relevant studies, please comment on the data from this or these studies and how the data indicate and quantify a mechanistic pathway from Atrazine's molecular site of action to histological and apical end points associated

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with adverse affects on amphibian gonadal development.
Please also comment on any dose response relationships associated with the steps in the reported toxicity pathway.
DR. HEERINGA: Doctor Furlow is our lead discussant on this question.
DR. FURLOW: So to begin with it's, when you're faced with the evidence that the Atrazine alone with Xenopus laevis and these nicely studies doesn't seem to have an affect directly on gonadal development, it's difficult to say, okay, well then what's the mechanism?
The prevailing working hypothesis often cited in the open literature is that Atrazine increases the activity of aromatase, gene expression activity during critical periods of gonadal development, shifting gonadal steroid synthesis in males from primarily testosterone to estradiol.
As we have discussed previously, both in the public comment and in Doctor Steeger's presentation, the previous evidence has been indirect, such as comparing the reported affects of Atrazine to the affects receptor antagonists and estrogen receptor agonists or reported reductions in plasma testosterone which actually appear to be the most consistently

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reported affect of Atrazine in the literature.
EPA correctly points out that the best evidence supporting the aromatase hypothesis remains the studies in cultured cell lines.
Direct evidence for induction of aromatase in vivo in tadpoles is conflicting and may be confounded by the low expression levels in the tadpoles and the same issues suggest to explain the variability in gonadal phenotypes observed with Atrazine.
There are a couple of papers regarding this issue that are not contained in the open literature review. But again these are using cell culture based assays and I will only mention them briefly.
Earlier this year Holloway, et al reported in the Journal of Applied Toxicology that aromatase activity, but apparently not gene expression can be induced and cultured, primarily in human granulosis cells two to threefold by Atrazine, so this affect is not solely limited to transformed cancer cell lines.
Secondly a pair of papers by Fan, et al, one in Environmental Health Perspectives and one in BBRC, presented data to demonstrate that Atrazine cane activate the aromatase gene expression in one dependent

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manner which is a morphonuclear receptor, known to be an important physiological regulator of aromatase.
The authors also suggest that aromatase acts as a drug ligand but the exact nature of the interaction is unclear at this time.
The concentrations used to induce aromatase activity in these line cell lines in these new papers appear to be higher than those reported to cause gonadal abnormalities in the open literature, although significant induction can be observed in both sets of studies with as little as 10 to -7 Atrazine.
In addition the dose response curves in both studies are monotonic rather than u-shaped as expected for a simple mass action driven interaction.
It is formally possible that Atrazine under certain conditions has affects on Xenopus gonadal that alternative mechanisms other than the induction of aromatase or its activity may be at play.
SF1 for example has other gene targets other than aromatase, it's expressed in the hypothalamus as well as the adrenal glands like gonads.
Again this point is highly speculative so at this point there is no new data on the potential mechanisms of Atrazine affects not mentioned by the white paper, other than the aforementioned cell culture

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experiments.
While these results should be considered by the EPA they are in and of themselves insufficient to explain Atrazine's potential detrimental affects on Xenopus gonadal development if they exist at any dose at all.
DR. DELORME: I agree with David's statements and I don't have much to add but I want to point out that if we just simply ask the question does Atrazine affect aromatase activity in amphibian tadpoles, I don't think the question has been tested sufficiently. I don't think that there sufficient data to either accept or reject that hypothesis.
But I agree that the only data that really support the idea that Atrazine can affect aromatase are the data from the cell lines, the cell culture.
DR. HEERINGA: Doctor LeBlanc.
DR. LEBLANC: I feel that David covered all of the issues quite well and I have nothing to add.
DR. HEERINGA: Additional input or comments from members of the panel on this particular topic, this particular question? I appreciate those contributions, Doctor Furlow.
Doctor Steeger.

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DR. STEEGER: So I understand correctly there, in response to the question there are no additional data other than the mammalian cell culture studies that were done at higher concentrations than what have been previously demonstrated to results in affects in amphibians?

DR. FURLOW: This is David Furlow. That's correct. You can get by ANOVA analysis anyway, affects at 10 to -7 but those are marginal. So you typically have to go to 10 micromolar but I didn't do the calculation, is that how many parts per billion and how many micrograms per liter, but that could be done.

DR. STEEGER: Thank you.

DR. HEERINGA: Doctor Portier.

DR. PORTIER: Doctor Furlow, you know, we had this discussion on these for transformation products.

Is there nothing, I mean what you talked about was the parent compound, right?

DR. FURLOW: Right. So far I didn't see much on the degredates. The SF1 data was screened against Atrazine and Simazine and there was interaction with Simazine as well. They did test 55 different pesticides and those were the only ones that showed statistical significance by their analysis and again

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the raw data wasn't available.

DR. PORTIER: A one-way ANOVA on 55?

DR. FURLOW: I'll show you, you can look at it and decide.

DR. HEERINGA: We'll have citations for those papers and that work?

DR. FURLOW: Yes, I'll add those.

DR. HEERINGA: Okay. At this point in time I think we're making very good progress so let me suggest that we take a fifteen minute break and we will reconvene at 10 o'clock.

(WHEREUPON, there was a recess.)

DR. HEERINGA: Welcome back everyone to the second half of our Thursday morning session of the FIFRA Science Advisory Panel meeting on the Potential for Atrazine to Affect Amphibian Gonadal Development.

One administrative note, the panel has been provided with a packet that I believe contains the draft manuscript or report of the re-analysis of the Carr, et al data from 2003, so that's available and I presume it's also part of the docket too for this meeting.

At this point we have made very good progress in the review of the charge questions. There are some additional questions that Doctor Steeger

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brought back this morning for clarification and we'll also get to those, certainly before the end of the discussion this morning.

But I'd like to move on to charge question number 12 for the panel. Ms. Peace, if you would read that into the record please.

MS. PEACE: In its 2003 white paper the Agency proposed a research approach using focused empirical laboratory studies based on the initial investigations with Xenopus laevis, potentially followed by selective confirmatory laboratory studies with frog species native to North America.

However the 2003 SAP did not identify any important differences between amphibian species to conclude that any affected development and/or mechanistic processes observed in Xenopus laevis would not be applicable to indigenous species.

Please comment on the Agency's recommendation that data derived from Xenopus laevis in the studies evaluated for this review are sufficient to conclude that additional testing with indigenous species is not warranted.

DR. HEERINGA: Doctor Skelley.

DR. SKELLEY: David Skelley. Let me start by acknowledging Doctor Steeger's comments this

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morning about the challenges that EPA faces in trying to maintain a healthy environment for the more than thousands of species that live out there, hundreds of thousands if not millions.

And having acknowledged that I guess our job as dumb scientists is just to give you our best read on these questions and allow you as risk assessors to decide which of our concerns and criticisms are worthy of notice.

So having said that I have to disagree with the conclusion that testing with native North American species is not warranted.

The Agency's decision is based on the presumption that Xenopus laevis is a suitable surrogate for native North American species. However there are reason to question such a conclusion.

Unlike North American species, Xenopus laevis is a fully aquatic amphibian in both larval and adult stages. Aspects of its biology are suggestive of pedomorphosis, that is the retention of larval characters in the adult form. And again this is unlike North American anurans.

These and other points were raised during the 2003 SAP meeting as well. I guess the Agency's question to this SAP suggests an interest in a

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more specific response and I'm going to rely on my co-discussants to add to what I'm going to say.

I'm going to focus on one example of a comparative study between Xenopus laevis and native species Rana pipiens that Doctor Bob Denver, a member of this SAP authored. The study is entitled, Developmental Changes in Interrenal in Anuran Amphibians, and I will include the full citation to this reference in my response, written response.

The research focused on the development of responsiveness to stressors by the hypothalamal pituitary interrenal or HPI axis.

During development tadpoles of different stages were subjected to one of two stressors, either shaking, physical agitation or injection of adrenal corticotropic hormone, ACTH or a control treatment.

The investigators then measured whole body corticosterone concentrations or sorry, whole body content as an index of HPI activity. The patterns of whole body corticosterone content during development differed strongly between the species.

Corticosterone content in Rana pipiens was low during pre-metamorphosis and pro-metamorphosis and then increased greatly during metamorphic climax.

By contrast, corticosterone content was

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at it maximum in Xenopus during pre-metamorphosis and then declines in pro-metamorphosis and then increased again during metamorphic climax and remained high.

While both species responded to experimental stressors, the pattern of response differed. As an example, elevation of corticosterone content in response to ACTH injection was maximal in Rana pipiens in pre-metamorphic stages and decreased in later stages.

In Xenopus laevis elevation of corticosterone in response to ACTH did not differ statistically among stages.

And I don't present this example to suggest that this bears directly on any specific hypothesis about gonadal development, but it does suggest that an axis that is involved in development, the HPI axis, is affected and affected differently in these two species by stressors.

In the following quotation from the discussion of the paper the authors compared the responses of their focal study species to other North American species that have been studied. They say, "While changes in whole body corticosterone content in Rana pipiens follows those observed in the blood of other species, (and that's North American species),

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Xenopus laevis exhibits a somewhat different pattern. Our findings with Xenopus laevis largely confirmed those of Cloross and colleagues who reported whole body corticosterone content to be highest at early lembut stages but decreasing to lower values during pro-metamorphosis?"

During this SAP we have heard evidence that the tendency to form testicular ovarian follicles may differ among populations within the species Xenopus laevis. Based on our knowledge of variation among species in response to environmental stressors, it is reasonable to predict that specific differences in response to stressors in important end points will also exist.

Concerns about ecological relevance to North American species and ecosystems prompted the 2003 SAP to suggest that studies of native species be carried out as early as possible and those concerns remain.

DR. HEERINGA: Thank you, Doctor Skelley. Doctor Green.

DR, GREEN: So to address the question specifically that additional testing with indigenous species is not warranted, I spent some time yesterday afternoon on the web.

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In the short amount of time that I had, in doing very simple searches like amphibian comparative toxicity studies on amphibian and Xenopus laevis versus Rana pipiens, and just those simple database broach net casting searches can turn up three to four papers on developmental differences between Xenopus laevis and Rana pipiens, neuro plate forms for example, differences in acidity of the water that would prohibit the development of Rana pipiens and not Xenopus laevis.

And so I think those differences are well characterized in the vertebrate and embryology literature that will take time to map out and form a comparative table. But it's certainly out there.

I also came across another useful URL which is a database and there is a movement about amphibians, there is a movement afoot to address the issues concerning species' differences in susceptibility to exposure to different stressors.

And I'd like to just read a short paragraph here.

Researchers are finding that there are wide variations in tolerance levels among amphibians, even between closely related species. And they cite a references, Bridges, et al in 2002 which I'll make

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available when we write our report as well as this URL. Therefore conclusions drawn from studies on only a few species cannot reveal the full effects of potentially harmful chemicals to amphibians in general. And this reference cited at the end of this sentence is, Diamhed and Mitchell in 2000.

