FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

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Reported by: Frances M. Freeman

CONTENTS

Proceedings.....Page 3

DR. ROBERTS: Good morning. I want to take the opportunity now to briefly reintroduce the panel. And let me as we have done in days previously begin with Dr. LeBlanc and ask each member of the panel going around the table to state their name, their affiliation and their expertise.

Dr. LeBlanc.

DR. LEBLANC: Good morning. My name is Gerry LeBlanc. And I'm a professor in the department of environmental and molecular toxicology at North Carolina State University. And my area of expertise is endocrine toxicology.

DR. KELLEY: I'm Darcy Kelley. I'm a professor of biological sciences on the faculty for the center for environmental research and conversation and a member of the Earth Institute at Columbia University.

And my area of expertise is sexual differentiation of the amphibian xenopus laevis.

DR. KLOAS: My name is Werner Kloas. I'm professor for endocrinology at University of Berlin.

I'm also heading the department of inland fisheries at the Leibniz Institute of Freshwater Ecology and Inland Fisheries. And my expertise is endocrine disruptors acting on sexual differentiation

and thyroid system in amphibians.

DR. GREEN: My name is Sherril Green. I'm an associate professor in the department of comparative medicine at Stanford University. My special interest and expertise is in the care and husbandry of laboratory amphibians, xenopus laevis and other species.

DR. COATS: My name is Joel Coats. I'm in the department of entomology, chair of the department, and professor of entomology and toxicology at.

DR. ISOM: State University.

My expertise is environmental toxicology, environmental chemistry of pesticides.

DR. DENVER: I'm Robert Denver. I'm from the department of molecular cellular developmental biology of the University of Michigan at Ann Arbor. And my expertise is developmental neuroendocrinology of amphibians.

DR. GIBBS: My name is James Gibbs. I'm an associate professor of conversation biology at the State University of New York's College of Environmental Science and Forestry. And my area of expertise is amphibian demography.

DR. RICHARDS: My name is Carl Richards. I'm a professor of

biology at the University of Minnesota Duluth, and I'm director of the Minnesota Sea Grant College Program. My expertise is aquatic ecologist and landscape ecology.

DR. DELORME: My name is Peter Delorme. I'm a senior environmental risk assessor with the Canadian Government, Pest Management Regulatory Agency. My area of expertise is aquatic ecology and risk assessment methods.

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

DR. MATSUMURA: My name is Fumio Matsumura. I'm a professor of environmental toxicology. I also serve as the director of our Center for Environmental Health Sciences. My area of expertise are molecular toxicology. And I also study some neural effect of the pesticides.

DR. THRALL: I'm Mary Anna Thrall. I am a professor of veterinary pathology at Colorado State University. And my area of expertise is veterinary clinical pathology.

DR. ISOM: I'm Gary Isom, professor of toxicology at Purdue University. And my area of expertise is neurotoxicology.

DR. HEERINGA: I'm Steve Heeringa, senior research scientist

with the Institute for Social Research at the University of Michigan. I'm a statistician. My specialization is in the design of research and in specialist research relating to human and animal populations.

DR. ROBERTS: And I'm Steve Roberts. I'm a professor at University of Florida with joint appointments in the College of veterinary medicine in the College of Medicine, and serve also there as director for the Center for Environmental and Human Toxicology. My areas of expertise are toxicology and risk assessment.

Before we proceed with the public comments this morning, at the close of yesterday, there was a little bit of confusion about the status of some research reports that were -- from research performed by Dr. Hayes while at Ecorisk.

Dr. Sielken has offered to perhaps clarify the situation on that. I would like Dr. Sielken to do that at this point for the panel.

DR. SIELKEN: Thank you, Mr. Chairman, and good morning to the panel.

Just to be as clear as possible, I did print out a copy of the clarification statement that I would like to make. And this is a clarification of the materials that I submitted yesterday to the record and to you all. That's my Texan coming through, you all.

On the morning of Wednesday, yesterday, Dr. Hayes made

reference to his study number 99XLATZ2. At the beginning of the afternoon session, I submitted four items to the EPA or SAP related to my review of the statistics in Dr. Hayes' report for that study.

These four items were as follows: And they are numbered here. I won't read the titles. But the first one was a document that was prepared for a meeting with EPA in June of 2002. And that document was briefly summarizing the comments that I had made in my presentation to Dr. Hayes on September 19th of 2000 at Berkeley.

There is no change in that. I'm just clarifying that that was the first thing that I gave you yesterday.

DR. HAYES: We didn't have a meeting in September --

DR. SIELKEN: That is the date that was on the front page of the presentation that I gave. It was at the meeting in Berkeley, whatever date it was.

DR. HAYES: It was January or February. It wasn't September.

DR. SIELKEN: I have been corrected. It was January. Boy. This is what happens when you go to bed at quarter to 4 in the morning.

It was at the one and only meeting that I attended in Berkeley with Dr. Hayes.

The second item was the overheads that I presented at that

meeting, whenever it was. It expressed my professional concerns as a statistician about characteristics of the study, including what Dr. Hayes described as "haphazardly selecting a sample to analyze for laryngeal size" instead of taking a random sample, the need to explicitly provide more of the numerical data and errors I found in a spot-check of the numerical accuracy of the report.

These overheads also contain the results of what I believe to be more appropriate statistical analyses of the data on laryngeal cross-sectional area. And the details of my findings are written out there for you.

Basically, what I found was that there was not a substantial reduction in laryngeal size due to atrazine. This was the same conclusion that Dr. Hayes had noted in the body of his report, but did not note in his summary.

The third thing that I handed out, which was probably pretty obscure to you, was the copies of the 24 transparencies that I prepared to show examples of some of the errors and inconsistencies I found in Dr. Hayes' Excel spreadsheets, worksheets, and which I had presented to Dr. Hayes at that meeting in Berkeley. Correct the date.

I just listed there some idea of what those errors and inconsistencies were. I had copied the transparencies that I used to

identify those things and had provided those to you yesterday.

Now, where the confusion really probably arises is with respect to Number 4. And the version of the final report signed by Nigel Noriega on June 23rd, '00, was the copy that I submitted to you.

The first three of the items that I briefly discussed above were reproduced from original copies that I had prepared and brought with me to this meeting.

I did not think that I needed to bring my copy of the final report that I reviewed. I didn't think I needed to bring those here since I assumed that there would be plenty of copies here and I didn't need to carry it in my luggage.

Therefore, I asked Ecorisk staff to provide a copy of the final report for me to submit. The Ecorisk staff provided the only final report that was signed. And I assumed that that was the same report that I had been looking at.

Unfortunately, the only version of the -- and unfortunately, the only version that the final report that was on the CD that was provided to the SAP members.

In other words, Syngenta provided to you the only signed final report.

When I asked the Ecorisk staff to reproduce the final report for

me, then that's what they did. They reproduced that signed report. And that's the same report that is on your CD.

That's the version of the report that was readily available yesterday since Dr. Hayes' statement therein, that there were no abnormal, undifferentiated or intersexual gonads were observed in any of the treatments or controls had been discussed over lunch.

So that was why that copy was just out and ready and ready to go.

During Dr. Hayes' presentation, he had shown a figure showing the distributions of laryngeal sizes in male frogs. I understood him to say that this figure referred to later studies, that is, later than this 99XLATZ2.

I remember that figure since I had referred to it extensively in my presentation at Berkeley.

Since I had given all the copies of the final report to the SAP staff, I left the SAP meeting during the last public commentor's comments to check that this figure was indeed in the copies of the final report I had submitted to SAP.

To my chagrin (embarrassment), I realized that the copy of the final report I had submitted could not have been the copy that I had reviewed for that Berkeley meeting since the figure of interest was

not in it and I knew it was in the copy that I had seen.

I understand that after I left the meeting, Dr. Hayes stated that the copy of the final report I had submitted was not the final version.

It seems that the signed final report that I copied and gave to the panel yesterday was not the final version. In fact, there is no final version. And there is only one signed and dated version. And that's the one I gave you.

Furthermore, all other versions of the final report I could find said that they were final reports. All the cover pages are the same. And none were dated except for that one signed copy that I gave you.

The only version of the final report that contained the figure of interest was an unsigned version of the final report with a handwritten note on the cover page stating that, "Cathy, here is a copy of the report that was sent to Ecorisk several months ago.

And it's signed Kathy and then the phone number.

Here the first Cathy is Cathy Benz, who is the Ecorisk quality assurance person. And the second Kathy with a K is Katherine Kim who is CEO of Sokoke, Incorporated, and also Dr. Hayes' wife.

This is the copy that one Kathy sent to the other Cathy.

I checked this version of the final report. And it did contain the figure as I recalled it. Furthermore, this version contained on Page 41

the erroneous correlation coefficient of minus 2.61 that I had remembered commenting on, since a minus 2 is impossible.

Therefore, I went to Kinkos last night, and skipped dinner, and had 26 copies of this version of the final report made from the CD that had been provided to EPA.

So there was a copy of that signed version in the miscellaneous materials that Syngenta had submitted to EPA.

Although this version contains the statement that there are no abnormal, undifferentiated or intersexual gonads were observed in any of the treatments animals and the figure as I recalled it and the erroneous correlation coefficient that I remembered, a closer examination of the figure, about 11 o'clock last night, revealed that the figure labels were slightly different.

The dots and the figure were still the same. But the axis label had changed. And there was a line in there for where the female mean was.

I said, well, this can't be the report that I reviewed either.

So of the reports that were available and that Syngenta had provided to EPA and they had provided only the final or what they thought to be the final to the panel members, it meant that there had to be another version somewhere.

I recall that at the time that I did my review, Keith Solomon, Dr. Solomon, had received an electronic copy of the version of the final report and of the Excel spreadsheet that Dr. Hayes had provided and that I had reviewed.

Therefore, this morning, instead of waking at my office in the middle of the night, I asked Dr. Solomon to print a copy of that version of the final report.

And I will be distributing copies of that to the SAP members as well as to Dr. Hayes as soon as they come off the printer downstairs.

This version of the report also contains the statement about no abnormal, undifferentiated or intersexual gonads were observed in any of the treatments or controls.

Let me make a personal apology for my unintentional mistake of submitting the only signed version of the final report -- or the only version of the final report that was signed by the study director to SAP, since, apparently, from Dr. Hayes' statement, it is not the final report.

I do apologize for any confusion. I have made in that box 26 copies of the version that Kathy sent to Cathy. And as soon as they come off the printer downstairs, I will give you the version that I received prior to the Berkeley meeting and that I reviewed at the

Berkeley meeting.

Just for your own edification, there is a not a great deal of difference in these reports, but there are some differences. You are welcome to review them at your pleasure and make your own decisions.

DR. ROBERTS: Thank you, Dr. Sielken, and thank you for your efforts to clarify that.

Dr. Hayes, I see you raising your hand.

DR. HAYES: I'll be brief.

DR. ROBERTS: That's fine. Please approach the microphone.

Let me just explain that what the panel is trying to do is obtain copies of reports of your earlier work with Ecorisk. And we're trying to get what represents the best -- a best representation of that research. And apparently, there are multiple versions going around. And if you could assist us by identifying which version you consider to be the most accurate representation of the research, that would be very helpful.

DR. HAYES: Yes. In terms of the gonadal abnormalities, I've already addressed that. I started out showing how we discovered the abnormalities. It was during that experiment where we were trying to measure steroids in animals. And we were sexing the animals without

buens (ph). And that's when it was discovered. And I reported that to Ecorisk, not to Syngenta, to Ecorisk in November of 2000.

And that's when we reanalyzed the data. So that is correct. I already addressed that.

The other thing I wanted to say, I think Bob Sielken has already said better than I can, you see the sort of level of confusion and the round and round they go about, there is never a final report because there is always we need to do this, you do that. And things never get signed.

That's exactly why I left the panel. We started reporting adverse effects as early as 1999. And because there was never a final report and things never got signed on and they would ask to change this and that and this version and that -- I think you see exactly why I left the panel.

So I won't comment anymore. It was exactly what was just described to you.

DR. ROBERTS: Okay. But we will now have several versions of that report. And if to the extent to which -- before we close public comment, if you could look at those and identify which one you think is the most accurate --

DR. HAYES: I can identify the last final report.

DR. ROBERTS: That would be very useful for us.

DR. HAYES: Also, let me say, by the way, the data that he, Dr. Sielken is talking about that he analyzed was not sent by me. I don't know where he obtained it. I did send data to Dr. Solomon and Giesy, but that didn't come from me.

DR. ROBERTS: Dr. Thrall has a question.

DR. THRALL: Dr. Hayes, would it be possible for us to have a copy of what you presented to us yesterday? Because that's probably what you're considering to be your final at this stage of the game. I think that would help us as we deliberate.

DR. HAYES: Yes. I don't think I have enough recordable CDs, but I can burn it on to a CD and make it available. Yes.

DR. ROBERTS: If a CD can be made available to Mr. Paul Lewis, our designated federal official, he can make copies of the CD -- and entered into the public docket.

DR. HAYES: Okay. And I'll look at this more carefully, but it does look like the final version. And it does look like it also has the raw data and all the numbers. I'll look through more carefully, including the numbers that Dr. Sielken just said weren't available to him.

DR. ROBERTS: Dr. Hayes and Dr. Sielken, both, we

appreciate your efforts to clarify this for the panel.

Let us proceed, then, with public comments. And as I have done at the opening of each of the public comment sessions that we have had, let me remind the public commentors and the panel that we are here focused on some specific issues related to potential developmental effects of atrazine on amphibians.

So we're focused on scientific issues because we are the Scientific Advisory Panel. We're not here to consider policy issues, legal issues and so forth.

So we could confine comments and our discussion to the scientific issues, I think that will be best. Let me see. Our first commentor that we have signed up for this morning is Dr. Jennifer Sass on behalf of Natural Resource Defense Council.

And she will be followed by Dr. Diana Post, just as a heads up, Dr. Post. She may not be here.

DR. SASS: Should I start? I was just going to introduce myself while this is --

DR. ROBERTS: Good morning, welcome.

DR. SASS: Thank you. I'm Jennifer Sass. I'm a Ph.D. with the Public Health Program of the Natural Resources Defense Council. It is an environmental nonprofit group. We're an advocacy group for

public health here in the U.S.

And we have been following atrazine very closely. In fact, it is because of a consent decree with NRDC that EPA is reviewing atrazine.