And then they go on to support differences that are known between various species of Leopard frog tadpoles and boyo tadpoles to different chemicals from copper to PCP to permethrin. Additional in formation in this particular URL, they do provide a very superficial overview of species' differences in response to chemical contaminants. There's nothing in this particular document that reviews the quality of the papers that have been cited here as references.

But they do bring up important points that there are, even within species, very different responses in terms of sexual development and LC50's to common contaminants, heavy metals, coal ash and whatever. Xenopus is frequently used in this list as is Rana species and the Bufo toad.

So I will make all this available when we write our report but I just want to reiterate what David said, is that I think that we cannot in good conscious say that studies with Xenopus laevis alone on

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the Atrazine of ours, to make the statement that studies on indigenous species are not warranted.

And that said, I can certainly be sympathetic with the Agency about the logistics and the practicality of trying to conduct these studies on species which may be in danger and certainly on species which would have to be wild caught and then protocols developed in the lab to try and grow them up and keep them alive during the studies.

As a laboratory animal veterinarian I recognize the difficulty with this and the mortality will be high and certainly it would not be good for the environment to go and collect native indigenous species and try to do this. The protocols simply aren't out there.

But nevertheless I think it would be appropriate to revise the wording on this particular point under 12a to reflect what I believe is the general consensus from the SAP, that additional testing would be highly desirable in native indigenous species.

And other than that I have nothing else to add.

DR. HEERINGA: Bruce Pauli.

MR. PAULI: Bruce Pauli, Environment Canada. I certainly concur with both Doctor Skelley's

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and Doctor Green's comments and I do also want to recognize for Doctor Steeger that amphibians are not typically included in risk assessments for pesticides and it's been an ongoing challenge to try and get that to happen.

On the sort of I guess biodiversity side of things we would be interested in having amphibians included in the pesticide regulatory system and I think it's to a great credit that there's so much attention being paid to amphibians in this particular issue.

Just very briefly, I would also like to comment that we can start to study native species, including Rana pipiens, Leopard frogs, we do now have a certain amount of understanding of these animals in the laboratory. We have ongoing breeding efforts for them going on as we speak so that we don't have to take animals from the wild.

And these protocols are being worked on and developed on an ongoing basis.

Certainly there are limitations in terms of the number of species that could be included in a pesticide risk assessment for regulatory purposes. I think we still need, in order to protect the resources that we have, to make sure that we include native species in those risk assessments.

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And for the remainder, just to preface my comments, for the remainder of my comments I'm going to be talking to a certain about differences in species' sensitivity.

Following on Doctor Skelley's talk and the information we just heard about differences in sensitivity, not in terms of a difference between Xenopus and native species, but we've actually done a little bit of work in looking at different native species.

Despite the fact that we can probably assume that some of the mechanisms are conserved and development pathways are similar between species, between Xenopus potentially and native species, we do have some data on native species and gonadal development.

And we have conducted a study which gave us some evidence that even native species can respond differently to compounds that influence gonadal development.

So this is not, we're not trying to address, I'm not trying to address here the difference in gonadal, in affects on gonadal development between Xenopus and native species, this is just between two native species. It's a paper that was published in

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2003 in Environmental Toxicology and Chemistry and I'll get the citation in my written comments.

In that work what was studied was gonadal differentiation in two native frog species. This was the Northern Leopard frog, Rana pipiens and the Wood frog, Rana sylvatica. And these two frog species were exposed to estrogenic and anti-estrogenic compounds.

Basically the study was conducted to try to determine whether or not in a laboratory situation, given exposure to an exogenous compound that might influence gonadal development, could we see impacts on the native species? And this was basically getting familiarity with these compounds that might influence gonadal development, differentiation in native species.

The studies assessed the response of the two native North American amphibian species to exposures to estradiol, Nonylphenol, and aromatase inhibitor and anti-estrogen. Various end points were assessed histologically and in the end it was concluded that the Northern Leopard frog in comparison to the Wood frog, Rana sylvatica, was much more susceptible to sex reversal and development of inter-sex gonads following these laboratory static exposures, than were the Wood frogs.

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The Wood frogs did show alterations in the gonads but these were much less traumatic than those that were seen in the Northern Leopard frog.

So we do have a basis of information with which to do some studies on native species. I think we're interested in obtaining information on native species, possibly more information on native species that are exposed to Atrazine in order to, as we say, try to potentially protect the environment from the possible affects of Atrazine.

Thank you.

DR. HEERINGA: Comments from other members of the panel on this particular question? Yes, Doctor Patino.

DR. PATINO: Reynaldo Patino. I would also like to qualify my comments by saying that I guess our job here is to have a scientific discussion about these issues and EPA's job is to just take what they think is appropriate in the context of their mission.

But I think I agree and also to some extent, perhaps a minor extent, disagree with at least one of the comments made, and that is that, you know, it is very, you know, it's not surprising that there are species differences. My understanding in this case was that the hypothesis being evaluated was one of

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feminizing affects and I do remember the table or the figure that was shown that on the scale of, when you look at that feminization, the sensitivity to feminizing substances, and I don't know what the substances were that were used in those studies, but there was a table shown that Xenopus was on the sensitive side. If you're looking for feminizing affects it's really not a hard species to see that phenomenon when you expose them to a feminizing agent.

So in that again I recognize there is species differences and depending on what your end point of interest is, Xenopus may be a bad species to be looking at, or not an appropriate species.

But in the context of feminizing affects what I saw in that figure that was shown, I think it was the EPA that showed that figure, that Xenopus seems to be a sensitive species if that is your interest.

Now the question I have, another question, a follow up I guess is if fishes are really the model that is used for determining aquatic affect, and I was not present, I was not part of the panel in 2003 but I do know, and again I don't know the quality of the studies because there's only abstracts, but at the last CTAC meeting in Montreal there were a number of posters that were presented by people from my own

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agency that showed that we're looking at Atrazine affects on fish and there were some affects if I remember correctly. But again I hesitate to rely on those studies because they have not been published.

But I'm just bringing them to your attention, that there are some studies, recent studies, that as far I can tell, I did a search, a recent search, have not been published but they're showing some affects of Atrazine I guess on fish reproduction using probably some models, you know, models that maybe are the ones that the Agency is using for assessing affects in an aquatic environment.

So I just wanted to bring that to your attention.

DR. HEERINGA: Doctor Steeger.

DR. STEEGER: Just to comment, the Agency is aware of the presentations that were presented in CTAC in Montreal last year. And on at least two occasions now we have requested access to the data to better understand it and we have not received those data.

DR. HEERINGA: Comments from other members of the panel on this particular question? Yes, Doctor Delorme.

DR. DELORME: Yes, I agree with pretty

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well everything that David, Bruce and Sherrill said, and I just want to point out that from the 2003 panel there was concern at that time that although Xenopus was a good model, there needed to be some sort of information to allow bridging to native species.

And I appreciate Tom, that surrogate test organisms are used, I work in the same area as you, but just a note that all those surrogates that are used currently are North American species.

And I also appreciate that we're probably on the front edge here for amphibians. The reality is as you've stated they're not part of the normal data packets that we would receive when we do our pesticide risk assessments.

We do use a number of assumptions in order to cover off amphibians in our risk assessments. But perhaps maybe it's time that we take a look at those assumptions and whether or not they're valid.

I know for example that within Environment Canada there is a researcher who is doing side-by-side acute toxicity tests between to see whether or not there is a concurrence.

So, you know, I appreciate that but I'm from the 2003 panel and if you go over the responses from the panel there are a number of indications there

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that stated that there was a concern there.

DR. HEERINGA: Yes, Director Williams.

MS. WILLIAMS: Thank you. Obviously amphibian, the whole arena of amphibians is one that's not been well researched, at least not for our regulatory context.

And it's something that we actually have on our mental research agenda that needs more research.

But I guess one of the things that I want to kind of probe here a little bit is whether you can help me understand why for Atrazine in particular this would be a recommendation, given that the statements that developmental pathways and mechanistic things are probably similar among frogs, amphibians, versus whether we're talking here about a more broad agenda of research on frogs and amphibians.

Because I guess what I'm trying to get a firmer grasp on is what additional testing would do in the context of the Atrazine action that we're studying and trying to take as opposed to what additional tests would do to give us more broad information about, you know, overall susceptibility of different species and subspecies to chemical stressors I guess is my question?

DR. HEERINGA: Director Williams, with

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that question are you referring to gonadal development and the apical end points that we've considered

MS. WILLIAMS: Yes.

DR. HEERINGA: or more generally in terms of reproductive success and population?

MS. WILLIAMS: Just the issue on the table here, gonadal development.

DR. HEERINGA: I think question 13 opens it a little bit more, but Doctor Green?

DR, GREEN: I'd like to clarify, you made the statement at the beginning that developmental processes were the same amongst all amphibians and I think the panel has just presented documentation that it's known that it is very different between different amphibian species, and within the species itself there are differences.

So why would that relate specifically to Atrazine and susceptibility differences? At some point along the way during metamorphosis there may be points where say Rana pipiens is much more vulnerable to a stressor such as Atrazine. They may stay in a particular stage such that the exposure is longer during that period which would results in changes in gonadal development. But you might not see it manifest in another species, or manifest in exactly the same

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way.

So I think that there is enough evidence and a long history in both Rana pipiens and Xenopus laevis development and embryological studies that they are very different within the amphibian class.

MS. WILLIAMS: Well I appreciate your explanation and just so you don't think I was making it up, I think it was maybe Doctor Denver who said, and I wrote it down when he said it, that developmental pathways and mechanisms are probably the same among species.

So maybe I took it out of context, I apologize if I did, I wasn't trying to imply something that wasn't said. I may have taken it out of context.

SPEAKER: Could I clarify?

MS. WILLIAMS: Sure.

SPEAKER: So we are, we including us, are descended from a common ancestor and

MS. WILLIAMS: That's one theory.

DR. HEERINGA: That question is not on the table.

SPEAKER: Okay, but that's one

MS. WILLIAMS: I apologize.

SPEAKER: the point is, and this was made in the 2003 SAP was that the panel at the time did

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not have evidence that there were significant differences between Xenopus laevis and native species like Rana pipiens that would preclude the use of Xenopus laevis as a model organism.

MS. WILLIAMS: Okay.

SPEAKER: However, it was also pointed out in the same paragraph that there weren't sufficient data to exclude the possibility that there were difference, important differences.

MS. WILLIAMS: Uh-huh.

SPEAKER: And as has been discussed here today, clearly there are.

MS. WILLIAMS: Thank you, I appreciate the clarification.

DR. HEERINGA: Bruce Pauli.

MR. PAULI: Bruce Pauli. I think also we were asked whether or not there is any specific reasons to look at amphibians in relation to exposure to Atrazine and I think that's what you were saying, are we generally talking about amphibians in the risk assessment paradigm or are we talking about a specific need to look at amphibians because there may be interest in them with respect to Atrazine specifically?