I want to first thank you all for giving your time to this extremely important issue. I know that you are all extremely busy. And I know that this is a tremendous amount of time, both in attending the meeting and in previewing prior to coming to the meeting. And I want you to know that we really appreciate your attention to this important matter.

I also want to thank the EPA for preparing the white paper. As you know, most of the studies, in fact, all of the Syngenta submitted studies were not published and, therefore, not available for peer review or public scrutiny. So we rely on the EPA scientists to review boxes and boxes of data that we would never even want to look at were it publicly available because of the time and the energy required.

So I do really appreciate the excellent review that the EPA has done in the white paper. Thank you.

I'm going to make my comments very brief. First of all, I want to point out that the charge questions to the EPA in the white paper I

thought were unusually, unprecedently vague and ambiguous.

And that's possibly because the charge questions were in an unprecedented interest of the White House -- actually passed through the White House for review prior to getting put into the white paper. And I don't think they reflect the white paper in terms of the scientific assessment.

So I would like to start out by redirecting a little bit. What I think that the key questions are that the EPA really needs to address in its legal obligations with the NRDC in reviewing atrazine -- in fact, the reason that this SAP is here today is because NRDC negotiated with EPA to send this data to a scientific advisory panel for review and since at the time the EPA was not going to look at the data.

And the key questions in that agreement were, does atrazine threaten wildlife amphibian populations. And does atrazine act as an endocrine disrupter.

Those questions are not in the charge questions anywhere. But those are the key questions that the EPA needs to answer to fulfill its legal obligations and to complete its assessments.

And those really are the questions that this scientific advisory panel can provide input and advice on because of the expertise that is here around this table.

I also want to point out that those are the key questions that are data driven. In other words, those are the questions that are going to be answered by a review of the science of the data that is available and the full body of literature. And from the answers to those questions, the next question will be, can atrazine be used safely.

At that point, the question that follows, which is not a question for the SAP, is at what concentration or dose or under what conditions can atrazine be considered to be safe. That's presuming that it can be used safely. Presuming there is some answer to that middle question.

At what concentrations or dose can it be used safe. In other words, from a policy point of view, we talk about a baseline or a no effect level or NOEL or reference dose and for human consumption.

Those are policy driven questions. Those are not appropriate questions for a scientific advisory panel. And in that light, I don't think that the white paper should be criticizing or critiquing or undermining the published literature because it doesn't provide that kind of an answer.

Those aren't answers that can be derived directly from the kinds of studies that the published literature were designed to answer, to tackle all kinds of questions.

So I would like to focus on the data driven questions and point

out that it is not a fair critique to say that a study doesn't come up with a dose response, therefore, the study somehow is insufficient to include in the review.

The NRDC asks that the scientific advisory panel provide a fair and complete review of the available literature with greatest consideration given to those data from robust and well designed studies published in the peer reviewed and scientific literature.

We ask that the amphibian data be evaluated as to its consistency with the whole body of available data, including mammalian, aquatic and mechanistic studies.

We ask that the scientific advisory panel provide its expert scientific opinion as to the effect of atrazine on amphibian health and to make recommendations as to whether or not it can be used in a manner that will not harm amphibian populations.

We believe based on the published literature and reviews therein that there is compelling evidence that atrazine is a multi-site, multi-species endocrine disrupter.

Despite variability between study designs, there are data for mutually consistent studies with sufficient statistical power published in the peer reviewed literature demonstrating that atrazine acts in mammals, amphibians and aquatic organisms through at least two

mechanisms of action to disrupt hormonal pathways critical to reproductive development and function.

These findings compel a determination that atrazine is an endocrine disrupter on wildlife and that its use should be banned or severely restricted. That's the position of the NRDC.

I've handed out my comments yesterday or the day before, how ever long we have been sitting here, on paper looking something like this. I handed out 25 copies. I hope you have them.

It provides more of a comprehensive literature review. In the back all references are there for everything that I'm talking about here. What I'm going to do is very quickly breeze through that. I'm not going to go into any detail on any study. They are not my studies. I can't defend or answer to them in any detail.

I just want to put the question of amphibian risk in the larger context of the published literature on atrazine.

What I want to point out in this graph, which, again, you have in the hand out is that there are a number of studies in rats, and there are a number of different studies that show tumor formation. But there are strained specific responses or rather differences between how strains of rats respond to atrazine.

So for instance, in one single study, a full litter resorption was

seen at low doses in the F344 strain, but not in the Long Evans and the Sprague Dawley strain, for example. And that was seen at 50 milligrams per kilogram when treated through gestational day 6 to 10.

There has been tumors seen in some strains of rats and not in other strains of rats and at different doses.

And those tumors have been reproductive organ associated tumors such as mammary and different reproductive organs.

There has been a suppression of luteinizing hormone and prolactin seen in Long Evans rats, but Sprague Dawley rats did not respond the same way.

Wistar rats, both males and females, showed delayed puberty. Females were less sensitive. They showed effects at 50 milligrams per kilogram when treated during the period of critical development of those organs, whereas males showed effects at 12.5. Much less.

I want to point out that I think that the published studies demonstrate taken together that atrazine acts as an endocrine disrupter in rats, but the different strains respond differently.

Strains differ in their response to different concentrations and also with different measured endpoints. This does not represent disagreement in the published literature, but, rather, demonstrates the complex action of atrazine like all endocrine disruptors on the

complex web of hormonal regulation. Don't think of these pathways as linear.

All results summarized below in this table are of statistical significance. I only picked what the authors themselves picked as their conclusions or results.

I think that in addition there are some multiple mechanisms that have been demonstrated. I don't think any of these have been demonstrated in many studies, but they have been demonstrated at least to be indicative or suggestive of wider implications in the luteinizing hormone and prolactin levels.

There has been some studies in whole animals. These have been done by EPA scientists. And in fact, it's some of these studies that the EPA is now using to set a no effect level for atrazine.

As well there has been some demonstration of aromatase activity. You have heard about that ad nauseam in this meeting.

I want to point out, though, that the Sanderson study that was referred to, the first publication actually had one of the Ecorisk people on that as authorship, John Giesy. And then later in the subsequent follow-up publication, his name was removed -- or he did not participate in the second follow-up study.

But he seemed to be in agreement with those findings, at least

the first time around. And there have been follow-up studies. So again, I don't think that that data is tight, but I think it is indicative.

Atrazine disrupts hormonal pathways in multiple species. In rats, there is evidence that prostatitis has resulted in the suckling rat pups when the mothers were treated with atrazine.

These are interesting studies because the atrazine does not seem to have come through the milk to the pup. It actually affected the mother, the dam, and then the pups were subsequently affected by alterations in the dam hormonal responses to atrazine. And this was by EPA scientists.

There has also been demonstration of reduced testosterone, reduced sperm motility and the delayed puberty in males and females in the Wistar rats. Again, this was in the EPA study.

In pigs, there is one. Delayed estrus after oral feeding of atrazine-laced feed.

And in alligators, you have already heard, there is some induced aromatase activity.

There is one paper I found in tiger salamanders that I thought was interesting because it showed that the salamanders were responding differently at different concentrations, that at lower concentrations development was delayed, but size and weight weren't

affected, whereas at the higher concentrations development progressed normally, but size and weight were reduced.

Again, I don't think that this represents disagreement. I think it accurately represents the complex reactions of biological systems to hormonal disruptors.

I think that the published literature is consistent in demonstrating that atrazine may disrupt hormonal pathways resulting in disruption of reproductive hormones and reproductive cycles.

While the Ecorisk people were digging up data last night, I was actually on the phone late last night with someone named Shana Swan (ph) who has just published yesterday in EHP on line a new study that where she collected, her group collected semen and urine samples from fertile men, about 200 each from Minnesota and Missouri, and shown that the risk of poor semen quality was elevated with several different pesticides. But with atrazine it was elevated 12 fold. And those are very statistically significant.

The populations are relatively small, but then again, finding a needle in a haystack. If this is able to be seen in such a small population, I think it is worth a follow-up. And so does she. This data will be followed up. But as of today, this is, I think, consistent with the body of literature that we're reviewing.

In frogs, there has been demonstrations in multiple species both in the wild and under controlled laboratory conditions.

The xenopus laevis, of course, is what the lab is addressing in large part. Also, in rana pipiens, lab and field work. Also, in bufo marinus, in field work.

The interesting thing about the bufo marinus work, and again, it is not publicly available, so I have not been able to review that, any published studies in any way, but my understanding from discussions with the author is that there is a built-in concentration gradient because of the frogs closer to the cane fields had more effects.

In other words, more females, skin coloration, than the frogs living farther away from the field. But obviously, I'm not the one to discuss that work.

I think that the ecological risks from atrazine are unacceptably high and that there is no relief in site as the EPA assessment now stands.

And I will quote from EPA environmental scientists, the risk quotients exceeded the levels of concern for chronic effects on mammals, birds, fish, aquatic invertebrates and non-target plants. The risks are possible at maximum and in some cases typical use rates.

So the EPA ecological assessors are extremely aware that the atrazine at current use rates under current use patterns is posing an unacceptably high threat to wildlife populations. And some of these wild populations include endangered species.

There are concerns, again, I'm quoting from the EPA scientists, for adverse toxicological effects on freshwater and estuarine plants and their communities as well as indirect adverse effects on aquatic invertebrates and fish populations at monitored atrazine levels in surface waters.

So based on real readings of real atrazine in the real world, there is real cause for concern according to the EPA ecological scientists.

The ongoing use of atrazine jeopardizes endangered species and their critical habitats. The exposure of aquatic communities to atrazine levels at 10 to 20 part per billion, this is based on an EPA assessment, can result in community level and population level effects.

This is significantly below the EPA's current proposal in its current assessment, which is to allow a seasonal average or 90 day average up to 37.5 part per billion before any action is triggered.

Up until 37.5 part per billion, the registrant, Syngenta, will

voluntarily do more intensive monitoring.

So other than monitoring, there will be no change in the use or use rate or use patterns of atrazine unless the 37.5 part per billion trigger level is exceeded for a 90 day average.

The U.S. Fish and Wildlife submitted comments on this. They claim that EPA's assessment underestimates the ecological impacts of atrazine in part because it does not consider sublethal effects on reproductive ability.

So to end and to thank you for your time, I want to point out that there are some very serious and more scientific questions that the EPA needs to address. And those questions are, does atrazine threaten wild amphibian populations and does atrazine act as an endocrine disrupter in wildlife populations.

These are data driven questions. And if the answer is yes, then the onus falls on the EPA to answer the policy questions of what doses and under what conditions atrazine can or cannot be used safely.

Thank you for your time.

DR. ROBERTS: Thank you, Dr. Sass.

Let me ask the panel members if they have any questions for you.

Dr. Matsumura.

DR. MATSUMURA: Regarding this new report on the human sperms, did those people really measure the level of the atrazine in sperms?

DR. SASS: In the fluid, is my understanding.

I spoke with the author last night and have read some of the various different reports of it. And my understanding is they measured in the fluid levels.

Atrazine was one of a small handful of chemicals that came to light as associated with this. Alachlor and diazonal metabolites were the others.

DR. MATSUMURA: Thank you.

DR. ROBERTS: Any others? If not, thank you very much, Dr. Sass.

It sounds like lots of folks were busy last night working.

Dr. Post has requested time to speak. I don't know whether Dr. Post is here this morning yet. If not, we can move to the next person and then come back to her.

I have Dr. Stephen Sheffield listed as requesting the opportunity for public comment. Is Dr. Sheffield here?

Come forth, please, and identify yourself.

DR. SHEFFIELD: Thank you, Mr. Chair. My name is Steve Sheffield. And first of all, I greatly appreciate the opportunity to address the SAP today.

In my mind, this is a very important issue. I would echo the comments of the previous speaker in acknowledging the amount of time and your willingness to serve on this panel. It is a very important issue. And I greatly appreciate all the efforts you are going to put into this over the next couple of days and the efforts you already put into it.

I should also indicate that I am an affiliate professor in the department of environmental science and policy at George Mason University, and that I'm actually providing these comments to the SAP as a professional wildlife toxicologist and private citizen, and that these comments shouldn't be construed as an official position of the university.

I should probably spend just a second giving you information on my pertinent background and experience. I have a Ph.D. in environmental toxicology from Oklahoma State University. I was on the faculty of environmental toxicology at Clemson University for four years. And I have been at George Mason for four years.

I have experience with both laboratory and field experiments

examining exposure and possible effects of pesticides on amphibians, including both frogs, rana species, hyla species and xenopus as well as salamanders, ambystoma species, and have coauthored a book chapter on multiple chemical stressors and amphibians that will be published by SETAC press this summer.

I have been following with great interest the atrazine amphibian issue as it has unfolded over the last several years. As a result, I was wanting to share with you my perspective on the subject. I will include some thoughts on atrazine in general, some comments on the studies highlighted in the white paper, some comments based on the peer reviewed literature and some recommendations and thoughts on the subject.

As a wildlife toxicologist, one of the things that catches my attention is the high volume pesticides. That's not to say that all of them are bad. I'm just saying that when I go about looking at things, looking at exposure and effects of pesticides on wildlife species, it the high volume ones that generally catch my attention.

In the United States, from the estimate of the literature and other sources I found, are roughly 75 to 150 million pounds of active ingredient annually over 40 years on up to 100 million acres of atrazine is applied or has been applied. It is ubiquitous in the

environment. It's found in all environmental media, including surface water, ground water, soil, sediment, air, including fog, and biota and it's atmospherically transported.

I think that this is an incredible volume of atrazine use and it's very difficult to comprehend that sheer volume of amount of use and the amount in the environment that I kind of liken it to the idea of trying to comprehend how much 100 million dollars is. I know I certainly can't do that. I have a hard time comprehending that number. That's why I'm saying that this is a very hard number to comprehend because it is so high.

Therefore, as Dr. Hayes made mention yesterday, control sites that are free of atrazine contamination are most difficult to find.

Further, peak application coincides closely with peak amphibian reproductive seasons. I see this as a scenario for potential trouble for amphibians, not just from atrazine, but from the various chemical mixture in which they are exposed.

Dr. Hayes' presentation yesterday, he had several different slides showing atrazine levels found in the environment. I don't need to repeat that. The levels that he has looked at and that some others have looked at are environmentally relevant, which is important.

I like to tend to discount some of the ones that look at really

high unrealistic levels and look at the ones that are just -- focus on the ones that are just environmentally relevant.

One of the things that I like to focus on a lot is exposure routes. And as far as exposure routes, I see amphibians getting exposed through oral, dermal and inhalation routes for atrazine. I don't know if there is any data on maternal deposition into eggs or not. But I imagine that that is possible. It does happen for other compounds.

It also needs to be considered the persistence of atrazine in the environment. The estimates range very widely. But it's generally relatively persistent. In aquatic systems, it can vary quite a bit.

In some of the experimental microcosms and mesocosms it does not have a very long persistence. But in some natural farm ponds and natural lakes it can persist almost a year. That's significant.

In terrestrial systems, one citation I found recently, the Talbert and Fletchall paper, 1964, atrazine persisted for 17 months in the soil at a two-pound active ingredient per acre application rate.

Now I'm going to move to a couple specific areas that I'm concerned with. One is bioaccumulation. At least one person so far in this panel -- in this proceedings has mentioned bioaccumulation.

The study by Allran and Karasov of 2000 found levels of atrazine in tadpoles that were six times higher than the concentration

in the test water.

That may or may not be a significant finding, but it raises a flag with me. It could possibly result in more continuous exposure of the target organs.

And amphibians are the only vertebrate taxa that I'm aware of that also accumulate organophosphate insecticides.

Another finding that could have a bearing on amphibians, given the suggestion that atrazine effects may potentiate or even synergize organophosphate effects.

As far as possible fitness effects of atrazine on amphibians, there is a couple different studies that have indicated possible fitness effects. And I'm very concerned about that as well.

The Allran and Karasov 2001 paper used exposure rates of 0 to 20 micrograms per letter. And frogs at the highest level stopped eating immediately upon introduction of atrazine and did not eat during the entire 14 day experiment, an anorexic like effect that could have implications for fitness in amphibians.

The Brown-Sullivan and Spence 2003 paper, which was a study that looked at atrazine and nitrate in combination, this particular study found that at 40 micrograms per letter of atrazine and 37 milligrams per litter of nitrate using an additive model, the snout vent

length at metamorphosis significantly decreased. They saw reduced growth and delayed metamorphosis.

This possible selective disadvantage for these frogs could result in fitness effects.

And finally, the Diana, et al., 2000 paper that examined effects of atrazine on amphibian growth and survival in artificial aquatic communities, they looked at hyla versicolor, which are the gray tree frogs, and atrazine concentrations of 0, 20, 200 and 2000 micrograms per liter.

They found frogs from two high dose groups were five percent shorter and 10 percent lower body mass at metamorphosis than those of the control and low atrazine groups.

Decrease in amphibian length and weight at metamorphosis can indicate a reduction in fitness in wild populations of anurans exposed to atrazine at these levels, although, I do admit that these levels, the 200 to 2000 micrograms per liter are not common, usually, except during the peak times when the runoff accumulates.

Now, moving to the studies that have been performed and published by Hayes, et al., first, I have to say that I was greatly impressed with the presentation given by Dr. Hayes yesterday. He was very thorough in scope and covered the contentious areas to my

satisfaction.

Overall, I would characterize these studies as very carefully conducted and analyzed, statistical sound and believe that the conclusions reached were reasonable given the data.

Do I find fault with some of his methodology? Yes. But not enough to discount the findings of the study.

Regarding the methodology, there is a few things that I would do differently. As an example, I would use glass containers instead of plastic. That's what I have used in any previous studies.

I would also use an atrazine formulation instead of technical. That's an personal choice of mine. Just as examples.

I don't understand the criticisms of their work because they suggested a mode of action for the effects seen or for failing to find an acceptable dose response curve.

Although they speculate on a mode of action of a particular fact, that's a normal part of a discussion section of any peer reviewed scientific publication.

The high incidence of males with gonadal abnormalities, whether it be testicular oocytes, intersex or hermaphroditic, how ever you want to say it, is of great concern to me. Particularly, if it's shown that these individuals are not fertile or otherwise

reproductively impaired, which has not yet been looked at.

From what I heard from the group on Tuesday, the intersex individuals do not get that way by themselves. It apparently takes exposure to an endocrine disrupting compound during gonadal development to make this happen.

In regard to the registrant studies, there was a relatively large number of studies that were funded by the registrant over the past year or two. Just as a personal comment or a personal observation, it was apparent to me that the studies were highly reactionary in nature. And reactionary not only to the results of Dr. Hayes, but also for inclusion in the atrazine IRED document.

Therefore, I see that they are put together and conducted rapidly. They contain many design and other flaws that limited their use and rendered them largely uninformative.

Further, only one of these studies is peer reviewed, the Carr, et al., paper. And I really hope that I get the ultra rapid turnaround this paper was afforded when I submit my next paper to Environmental Toxicology and Chemistry.

As far as field studies, Dr. Hayes beat me to the punch on this one, but I'm going to expand on his comments from yesterday.

The study by Reeder, et al., 1998 appears to have been

completely written off as a study with nonsignificant data. However, a point that seems to be overlooked on their work is the fact that although they found significant correlation between PCB and PCDFs and sex ratio reversal, they state that there was an association approaching significance, the P value of 0.07 between the detection of atrazine and intersex individuals.

The failure of this study to find a significant correlation for this was pointed out in subsequent studies on this topic, including in the Carr, et al., 2003 paper. However, I have to point out that I'm very comfortable in assigning a significance level of P equal of 0.1 for field studies such as this due to the large inherent variability and my willingness to accept a slightly higher error in these cases.

In fact, this is a common statistical practice for field studies, including the set of field studies conducted at the EPA Corvallis mesocosm facility over the past 10 years, of which I have peer reviewed many of them for journals.

Therefore, I would ask the panel to consider the use of P equal 0.1 significance level in field studies when appropriate.

I'm not saying it's always appropriate. I'm saying that it's very commonly used in field studies because the variability is so high that you are willing to accept a little bit more error to try to tease out what

is going on. That's what I'm saying.

As far as endocrine disrupting abilities of atrazine, I strongly agree with Dr. Matsumura's assertion the other day that we should be looking at the hypothalamic pituitary interrenal axis in amphibians, a system that is involved in the stress response, metamorphosis, feeding and mating.

This is where the endocrine disruption action is in mammals, and I have a hard time figuring out why we are not looking at this in amphibians.

As we heard on Tuesday from the registrants, these hormonal systems are evolutionary highly conserved through the vertebrate taxa. So we can reasonably expect that the amphibian HPI axis system would closely resemble that of birds and mammals.

Therefore, in addition to the examination of the hypothesized mode of action involving aromatase, we should be looking at possible impacts on ACTH, gonadotropin releasing hormone, FSH, LH and prolactin as well.

The HPI axis has been looked at in amphibians by Gendron, et al., 1997, who examined the functional integrity of the HPI axis in mudpuppies exposed to organochlorines in the field. They found contaminant-induced disruptions within the HPI axis in mudpuppies

collected at most of their contaminated study sites.

There has been one mention so far in proceedings about immunotoxicology. I'm very interested in that area. The Christin, et al, paper, 2003, that was published in the most recent issue of ET&C, Environmental Toxicology and Chemistry, exposed juvenile rana pipiens for 21 days to a mixture of six pesticides, including atrazine, and then challenged with a parasitic nematode.

They found that pesticide mixtures caused diminished phagocytosis and splenocyte numbers, thereby suggesting a compromised immune system in these frogs.

I have three other considerations that I want to mention. One is an interaction consideration. Both potentiation and synergy. I alluded to this study earlier, the Belden and Lydy study, 2000. Exposure to nontoxic concentrations of atrazine cause potentiated toxicity of OPs, of organophosphate insecticides in amphibians.

In a previous study by Lydy in 1997 showed the toxicity of atrazine and organophosphates was synergistic to invertebrates. The ubiquity of atrazine in the environment makes me wonder how much additional damage of the OPs are causing amphibians due to an interaction such as this.

And another paper by Howe, et al., 1998, there was an atrazine

alachlor mixture that was more toxic than either of the two compounds separately.

As far as formulation, this is a personal bias of mine, but I would submit that there is no such thing as technical grade atrazine in the real word.

The real world, which is the crops and other plants in which atrazine is applied, receives various formulations of atrazine. I believe that it should be formulations of atrazine that we should be examining and not technical grade.

I'm fully aware of the arguments for using technical grade pesticides in experiments, but in my mind, that has little practical use if it's not the exact chemical in which the organisms are being exposed in the field.

And my other area of consideration is atrazine metabolites and degradates. These compounds such as hydroxyatrazine and de-ethylatrazine and others should not be ignored in any amphibian studies. These compounds are also ubiquitous in the environment and may be exerting some effect.

So the way I see it, the major studies that need to be done, are intersex frogs fertile? To me, that's the Number 1 thing from the two days and part of a third day that I have seen so far. That's the

question I think needs to be addressed immediately. Are these intersexed frogs fertile.

If yes, is there a problem. If no, you have identified a big problem. And then where do we go from there.

Number 2, as I alluded to already, the HPI axis in amphibians is something that is in bad need of being examined.

Number 3, I strongly suggest including a salamander species in the testing that will be done.

A study of mine that will be published later this year found that salamanders are on the order of two to four times more sensitive to an organophosphate insecticide than three species of anuran frogs including xenopus.

In this study, I used the marbled salamander, ambystoma opacum, and found it to be an ideal test species for studying possible effects of contaminants. It is an autumn breeder with relatively large clutch sizes, larvae that are relatively easy to raise in the lab and eggs whose development can be started on demand.

Number 4, the use of xenopus as a primary test species. I would point out to the panel that there are inherent strengths and weaknesses to going this route.

The strengths are well-known. But in addition to being a

non-native species and only related to our native species at the order level, there is an important life history difference between xenopus and native anurans. The adult xenopus are 100 percent aquatic, whereas native anurans can spend significant time on land. Thus, differential exposures to atrazine can occur.

Native species leave the water and feed on terrestrial pray items, move through sprayed vegetation and can pick up both dermal and inhalation exposure that could be more significant or at least different than in water.

For example, a frog moving through a freshly sprayed field could be exposed to atrazine through oral, dermal and inhalation routes where the atrazine would not be diluted as it would be in water.

This is the major point. These two differences can make extrapolation between xenopus and native anurans less reliable.

Number 5 is the tolerance resistance question. I noticed with interest that this has been touched on already in these deliberations. It is important to address this issue because this might be contributing what is apparently being seen in the field, the populations of frogs in heavy atrazine use areas are still there.

In areas where heavy atrazine use has gone on for 40 years, such as some of the areas we have heard about already, that is more

than enough time to have had enough generations in which to evolve genetic resistance.

Other vertebrates have done it. Mainly, muroid rodents and gambusia, mosquito fish. But I submit that the tolerance resistance question is very important to this. Particularly, in looking at why all these frogs are still there after 40 years.

And Number 6, population level effects. Certainly, this could be argued to be the ultimate question to examine. However, I firmly believe it may take many years to possibly see an effect at this level.

If some males are losing reproductive function, other males may hop right in and be more than happy to increase their contribution to the gene pool. If fertility is negatively impacted in intersex individuals, intersex males die and are lost to the gene pool without contributing.

As the frequency of intersex individuals increases in the population, the effective population size should decrease as fewer individuals are contributing to the subsequent generations.

Also, as frogs die out of a particular area, other frogs may come in and colonize, recolonize the area, making it likely very difficult to detect a population crash.

So if this scenario could be maintained for a relatively long

period of time, a population level effect may take many, many years to be detected.

My final thoughts. I have a couple final thoughts here and I'll end. The static renewal verse continuous flow-through tests. I have modified this -- I've added this since the deliberations have started.

I strongly agree with Dr. Hayes and others on this. I believe for these tests it is imperative to mimic as closely as possible the natural conditions of the test organisms.

Native anurans and xenopus tend to be found in pools, ponds, vernal pools and ephemeral standing water such as puddles and ditches, not flowing waters like streams and rivers. And if they are found in streams, which I have found some in streams, they are likely found mainly in the intermittent pools within streams and not in the moving water.

Also, cost is a major factor in this. As being a researcher at a university, static renewal is relatively inexpensive and continuous flow-through is so expensive as to be prohibitive to most researchers.

I do agree that the flow-through systems successfully deal with the water quality issue, but it's probably not worth the trade-off.

Second, I'm not going to spend any time talking about this other than just mentioning it because it is getting away from things a bit.

But it is proposing that the EPA consider some sort of action the auspices of the Clean Water Act such as the National Water Quality Criteria for Amphibians.

Something like this was proposed previously in the 1990s, but no water quality criteria exists for amphibians in the United States. And it's automatically assumed that criteria for fish and human health are adequate to protect all aquatic species, when, in fact, this assumption has yet to be tested.

Finally, regarding the white paper and the charge to the panel. I was impressed with all the work done by Dr. Tom Steeger and his colleagues for this white paper. And I commend Tom and his colleagues for their effort.

However, when I got to the eight questions at the back of the white paper, that was a different story. To say that I was sorely disappointed with them is an understatement.

In my opinion, they were poorly written and off base from where I think this SAP should be headed. I say this in part because they failed to address one of the most fundamental questions one could ask regarding atrazine and amphibians. Is it an endocrine disrupter in amphibians.

Why is there no question that states, does the panel think, given

the data available from the 17 studies, atrazine is causing some level of endocrine disruption in amphibian populations. And does this effect on the endocrine system have the potential to translate into population level effects.

That concludes my comments. Thank you very much. And I wish you the best of luck in the next two days in your deliberations.

DR. ROBERTS: Thank you, Dr. Sheffield.

Are there questions from the panel? Dr. Green, then Dr. Thrall.

DR. GREEN: I have two short questions. You refer to the paper by Belden and Lydy of 2000, exposure to nontoxic concentrations of atrazine cause potentiated toxicity of OPs in amphibians.

Do you recall just ballpark what these nontoxic concentrations of atrazine were?

DR. SHEFFIELD: I don't, but I believe I have -- no, I don't have the paper with me. I don't know what the compounds were. But I know that the two OPs tested were chlorpyrifos and diazinon. Those effects were potentiated with the atrazine exposure.

DR. GREEN: The second question I have is could you clarify the difference between technical grade atrazine and other formulations of atrazine?

DR. SHEFFIELD: Yes, absolutely.