And I think the latter is the case. I think we have gone through, there is suggestive

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evidence I might call it at this point, that because these animals appear to be able to be influenced by their exposure to exogenous compounds that would influence their sexual development and differentiation. And there is a possible unproven mechanism through which Atrazine might influence this, either aromatase or alpha reductase or something like that.

We have a specific interest in examining these species that are possibly sensitive to this insult as their response to exposure to Atrazine.

MS. WILLIAMS: Thank you.

DR. HEERINGA: Doctor Delorme.

DR. DELORME: The risk assessment that we use suggest that in order to have affects you have to have exposure. I don't think there is any argument that large portions, or a lot of the water in the United States and in areas of Canada have an Atrazine presence. So I don't think it's a potential for exposure, there is exposure there in a lot of frog habitat.

And perhaps, it's perhaps an unfortunate coincidence that it's Atrazine in frog gonadal development, but I mean as I said before I think we're, you know, on the front edge of something.

MS. WILLIAMS: May I?

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DR. HEERINGA: Director Williams, please.

MS. WILLIAMS: Thank you. Yeah, I obviously don't argue that at all, that's been an issue for a long time. It's in a lot of different places in the water habitats.

I guess more specifically my question, maybe I stated it too broadly, was that we obviously have a concern and there was hypothesis that Atrazine was going to result in certain affects in amphibians and we've tested the hypothesis and our conclusion anyway is that the hypothesis was not supported by the data.

And so I guess what I'm wondering is kind of, if you go down that line, then I mean if we do another frog and it's not, the hypothesis is not supported are we going to, is it going to be suggested that we do yet another one and another one?

So I guess what I was trying to get at in my own mind was maybe the question is why maybe it's even more basic, why is what we have done to try to prove or disprove the hypothesis inadequate? And I guess I'm hearing that there are species differences that people are concerned about and there are new data or information that shows that perhaps the developmental pathways and mechanisms are not the same

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now.

So maybe I did get my answer. I wasn't meaning to suggest that, gee, why are worried about Atrazine in frogs?

So I apologize if that's the impression I left.

DR. HEERINGA: Steve Heeringa, let me make a comment. I think a number of us have commented that this is really the scientific process at work and in my own personal judgement it's probably about as good as it gets in terms of the sequence of what we're doing. But it is a process that continues.

And I think if you come to a panel such as this and ask, is the door closed, is the book closed, it's like saying is the scientific process terminated? And it doesn't.

And so I think the types of answers that you're going to hear from us represent, you know, pursuing that scientific process beyond the intermediate results. And we certainly have an excellent set of intermediate results.

And then the next question, is that the end of the process? No. And from our standpoint, from a regulatory standpoint you'll have to make decisions that incorporate the best available science at this

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point.
MS. WILLIAMS: Absolutely. My reason for asking is to try and frame kind of your, this group's perception or thoughts on what the degree of uncertainty is in all of this so we can make that kind of a decision. That's why I'm probing along those lines.
DR. HEERINGA: Yeah, excellent, no, that's, and that's clearly something that you need to incorporate.
So any comments, Doctor Skelley, on the magnitude of the uncertainty associated with the position?
DR. SKELLEY: David Skelley. So I did try to pick the example that I focused on carefully.
And to break it down, what Doctor Denver's study shows, and I'm sure you will correct me or hit me if I get this wrong, is that you have a hormonal axis in which the response to a stressor in one species shows a different pattern of sensitivity in another species and we also have a developmental time line when gonadal differentiate, limb differentiation, all that stuff happens in certain windows.
And so if the sensitivity and gonadal development line up differently, you could get a

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different response, perhaps a qualitatively different response. So we have evidence of that and that's the state of scientific knowledge.
Now, that doesn't bear directly exactly on proposed Atrazine pathways that ecologists understand, at least what I understand. Perhaps someone else could comment on that I guess. But it, to me that raises concerns that the biology of these species and Xenopus relative to North American species in particular is different enough so that I can't agree with the Agency's statement.
DR. HEERINGA: Other comments particularly relating to Doctor Williams' interest in assessments of the degree of uncertainty with this? Doctor Furlow.
DR. FURLOW: Well just a quick point and just to amplify something Doctor Skelley said, and that is there's a difference between how concerned the basic mechanisms are and how sensitive the animal might be to different exposures and the windows.
Just to make that clear, right, that we can say, yeah, I mean the basic, the SF1 and all these things, right? So all these activation pathways and biochemical pathways that say, am I going to be a testes or am I going to be an ovary, yeah, I mean the

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best evidence says they are concerned, but how sensitive those pathways are to pertivations and at what window, can differ, right?
So I just want to make that point.
DR. HEERINGA: Doctor Bucher.
DR. BUCHER: So coming at this from the mammalian physiology perspective I'm sure that everybody who has looked at Atrazine and the cancer data can clearly show the, or understands all of the work that's gone into determining the differences between the carcinogenic response of this rat versus the Fisher rat and the mouse.
So I think all of that literature is pertinent here. It simply points out that strain specific differences of response certainly exist for Atrazine.
DR. HEERINGA: Doctor Delorme.
DR. DELORME: I think that you hit it on the head when you brought in the word uncertainty.
For me that's what this is all about. How uncertain are we with the assumption that Xenopus is a representative model of North American amphibians?
And to my mind there is a lot of uncertainty there. As an environmental risk assessor in pesticides, you know, I find that there is a lot of

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uncertainty there, certainly for the other surrogate species. We have a body of data that we can go to and look at to compare a species' sensitivity distributions to look at relative responses to different groups of pesticides or chemicals.
So we can go to that and draw comfort from that or draw certain assumptions from that.
We don't have that in this case. And I think that's where some of the concern comes from, from this group, coupled with widespread occurrence of Atrazine in water, coupled with the fact that Xenopus is not a native species.
I think that some of that came out in the 2003 SAP as well.
Now, is there a way to deal with that uncertainty other than going and doing more tests? Possibly. Safety factors and what not, that's something that is part of the risk assessment process. It's not something that's necessarily part of the science process although it has been subject to SAP's before on the human health side.
So there are things to consider.
DR. HEERINGA: Doctor Green and then Doctor Steeger.
DR, GREEN: I just, this is more of a

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personal feeling as a scientist. You know, the original Hayes' papers reported affects in both Xenopus and Rana pipiens, correct? And after sitting through and listening to the data and looking at the DIC study, I feel fairly comfortable about the results that have been reported for Xenopus laevis.

And they're certainly not an endangered species all over the world and they certainly have been exposed to Atrazine in the wild. And there were problems with the study that we reviewed in Doctor Hayes' original study for both the experimental design for both Xenopus laevis and Rana pipiens.

When it comes to Rana pipiens I'd have to echo the sentiment of Peter, that I have a real sense of uncertainty about the original data and because it hasn't been tested or investigated further, I still am very uneasy about leaving it as what we found in Xenopus laevis would apply to Rana pipiens. They are a threatened species in certain parts of the world and they are exposed to Atrazine.

And is there a way to address that question now? What about Rana pipiens? Without spending three years and thousands and hundreds of thousands of dollars repeating these experiments. And so I'm thinking about that and we have a few more hours

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in the day, so if there's a way that perhaps, and I could only make the recommendation that with the help of statisticians, looking at the most reproducible, the most solid experiments that we could and the information that we got from the Xenopus laevis studies, maybe repeat those studies in a small subset of Rana pipiens.

DR. HEERINGA: Doctor Steeger.

DR. STEEGER: I can appreciate the concerns that have been voiced and recognize that the SAP is providing their scientific perspective on what would be the right thing to do.

I'm a risk assessor, I'm a biologist, I'm not a risk manager. We only tell them, we tell risk managers what our assessment of the biology and the environmental fate of a compound is.

And we also define, or try to define what kind of uncertainties there are with those estimates of risk and the effects. And in doing so we try to define additional, what data gaps may exist and what kind of studies would be necessary to address them.

And in 2003, working with the Office of Research and Development we defined some study elements that would address the sources of variability and

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potentially answer whether Atrazine exposure could affect amphibian gonadal development.

The protocol the Agency doesn't tell a study, it doesn't a registrant how to develop a protocol. We can suggest design elements but we cannot dictate to them what they ultimately do. That is their choice. Whether it flies afterwards is our choice but in this, in the case of this study we presented the registrant with a number of design elements that we would like to see incorporated. We worked very closely with them to make sure that they were incorporated, even though that's not what we traditionally do, but we did. And in that process it took two years to develop a protocol and test it, that would work on a regularly tested laboratory species.

And my concern is that I understand, I'm fully aware or cognizant of the idea that the SAP recommended in 2003 that indigenous species be tested. But after standing in those labs for the umpteenth time, listening to yet another problem that has come up with a regularly tested species, and recognizing how it would impact the outcome of the study, I thought, gees, if they bring up the idea of another species I am hoping that if we proceed down this track there will be some willingness to provide input on how to actually

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conduct the study with an indigenous species that will have mortality that falls within the range that's acceptable to this Agency and provide data that has some hope of being used to regulate.

I do not typically comment or commend a registrant on the conduct of a study but the contract labs that were used for these studies in my opinion did an excellent job in starting from scratch and pulling together a GLP study that may serve as the paragon of amphibian studies for looking at this particular end point.

That might have been the luck of the draw. Whether they could pull it off for a native species has yet to be determined. But I suspect that if it took two years to pull this regularly tested species study off, I can't begin to guess now many years it might take to pull off one with a native species.

DR. HEERINGA: Well let me throw that challenge back to the panel. It's a fair question. I mean clearly scientifically and ecologically there is a strong interest or willingness to sort of extrapolate from Xenopus to all native species.

But the next question is, if we were to propose additional research is it feasible to conduct

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an experiment say without confounding mortality? It's an experiment of a type and quality that we've seen with the Xenopus study.

Doctor Green?

DR, GREEN: I won't belabor the point but Rana pipiens is a well established laboratory animal, frog model. It was only eclipsed in the 1980's by Xenopus laevis when cancer research and vertebrate developmental embryology studies came to the forefront in terms of funding.

So I do believe that there are well established protocols for Rana pipiens in the laboratory and Doctor Pauli might have some that he'd be willing to share.

Other species, aside from that I do agree, you know, it would take longer than two or three years to even set up the protocols such that you'd have enough live frogs at the end of the day you could experiment, only manipulate.

But I think Rana pipiens would not be out of reach in terms of what we know about them in terms of housing and husbandry and SOP's for their routine care.

DR. HEERINGA: Bruce, do you want to weigh in on this? Is this pie in the sky or is this

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MR. PAULI: Well I probably would lean towards Doctor Steeger in considering that a completely daunting task to established Rana pipiens in a manner similar to what was done with the DCI studies that we're currently evaluating here.

They will behave for you in the limited experience, relatively limited experience that we have with them. We have not, and I should emphasize this, ever had them in a flow through apparatus. And that is probably one of the things that would cause, you know, a fair amount of delays in terms of getting these things established.

In the current, with luck in the current set ups that we have, we have reasonably good success in both attaining fertilized egg masses in our laboratory, that's one lab only and taking animals from that stage through metamorphosis.