As I started into this field, it just made sense to me that if you are going to study something in the lab, why not study something that has application.

As a toxicologist, the tendency is to use something that is pure or as close to pure as you can get so that you can actually say this is causing the effect. You don't want these other things in there mixing up with it.

But in pesticides, the stuff that is used in the field are called formulations. So they take the technical grade and they make formulations out of that so that you get a various percentage of the active ingredient in the formulation.

For example, diazinon, a compound that I have used quite a bit. 50 percent, an emulsifiable concentrate of 50 percent is used. And the other 50 percent is inert ingredients. So they will put other compounds in there to dissolve the diazinon and other things needed to make that formulation go on that particular crop or whatever application is being used.

So the technical grade is the pure or as pure as it gets. Sometimes technical grade is only 95 percent, sometimes it's 99 percent. It usually ranges in that area. Formulations are what is used

in the real world. And For atrazine, there is many different kinds of formulations.

I don't know exactly what the active ingredient percent is in these formulations. Offhand, I don't know what it is. But it is some percentage. So what there is, there's other compounds, inert compounds in with the atrazine.

And they may have an effect on their own. Some of the compounds in with diazinon that's mixed in as an emulsifiable concentrate are known teratogens and other things like that.

So you are moving away from getting a look at a pure compound, but you're looking at something that is environmentally realistic because that's what the amphibians are getting exposed to.

Does that help?

DR. ROBERTS: Dr. Thrall, then Dr. LeBlanc.

DR. THRALL: I was particularly interested in the immunosuppression aspect of atrazine that we haven't touched on very much here. And I was wondering if you -- I'm not familiar with the Christin 2003 paper you alluded to. I wondered if you could give me a little bit more detail on that, the pathophysiology of the nematode and how the decreased spleen function interacted with that pathophysiology.

DR. SHEFFIELD: That's a really good question. I have the paper with me. I don't remember all the details of it because it just came out. But I will tell you I noticed, because I have done studies like this before, what they did, they used the pesticides as a -- they exposed the organism to the pesticides. And they used a parasite as a challenge to the immune system and tested it that way.

And they used a mixture which included atrazine. Like I said, I have the paper with me. I can provide you a copy of that paper if you want to look at it. I don't remember all the details offhand because, like I said, it just came out.

DR. ROBERTS: Dr. LeBlanc and then Dr. -- Dr. Green, do you want to follow up to that question?

DR. GREEN: I want to follow up.

I also have that paper, Dr. Thrall. I think relevant to this discussion about atrazine, it was used in the mixture to treat these rana pipiens or various rana species. And then these species were inoculated with lung worms.

The conclusion by the authors, and there were two papers, the first paper just stated that it appeared that in the presence of atrazine in particular, the virulence of the pathogen was enhanced, and that it accelerated their life cycle such that they matured and were present in

greater numbers in the atrazine treated frogs. And they speculated that it might have something to do with immune suppression.

The follow-up paper by the second group came back, repeated the same experiments and did T lymphocyte function tests. They show lymphocyte suppression, and they propose -- I don't believe they followed up with this, that there might be B cell suppression and immunosuppression of immunoglobulins that would protect against parasites.

But neither of those two papers had any data that documented that the frogs were compromised in growth or that they died earlier because of it.

So they didn't extend to make any speculation about what this might do to an amphibian, a wild population that was exposed to atrazine.

But I think we have both of those papers here if you would like to look at them.

DR. SHEFFIELD: Thank you, Dr. Green.

The one other comment I make on that is that I think another thing that would be useful instead of using the parasite, and it's something that I've done in the past, is use a pathogenic challenge. That way your endpoint is mortality.

So you get to see immediately what the effect is. So you don't have to drag it out and look if the parasite is going to kill the animal down the line or not.

DR. ROBERTS: Dr. LeBlanc and then Dr. DeLorme.

DR. LEBLANC: You noted that atrazine has a bioconcentration factor of six in, I think, frog tadpoles.

In looking at that number, my interpretation would be, well, at least one thing we don't have to worry about is atrazine's ability of propensity to bioaccumulate in these animals.

But it raises a flag in your mind. And I just wondered if you could expand on that a little bit. Is there something unique to amphibians and bioconcentration that we should be aware of? Or is six really a significant number?

DR. SHEFFIELD: I don't know. It just struck me as something that I thought was -- I guess maybe it was because of the fact that I had the other paper in mind and I was thinking about the Lydy work that showed the potentiation and synergy of atrazine with the OPs.

The organophosphate insecticides are also very ubiquitous in the environment. That's getting away from the purview of this panel, but for me, anyway, it has implications.

If there is potentiation and synergy effects from atrazine

exposure dealing with the OPs, that, in my mind, is significant.

That's what it was -- I don't think the six is alarmingly high or anything. It indicates that they do accumulate it and because I know that they are the only taxa that accumulates OPs also.

So there is something unique about amphibians that the other vertebrate taxa don't have.

DR. LEBLANC: Atrazine is pretty water soluble, and six is pretty nonsignificant. So I think in that respect, at least, the amphibians are probably responding like everybody else and they are not accumulating the material.

DR. ROBERTS: Dr. Delorme, then Dr. Skelly.

DR. DELORME: With respect to your concerns about formulation, are you aware of -- versus technical product, are you aware of any studies that show concentrations of the formulants that are mixed with atrazine in the end-use products and their concentrations in water?

Because, obviously, atrazine isn't applied directly to water. It is applied on fields. There is going to be differential degradations and the ratios may change.

Are you aware of any data that would indicate what of the formulants get into aquatic systems?

DR. SHEFFIELD: Not offhand I'm not. I'm not sure exactly what they use to make the atrazine formulations either. Sometimes -- I don't know with atrazine, but sometimes a lot of chemical companies are very sensitive as to what they put in there. And that's not public information. I don't know if that's the case with atrazine or not.

So I don't know what that is. And I don't know how it differentially divides. Once it is applied and it starts its fate in the environment, I don't know how it divides up or not. All I'm maintaining is that I have seen many studies that have shown, that have looked at technical grade pesticides and other studies that have looked at the same pesticide with formulations and found very different results.

So I'm saying as a real world type situation, I think the formulations are important to look at regardless of how they divide out. The chemical pool is still going to be there in the amphibian's habitat regardless of how they divide out. The pool is still going to be there of all the different chemicals that are in the formulations.

Whether some of those chemicals disappear faster than others -- they probably do. They have different fates because they are different chemicals.

I don't know. I don't really know what else to say on that one.

DR. ROBERTS: Dr. Skelly?

DR. SKELLY: Dr. Sheffield, several other people that have presented to the panel have given the impression that field studies should be of kind of secondary importance or they are messy or something. And you are one of the first people to say that field studies may be of primary importance.

And I wondered if you could comment further on that and specifically talk about the distinction between observational field studies and field experiments in this sort of work.

DR. SHEFFIELD: Well, what I would say to that is that I'm of the opinion that this is -- this and many other pesticide questions are very complex. It is not going to be answered by any one set of studies necessarily.

You have to use an integrated lab and field approach to this thing. And you may have to do something in the lab, then go to the field and at the same time go back in the lab and do something further. And then maybe you have to go back in the field again.

It is not anywhere near -- there is no road map written for this. It is kind of like what you find by trial and error, by experimentation in the lab you can use to translate to what you might want to look at in the field.

Plus, you can do some things that Dr. Haye's group and others have done with just looking at -- going out to field sites that are contaminated.

They didn't -- they picked their sites based on atrazine sales and then went there and looked at various sites. You can do a little bit more as far as preselecting sites or actually setting up experiments, experimental ecosystems, for example.

There has been some work on that done. I would like to see some more on that with this particular question, setting up experimental ecosystems. At that point, you have a lot more control over your system and it is in the field.

I would say that the field studies are definitely harder to deal with. They are harder -- there just is a lot more things that can go wrong. But they are very useful.

And I my emphasis on that may have been simply because of the fact that I have a slight bias towards -- and a lot of enthusiasm, towards field studies.

They are a group package. They have to go together. And just doing one at a time is not necessarily anything. I mean, they can overlap. There is no written rules for this.

Basically, it's adaptive management. You are going along. You

find something. You adapt. You figure out what to look at next. If that question could be best asked in the field, then that's where you go. And if not, then you do more lab work.

So it is, like I said -- in some of these areas in wildlife toxicology, you are going down a road that has not been paved yet. It is adaptive management.

But the field studies are important. But like I said, there is a lot more inherent variability. You can possibly accept a little bit more error because of that inherent variability. They are harder to do, so they haven't been done as much. But they are valuable.

DR. SKELLY: Thank you.

DR. ROBERTS: Thank you, Dr. Sheffield. We appreciate you coming in and sharing your comments and thoughts with us this morning.

DR. SHEFFIELD: Thank you very much. Good look to all of you in the next two days.

DR. ROBERTS: Next public commenter is Mr. James Tozi (ph) on behalf of -- I'm not sure what the note says. But I'm sure he will tell us.

And he will be followed by Mr. John Hall.

MR. TOZI: Thank you, Mr. Chairman. Good morning. I'm Jim

Tozi with the National Business Services. And with a title like that, given the chairman's remarks that you want scientific remarks, you may say there is somewhat of a divergence there, but I assure you there won't be.

One of our main roles is to ventilate to the public the scientific basis or bases for regulatory decisions.

And the question is how do we do that. We do that by the use of a web site called Cyberactivist.US. You might not have heard of that site, but if you go to your web master, it is probably by traffic gradings the highest, if not one of the most highly trafficked, web sites in the world on the very specialized area of regulatory policy. And it is used throughout the world.

Now, questions given that, why am I here. I think this panel, and it just happens to be that you drew this card, that subsequent to the passage of a very important act, the Data Quality Act, and we look throughout the entire government for proceedings, this proceeding is the first major proceeding that's going to be subject to the Data Quality Act when the agency goes to use it.

And I haven't been here all the day, so you might have heard some of this before.

Now, keeping with the chairman's charge, I'm not going to get

into law because I don't think the law is any different than which most scientists do anyway.

The basic two important portions of the law says the data used by the government and regulatory decisions first must be reproducible and second it must be transparent.

And many scientists when a law passes, big deal, we do that all the time. Well, some of the regulators thought it was a big deal. The scientists did not.

So I just want to say that those two standards, reproducible and transparency, are going to be judged on anything that comes out of this whole proceeding.

What does that mean? That means any third party such as I, on National Business Services, or anyone that testifies, including this committee, you can write and say anything you want. I can write and say anything I want. It need not be reproducible and it need not be transparent.

However, if the agency is going to use anything that I give in this proceeding, anything any other third party gave or whether they are going to use your report, it is going to have to meet those two standards.

If it does not, any third party can move to strike that

information from decisionmaking in the government.

Now, a point that I wanted to emphasize here in terms of what we're going to do to advise this committee, we are very interested in this proceeding because of the data quality aspects.

But I think you can't look at data quality unless you have data. And so, we are going to put on this cyber activist web site Monday evening a request that petitioners have made from EPA on the data on this proceeding.

I haven't looked at all the data, so don't ask me about all the details, but I'm advised that -- and now would I want to compete with you on what the data means. But I'm advised that there's petitioners that have asked EPA for the data and it comes out in these kind of categories.

There were seven data sets, I understand, but this will all be on the web site, five of which were given to people, some of which were encryptive, some of which was withheld.

We're not going to take a position on these matters on the site. That complete data set will be put up on Cyberactivist.US, and it will be out for public comment.

And the form, you are familiar with forms, it is not a web site. It is a form and it is live so any person in the world, anyone in this

panel, anyone in this room can look at that data set and make a comment on it in realtime, which we'll be expecting a lot of comments.

And I will tell you when we put it up, we're going to urge people to ask three questions when they look at this data set. First, on the data received, was it reproducible. Second, how does use of encrypted data fit into regulatory proceedings. And third, when the federal regulatory agencies withhold data, how does FICA (ph) committees and how do people handle this.

We think by putting it on Cyberactivist.US and the charge of this committee as well as the composition of this committee on the web site, we think we'll be able to ventilate way beyond this proceeding an importance how this type of information is used by influential bodies such as yours.

We urge you to look at that site and the comments we'll receive. Some of them, we've received thousands of comments. Thank you very much.

DR. ROBERTS: Thank you. I think there is a question from Dr. Heeringa and then Dr. Green.

DR. HEERINGA: Mr. Tozi, as a scientist operating in a university environment, I believe in full and open disclosure on this

data, obviously, there are proprietary interests, so and I commend you on that.

Just a comment too that data alone aren't sufficient. You need documentation to accompany it. I presume that part of your posting of these data will be to post the relevant documents that describe the study designs and protocols used to collect the data too. Because as you open that up to analysts, it is going to be very, very critical. And I think particularly in the case of the data sets we're looking at here, that those documentations of procedures and assessments be available simultaneously too.

MR. TOZI: Let me ask you one question on this. I think that's very good. What I would encourage when we post these that anyone that has something that they want, and it is very easy, you just press, click on a button and you put an attachment and it goes right up, we will put some of that up. The initial things are going to be what was response to the FOIA.

But I agree with you. There are going to have to be additional postings to interpret that. I have looked at some of the data. It is not my technical expertise to say what is there, but I agree with you.

We would encourage anyone that is involved in this proceeding, whether around this table or in the room, that have relevant

information, because I will tell you that a lot of other agencies way beyond EPA -- which, by the way, EPA on third party data and the Data Quality Act is probably one of the leading agencies.

You can criticize some stuff they have done. And I know the agency from when they were born, they are doing a very good job. I agree with you. And to the extent I can get that kind of information and people want to put it on, we welcome them.

DR. HEERINGA: One more additional comment. Just a technical question for people who would access this. What data storage format will it be in. Will it be in Excel spreadsheets? SPSS Sass? Raw data?

MR. TOZI: I can tell you how you do it now. You register on the site. You type something in. You press a button. I think it comes all out on HTML. There is attachment. The question is how do you get that attachment on there.

Generally, the people that put the attachments on now either have it on their web site and they cut and paste it over or they send it to us and we'll scan it in.

We prefer you have it on your web site because of the amount of traffic.

DR. HEERINGA: Thank you.

DR. ROBERTS: Dr. Green had a question.

DR. GREEN: Yesterday and today several presenters have alluded to encrypted data, which I'm not clear what data people are talking about that has been encrypted.