It's doable but again, these are static renewal experiments of a rather small nature given the resources that we have to do these experiments.

We have done it, we've done it on an annual basis for the last eight years. They, given good husbandry, acceptable laboratory conditions, you can take a lab, Rana pipiens tadpole through metamorphosis with fairly good success and acceptable

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mortality.

DR. HEERINGA: Doctor Skelley.

DR. SKELLEY: David Skelley. So in the last decade or so my laboratory has worked on, I think I just counted seven different native species, in all cases we're dealing with wild collected, usually embryos and reared in the laboratory.

I don't think the challenge, at least in the static renewal context is particularly tough in getting them to survive and rearing them to metamorphosis. I think that the protocols as Bruce Pauli mentioned, I think the protocols, or I guess it was Doctor Green mentioned, the protocols are out there to do that part, excuse me.

The distinction is in the ability to start experiments at any time of the year. That's routine with Xenopus. That's a bit more challenging with the native species. It seems to me that that would be the big challenge, not the actual laboratory rearing and feeding and so on.

DR. HEERINGA: Doctor Handwerger.

DR. HANDWERGER: I'm just wondering, what would happen if we repeated this whole study in Rana pipiens and came back to an SAP meeting in four years, three years, would we be then asking again the

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question, well, is this data adequate, do we need to do another species? You know, there are thousands of them and we've now done two or are some of us going to question the fact that we need to do a third and a fourth and a fifth? I mean where do we end, what is the point at which we're all going to be satisfied that Atrazine use does or does not have an affect?

So, you know, as a biologist, as a pediatrician and I, you know, I always to see completion in many, many things done, but only if at the end you can make a definitive statement.

And I don't know how we're ever going to be able to generalize to all amphibians whether we studied Rana pipiens or not, because we're still going to have this same fundamental question that there is variation. And maybe we just didn't pick the particular, you know, strain or whatever it is that's going to be susceptible to Atrazine.

And I'm sure that if you look, if there are thousands and thousands of strains, you'll find one that probably is susceptible just as there is probably one susceptible to glucose and anything else.

So I think the real question is, what is the end point? I mean if we do Rana pipiens are we going to be satisfied with that? Is that going to

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answer the question?
And I don't know the answer to that.
You people probably should know. I only work with homo
sapiens.
DR. HEERINGA: Doctor Miller.
DR. MILLER: I just want to make a brief comment that I am part of a research team that has had very good success taking through metamorphosis bullfrogs and, you know, I'm sure everybody has their opinion on using bullfrogs. But we have done that in flow through systems as well as static systems.
And in regard to bullfrogs there is a lot of success with Rana culture systems and I know that's not laboratory approved necessarily, but as far as growing them out there's a lot of information there.
DR. HEERINGA: Doctor Steeger.
DR. STEEGER: I think continuing on on the discussions with the species, native species testing, we've seen from the work that Doctor Hayes has done that even within Rana pipiens he's demonstrated affects in one case but in the next there is no affect.
And so we go back to the, you know, being hit or miss on whether we've selected the correct strain, so the logistics of pulling this off I think again are very daunting for a study that would meet the

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standards that EPA looks for from studies submitted for regulatory purposes.
Again, as Doctor Skelley has noted you have a limited time window in which to work with the animals. Their period for metamorphosis could be protracted and as the studies are extended in time the potential for errors occur. And as you've seen in the studies that were conducted for the DCI, and these were conducted by very well known contract labs, errors happen.
And the likelihood of them happening increases as the time of the study extends.
And again you're right, the Agency doesn't have to embrace your recommendations, they're just simply opinions. But we do take them to heart and my job is to try and put those recommendations into something that's concrete and workable that we can then hand over to the registrants and say we want to see these incorporated. And we also have to explain them to the Office of Management and Budget as to how do these changes, these additional studies affect the risk assessment decision to warrant the regulated community having to generate those data.
And where will this stop? Those are questions that I will have to answer before it even

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goes out the door. Does this study have any likelihood of success or are you just having someone spend millions of dollars to prove that something can't be done to your satisfaction?
I only mention that because those are the realities that I have to face in moving forward with working with the recommendations that the panel makes. And I mention it to bring some sense of reality to where regulatory use of the information deviates from the science itself.
DR. HEERINGA: Thank you, Doctor Steeger. And I believe too. That's what I want to spend the little time we have as a panel trying to separate the sort of scientific motivation for these recommendations from the current practicality and logistical difficulty of maybe doing it.
Because hopefully that benefits the EPA in their consideration too.
Doctor Portier?
DR. PORTIER: As I listen to the discussion you've got to throw out a challenge that says, you know, how do I design better experiments to address these things?
And then Doctor Handwerger keeps coming back saying, how do we make the decision in the final,

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you know, do we experiment forever?
And I think EPA has developed protocols to handle these kinds of things for some of the animal studies where they go to more physiologically base models, right? They use the basic research and the understanding of what's happening in the, in specific organisms and they incorporate variability at that level to be able to handle the broader class of animals.
The problem I see with the amphibians and the frogs that we're talking about here is that we haven't done the basic research to understand the processes to be able to help you design the kind of confirmatory experiments that you need to close the door on some of these issues.
And, you know, for a lot of this I feel like there's a failure in our society to fund that basic research, that basic physiological research that'll help you answer these questions.
And I've been sitting here hoping that one of these guys would say, well, we've got this model of a frog and I haven't heard that and I've asked that individually and I still don't hear that.
DR. HEERINGA: Doctor Skelley.
DR. SKELLEY: David Skelley. First I'd

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like to reinforce Doctor Portier's comment that there is not enough money for research on basic biology frogs.

DR. HEERINGA: That's the other second major recommendation we'll submit.

DR. SKELLEY: Absolutely. The second point in response to the comment, when will there be enough species?

I think I'd look at that differently at this point in the development of EPA's interests and effort with amphibians.

If looking forward you wanted to bet on a horse that's going to help us understand risks to North American species and North American ecosystems, should we continue to invest in the Xenopus model which everyone agrees is very well characterized, or should the investment be made to switch to a North American species?

It's my sense, and I'd be interested to hear what other panel members think, that the effort expended to develop a North American model makes a lot of sense in the context of Doctor Delorme's comment, that other surrogate species that are used in North America, both in Canada and the United States, are North American species.

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We have some very nice frogs in this country and I know that we can work out the details to figure out how to do good lab based culture and then lab based experiments that get results that everyone can be proud of.

And I think there is some hope that those results will be more generalizable to other North American species.

I won't raise the common dissent issue again, but certainly Rana pipiens as one example is a member of the dominant frog family in North America and Doctor Denver's paper that I quoted from earlier noted that for that particular physiological pathway, the results were congruent, not just within Rana, but between Rana and another family of Spadefoot toads.

So, you know, the little bit that we do know suggests that there is going to be more congruence and that if the future some SAP comes back at you with the interest in doing more species, if the work is being done on a North American species I think you'll be in a much stronger position to push back.

DR. HEERINGA: Doctor Green and the Doctor Chambers.

DR, GREEN: And just for the record, it was the original recommendation of the panel in 2003

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that Xenopus laevis be studied as well as Rana pipiens and I believe we recommended that those field, or those studies on that particular species, Rana pipiens, be taken up immediately, because at that time we recognized the utility of looking at a frog species that is on this continent and is indigenous to this country, or North America.

So where does it end? That's a really tough question but I think I'd have to concur with David in that we'll be in a much stronger position when the compounded question is tested on a species that is relevant to agriculture and the ecosystems in North America.

DR. HEERINGA: Doctor Chambers.

DR. CHAMBERS: I think Doctor Skelley made some very, very good points a few minutes ago and, you know, certainly it seems like ultimately we need to study some frogs that are more relevant to our situation here in North America.

However at this point in time if those procedures are not established enough to give you the type of data you need in a regulatory context to have the same sort of quality that you're getting out of mice, rats, rabbits, Xenopus and all, then it seems like it would premature to demand those types of

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studies until the protocols and the procedures were really established enough to have confidence that the studies could be run accurately.

DR. HEERINGA: Yes, Doctor LeBlanc.

DR. LEBLANC: During the 2003 SAP meeting I don't think anyone was thrilled about doing the proposed studies with Xenopus, but I think we all recognized that it was the available model, that the studies could be done in. And we threw in that caveat, we need to look at an indigenous species as well.

My recollection was not, and but I could be wrong, was not that we do it immediately but that we do it as soon as possible. And I think the two are different. I think we recognized that it wasn't possible to work with a Rana species.

So I think the point is the Xenopus species is currently the most appropriate species but a significant level of uncertainty remains, having tested only that species.

As to how many species do we test before we get the answer? If we keep getting negative answers I suppose that's comforting but again it's hard to prove the negative.

So that's an Agency decision, when do we have enough information that we can say, okay, we have

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enough information to make a valid decision?
And I think one of the things that everyone's struggling with right now is the Agency is struggling with the issue of whether or not that decision can be made with only a single species and not just a single species, but a species that's not necessarily representative. Or the uncertainty with respect to the degree to which it's representative to North American species.
DR. HEERINGA: Steve Heeringa. Just a personal comment again going back to my earlier comments about the scientific process.
Our discussion here is post hoc of some findings that are predominantly null with respect to the affects of Atrazine on Xenopus. If we had conducted the experiment and the experiment had turned out to prove major dose response affects, substantial affects I think conclusions to progress forward would have taken a different path.
But in the presence of a predominantly null results from a well designed experiment on Xenopus, now we're still left with this secondary question, it's a step forward.
So again I think the steps taken in this process in terms of the resources expended and the

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effort put into that protocol were appropriate.
But now that we are sitting here with again a predominantly null result on this particular hypothesis test for Xenopus and the door is not completely closed, but it then leads us to this other question of the species difference.
Doctor Bailey.
DR. BAILEY: Ted Bailey. I am listening to the discussion here about this species or that species. I don't even know if this is possible, but if you design an experiment maybe you should have two or more species in it so you can make this comparison among species.
That would be better than doing one experiment with this species and a second experiment with that species.
DR. HEERINGA: Doctor Delorme.
DR. DELORME: I think I'd have to agree with Doctor Heeringa's remarks that if we had had positive results would we have had a different outcome on this? And it's a difficult question but I think the fact remains that there is a considerable amount of uncertainty and I think that's what's causing some of us a little bit of angst.
And in the end EPA is the one and the

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Agency is going to have to deal with that uncertainty.
As I said before there are other ways to deal with it. Certainly we deal with it when we use other surrogate species. It's not to say that in the future data is not going to be developed for Atrazine on other data species voluntarily. Who knows?
But also for other chemicals or other pesticides as well. And as I said in my earlier remarks which were actually part of my answer for question 13, right now we lack a good database that we can go to and draw comfort from to support the fact that Xenopus is a good surrogate species.
I don't think we're saying that it's not a good surrogate species, I think we have a considerable amount of uncertainty of where to place it with respect to the native species. And given the widespread contamination of water with Atrazine, you know, is that a reasonable conclusion to make based on a single species? I'm not sure.
DR. HEERINGA: Thank you. At this point I would like to move on to the final charge question if we could. And we'll have a chance to come back for a general closure and comments as well.
So I believe it would be Doctor Irene.
DR. IRENE: Charge question 13. Based on

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the available data provided by the DCI studies, the Agency has concluded that Atrazine does not adversely affect amphibian gonadal development. The Agency has further concluded that no additional studies are required to address the hypothesis that Atrazine adversely affects amphibian gonadal development.
Please comment on the Agency's recommendation that the current body of data is sufficient to refute the hypothesis that Atrazine by itself can adversely affect amphibian gonadal development, and that no additional data are required to address this hypothesis.
DR. HEERINGA: Doctor Delorme.
DR. DELORME: I think we just spent about a half hour answering those questions.
Anyways, I'm just going to reiterate some points that I wrote down and then I think the discussion will continue. I believe I'm going to have a fun time writing this one up.
I think that the data is sufficient to refute the hypothesis that Atrazine by itself can adversely affect Xenopus laevis gonadal development.
I think the real question is if the data are sufficient to extend this conclusion to all amphibians at this point in time with that uncertainty.