Is it available to the SAP or could you clarify that?

MR. TOZI: Thank you. One of the people told me I was supposed to say that. Having worked in OMB for years, some things I don't address, maybe because I don't want to.

They asked me this, yes. From the FOIA requests that I understand that people filed out of these seven -- I just say I haven't gone through all the data, five data sets came back, four of which are data.

When I say encrypted, I understood, but others in the room would know more about this than I, the term encrypted means it was in some machine code that you couldn't interpret.

So when they put that up, I want to ask them -- they will identify which one was encrypted. What it meant. But I got from the conversations that unless you had some transition or translation codes, you could not interpret the data. You needed some other kind of information to turn it into meaningful data.

DR. ROBERTS: Are there any other questions?

If not, thanks very much for coming in and your comments and letting us know about that.

Mr. Hall has requested the opportunity to speak to the panel. As a heads up, he will be followed by Mr. Botts.

MR. HALL: Good morning, Mr. Chairman, members of the panel.

My name is John Hall. I'm with Hall and Associates. I'm here today representing the Kentucky Corn Growers Association.

By way of background, I'm an environmental engineer and an attorney. I suppose maybe from this panel's perspective that puts two strikes against me since I'm not a scientist per se.

In general, I have spent the last 20 years of my career dealing with water quality related issues under the Clean Water Quality act, development of water quality standards and the like.

The information that has been presented over the last two days, as you are aware, not only has an effect on the reregistration of atrazine, but also affects what EPA is doing in terms of its water quality standards development for atrazine.

I come from that perspective in terms of my review as to the information presented. In the water quality standards issue area, they generally look at impacts in terms of what I will call the big picture.

They don't typically parse out mechanistically why an impact occurs, what the exact cause of the effect is, but they look at whether or not there is a significant effect on growth, reproduction or survival, the three main endpoints that are often the, if you will, the end result of most research to decide what the real world impacts are.

I have just a couple comments with regard to the information presented and some comments regarding an earlier presentation to this panel.

Dr. Hayes' presentation in my mind raised at least one issue. I guess others have thought it raised several. His analysis focused on abnormalities in gonadal reproduction.

And without looking at the environmental significance of that, the question I had was that these abnormalities were classified as such because apparently they weren't seen in certain controls during the study. But that in possibly later studies that were conducted, did find some of these abnormalities in the controls.

And therefore, I guess there is at least a question in my mind as to if the later studies exhibited some of these abnormalities, should the results of the earlier studies that classified certain things as abnormalities kind of gone back and corrected those charts. If that, in fact, was the case.

The other thing I would note is that in terms of at least statistically, by classifying abnormalities as something you don't see in controls, you automatically get a zero abnormality in your control group.

And then when you do your statistics and comparison to that, very often you can get anything being mathematically significant in terms of a difference compared to a zero effect on the control.

I would note, though, that at least out of all the studies that I have heard about today and in the prior days, that the environmental significance of any of these effects that have been noted is not demonstrated.

I have not seen any information presented that these effects actually cause impacts on frog populations, which in the end is I think what we're really concerned about.

Let me just move on to a couple other points. In the NRDC presentation, they indicated that the charge to this panel was misplaced. I could not disagree more.

The charge to the panel is not misplaced. What we have is different agendas occurring on from different parties that I believe are a little misplaced.

NRDC's view is that endocrine disruption equals ban. In other

words, any level of impact you find regardless of its significance to the real world triggers a ban.

And that trigger on endocrine disruption shouldn't be ecological significance. It is simply statistical significance.

That is not the standard. That is applicable under any federal law I am aware of.

The Clean Water Act requires you to avoid significant adverse impacts on aquatic life. And the endpoints that EPA has used for three decades are growth, reproduction and survival.

In other words, does this have a real world effect in the real world. You may find an effect, a histopathological effect in the organism. But the question is does that cause any significant adverse effect later on such as populations are affected -- unless you don't classify something as a substantial adverse effect and regulate it.

OPP, the pesticides office, uses a no unreasonable adverse environment effect endpoint. Again, it is a similar type of legal standard.

All these programs in summary look at population-based impacts. They require documentation of those impacts and not merely speculation that the impacts occur.

And that, of course, is the charge to this panel. And I think

you will serve us all greatly by answering the question of, whatever impacts these studies have shown, whatever they are, is there any information that shows these impacts are demonstrated to impact these organisms at a population level?

If the answer is no, then you don't regulate the pollutant -- the constituent more stringently at this time. You may research it some more if there are questions that you have, but you don't decide that you are going to cancel a registration over something that is not connected to a real impact.

I will tell you one thing that was said in the earlier presentation that is clearly misleading and incorrect.

NRD stated that EPA's report confirmed that there were real readings and real world concerns showing significant risks. It is simply not true.

What EPA does when they do these risk assessments, I call it triage, I guess is the best way to look at it, you go through doing a very gross level of review and decide, my god, if you can pass that test, you are clearly not an effect.

That's what they call a Tier 1 assessment. And they make multiple, multiple worse case assumptions. And if you can pass all those worst case assumptions, you are out the door. We leave you

alone.

If you can't pass the series of worst case assumptions, they go to a higher level review. They don't declare that they have determined something that is causing significant real world effects. You move on forward.

One example, and NRDC relied on it in notifying the EPA, they intended to sue them under the Endangered Species Act on this issue.

EPA in the report took data from what they called estuarine areas. The entire Chesapeake Bay. They took data from areas that were even 30, 50 miles upstream in non-tidal waters, clearly freshwater systems. And in their report, they classified that as estuarine data.

You would have thought that the Chesapeake Bay had 30 parts per billion of atrazine occurring in it. In fact, it does not.

Well, if you read the EPA report, and not very carefully, what you think is the Chesapeake Bay has these enormous high concentrations of atrazine.

When you look at the underlying database, you find the concentrations of atrazine in the Chesapeake Bay proper, around the order of 10ths and 100ths of a PPB unmeasurable levels.

So in other words, you have to look a little more carefully at

the data before you decide that what is in that EPA report has demonstrated there is real readings, real world concerns before you make claims that leather back turtles are adversely affected in Chesapeake Bay.

I guess I would like to end with I applaud what this panel is doing. I think you will do us all a great service by clarifying what the real effects are, what the information really does show and whether and how we should move on to do further evaluations.

I think it would be extremely helpful if this panel confirms what I believe is a correct assessment on EPA's part that no one has demonstrated a real world connection to population-based impacts from these endocrine disrupter endpoints.

That is not to say no one ever will. That is just to say that it has certainly not been demonstrated to date.

Thank you very much. If there's any questions, I'll be happy to take them.

DR. ROBERTS: Thank you, Mr. Hall. Let me ask the panel if they have questions.

I don't see any. Thanks very much for coming in and sharing your comments and thoughts with us.

MR. HALL: You are welcome.

DR. ROBERTS: Mr. Botts is up next. Welcome, Mr. Botts.

MR. BOTTS: Thank you, sir. My name is Dan Botts. I'm the Director of the Environmental and Pest Management Division of Florida Food and Vegetable Association.

And as such, we're a voluntary trade association that represents the producers of about 60 different commodities in the state of Florida, including sugar cane, sweet corn and sod, all of which use atrazine.

But the reason I'm here today is because I have been following this issue for at least 10 years, even before the special review was issued in 1994. And have looked forward to some resolution at some point down the road in determining if, in fact, atrazine is safe, but more importantly, that triazines as a class of compounds. Larger acreage use of triazines in the state of Florida is simazine use on citrus.

And because of that, your deliberations here today pave a pathway of how this whole class of compounds will be dealt with. And not only this class of compounds, but others as we move into the new process of determining endocrine disruption impacts and how to regulate those impacts for the office of pesticides programs.

I look forward to your deliberations, and looking extremely

forward to your final report, having sat through many SAPs over the past since 1997 after FQPA passed on all kind of issues, everything from modeling for OP, cumulative exposure to OPs and dose response curves for other issues.

I'm extremely encouraged by the discourse that has already occurred among the panel members and between the panel members and the people presenting. It is a much more engaged panel than a lot of SAP panels have been in the past.

And I will keep this extremely brief for that respect. I know you are already a half a day behind schedule. It's going to be extremely time consuming to go through the questions that have been presented. I would first applaud the agency for taking a very controversial set of data and the information and compiling it and looking at it in a manner to put forward the review that they did in their white paper and framing the issue to go forward.

And I'll be honest with you. I didn't read the charge of the questions until the plane ride up here, so I don't have as great a familiarity with the intent or tone of the questions as some of the other previous speakers have.

But after listening to the comments and conversations, I am sure that any issue that might be floated out in the vagueness or the

generalities associated with those questions will come forward as this committee deliberates.

I would draw one thing to your attention, though. In the presentation yesterday afternoon, there was one slide that was put up by Dr. Hayes relative to several epidemiology studies relating to the human health effects and potential carcigenocity of atrazine.

This committee can be informed by the deliberations of the SAP panel which was held in June of 2000. I'm sure that report is up on their web site, and would be available for you.

Most of the studies that were referenced in his presentation yesterday were discussed at length in that document. Whether they are relevant or not to deliberations of this panel I would suggest are probably not the case.

Some of the mechanistic issues that might have come up in the previous part on determining the cancer risk associated with atrazine probably are.

There will also be an SAP panel later this summer to deal with epidemiological information relative to cancer, and those discussions would probably be more appropriate at that point.

With that, I encourage you to move forward since you don't have to listen to us in the public anymore and can deliberate among

yourselves. And I look forward to your discussions over the next day and a half.

DR. ROBERTS: Thank you, Mr. Botts. We appreciate you coming in and sharing your thoughts and comments. Let me ask the panel very quickly if they have any questions for you.

I don't see any. Again, thanks very much for your thoughts.

Dr. Post had requested the opportunity to speak. I asked if she was here earlier. Is she here now?

This concludes the list of people who have asked previously to speak. Let me ask now if there is anyone in the audience who has not had an opportunity as yet to address the panel and would like to do so.

This will be your last opportunity to make a public comment. Because when the public comment session closes, the next item of business will be for the panel to begin their deliberations.

So final call to the audience. Is there anyone who has not yet had the opportunity to address the panel that would like to speak?

Seeing none, this closes, then, the public comment portion of the meeting. Let's take a break for about 15 minutes and reconvene, and the panel will then begin their deliberation of the questions.

(Thereupon, a brief recess was taken.)

DR. ROBERTS: Before we begin deliberation of the questions

posed by the agency, our designated federal official, Mr. Paul Lewis, has a few announcements about keeping the docket squared away, some announcements about submission of documents.

MR. LEWIS: Thank you, Dr. Roberts. Just briefly, during the break we distributed to the panel two pieces of information. One is a CD that was provided by the EPA Office of Pesticides Programs of additional data for the panel to review, to consider. In addition, additional data, additional clarification provided by Syngenta based on comments they made yesterday.

The material is available to the panel now. In addition, we will make it available in the public docket.

DR. KELLEY: Do you know where this came from?

MR. LEWIS: Let me give this back to the chair.

DR. ROBERTS: The question came from Dr. Kelley regarding a CD that we were distributed, and she wanted to know the source. And it is the one that says Hayes data sets.

DR. STEEGER: The data sets on that CD are all of the data that Dr. Hayes has provided to support his report to Syngenta, the report that was distributed earlier today.

It also contains data to support the standard operating procedures that he developed in his lab to determine feeding rates.

There is an additional file on there that provides the password for the one password protected data set that is contained among the seven.

DR. ROBERTS: Thank you, Dr. Steeger.

We have a follow-up question by Dr. Kelley.

DR. KELLEY: For clarification, so the feeding data which were alluded to yesterday, does this just include the methodology for gathering them and not the results? Or does this include the results of different feeding regimens?

DR. STEEGER: My understanding is they are the results of different feeding regimens to determine what would be the ideal feeding rate for xenopus in Dr. Hayes' lab.

DR. KELLEY: Thank you.

DR. ROBERTS: And just as a general comment, the panel very much appreciates the data and the reports that were submitted during the course of the meeting and the comments.

We will try and consider and utilize those in our deliberations as best we can. But time is very short for us to be able to consider those. So we'll do the best we can with it.

Yes, Dr. Steeger.

DR. STEEGER: I want to make one more comment about the

encryption of the data.

As Dr. Hayes indicated in his presentation, his treatments are color coded. One of the data sets is codes dot X L S. It's an Excel spreadsheet. It provides the way of associating the colors with the actual treatment levels.

DR. ROBERTS: Thank you, Dr. Steeger.

Dr. Bradbury, the panel has received a great deal of public comment over the last couple of days, a lot of information.

We appreciated the opportunity to hear from the investigators and asked them questions. We now turn to deliberation of the questions posed by the agency. And I wondered if you had some introductory remarks or comments you wanted to make to help us make sure we have the right focus as we begin to that.

DR. BRADBURY: Thank you, Mr. Chairman. I appreciate the opportunity.

What I would like to do is just spend a few minutes to recapping where we have been over the last few days and perhaps help set the stage for moving through the questions.

What I would like to do is sort of again get us back into the context of the science and the risk assessment issue that we're facing and to put this question in the context of the agency's ecological risk

assessment guidelines, the process whereby the science is evaluated to help inform the regulatory decisions that the agency needs to make.

We talked about this on Tuesday morning, that one of the important phases of an ecological risk assessment is the problem formulation stage. It provides the context, it provides the foundation for proceeding further in the actual risk assessment.

The risk assessment, of course, is designed to help inform the risk management decision, science for a purpose. The science for this purpose is to help inform decisions about the potential risk of atrazine to amphibians.

As we discussed on Tuesday morning, ecological risk assessments tend to be an iterative process. And some of the speakers over the last couple days noted the risk assessment that has already been completed in terms of the potential effects of atrazine on aquatic community structure and function.

As part of that it iterative process, questions came up from the public, from NRDC and from others. And in the context of interacting with our agency's risk managers, it formulates another question.

The question being whether or not atrazine can cause developmental effects on amphibians, and, if so, what could be the consequences if that effect occurs.

As one moves in theory from problem formulation into the analysis phase, that's where one blends the exposure information with the effects information and takes that into risk characterization where, in fact, we attempt to provide an estimate of the magnitude and likelihood of potential adverse effects. If you will, try to establish the probabilities that certain events can happen at different atrazine exposure concentrations.

The goal of the risk assessment is to provide that exposure response profile and articulate what the ecological significance of that exposure response profile is. And then communicate that to the risk manager.

And as we have heard over the last few days, there is a number of issues that come into play in making a decision about the registration and reregistration of a pesticide. Science is only one aspect of the overall decision. There are many other factors that go into making a regulatory decision.

The challenge for the scientific community, not just the scientists in the office of pesticide programs and the office of research and development, but the scientific community that spans academia industry and public groups, is to ensure that the science that goes into making these decisions is clear and transparent and provides

the risk managers an objective understanding of what we know and what we don't know so that certainties in their decisionmaking is clear to all concerned.

So that's the context. We're inside the box. We're inside the box of science, but it is science for a purpose and it is science that has urgency. Decisions have to be made. Making no decision is, in fact, a decision. And it is a decision that is made in the context of whatever scientific uncertainty or certainties we have at the time.

So with that in mind, let us just walk through again a little bit of context on the road map that we're working through. Let's take a look at problem formulation again. And that's really where we are right now.

The white paper is, in fact, a problem formulation. Problem formulation is where we integrate available information to try to establish some sense of risk assessment endpoints in the context of environmental management goals and start to articulate what those measures of effects could be to make estimates about those risk assessment endpoints.

Based on the integration of available information, we focus on the formation of risk hypotheses and try to develop a conceptual model that could be used, a working hypothesis, if you will, to relate

the information that we do have in terms of exposures and effects to then set up the analysis plan for actually undertaking the risk assessment.

It is very critical in a risk assessment that has a lot of attention and has a lot of implications to use this problem formulation step to its utmost, to have it be rigorous, to gain the input from the scientific peers, to ensure that we've thought this thing through the way it needs to be thought through.

At the end of the analysis phase, there are sort of three broad paths one could imagine going down. One possibility is that through the analysis of the available information and dialogue with the risk managers it could be concluded that there is no need to do a risk assessment, that there is sufficient certainty in the information, in the context of the certainty that is required to make a regulatory decision that there is no need to do a risk assessment. That's one possible outcome of problem formulation.

Another outcome, possible outcome of problem formulation would be that, in fact, there is sufficient information to formulate the working hypotheses in the conceptual model. The analysis plan lays out how to use the available information to proceed with the risk assessment. Acknowledgment of potential uncertainties are

recognized in the analyses plan, but the decision is that one could go ahead and actually start doing the risk assessment to move into the analysis phase and begin ultimately to characterize risk.

A third outcome of problem formulation could be that there is sufficient information to formulate a reasonable conceptual model to formulate reasonable risk hypotheses, to formulate a reasonable working hypothesis and to develop an analysis plan that outlines the amount of information that is available and outline the data gaps that are facing the ability to proceed with the risk assessment with varying levels of certainty.

That's sort of where we're at right now in taking a look at the white paper, the problem formulation and gaining your insight and input and wisdom on the paths, the possible paths to go forward after problem formulation.

Is there sufficient information to proceed with the risk assessment and quantify to varying degrees the probability of adverse effects to amphibians in terms of development based on varying atrazine exposures?

Is there sufficient information to say there is no need to go forward; there is no plausible risk hypothesis for the potential of atrazine to cause adverse effects on amphibian development or are we

somewhere in between. That's the ultimate question before us. And a series of questions to help us get to where we need to get.

One thing I want to point out is that in the process of going through problem formulation and ultimately getting to risk characterization, we're talking about uncertainties, we're talking about what we know, we're talking about what we don't know and we're pulling that information together.

There has been some discussion that it is a weight of evidence approach. In fact, it is not a weight of evidence approach. In fact, the agency's ecological risk assessment guidelines are very clear that it is not a weight of evidence approach.

In fact, it is termed the lines of evidence approach. And to quote from the agency guidelines, the phrase, lines of evidence, is used to deemphasize the balance of opposing factors based on assignment of quantitative values to reach a conclusion about a "weight." in favor of a more inclusive approach, which evaluates all available information even evidence that may be qualitative in nature.

So the point is we're not balancing pounds of information. We're looking at lines of evidence. If I could be so bold as to change the analogy a bit from the risk assessment guidelines, you may want to think about it as pieces of evidence, pieces of information and

think about how we might try to build a puzzle. Think about building a jigsaw puzzle with lots of pieces on the table, all different shapes, all different sizes, all different contours. Some with many edges, some with a few edges.

Are all those pieces of information on the table even part of the same jigsaw puzzle? If they are, how many of those pieces on the table can one start to put together to start to build the picture? Is there enough pieces on the table that are starting to connect to each other to actually see what the picture is and to talk about the picture and describe the picture in great detail?

Or is it apparent that the pieces on the table don't even allow one to start to put the pieces together? Is it even possible that the pieces don't even belong in the same puzzle? Or are there enough pieces starting to come together that one can start to imagine what that puzzle could look like if one could get more pieces and can start to see what kind of pieces would be the most critical to start building the picture, to start painting the picture.

I think the other idea in terms of not using the term weight but using the term lines or pieces of evidence is that some pieces in this puzzle may be very, very small, but have lots of edges and they connect lots of pieces that are on the table. They may be very small,

but they may be very critical.

I think another aspect of thinking about this in terms of painting a picture or putting together a puzzle is that as we have certain pieces come together, there may be some gaps between some of the pieces. But depending upon the shape of the pieces and how those pieces are coming together, it may be very relatively easy to imagine what that missing piece would look like, i.e., we might be able to extrapolate to what that missing piece would look like, a reasonable level of confidence.

In some cases the edges of the pieces may lead us out into parts of the universe we haven't been before, and that may require actually getting some pieces to help put the picture together.

So it is an inclusive process. There is no right or wrong. There are no winners or losers in this process. The only winners are the people of the United States getting the kind of information it takes so that the risk management decisions can be informed.

The only winners in this operation, in this endeavor is a science, and the science being blended together to maximize all the information possible to make the most informed decision that we can make.

In going through problem formulation, one sort of gets the

impression it's a linear process. It is not. You sort of take different information and you work it through. Let's just recap real briefly sort of where we are coming from in the white paper.

We initially laid out on Tuesday morning the statement of the risk management goal, the environmental management goal as well as the risk assessment endpoints, and those being the reproductive and recruitment capability of native anurans. I don't think I need to go into great detail on that.

But what I would like to do is sit back and think about what that means in terms of being able to make some estimates about reproduction and recruitment of native anurans. That's a big question. That's a question that brings in all sorts of fields of biology, landscape ecology.

Problem formulation helps us get started on the process of putting this information together to answer that question.

When the agency has to take on chemical risk assessments in the ecological realm, we take on a challenge that, with all due respect to the human health risk assessors of the agency, I think we have a bigger challenge and a more exciting challenge, because we have to work across many layers of biological organization.

And the examples of the levels of biological organization on

that slide don't even capture all the levels of biological organization as Carl Richards and others on the panel know, but to at least get it all to fit on one slide, I think I can get the point across.

This concept on this slide has been published by many folks in many different venues. But I think it illustrates very nicely some of the challenges in the integration of information that is required as one goes forward in a risk assessment.

One could enter our examination of a question at any level of biological organization, but the concept is that in general as one goes down levels of biological organization, one creates greater understanding of the potential or actual interaction of a chemical with a biological system.

But certainly as one goes down in levels of biological organization, our understanding of the relevancy of those events at the population or community or landscape level become less and less certain.

So, in fact, doing an ecological risk assessment is a blending of many levels of biological organizations to inform, to help us understand what is going on and to help us understand the relevancy of the ecological risk assessment.

I think this panel is a nice example of the kinds of skills and

disciplines and professions that need to come together to understand in the problem formulation context of where we are and where we need to head.

So if I could try one more analogy. We talked about pieces of a puzzle in terms of creating that puzzle and creating the picture to understand where we are and what the world looks like. We also could think about it in terms of threads and how threads get woven together to create a tapestry, a tapestry that has texture, a tapestry that has a lot of vibrant aspects to it.

And it means we're blending in the skills and talents of folks that are experts in molecular biology and interactions of chemicals with receptors or enzymes as well as interacting with folks that are experts in landscape ecology and understanding how spatial and explicit descriptions of habitat are critical for understanding the population demographics of amphibians or other species.

So our challenge is how to weave these threads together to create a coherent picture that blends understanding and relevancy.

So that as a context, I would like just a couple more minutes if I can, Mr. Chairman, for Tom, Tom Steeger, and I to just touch base again on the integration of available information for this specific risk assessment, problem formulation for atrazine, touch on the conceptual

model again just to touch base and kind of get us all reequilibrated and then to just touch base on the first aspect of our analysis blend for the problem formulation.

DR. STEEGER: Over the past two days, panel members have received range of input from researchers engaged in studying the effects of atrazine on amphibian development.

Panel members have been provided copies of each of the studies discussed and EPA's assessment of the studies.

To a large extent, the presentations have focused on the results generated from research efforts. As has been pointed out by a member of the SAP, it isn't sufficient, though, to look exclusively at the data. But rather, you have to also consider the study design and study conditions in which the data were collected.

As Steve Bradbury, Joe Tietge and I have pointed out, the agency follows a process for conducting ecological risk assessment. The initial stage of ecological risk assessment is problem formulation where risk assessors work with risk managers to integrate available information on a chemical to define potential hazards, their impact on assessment endpoints and the uncertainties associated with the measurement endpoints used to identify potential hazards.

Typically, the agency relies on guideline studies to assess

risks. However, open literature and nonguideline studies are also considered.

Consistent with the agency's process for evaluating studies, studies were evaluated using the following criteria: Experimental design, study protocols, protocols and quality assurance, the strength of the cause effect relationship, whether there was a dose response, whether the observed effects have plausible mechanism of action that is consistent with what is known about the chemical, and, finally, whether the measured effects are ecologically relevant.

Did we know what to expect? No. While the agency routinely receives studies that have a very standardized protocol and established databases on which to gauge the conduct and outcome of the studies, the studies under current review on amphibian effects represented a new area of information.

Did the agency have expectations on whether the data were realistic. As Dr. Giesy testified, the data are what they are. Agency reviewers, though, examine how the data were collected and analyzed to determine whether they would have come to the same conclusions as the study authors.

The agency, however, is looking for input from this panel on whether the data provided from the current suite of studies are

reasonable for gauging the effects of atrazine on amphibian development.

Also, there was some discussion about the agency's unrealistic expectation of a monotonic dose response curve. The agency is not focussed on the shape of the curve as much as on the consistency of the dose response.

We do not insist that a chemical exhibit a monotonic dose response, but simply that we are able to understand and project what type of response might be expected in a consistent fashion from exposure to particular levels of atrazine.

By now, the panel is painfully aware that the agency has reviewed seventeen studies that were received as a February 28th consent decree deadline. 12 of the studies were submitted by the registrant. Five were drawn from open literature. Seven studies were conducted in the laboratory and ten were conducted in the field.

While the presentations by researchers over the past two days have focused on study results, the agency has a number of evaluation criteria that extend beyond the data and examine the conditions under which the data were collected.

As I indicated, the data evaluation records for the 17 studies focused primarily on methodological inconsistencies that were

considered to have potentially critical impacts on the study.

While Dr. Sielken was correct in pointing out that collapsing replicates can potentially increase the likelihood of a type one error, that is, drawing a conclusion that there is a statistical difference when one does not actually exist, agency reviewers conducted statistical analyses based on both the original study design and on collapsing the data sets in order to explore whether statistical relevancy could be extracted from the highly variable data sets.

However, it is important to keep in mind that regardless of what approach was used to view the data, confounding effects across all of the studies limited, if not precluded, the utility of the data regardless of how they were analyzed.

Thus, atrazine contamination in the controls, poor water quality, poor growth and development and survival, high variability in endpoint measurements, lack of reproduceability and unresponsive positive controls were recurrent themes that were considered critical.

One question that was posed on Tuesday was whether panel members should use any of these studies in determining whether there are sufficient data to gauge whether atrazine is impacting gonadal development.

However, we look to the panel's collective expertise on

amphibians' endocrinology, plus extensive laboratory and field research coupled with the understanding of the science of risk assessment to provide feedback to the agency on that very question.

The agency views that the studies do have utility in helping to identify potential effects on amphibian development. Additionally, the studies provide insight on the sources of variability and they provide insight on the appropriate test species and study conditions that may be utilized in future studies.

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

I suspect that it is often times frustrating for the public to recognize that the agency is not omniscient in its understanding of the available research on a particular subject.

While certain background information would be particular relevant to the measurement endpoints under consideration, the agency may simply not be aware of its existence.

Additionally, because of research limitations, the agency has come to rely on the testing of surrogate species to be representative of the effects of a very broad range of organisms. Thus, while it might

be ideal to test actual species that may be likely to be exposed to pesticides, these data are not typically available.

Additionally, surrogate test species are not selected based on their sensitivities to chemicals, but rather on their ability to survive under laboratory conditions.

Also, while laboratory test conditions may not be deemed as ecologically relevant, they are intended to provide sufficient control over environmental conditions to permit better elucidation of potential treatment effects.

Steve will now recap on the approach that the agency is going to recommend to address the current uncertainties.

DR. BRADBURY: Following up on what Tom just indicated, a written description of the risk hypothesis and, as the guidelines recommend to the extent possible, try to visualize what the risk hypothesis is in a conceptual model. And the white paper provided an image similar to this.

Before we get started on talking about this conceptual model, I'm reminded of a quote, I think it is by George Fox that says, all models are wrong, but some are useful. And I'm really used to that phrase. My research is more on quantitative structure activity relationships. And we're always dealing with the fact that you're

trying to predict what you don't know.

So all models don't capture reality. But if used in the proper context, they provide insight into how to move forward.

I think another way to view it and perhaps a less harsh way would be to say that all models have limitations, but all can be useful if placed in the proper context.

I think a lot of what we heard over the last few days and we'll be discussing over the next day and a half will be the context of models, be they biological models, be they experimental models, be they representations of the field to try to capture what all of the ecosystems in the country do, they are models. And they are models used to try to help us find a path to move forward.

So in that spirit, a conceptual model is a working hypothesis, but you have to start somewhere. You have to start somewhere.

So in our conceptual model, we're focusing on that top, for me, the top left-hand corner of our conceptual model. That's the focus and we're going to move forward. We have talked about the idea of linking the potential molecular effects to the effects in the working hypothesis in elevated E 2 (ph) to the concentration on or the focus initially on gonadal effects in males which would then lead to the issue of impaired fertility, reproductive and success and then to our

risk assessment endpoint.