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We acknowledge the use of surrogate species as an efficient and logical approach and generally accepted. We further acknowledge that current toxicity data related to amphibians is not a specified data requirement. So we are really on the front edge here.

And there are certain challenges faced by the Agency and the registrants with respect to conducting amphibian studies.

But in essence as we've already stated we're addressing concerns related to the uncertainty of refuting the hypothesis.

Unlike other tests or other test organisms a body of knowledge and research related to these types of affects in other amphibian species for a range of chemicals does not exist to support the assumption.

So if you make an assumption that's based on uncertainty and some of the factors that we've already talked about is the relative sensitivity of different species is unknown, the different ecologies of species and how they're going affect outcomes, the nonrepresentativeness of Xenopus to native species, that's atypical ecology, totally water living. And specifically with respect to Atrazine as I previously

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pointed out, the widespread occurrence in water.

So there's all these factors that are going into this that are adding to the uncertainty.

You know, I wrote down here before the previous discussion that at a minimum and consistent with the recommendations of the 2003 SAP, the Agency should consider tests with a native species for accuracy.

And I think we recognize the difficulties in conducting studies given our current level of knowledge and technical expertise.

And as I said just before we started answering this question, I think in the future we're going to see some of those techniques and methods and protocols develop for native species, we're on the front edge of that.

And perhaps in another 10 or 15 years there will be more evidence, pro or con for this issue. It's going to take time, I recognize that.

In the interim I'm not sure but I'll go to the other discussants, there are several of them on this and see what their input is.

DR. HEERINGA: Doctor Green, Sherril Green.

DR, GREEN: I think I have nothing to add

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at this point.

DR. HEERINGA: Doctor LeBlanc?

DR. LEBLANC: I agree with everything Peter said. The only thing I want to emphasize is that any well executed study succeeds in answering the questions that are originally posed for which the protocol was defined to address those questions and at the same time raises new questions.

And I think that's what we're seeing here.

And it then becomes a judgement call on the part of the risk assessors, the regulators as to whether the strength of the answers that were answered are sufficient to make a judgement, or whether the unanswered questions are sufficiently important that more studies are warranted.

And that's a judgement call. It's a very difficult question to answer and I know it's one that you're, as the Agency is asking for advice on now from the experts in the field. But it is difficult to answer.

I think that many uncertainties were raised over the course of this SAP meeting and really the only one that I felt that the committee felt was of significant uncertainty is the issue of species

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sensitivity. And I think with respect to the conclusions of the DCI studies we can state with some degree of certainty that Atrazine does not affect gonadal development in Xenopus laevis at concentrations as high as 100 parts per billion.

We can't make judgements about concentrations higher than 100 parts per billion in Xenopus laevis and we can't make judgements about concentrations lower than 100 parts per billion in other species because we simply don't know.

And again that's a judgement call. One has to look at the information that's available with respect to species' differences in sensitivity, the likelihood that there might be an affect. Certainly the most scientifically sound and comfortable approach is to test another species and see what the information says. If that's not practical, if it's not feasible, if it's not tenable at this point in time for scientific reasons, then alternative approaches need to be taken.

And for example Peter mentions safety factors as an approach that's often taken.

DR. HEERINGA: Doctor Skelley.

DR. SKELLEY: David Skelley. So I agree with the comments of the prior discussants and I just

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want to add one point along a different line.
The 2003 SAP considered two primary lines of evidence in making its recommendations. The first involved laboratory based evidence that Atrazine exposure was related to abnormal gonadal development and other responses, and that's what we've spent most of our time talking about.
The second line of evidence was based on the detection of gonadal abnormalities in wild populations of amphibians. Since 2003 very little new evidence has emerged to evaluate the role of Atrazine or other stressors in producing these abnormalities which are heterogeneous in space and in some cases related to gradients of exposure to Atrazine or other presiticides.
Given the possibility of inter-specific differences in response to Atrazine exposure, the lack of study on native North American species means that the role of Atrazine in producing abnormal development in field populations of native North American species remains unknown, or at least uncertain.
Even if the Agency concludes that laboratory studies provide no basis for further exploration of the Atrazine hypothesis, these observations of natural populations remain unexplained.

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They are still there and we still don't know why.
And in thinking about the second line of evidence I'm reminded of the story of the man who is searching under a streetlight for his, on the ground and somebody else walks up to him and says, well, what are you doing? He says I'm looking for my car keys. And the person asks, well, did you lose them over here? And he said, no, I lost them over there but it's really dark over there.
And I think that while it's, it can be comfortable to stick with a model system, if we're really going to figure out what's going on in North American ecosystems we're going to have to go where it's dark and scary and maybe a little more difficult.
DR. HEERINGA: Doctor Portier.
DR. PORTIER: I looked at this just slightly different. When you look at the statement it's pretty broad and you talk about adversely affect, and that, and what I've heard is that there are direct and potentially indirect affects. And so I think we have a pretty clear statement about a direct affect. But these indirect affects which I break into two parts, these degredates or transformation products, there seems to be a lot of uncertainty with what's going on there and it ties in with whether you're

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dealing with a flow through or a static system.
And the other one that was mentioned, and we really haven't talked about is the potential for a synergistic affect of this chemical and/or its degradate with other chemicals in the environment and what is happening there.
And I put a note here, cocktails based on water quality data from ag fields. So we all know that the water that's in the environment is not just parent Atrazine in H2O, it's a cocktail. And the concern that I have is that while we've tested direct affects, we haven't even begun to look at any of these indirect affects.
And with a broad statement like that it excludes all these indirect affects. So I'm just kind of putting on the table that I think a limitation in our hypothesis that we can make a positive statement on should have the word direct in it.
DR. HEERINGA: Doctor Schlenk.
DR. SCHLENK: Well this has been a very interesting discussion this morning Dan Schlenk, UCR.
I wondered again, this is, I'm more of a fish toxicologist but I wonder if we had these discussions 50 years ago when we were talking about utilization of the Fathead minnow if this was something

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that was bantered about quite a bit.
And, you know, the Fathead minnow is obviously a surrogate model, it's a native species, but if I want to look at genetic toxicology or how things affect the geno I'm looking at the Zebra fish. The Zebra fish is a well characterized model.
Now, would EPA use a toxicity test from Zebra fish to set a standard or to make a decision? I don't know.
I think the model that you select is based upon the question that you're asking, it always is.
And in this particular case I think the question you're asking is, are North American amphibians going to be affected? And in order to do that I think you have to in my opinion, have a comparison between your exotic species answering the question that you are, and in this particular case, is gonadal development, that question. This particular exotic model actually answers that question I think in that regard.
Can you apply those data to native American species? Maybe, but you can't say for sure until you actually test it in the North American species.

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So again I would concur again with what Peter sort of indicated in his responses that, you know, I think you have to kind of change the question a bit.

It definitely affects Xenopus gonadal develop or it does not affect Xenopus gonadal development. But does that mean that's it and the door is closed? I can't agree with that.

DR. HEERINGA: Other discussants, then Doctor Bailey.

DR. BAILEY: Yeah, I agree with Ted Bailey I agree with the previous comment. And again if you're going to make that comparison I think it will have to be within the same experiment. Otherwise you have what statisticians refer to as disconnect experiments in which you can't make any comparison. And if we can't do that we'll be back the next time we're here in the same position of trying to decide if you can have any test about, no difference between species.

So it's a very difficult problem to handle.

DR. HEERINGA: Comments from other panel members on this. Doctor Delorme.

DR. DELORME: Just following along on

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Doctor Heeringa's earlier observation that had the results been positive would we be as concerned?

Perhaps the panel would be concerned and maybe the registrant would have been a bit concerned and maybe at that point they would have said, oh well, maybe we should do this in a native American species to try and see whether or not there is concurrence of affects.

Just an observation. I mean there are different ways of looking at this. You know, it's a conundrum, I acknowledge that.

And again it's just the in part I think a coincidence of events that's happened. Certain papers that have been published in the past number of years have focused on frogs and frogs are now on the radar.

And I think from a broader context or a longer term perspective, you know, amphibians are, you're going to have to do something with them in the future.

At the very least we're going as regulatory agencies, I think we're going to have to go back and look at the assumptions that we can use fish as surrogates, at least for the in life water stages of amphibians.

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And but as I said it's on the public radar, it's on the scientific radar and, you know, our job is to do the assessment and make sure that the assumptions that we make here are valid.

And our assumptions with respect to uncertainty and our ability to make conclusions based on surrogate species I think are going to come under scrutiny probably.

DR. HEERINGA: Steve Heeringa, my comments earlier about the sort of post hoc nature of our conversations, I think obviously as a statistician we want the hypothesis to be followed through regardless, but from a regulatory standpoint and a practical decision making standpoint, the post hoc sort of view of this is different depending on the outcome of that first experiment I think.

So there's a little difference between the decision process and what would be a pure scientific process.

Additional comments? Doctor Green.

DR, GREEN: Could I just raise the question, when you referred to had the outcome been different in Xenopus laevis, do you mean that for certain these experiments would have been repeated in Rana pipiens?

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DR. HEERINGA: If the outcome had been different

DR, GREEN: Positive.

DR. HEERINGA: positive.

DR, GREEN: So we were able to show affects of Atrazine on Xenopus laevis gonadal development.

DR. HEERINGA: That point I hadn't actually considered, but I think the framework of the discussion here in the face of a positive, a strong positive affect of Atrazine on Xenopus laevis, that in fact the whole conversation would have been a review of that study and the EPA, I'm speculating that the EPA offices would have moved ahead on the basis of our judgement about the quality of that Xenopus data.