As we discussed in the white paper, the analysis plan of moving forward is a phased approach. Again, the idea that one doesn't have to know everything to know something to then moved forward in a risk management criteria.

This then lays out the first part of our analysis plan, which gets at taking a look at the apical effects of gonadal development in amphibians, provide the logical point to break through that logic train that's in the conceptual model. Again, to go back to the earlier slide, understanding combined with ecological relevancy.

This working hypothesis starts off with sort of posing the question on ourselves, can we establish with greater confidence the potential of atrazine to cause developmental effects, start to get a handle on what the stressor response profile is for that effect as a launching pad to either simultaneously look for ecological relevancy as well as mechanistic.

That's what we'll be looking forward to hear, the panel's discussion on the pathway of moving forward.

The phase one aspect of the analysis plan we feel is the most important part or at least would hope that the panel would spend a fair amount of time on what we're proposing as phase one. Or if you feel

there is a different phase that we should start on, of course we'll be very happy to entertain that.

But at least from our perspective as a working hypothesis for you all to test, our phase one component then is focusing on whether or not atrazine exposure results in gonadal effects in males and perhaps females, and again, to try to determine what the dose response relationship is.

We have no preconceived notion of what a "right" dose response relationship is. That's not the issue. The issue is what is the dose response relationship and what kind of confidence do we have in quantifying that dose response relationship.

In the context of the phase one studies, we indicated on Tuesday morning a number of issues in terms of getting started. We started a little bit of discussion on Tuesday morning about the choice of the biological model to get started on. There probably will be a number of biological models we can consider.

Again, all models have limitations, but all can be useful if placed in the proper context, the context of understanding chemical toxicity, the context of studying ecological relevancy.

There is probably not one model that can answer all our questions. But what are the types of models we should use for the

different types of questions at hand.

We spent a lot of time as Tom summarized in looking at the available information in terms of some standards that exist in the scientific literature as a function of scientific bodies, in terms of some of the basic conditions that are required to do aquatic toxicology testing.

The history of aquatic toxicology is about 35 year old now. Over 35 years there has been a lot of advancements in the technology of how to do aquatic toxicology testing. There has been a lot of work to describe what the conditions of organisms should be expected and the terms of doing those tests.

And in this context, the American Society for Testing Material has established standards, expectations of quality in terms of undertaking an aquatic toxicology test, including test with amphibians. We feel that's an important criteria to take into account in terms of doing aquatic toxicology.

This will be one of the exciting parts of the discussion, because the methodologies used in aquatic toxicology may not be the same methodologies that are used in developmental biology and they may not be the same methodologies that are used in field biology protocols. We're going to have to weave that tapestry, weave those

threads together to try to create a vibrant textured tapestry.

Obviously, you know where we're coming from in terms of how we think there is a way to approach maintaining appropriate quality in the bioassays and combined with keeping track at the end that's going to be required to test some of these hypotheses.

We obviously have a proposal on the table in terms of using a flow-through technology to meet those ASTM standards. That doesn't mean that all tests that could be done to answer these questions have to be done with flow through. In our opinion, the most important criteria is going to be whatever method is used, it has to meet ASTM standards.

Whatever is needed then to answer the statistical power issues, let it be what it will be, but ASTM standards we feel are important because they are based on 35 years of aquatic technology and we're somewhat nervous in backing away from what people have been developing over the years of aquatic toxicology, to not belabor the point, some of the data quality indicators that we discussed previously and which I'm highlighting just in the last few minutes.

So with that, we'll wrap it up. Again, just to bring it back home to the risk assessment issue at hand, is there sufficient information available to describe the certainties? What are the certainties? What

are the uncertainties?

Risk managers can use that information to decide if we should go ahead and do a quantitative risk assessment or if no risk assessment needs to be done. Under the assumption there may be a request for greater certainty in performing a risk assessment for atrazine, we laid out an analysis plan to describe what we considered would be important information to reduce the uncertainty.

We look forward to the dialogue with the panel.

DR. ROBERTS: Thank you, Dr. Bradbury. I think your presentation and your comments by Dr. Steeger are very important to help us refocus as we begin our deliberation of the questions posed by the agency.

A considerable amount of effort has been taken to put the available information regarding potential effects of atrazine on amphibians before the panel. It is now time for the panel to go to work and offer our best scientific evaluation and recommendations.

I would like to go ahead and take the first question and ask either Dr. Bradbury or Dr. Steeger if they would pose the first question to the panel.

DR. BRADBURY: I'll turn it over to Tom to read the questions.

DR. STEEGER: First question.

In reviewing the available laboratory and field studies, the agency used a number of criteria to evaluate individual investigations. Criteria such as experimental design, test protocols and quality assurance information were used to ascertain the reliability of the generated data in terms of its ability to adequately assess the hypothesis that atrazine elicits developmental effects in amphibians, and, if so, the nature and strength of associated dose response relationships.

Then part one of a two-part question.

Does the SAP have any comments and recommendations regarding the EPA's approach and criteria used to evaluate the studies?

And secondly, given the evaluation criteria employed by the agency, please comment on EPA's overall characterization of the currently available studies.

DR. ROBERTS: Dr. Kelley, as lead discussant, I will ask you to start out on this. I will leave it to you. Do you want to take both parts together or do you want to have discussion on Part A and then discuss Part B?

DR. KELLEY: What I thought I would do is to read a response to the question that I have written after discussion with several but

not all panel members, and it includes both parts. And then ask the panel for comments on what I have written so that it can be revised to more accurately reflect the viewpoint of the panel.

Let me ask the panel to begin with whether that's an acceptable procedure to them.

DR. ROBERTS: Sure.

DR. KELLEY: And let me just tell you. Feel free to disagree violently. Not a problem.

DR. ROBERTS: I don't want them to disagree violently.

DR. KELLEY: Violently in the academic sense. That is to say how could you possibly say that. That would be violent for an academic.

DR. ROBERTS: Proceed then.

DR. KELLEY: Bear in mind we may amend this. All right?

We felt that the review was thorough and the conclusions were appropriate, given the data reviewed by the EPA. And here I'm referring to the seventeen studies.

We agreed that additional studies are warranted. There are several studies supported by the registrant or by other agencies that indicate that atrazine can cause developmental abnormalities. On the other hand, a range of abnormalities are reported and they are not

consistent from study to study (bearing in mind that the literature includes several anuran species.)

Further, though not considered in the white paper, the findings are consistent with studies in other vertebrate, both aquatic (e.g. fish) and terrestrial (some rodents.) Given the conservation of many basic pathways for endocrine regulation, these studies are relevant to this white paper focused on anurans.

Comments from the panel?

DR. ROBERTS: I have one and then I'll open it up to other commenters. A asks regarding the approach in the criteria used by the EPA to evaluate those studies.

And maybe I missed it. But did you basically agree with their criteria?

DR. KELLEY: I thought -- yes. The review was thorough. I think that's implicit. So let me add a phrase saying that the criteria were appropriate.

DR. ROBERTS: Yes. If you believe so. I think we should make that explicit in the response.

DR. KELLEY: I'll add that.

DR. ROBERTS: And then Dr. Coats. You had a comment.

DR. COATS: Yes. I'm a secondary discussant on this. I would

like to read my opinion which is certainly consistent with the opinions expressed already by Dr. Kelley. The agency's approach on criteria are valid and address a number of very important points from the exposure side of the risk assessment, which is where some of my concerns are.

Number 1, the data evaluation reports could include a description of the analytical methods, that be ELISA or chromatographic methods to enhance the completeness of the reports and their interpretation.

Measured concentrations which are extremely important of the chemical in the water need to be obtained and any potentially bioactive metabolite should also be quantified as mentioned in the white paper.

In some research papers, the exposure concentrations are nominal with no measured concentrations provided or recovery confirmed given, but no values.

The importance of having measured concentrations could be addressed more directly in weighing the validity of the work.

Thirdly, the significance of the exposure method was pointed out, including some of the shortcomings on the static renewal systems. The ASTM standards for flow-through systems should be

followed and the method strongly encouraged.

Fourth, body burdens must be measured in the organisms to reflect the degree of exposure and help explain the mode of action. Once again, bioactive metabolites also should be included.

Determination of residues in specific tissues would also be very valuable, but at the very least the whole body residues could confirm the exposure.

On the hazard side of the risk assessment equation, it is critical that the dose response relationships be shown as pointed out by the agency. Even if it is an atypical inverted U shape, nonmonotonic response, a dose response curve can be generated if appropriate concentrations are tested.

This of course would help delineate safe versus unsafe concentrations, but also could assist in elucidating mechanism of action.

DR. ROBERTS: Dr. Coats, I think I heard three different kinds of comments there. One was perhaps some deficiencies in reporting among the existing studies.

Some were aspects, experimental aspects that perhaps should be addressed in other studies. But the question really asks about the EPA's characterization of currently available studies. And I think

some of your comments touched on that.

But I want to be sure that when we respond to this question that we sort of don't mix other things, other points that we want to make in with it. So I think it is going to be important that we give it as clear a response as we can to be about things that you feel and other panel members feel that any deficiencies in their characterization where there are things that they didn't describe or that they inaccurately described that perhaps could be tweaked in the document.

Dr. Kelley?

DR. KELLEY: I actually did in my written comments separate out A and B, I see here. And so I did have an additional comment, which is that the design of the experiments reviewed by the EPA and the analysis of the data were flawed in many instances, which was the EPA's conclusion.

And then this relates to the dose response -- if there is a threshold effect for atrazine, that threshold is not firmly established.

With respect to dose response, any requirement that functions be monotonic are clearly inappropriate. And as we've heard, I think the EPA would agree with that.

So I think these were in discussions of the panel members among the most important issues that came to mind, the threshold

dose for observing an effect if, in fact, an effect can be observed strikes us as a very important piece of information to be gathered.

DR. ROBERTS: So you think that perhaps the report as the EPA said should clarify that in their criteria to evaluate the studies there was not an insistence on a monotonic dose response relationship. Is that correct?

DR. KELLEY: And I think actually that it is just a wording matter. I think use of the word threshold is useful here, unless it has some technical meaning that escapes me. So threshold is the question here.

And then of course it will have a shape, and we can't predict in advance what shape that will be.

DR. ROBERTS: Dr. Green.

DR. GREEN: I'm the third discussant on that question. And following up on what Dr. Coats just read to you, I think what we were trying to convey is that we would like for the EPA to include in their approach and criteria for evaluating the study the fact that so few studies, if any, evaluated water levels and tissue levels in the animals.

And that should be emphasized in an EPA report describing their approach and criteria and noting that so few studies did that with any. That would be quite helpful.

DR. ROBERTS: Thank you.

Comments from other panel members?

Dr. Matsumura?

DR. MATSUMURA: I really appreciate what Dr. Kelley said about the comparative aspect in comparing the action of atrazine to other organisms, particularly in the vertebrates, so that we formulate some idea what happens in other organisms. I think this document could have more information on the other organisms. Some people have pointed out too.

And regarding the question that Joel Coats raised, you are using the water concentration of atrazine. In some setting, it is just crazy. As Dr. Hayes pointed out, the next day when you come a concentration may be quite different if it has rained the next day or dried up.

So criterion that one should use really is the residue levels in the frogs themselves, including metabolites.

So I know our chairman don't want to mix up with later questions, but still other criterion we should include whether residues can be found.

In the Reed study from Illinois, they were measuring the residues. And really using the water concentration is not a really

good method. We have to identify the residues, including the metabolites. Even if it is sort of ephemeral, still we have to do that.

DR. ROBERTS: Dr. Richards?

DR. RICHARDS: I believe that in a general way that the agency's review of these is pretty much in line, particularly with respect to the field studies. I think that they have done a fairly reasonably qualitative look at availability of the studies and the data and information that was provided.

DR. ROBERTS: Are there other criteria that you would like to see added or articulated?

DR. RICHARDS: I think that we will address that in other questions, specifically.

DR. ROBERTS: Okay. Dr. DeLorme?

DR. DELORME: I just wanted to concur that for the most part the criteria they used were reasonable and that they are reflecting the need for sound science and consistency in the science used to conduct ecological risk assessments. That's their job. And they want to make sure that the science that they are using and the studies that they are using are sound.

With respect to the overall characterization of the studies, I found it to be reasonable. Certainly, there were some minor points

that we might disagree with on specific studies, but the overall characterization, I think they did a good job. They have identified their concerns with the studies from a risk assessment and from a scientific perspective.

They have also identified any conclusions or information or contributions that they felt the study made. And they have also identified uncertainties that result from the studies. And I think they have done a reasonable job in doing that.

DR. ROBERTS: Dr. Skelly.

DR. SKELLY: Overall, I think the EPA did a good job of characterizing the studies. And I just want to make a comment about how studies were divided into categories.

The distinction between laboratory and field studies was made. And in a couple places in the report, I don't know whether it is referring to all of the studies that were submitted, but they were characterized as experiments.

I just would like to point out that the field studies that are included here include something called a microcosm experiment, which, in my experience, is actually a mesocosm experiment. That's probably just technical.

But mesocosm experiments are not field experiments. I think

that's an important distinction when we're thinking about this. And most of the field studies were observational, which isn't a criticism, but I think the criteria for evaluating a field observational study, a field experiment, of which from what I can tell there are none here, and a mesocosm experiment might be distinct.

DR. ROBERTS: So some of the terminology may need to be tweaked a little bit in terms of how they are described or classified, is that your recommendation?

DR. DELORME: So that the terminology might be tweaked and also the criteria for evaluating things like the distinction between a field observational study and a field experiment in terms of what sort of expectations you have for variation and environmental conditions could be different.

And I think we're going to talk about that a bit more when we get to the next question.

DR. ROBERTS: Thank you. Just a heads up for Dr. Kelley --

DR. KELLEY: Would you like me to put that wording into this first thing? I'll put it in. You can check it later.

DR. ROBERTS: Just as a heads up for Dr. Kelley as the lead discussant, when we finish our discussion, I'm going to ask you to sort of give me your sense of the capsule summary of this.

DR. KELLEY: Thank you.

DR. ROBERTS: Dr. Denver.