Now whether that would have led to other decisions, I don't know and that's probably neither here nor there. But I just wanted to make that point, that, you know, in the discussion and the motivation in terms of this additional step and apparent recommendation from this panel that we not think about Xenopus as a complete surrogate for the native species and that additional work on native species is warranted, I'd say we're looking at that decision after a set of experimental results and it has to have been

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different even when we looked at it in advance.
DR, GREEN: Maybe I could address Doctor Steeger. What is the standard protocol for the Agency if you do have a positive study say in one species, do you routinely move into a higher species?
For example if there are positive results in rats or mice, do you then repeat the experiments in dogs?
DR. STEEGER: Each pesticide has a standard set of data that are required of the registrant to submit for the purposes of registering the pesticide.
The human health studies contain a battery of tests across a number of species. As I indicated though for ecological risk assessments, while we make use of the mammalian tox data we have a very limited number of species, two birds, two freshwater fish, on invertebrate, one freshwater invertebrate and up to three saltwater invertebrates and one marine fish.
That's it. We do not, we have to draw our conclusions on ecological risk based on that base set of data and what we can glean from the open literature. Whether it concurs, conflicts, with our understanding of the acute and chronic toxicity of the

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chemical.
DR, GREEN: Thank you.
DR. HEERINGA: Additional comments on this particular charge question? Doctor Steeger, I think the panel has been fairly clear that there is an element of uncertainty that remains that will have to be considered. I hope we provided enough guidance for you to sort of calibrate that level of uncertainty, at least at a gross level.
DR. STEEGER: I do have some follow up questions beyond the ones that I asked this morning.
DR. HEERINGA: Okay.
DR. STEEGER: I'd like some clarification on the issue of the degredates.
Is the panel concerned about the exposure to the degredates themselves or the fact that it was a flow through as opposed to a static study?
DR. HEERINGA: I had that as point 3 from this morning but maybe it's different.
Would somebody on the panel, a member of the panel like to respond to that particular issue of the degredates and the flow through versus static test experimentation?
Doctor LeBlanc, will you lead off?

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DR. LEBLANC: I can try but I don't know that I'm the best person to respond because I wasn't terribly concerned about it.
But it was my understanding that not so much I don't think the issue was so much whether or not the degredates are themselves problematic because I think the issue was raised and I think it was resolved that if indeed the degredates are responsible for toxicity, then toxicity would be observed in the in vivo studies.
I think the issue was, as I perceived it, was perhaps ambiguities between flow through and static renewal studies could be explained in part due to degredates. That is, degredates are accumulating in the static renewal conditions and as such animals in those conditions are exposed to higher levels of degredates that might be biologically active and that might be why we're not seeing it in flow through studies.
That was my understanding. So if, and again if I'm wrong my personal take on that is I'm sure it's relevant because it seems to me that's an artifact of experimental design and that in my opinion the flow through study is a better design.
DR. HEERINGA: Doctor Denver.

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DR. DENVER: Yeah, so the issue that was raised I think yesterday and discussed was that we don't know if degredates or metabolites were accumulating in the static renewal, that were not present in the flow through.
But the other issue that was brought up was that we don't know if there are affects of individual degredates or metabolites on amphibian gonadal development.
There is literature that suggests that there are affects of these degredates on, as I mentioned prostate and pubertal development in rats and there are some other studies that suggest that there may be affects.
So that's why that issue was raised, that we don't know whether there are affects in amphibians. And we also don't know whether the affects that were potentially seen in the static renewal, whether you believe those affects or not, were caused degredates that could have accumulated.
So I don't know how to directly answer your question since we don't know whether these degredates or metabolites have any impact on amphibian physiology or development.
I guess that there are two questions

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there.
One is whether they do impact amphibian development and the other is whether affects that may have been seen in the static renewal, or people may see in the static renewal, are due to that accumulation.
So those are two, I guess two different questions.
DR. STEEGER: Are we agreed that because we have data demonstrating that the three primary degredates, DACT, DIA and DES, I'm sorry, it's just too much to mention those chemicals again, that they do form in vivo and that the mass balance if you will of chemical in and chemical out would have been occurring in the flow through study in the Xenopus and presumably that those animals would have been exposed to the degredates as well as the parent in the flow through study, and that the study would be accounting for potential affects of the degredate plus the parent in the Xenopus study?
DR. DENVER: Well I guess the question that goes back to the static renewal is one of concentration and if they have accumulated to a higher level than they would have in the flow through system and does that have an impact?
But I don't know the answer to that, but

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that is the question that is posed.
DR. CHAMBERS: This is Jan Chambers, I have a response to that too.
There's a little bit of a semantic problem here I think, because those of us who study metabolism would call those metabolites and degredates would be environment breakdown products in my opinion.

But as was mentioned yesterday I think the animal if it's producing metabolites is going to be exposed to those in the study and therefore if they are exerting any toxicity, then the in vivo study would demonstrate that.
However I think the concern level on this needs to be leveled against what the environment is accumulating. I assume you're doing some environmental monitoring studies on the parent compounds and the environmental degredates. If the degredates are prominent then there may be a greater level of concern. If they're pretty diluted then I don't know how much of a concern there needs to be about that.
But the metabolites really should take care of themselves in the in vivo study as being present in the organism.
DR. STEEGER: I understand this is Tom

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Steeger our understanding is that one of the primary routes of degradation in Atrazine is by biotic degradation and that the degredates are equivalent to the metabolites that the animal was forming, so that the exposure through a flow through study to the metabolites would likely be as great as they would have been in a static system, because they're the same compounds.
DR. HEERINGA: Doctor Delorme and Doctor Green.
DR. DELORME: I think if that's the assumption you're making you need to look at it with respect to what you see in the environment, okay? And I think that we've already made that comment.
And, you know, I think that Doctor Chambers has covered it off, saying that with respect to the metabolites, i.e., the in organism produced degradation products, it's encompassed in the design.
But I think the concern is those degredates probably have different physical chemical properties than the parent compound. They may be more or less persistent and therefore there could additional concentrations in the environment that come from either bacterial degradation or other biotic transformation.
And it has to be considered.

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DR. STEEGER: This is Tom Steeger again, so you would not be recommending that metabolites, if you indeed found that they were higher concentrations of the degredates in the environment, that a static renewal study would be required to look at that, you could do it with a flow through study.
DR. DELORME: I'll have to think about that. It just depends, Tom. You really have to look at the first part of the risk assessment framework is the exposure, right?
And given that we know Atrazine does, is out in the environment, are the degredates accumulating a little bit, are they sticking around a little bit longer than the parent? In which case they may have reached an elevated concentration than you would normally find.
I don't know, you have to look at the data.
DR. STEEGER: Right, but the question for approaching the study itself
DR. DELORME: Uh-huh.
DR. STEEGER: did not require a static renewal study to accomplish
DR. DELORME: No, not necessarily. If you had access to the degredates and the chemicals you

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could expose them to them.
And I think that earlier I suggested you might want to even look and see if there's any receptor assays that have been done to see if there's even any indication that they could interact with the endocrine system.
DR. HEERINGA: Doctor Isom and then Doctor Green.
DR. ISOM: I just might add, I think in the 2003, in our discussion at that panel meeting there was some concern about interaction of the degredates of the metabolites with the receptor system, estrogen receptor and there were some comments. They may even be anti-estrogens in activity. There was some, I think that was written up in the report also.
And there was so much concern about that comment on it that it was recommended that that be studied in a little more detail, or at least come data be generated there.
DR. HEERINGA: Doctor Green.
DR, GREEN: Just a point about the flow through system. In terms of degredates and metabolites and exposures, I feel pretty confident with a flow through system you have exposure and those exposures are occurring in the absence of additional stressors

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that relate to poor water quality, which certainly would maybe I wouldn't say certainly, would have the potential to intensify negative affects of the parent compound, the metabolites, the degredates.
So the fact that the studies were conducted in flow through systems I think the first study is a good thing, because we have a pretty clear picture of exposure in the absence of say nitrate and nitrite, ammonia and anything else.
So moving into a static system now where those compounds may hand around just a little bit longer, but in the presence of additional water quality parameters that are already known to stress frogs in captivity in the laboratory environment, might enhance the affects that they have.
And those affects would in terms of water quality may be closer to what happens in the environment in a static system versus the beautiful water quality that you get in a nice well managed flow through system.
DR. HEERINGA: Doctor Steeger.
DR. STEEGER: Not to beat this poor dead horse to death, but the difficulty that I have is that the Agency relies on very strict standards for conducting studies. And those studies are under, are

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derived from ASTM guidelines and ASTM guidelines, the most modern ones relay on flow through conditions because you are able to eliminate the confounding effects that can influence the outcome of a study.
At some point you lose the ability to detect whether it's the chemical or all these other factors.
And where we digress into how much our standardized studies reflect reality, that's an uncertainty that we, EPA staff scientists wrestle with on a daily basis.
But in order for us to move forward and be able to say with some reason of certainty that it is the chemical and not environmental factors that are causing the affect, we relay very heavily on flow through systems and not the static.
And that's why I raised this issue as if you felt that it was necessary to address the degredate issue, does it have to be addressed under static flow through conditions, or static conditions, because that is not consistent with our process.
DR, GREEN: Yeah, and I agree and that point is well taken. And I think if there is a way to expose them to degredates in a flow through system that would be ideal because then you eliminate all the other

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possibilities with all the water quality issues that come up otherwise.
DR. HEERINGA: No comments on this point? Yes, Doctor LeBlanc.
DR. LEBLANC: Just a quick comment. In reading over the charge questions I think the Agency was very careful to whenever they made a statement with regards to conclusions based on the DCI study and Atrazine and its affects on gonadal development, they seem to have gone out of their way to always state Atrazine, by itself, and you know, I think that qualifying statement is very, very important.
There are a lot of other, as I mentioned earlier, considerations that could impact the affect of Atrazine on gonadal development but I think it's beyond the purview of this SAP.
We can discuss them but I don't think it's part of the charge questions.
DR. HEERINGA: Doctor Steeger, we have several other issues, I wrote, maybe we could turn to the issue of the reliability study or would you have an order that you would like to pick up these final points?
DR. STEEGER: Tom Steeger. We can, I have hard copies of each of the items that were brought

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DR. HEERINGA: Great.
DR. STEEGER: up this morning.
DR. HEERINGA: Okay, good. Those will be distributed.
DR. STEEGER: I'm wondering, Doctor Heeringa, if it would better if we wait until after lunch to get into this?
DR. HEERINGA: Doctor Portier just suggested the same thing and since we have confirmatory replication, let's do that.
I have twenty minutes of twelve. Let's rejoin here at 1:00 p.m. if that suits. A good suggestion for everybody. A very productive morning. I think we'll be fresh to pick these up and any final closing items.
Thank you, Doctor Steeger. We'll see everyone at 1:00 p.m.
(WHEREUPON, there was a recess.)
DR. HEERINGA: Welcome back everyone. I invite you to return with us to the final I think afternoon session of our multi day meeting of the FIFRA Science Advisory Panel on the topic of the Potential for Atrazine to Affect Amphibian Gonadal Development.
At this point we have completed our

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panel's response to the 13 charge questions but we are revisiting some points related to the earlier responses, points of clarification.
And Doctor Steeger and the scientific staff of EPA have provided us I think with a list in writing of some of those questions.
And one of them we had addressed prior to the break which related to the degredates I believe of Atrazine and the potential experimental process that might be applied to study their affects. And I think the panel was quite clear in its responses to that particular follow up question.
Doctor Steeger, let me have you take them in the order that you'd like. There are some residual questions that you have, so if you would just point us to the question you'd like to address and we'll pick it up.
DR. STEEGER: Let me just start at the top of the page. That's with respect to question number 8. I'm unclear whether the panel in its final recommendation to the Agency is to require their review of the sub-samples of slides from the DCI studies or whether that is simply a added benefit that could be derived to help reduce uncertainty.
If it's the latter, if you feel that

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there is an actual need to conduct this pathology review board, is that based on uncertainty regarding measurements that were made or observations that were made relative to the apical end points or is it relative to secondary measurement end points such as aplasia and mineralization?
If it's the latter, has the panel determined the biological relevance of the secondary measurement end points and/or, how much would these secondary end points really have to change before conclusions regarding the apical end points would be affected?
DR. HEERINGA: Doctor Miller, would you like to address that first?
DR. MILLER: Debra Miller. Yeah, basically what we're going to do is recommend that you bring in two additional pathologists.
And the main reason for this is because you're doing it for regulatory purposes and you wanted to follow the general laboratory practices with quality assurance.
And to do that it's a good idea to bring in two additional pathologists.
And the sub-sample that we are talking about is the sub-sample of whole animals. And you'd

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probably need some statistical testing to determine the proper number that will give you what you need as far as the number of animals to look at.
And then in that sub-sample you take the whole slide set from those animals and using those same slides you have two other pathologists review everything. We're not breaking it out into, you know, primary or secondary things. Everything that was reviewed from those slides should be reviewed again for quality assurance. And should be read and the lesions scored in the same manner, using the same parameters.
And then as far as biological significance of the secondary end points, do we know that they are biologically significant or at what point they're biologically significant? We can't always say that but we also cannot say that they're not, because we don't necessarily know.
And until you look at them and include those scorings in your analysis, we're not going to know. So we need to see, how do the different scores factor in and do they relate to anything?
And then also just to go back to that, at what point are they significant? Until you test function we also don't know.
So you need to do both.

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DR. STEEGER: Thank you.
DR. HEERINGA: Doctor Miller I think was the lead discussant on that question too, so that response would reflect at least the tenor of the current collective response from the group.
Any other contributions from panel members on that particular question of clarification?
Go on to the next question, Doctor Steeger.
DR. STEEGER: With respect to question number 1, yesterday's discussion sounded as though panel members concurred with the Agency's evaluation criteria for open literature.
These same criteria were applied to the registrant's submitted studies as well. The panel also seemed to agree that the open literature consisting of both laboratory and field studies did not across multiple evaluation criteria meet the standards of acceptability.
It was unclear after yesterday's discussion though, whether the panel believes that the open literature continued to have some utility in refuting or confirming the hypothesis that Atrazine exposure causes affects on gonadal developmental affects.

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Yesterday Doctor Carr from Texas Tech University and Doctor Jeff Wolfe from Experimental Pathology Laboratories provided a brief overview of the re-analysis of the tissues which were initially reported as inter-sex animals. This re-analysis concluded that none of the animals originally reported as inter-sex were indeed inter-sex.
Therefore to our knowledge the only literature reviewed to date claiming to result in inter-sex is that of Doctor Hayes.
If the panel believes that open literature has some utility relative to the data call in studies, do they believe that the multiple lines of evidence are consistent with the outcome of the DCI studies indicating that Atrazine is not affecting amphibian gonadal development? And I understand that that would be more refined now based on earlier conversations today that Atrazine does not affect amphibian gonadal development in Xenopus laevis at concentrations up to 100 micrograms per liter.
DR. HEERINGA: With those qualifications as Doctor Steeger has just presented them, would anyone from the panel like to comment on this point in response to that question?
SPEAKER: Could I just get a

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clarification please on those data?
The re-analysis that was conducted on the Carr data set was on the animals that were originally classified as inter-sex. And there is another, according to the paper there is another data set which discusses discontinuous, a measure or at least a categorization of in my understanding, abnormal gonads which were classified in the study as discontinuous gonads. And it's also a significant end point in the study.
I was wondering if those data were, they were not re-analyzed because they were not classified originally as inter-sex. The data stands as a data set that suggests that there is some gonadal abnormalities in those animals at significance level of 25.
I just wanted to clarify if those two data sets are still being treated separately?
DR. STEEGER: We could have Doctor Carr or Doctor Wolfe come back up and present a more detailed presentation on what their analysis consisted of if the panel would benefit from that.
DR. HEERINGA: I'm turning to the panel here, Bruce?
MR. PAULI: I guess so, if we can just have a determination that, maybe even a clarification

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of what this abnormality, which I think it is, represents. It's a discontinue the categorization of those gonads was discontinuous and it's a separate data set. It's a separate data set from the inter-sex animals.
DR. CARR: I'm Doctor Carr, Texas Tech University. To answer the first question, we did not pull the slides that were from animals that were identified by gross morphologies, discontinuous testes and have those analyzed by EPL, just the animals that were originally scored as inter-sex.
And part of the rationale there was to try to harmonize terminology from 2001 which was when our study was done with some of the newer findings on how the term inter-sex is used.
The question about what discontinuous gonads were at the gross morphology level, the original description in the paper discussed this and it really has to do with uniform shape of the ovary, the ovary in Stage 66 animal is longer than the testes. Whether there is a uniform shape throughout the gonad at the gross morphology level in either the testes or the ovary.
So there might have been for example a testes with a small butt of tissue at the end or

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something that the readers felt was just not uniform in shape as a discontinuous gonad. And those were based on two naive readers who went through all of the gross morphology of the animals that were studied.

MR. PAULI: Bruce Pauli, so just to clarify then, those readers compared to the control gonads or their understanding of what a controlled gonad would look like, they might classify it as abnormal?

DR. CARR: They would not compare them to the controls because they were blinded to the treatments. They would just identify whether they look uniform in shape or were discontinuous as kind of an absolute.

You know, part of your identification is male or female and at the gross morphology level it's a pretty easy thing to do in Stagee 66 animals. So was the ovary normal looking in terms of its uniform shape, was the testes normal looking in its uniform shape or were there things that looked like they were butting off or discontinuous in the gonad.

It's not an end point that we know has any biological relevance at the point, at the time but it was something that they did write down and score in the raw data when they evaluated it.

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MR. PAULI: And yeah, I guess I'm happy with that. It clarifies that that's a separate data set and it's basically a, I guess you could say a qualitative score of abnormality and it's, there is a dose response in that data set with significance at 25.

But it is as you say a qualitative score based on a blind reading of

DR. CARR: Right.

MR. PAULI: the gross morphology of those

DR. CARR: Right.

MR. PAULI: -- gonads.

DR. CARR: Right.

MR. PAULI: And it's the inter-sex animals only that were reevaluated.

DR. CARR: Well

MR. PAULI: Those animals that were identified through gross morphology as potentially ambiguous sex.

DR. CARR: Right.

MR. PAULI: That were reevaluated at DPL.

DR. CARR: That's correct.

DR. HEERINGA: Other questions for Doctor Carr at this point on the research and the re-analysis or the review? Thank you very much, Doctor Carr.

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Back to the question. Bruce, have you had time to can we put you on the spot here?

MR. PAULI: Bruce Pauli. I guess the thing that I was maybe doing there, maybe not effectively, was there is certain I think when on Tuesday we discussed the possibility that there are some, what I'm calling suggestive evidence I guess, that there are some things going on.

And when the data was presented yesterday as a re-analysis of the inter-sex animals to take that bit of evidence away from consideration, I think it in my opinion it was important for me to try to understand what that actually meant in terms of this study and whether or not I know that we've already discussed this particular study and the fact that there might have been some water quality issues with it my interest I guess was to say, is there any other confirmatory or even suggestive evidence out there that would provide some information on whether or not there is an affect in amphibians?

And I think in this case there is data from one other study I suppose in my opinion that might have some suggestion that there is something going on in terms of exposure of these animals to Atrazine. And just to get some clarification on how that data set was

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more recently assessed was good for me to hear.

Thanks.

DR. HEERINGA: Additional comments from panel members on this particular question as to whether beyond the Hayes studies, whether there is any other evidence in the open literature that you would like to bring?

Doctor Steeger, I don't know if we have actually addressed this.

DR. STEEGER: So is that concurrence that the open literature is not, has little utility in refuting and confirming the hypothesis?

DR. HEERINGA: Bruce, I think

MR. PAULI: Bruce Pauli. I guess, yeah, I mean we've already agreed that the open literature has flaws and we agreed that the way that you evaluated that open literature was appropriate and that there are some methodological issues and things like that in the open literature.

So I guess in my opinion alone we'd have to agree that there aren't any open literature studies which would be useful to you based on the evaluation of the literature that you do.

Any further ones I'm not aware of. I was just trying to get a clarification on this one

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particular study which is as has been noted, is oft cited as something that provides to a certain extent a little bit of confirmatory evidence to Doctor Hayes' studies.

And to see that there is an affect, a significant affect in this study while at the same time recognizing that it isn't completely in consideration because of the methodological or the data quality issues, I think is something that I would like just to recognize, that the data set is there.

There's been a reevaluation of that data but not the entire data set which took away from what we're dealing with here is a question of whether or not we are seeing inter-sex ova testes or testicular oocytes in these animals exposed, whereas there is another question, can you see gonadal abnormalities? Does Atrazine affect gonadal development?

And I think in this case there is some suggestion that it did affect gonadal development. We've taken away the inter-sex animals by the re-analysis but there is some suggestion that there is some affects on gonadal development.

And then again, then we have to bring in these qualifications in terms of the way the study, the way the methodological flaws of the study or the data

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quality, the water quality issues had to be factored into the assessment of that.

So in the end I think because we have accepted the fact that the review and assessment of the studies based on the criteria that were applied to them were acceptable, then we're left with the one study, the DCI study to determine whether or not the hypothesis is true, that there is no affect on the production of ova testes in Xenopus laevis by Atrazine at the exposure concentrations that were assessed.

DR. HEERINGA: Would any other panel members like to contribute on this particular topic? Yes, Doctor Schlenk.

DR. SCHLENK: Yeah, I mean Dan Schlenk here I think as memory serves I think Doctor Denver had mentioned something about the fact that we wanted to not throw the baby out with the bath water, that I think some of the studies that were present should not be disregarded entirely, but be utilized as a comparison after the fact.

Correct me if I'm wrong, that's sort of what you had mentioned before.

DR. HEERINGA: Doctor Denver.

DR. DENVER: No, that's right, that was, the point that I was trying to make is that the fact

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that a study doesn't adhere to EPA standards for GLP it opens important significant questions about the validity of the findings.

But the point I was trying to make is that those 30-odd studies have, potentially have some data in them that could form a basis for developing hypotheses, not confirming or refuting the hypothesis.

I'm not sure if I'm being entirely clear. Is that

DR. STEEGER: Yeah, I this is Tom Steeger I understand what you're saying and yes, and that's why we're here is because there were sufficient data to formulate hypothesis, but at this point it's the Agency's position that based on those available studies and the flaws that were identified in them or the limitations I should say that were identified in them, we are in a position that we feel that the only study that we can use to test that hypothesis that Atrazine exposure results in affects on Xenopus laevis at concentrations between the level of detection and 100 micrograms per liter, have to be based on the studies that were responsive to recommendations made to the registrant in 2003 by both the Agency and the SAP.

DR. DENVER: Yes, and with that definition I agree.

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DR. HEERINGA: Next point, was there a

DR. STEEGER: That was it I believe.

DR. HEERINGA: Okay. Okay, at this point I think that we have addressed each of the charge questions but what I would like to do before we wrap up this meeting is I would like to go around the panel.

Doctor Portier just pointed out to me, I think in your notes, Doctor Steeger, just to make sure that we've covered everything, on the second page of your notes in reference to number 11, question number 11 follow up I have it as the tiered stage of testing from 2003 indicating going forward with mechanism studies only if apical affects were observed.

Does the SAP still support that recommendation?

DR. STEEGER: It's my understanding that, and correct me if I'm wrong, that the SAP does still support that recommendation.

DR. HEERINGA: Is that the consensus of the panel? Would anybody like to yes, Doctor Green.

DR, GREEN: Honestly I have to review the tiered stage testing that we proposed form 2003, so if you could give me just a minute to look at that, because was testing in indigenous species part of that tier?

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And I can't actually recall, I'll have to dig that diagram out. So otherwise I guess, is that part of the apical affect that we'd be looking at, the response of that species, the Rana pipiens?

DR. STEEGER: The mechanistic studies were proposed as a tier two study. The testing of an additional species for whether there is an affect or not would be a tier one study.

DR. HEERINGA: Okay, I think individuals are thinking here. Yes, Doctor LeBlanc?

DR. LEBLANC: It's certainly my understanding and I think it was the agreement of the SAP that tier two testing was warranted only if affect were observed in a tier one.

DR. STEEGER: Thank you.

DR. HEERINGA: Yes, Doctor Patino.

DR. PATINO: Reynaldo Patino. I have already said I was not part of the 2003 SAP but just generically I can say too that, just confirm that if there is no phenomenon to study the mechanisms, there's no reason to study mechanisms. I mean that's as simple as you don't have to explain why.

DR. HEERINGA: Okay, at this point oh, Doctor Denver please.

DR. DENVER: I have just one additional

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general comment. And that is that in light of the re-analysis I do hope that the published record will be corrected.

DR. HEERINGA: There was a distribution to the panel this morning under a cover from Syngenta and Doctor Carr as well with the I think draft report and I don't know whether that's actually a manuscript or just a report at this point.

So it's a good point.

At this stage what I would like to do is to go around the panel just to see if there are any additional closing comments that the panel would like to make based on their participation in this panel meeting or the materials that you have seen.

Maybe we can begin with Doctor Furlow.

DR. FURLOW: Right, so just to summarize some of the thoughts I've had earlier, the testing system that was devised and funded by the registrant is in fact impressive in its ability to maintain the animals in a healthy state and to get them through metamorphosis, and I think has real potential to serve as a paradigm for testing in at least one amphibian species.

One I guess nagging issue that I guess is mollified a little by the statements by the EPA that

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they will consider new data as it comes, but this is always an ongoing situation and I suppose if not us, someone will hold you to that I'm sure.

MR. WILLIAMS: I think our law holds us to that.

DR. FURLOW: Yeah, exactly. So, that there were observations that were not consistent between the two laboratories, but were in fact reminiscent of some of the findings that the earlier Hayes' studies had examined and reported on in terms of pigmentation and translucent gonads that we couldn't assign to a phenotype, but that's because we don't know enough about what that means.

You know, one can't help but think that it is still formally possible that above the 100 micrograms per liter that something is going on and I understand that at least that, you know, with this flow through system that this was a system, a situation where the animals were not particularly sensitive to Atrazine, and that's fine.

You can argue either, there's no evidence one way or the other to support that at this point.

But I just hope that, you know, the EPA and as you say, the law requires you to do so. We'll

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keep an open mind and keep looking at the open literature to see if that in fact higher levels may in fact cause gonadal issues, whatever that may mean for the animal and that if surface water or drinking water reaches those levels, despite the best practice management issues that I believe was sincerely presented by the Farm Bureau, et cetera, despite, you know, their best practices, you know, these things happen.

So I wish, I guess that sums up most of my concerns.

DR. HEERINGA: Thank you, Doctor Furlow. Doctor Denver, any additional you take a pass. Doctor Skelley? Bruce Pauli?

MR. PAULI: I agree with that and I think there's, I think that the 2003 white paper statements where there's insufficient evidence to either support or refute the hypothesis that Atrazine has affects on amphibian gonadal development, and we're now basing a decision or an evaluation on whether or not Atrazine alone causes gonadal inter-sex in Xenopus laevis and in the DCI experimental setup and there are slightly different things there.

And I think I agree with and I like Dan Schlenk's, Doctor Schlenk's statement that we don't

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want to throw out the baby with the bath water and I think I would concur that as evidence or as new studies are published, that it will be interesting to see how we can use those open literature studies and further assessment of environmental impacts.

DR. HEERINGA: Doctor Green? Doctor Isom?

DR. ISOM: Right, I'd just like to make a comment and commend the EPA and the registrant for conducting the studies and interpretations of them. I think that the conclusions are very logical and certainly have a great deal of bearing on future types of analysis.

With that caveat though I'd like to point out that there is kind of a, I guess uneasiness among the panel of a clear interpretation that answer the questions directly, the main question of whether there was an affect or was not.

I guess from just observing this as a scientist that we're really kind of stuck at a point where we need some really good basic science and we need to continue to monitor the field studies which I think have an important contribution in any of these pesticide management and decisions.

And we kind of got away from that, but

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in the past the SAP has considered toxicological evaluations of pesticides in the field studies so I guess you could say post-marketing did play an important role to continue to follow the toxicological analysis of the pesticides, and certainly that would be true here.

DR. HEERINGA: Thank you very much, Doctor Isom. Doctor Handwerger.

DR. HANDWERGER: I'd just like to say how much I appreciate the difficult position that you're in.

It's so, on the one hand reassuring to have negative data but negative data is often so difficult to interpret because there are so many reasons why it could be negative. I really think it's so difficult to make regulatory decisions based on negative data.

I mean in the field of good old homo sapiens of the position of the FDA approving a drug after it's gone through 500 patients and having the 501st and 502nd patient developing fatal complications.

It's really a very difficult position, giving you a little bit of a hard time. I hope it'll be taken in perspective. We realize the tremendous pressure on you and the difficulties. And the

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selection of an appropriate model is always a difficult situation. I've never seen a model system that couldn't be criticized except the actual species you're pertaining to, and then you can always find something wrong with that.

Hypotheses are only there to be tested and they're only as good as the next piece of data that comes along.

So thank you very much for this meeting.

DR. HEERINGA: Doctor Schlenk? Doctor Portier? Doctor Patino?

DR. PATINO: Reynaldo Patino. And I would just like to reiterate some comments I think I made earlier. And in the context of the way I understood our charge, my charge was to address or assess the evidence for Atrazine affects on amphibian gonadal development.

And, you know, using the I didn't comment when the question number 13 came up, but using the way the question was posed by the EPA that the first conclusion being that Atrazine does not have adverse affects on amphibian gonadal development, I think as a scientist I cannot answer that question in the, positively or negatively, I don't think there's sufficient evidence for that.

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But if you phrase the question as we discussed yesterday, does Atrazine affect Xenopus gonadal development within the range of concentrations tested, as it says during Stage 66, the answer is no, there is no evidence for that.

So I just wanted to make sure that I can answer some questions but I cannot answer others. It depends on how the question is phrased.

DR. HEERINGA: Doctor Delorme?

DR. DELORME: I just wanted to echo Doctor Isom's comments with respect to the quality of the review and the quality of the study that was done.

I think it's rare and given that this was the first attempt I think Syngenta should be commended as well as EPA for their review of the information and the presentation.

I also appreciate the position you're in with respect to trying to deal with the uncertainty and I look forward to discussing it with you later.

DR. HEERINGA: At this point I think that we've reached at least the end of our general input. I'll turn back to Doctor Steeger to see if there are any final closing comments or questions of the EPA scientific staff.

DR. STEEGER: I just wanted to take this

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opportunity to thank the SAP for their time and dedication to helping provide input to the Agency on what is a very important issue for us and we look forward to reading your final report.

Thank you.

DR. HEERINGA: Thank you, it's Steve Heeringa here. On behalf of the panel I believe this has been a very productive three days and I want to thank the panel members, members of the EPA scientific staff for all of their contributions, the public commenters, representatives from Syngenta for their detailed presentations.

At this point in time the panel will compile its minutes of this meeting in the form of an edited report which should reflect the discussions and the comments made during this meeting. It shouldn't reflect things that weren't covered or you shouldn't expect to see a point of view stated that was not expressed in these meetings. That's the nature of the open meeting setup that we have for this Science Advisory Panel.

I want to again thank everybody for their participation and obviously this is a large issue, a very important issue to the Agency and also to the industry as well and to the general public in the

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United States and Canada. And I thank our Canadian representatives for participating in this process as well, it's very, very helpful to have that perspective.

So at this point in time before we close the meeting I'd like to turn back to the Designated Federal Office, Joe Bailey, to see if there are any final closing administrative comments.

MR. BAILEY: No administrative comments. In closing I just want to thank everybody for their participation, in particular the public commenters who came forward offering remarks and to EPA for their thorough compilation of the background materials and the presentation slides.

I want to thank the panel for agreeing to take the time out from their busy schedules to do the work that's necessary to come to the meeting in such a prepared state as you were. So thank you very much.

And I look forward to working with you on completing the final meeting minutes. And they will be completed within 90 days after the meeting and will be available both in the docket and on the SAP website.

And finally I'd like to thank Doctor Heeringa for chairing the meeting for us.

DR. HEERINGA: Thank you. With that I'm

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prepared to call this meeting to a close.

Again, thank you everyone and safe travels.

(WHEREUPON, the Meeting was concluded at 1:40 p.m.)

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CAPTION

The foregoing matter was taken on the date, and at the time and place set out on the Title page hereof.

It was requested that the matter be taken by the reporter and that the same be reduced to typewritten form.

Further, as relates to depositions, it was agreed by and between counsel and the parties that the reading and signing of the transcript, be and the same is hereby waived.

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I further certify that the inspection, reading and signing of said deposition were waived by counsel for the respective parties and by the witness.

I certify that I am not a relative or employee of either counsel, and that I am in no way interested financially, directly or indirectly, in this action.

MARK REIF, COURT REPORTER / NOTARY
SUBMITTED ON
October 11, 2007

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