DR. DENVER: I just want to support a point that Dr. Coats made. And that goes to the criterion used to evaluate especially the endocrine data and the need to really validate the assays that are used, especially the ELISA assays. And that will come up in another point later and we'll discuss it further. But I think that that can be raised at this point also.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: First of all, I felt that the criteria certainly was appropriate, and I was very thankful of the EPA for making our life a lot easier, I think, in reviewing this document and assisting us in that manner.

I would like to comment on a somewhat minor but I think important terminology consideration. Particularly, in light of the fact that we have had discussions relating to aqueous, measuring aqueous concentrations versus measuring tissue residues.

I certainly think tissue residues are important, certainly from a mechanistic standpoint, to understand what the burdens are and the effect that organs might be carrying in terms of atrazine loads.

Concentrations, aqueous concentrations are important because

the bottom line is that's probably how the regulatory agency is going to make decisions, based upon exposure concentrations and not true doses that the animals get.

Throughout the document, there is discussion of dose response relationships, and I would just advise that consideration be given where appropriate to use the term concentration response relationship and use dose response relationship where appropriate, but don't interuse the two.

DR. ROBERTS: I agree. Any other comments? Dr. Delorme.

DR. DELORME: I just wanted to add something. Dr. Matsumura and I believe Dr. Kelley indicated that one of the criteria that they felt might have been addressed was inclusion of data on other vertebrates.

I think that was -- especially as goes to ecological relevance, it might be -- had some consideration.

DR. ROBERTS: Other comments?

I'm thinking about, trying to think about whether or not I agree. I think their intent was to at least, I think what this statement is, to summarize studies on a particular topic.

I think at some point you have to try and perhaps put that in context, in broader context. I think at that point you would look for

analogy from other species.

But I'm not sure I would criticize this summary for not going -- making that broader look. I think it had a very specific purpose, not to say those other comparisons might not be important in terms of understanding potential effects, but I thought it was very well focused.

Personally speaking, I was satisfied with it.

Any other comments? Dr. Gibbs.

DR. GIBBS: Just one quick question in terms of the case-by-case treatment of all the studies. I felt that on the possibility for type 2 statistical errors to be committed in all of the studies wasn't very frequently considered.

I think the focus was on the statistical significance reported, but I didn't -- and clearly EPA is aware of the problems with sample sizes. But I felt many of the studies had serious issues with not being able to detect effects should they have occurred. And I just felt across studies that that consideration of type 2 errors wasn't particularly prevalent.

DR. ROBERTS: Dr. Heeringa.

DR. HEERINGA: I just add my support to that comment. I think -- as I reviewed these studies and the EPA's assessment and

review of them, I generally agree with the conclusions, but I also support the last comment, too, that I think if we look at these and the other contributing sources of error we have talked about, that many of these studies are underpowered to detect the type of effects that we are measuring.

In spite of some of the identified measurement, contamination or quality problems, all of these contribute potentially to biases in results, but there are other sources of variable errors that I think really would lead us to assume that even given nominal sample sizes that a lot of these studies are underpowered.

And a recommendation I'll make later on is that, if anything, when we start into new studies, if they are conducted, that they be overpowered to start with simply so we don't find ourself in this quandary of being right on the edge of type 1 versus type 2 error problems.

DR. ROBERTS: Any other comments or responses to 1 A and 1 B?

Dr. Kelley, I know you have been taking notes. To the extent possible, can you try and summarize your discussion?

DR. KELLEY: Yes. I'm not totally up to speed on Dr. Skelly's comments. But I will try to summarize them.

So in general, the panel felt that both the evaluation criteria and the thoroughness of the EPA's white paper were appropriate.

The panel will get to the hypothesis in a moment. But the panel did support the hypothesis that there was enough data out in the literature given this evaluation to proceed with evaluating the hypothesis that atrazine may contribute to developmental abnormalities in amphibia.

Some concerns were raised by the panel with regard to the evaluation, areas of emphasis that the panel felt should have been weighed in more heavily than other areas. That includes possibility of type 2 errors, which is, for the public, also my stat students always get this confused, but the inability to detect effects that are really there for whatever reason. And some of the possible reasons were outlined here. So that was one area of concern.

Another area of concern was the ability to compare across studies between nominal concentrations of application of atrazine and actual tissue concentrations of atrazine.

And the third was that the characterization of the field data in terms of what was actually obtained was felt by panel members to be, how can I say, somewhat superficial, I think would probably be the best.

DR. GREEN: Observational.

DR. KELLEY: Observational. Thank you.

Although, there were experiments in those field data, right, so it wasn't completely observational. The microcosm experiments did have experimental and control groups.

DR. ROBERTS: Dr. Skelly can, I think, fill in on that.

DR. SKELLY: We can talk about it afterwards --

DR. KELLEY: Anyway, he's going to fix my terminology here.

DR. SKELLY: The point was that a mesocosm experiment is not really a field experiment. There is an important distinction.

DR. KELLEY: Yes. So that the use of the technical terms.

In a field experiment, you don't take the animals out of the field and throw them into a tank. You manipulate them in their environment in situ. You get rid of the red on the red wing black bird's wing, right, or something of that sort. That would be a field experiment, as opposed to bringing them into a mini lab. So there was some concern about that.

But these concerns were felt by the panel to be relatively minor, and to not abrogate the conclusion of the panel that the initial analysis by the EPA was thorough, that the criteria were appropriate and that the evaluation was a complete one.

DR. ROBERTS: Would anyone like to edit or make suggestions regarding that?

Dr. Bradbury, is our response reasonably clear?

DR. BRADBURY: One question of clarification.

In interpreting or evaluating future studies, am I to understand the consensus of the panel that future studies should have measured aqueous concentrations of atrazine at a minimum and, depending upon the nature of the hypothesis being tested in a given study, perhaps tissue concentrations as well and perhaps activated metabolites?

DR. KELLEY: I think actually the panel might feel a bit more strongly than that. It might feel that measured tissue concentrations of atrazine is not a perhaps but should be a requirement going forward.

But I don't want to speak for the panel as a whole.

DR. ROBERTS: Dr. Green.

DR. GREEN: I still think in the laboratory environment that water concentrations of atrazine are important to know through the experiment.

DR. ROBERTS: Others want to weigh in on this? Dr. Coats and then Dr. LeBlanc.

DR. COATS: I want to reemphasize. I believe that the tissue

concentrations would be very helpful and water absolutely needs to be measured.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: It is my opinion that measuring aqueous concentrations is mandatory and measuring tissue levels is desirable based upon the questions being asked in a given experiment.

DR. ROBERTS: And speaking for myself, I would concur with that as well.

Anyone else want to weigh in?

Are there any other follow-ups or clarifications?

Dr. Steeger.

DR. STEEGER: I would like to make one additional comment for the benefit of many of the researchers.

Dr. Heeringa is correct that type 2 errors are a critical consideration in the design of studies. Many of the researchers did attempt to design their studies with that in mind. The data evaluation records that were prepared didn't fully capture those efforts, but I think that a genuine effort was made.

Unfortunately, as things progressed in the labs and things didn't turn out as well as they had hoped, the study designs didn't support the data in terms of controlling some of the variability that really got

out of hand.

DR. ROBERTS: I believe Dr. Heeringa wanted to respond to that.

DR. HEERINGA: Thank you very much. I agree completely. I didn't intend to say that, you know, forehand that there was inappropriate planning in terms -- I think across these studies that adequate planning went in.

But as you say, during the course of the studies as we learn more about some of the measurement problems and some of the other issues that came in, that clearly this caused those sort of a priori expectations to be modified and in a way that my recommendation is really that for the future that we can anticipate these things will reoccur to varying degrees, and we may have even other unmeasured sources of error in the experimental process.

And instead of sort of delaying the result of this, let's sort of design with a margin of error on these type 2 error problems so that we can accommodate them as they arise in laboratory and testing situations.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: I would just like to revisit aqueous concentrations again for a moment. Certainly, in many of the studies

that I have read, it appeared to me that concentrations of atrazine were measured only in solutions that were freshly prepared.

And certainly depending upon the delivery system that's selected and the design of the experiments, ultimately, I think consideration should be given to make sure that concentrations are measured at times at which you would expect high levels of atrazine, but also the lower levels that might be resulting in these treatments.

DR. ROBERTS: Any other comments or points to add on 1 A and 1 B? We also had a 1 C.

DR. BRADBURY: We have a 1 C and a 1 D.

DR. ROBERTS: I don't have a D on mine.

DR. STEEGER: Actually, it's pretty much like 1 C.

1 C is, Please comment on the availability, as of February 28th, 2003, of additional relevant studies in the open literature that were not addressed in the white paper.

And 1 D is, Since February 28th, 2003, is the panel aware of any studies that would be relevant?

DR. ROBERTS: We have both of those. The second one, at least on mine, was not separately marked as D.

Let me then go back to Dr. Kelley.

DR. KELLEY: Yes. So the review of the panel, while very

thorough and complete, was narrow in its focus. So the question is what kind of guidance would the EPA like from us about what we consider to be the relevant literature.

Let me tell you what I have in mind. So omitted from this were several studies that I found in the open literature on effects of atrazine, usually at very high doses on early mortality, say, in xenopus, tadpoles and some more recent effects on slightly later animals. So those were omitted.

The EPA analysis raised issues of consistency in the data and consistency of things like the stages of sexual differentiation. Yet, of course, there were none of the background papers on sexual differentiation in that panel. So those are two areas.

And the third is of course the issue that the panel has raised that are relevant to things like the mechanism of action of atrazine if it, in fact, has an effect that might be addressed productively by references that included work on other than amphibians.

So I'm in the midst of collecting a series of papers of that sort. But what I would like to know -- but I have to say I didn't find a paper that you guys had left out on effect on gonadal development in amphibia due to atrazine. I haven't found such a thing yet.

So I do believe that in terms of the focus of review that you

didn't omit any obvious and glaring paper. But in view of the panels's desire to provide scientific guidance to the process going forward, I think that there are probably a number of studies that we would like to bring to your attention.

DR. ROBERTS: Other panel members?

Dr. Matsumura.

DR. MATSUMURA: I would like to ask the panel to consider to add the paper by Bevan appeared in the EHS regarding action of alkylphenols. Of course it is not the atrazine, but this particular paper really shows what the estrogenic effect of the alkylphenol really look like.

It is summarizing their work on some of the early stages of development, and the curvature that develop in the tadpoles are very clear on the melanocyte site formation. You can see what real estrogenic compounds could look like.

This is the one endpoint studied in the xenopus. And it is a very clear cut effect that you expect.

And knowing that alkylphenols, particularly in nonylphenol can be found in many detergents, including the triatonix 100 and all those agents that you use in the lab washing your cages as well as in the formulation in some of the pesticides.

We should really show that this is a kind of thing, if it is a direct effect on the estrogen receptor, this is what you expect. So I would like to suggest that.

I also would like to suggest that the Christin's papers that Dr. Green mentioned too and, yes, we are looking very narrow way and there are other effects of the atrazine on the immuno competency. So that I would like to suggest.

DR. ROBERTS: Dr. Isom.

DR. ISOM: I would just like to point out for the record that the Hayes paper in EHP came out in April 2003. It has been referred to several times throughout the proceedings. And that is actually a detailed publication of the nature paper that appeared this past year.

DR. ROBERTS: Dr. Steeger.

DR. STEEGER: The requirement for the review is that studies had to be submitted by February 28th. You are correct that the published hard copy of that report came out in EHP in April.

However, the online version was indeed published or available in October. And that was the version that was reviewed for the white paper.

DR. ROBERTS: I think Dr. Isom's response is of 1 D.

DR. STEEGER: That April publication is identical to the

October one.

DR. KELLEY: Could I ask my question again of the EPA?

What sorts of guidance would you like to have? Clearly, we can give guidance going forward. I still see no -- within the very narrow focus of what you reviewed, I don't think you missed anything, but we have compiled a bibliography that we thought would be helpful.

Would you like that? Or do you want to sift through it?

DR. BRADBURY: This is sort of an awkward question to answer because it presumes the path the panel may or may not go down.

Hypothetical. If the panel is thinking about a pathway about gathering additional information, my sense is that some of the information you are talking about would be very instructive in thinking about the design and nature and aspects of other information that would provide greater insight either to do a risk assessment today or insights into what kind of information and pathways to gather new information would be helpful.

So my feeling is that, yes, it would be very helpful is more in the context of the proceedings --

DR. KELLEY: But what about my question about the omission of acute toxic effects of atrazine on very early tadpoles from the

bibliography?

DR. BRADBURY: I'll turn it over to Tom some, but in looking at gonadal developmental effects and I think a sense of what those exposure concentrations were sort of kept us focused in that context. Putting it all into the context of full dose response and full effects endpoints, that could be helpful in the white paper now because it provides some founding in terms of effects of exposures.

DR. STEEGER: From my perspective as a scientist, I would very much appreciate to have access to that information to improve our understanding and representation of the effects of atrazine.

DR. BRADBURY: In the context of getting through --

DR. ROBERTS: Right.

DR. KELLEY: I bring this up because, of course, mortality was an endpoint that was studied in all these studies.

And even though the doses in these acute studies were much higher and the mortality was much faster, still, in all, that's the compound that you are interested in and the species that you are interested in.

So their exclusion seems to me inappropriate even if they are not directly relevant to gonadal development because they die before they had any gonad.

DR. ROBERTS: Perhaps the panel could recommend that they be included if for no other reason to help provide a perspective for the doses at which developmental effects may or may not occur.

For suggestions on other papers, I think we should clearly identify that if the agency is interested in studies that do not relate specifically to gonadal development but may in fact inform -- by analogy help understand whether or not a potential phenomenon occurred, here are some examples that we can point out so that it's very clear that we don't necessarily consider them omissions for the focused purpose of this paper, but they may be useful for the ecological risk assessment.

DR. KELLEY: Let me tell you how I would like to proceed. What I would like to do is to complete my list of additional sources of information that would be helpful categorizing the papers as we have just discussed under different categories, studies and other species, acute effects on toxicity, studies that might be useful if a risk assessment goes forward and why they might be useful.

And I will compile that list over lunch, and I will get it printed up and I will distribute it, and then I will allow -- of course I will allow -- the panel will make me make sure that we haven't missed studies that individual panel members have discovered. And then at

that point I think I would be able to sign off on both 1 C and 1 D.

At this point I would just like to revisit them afterwards.

DR. ROBERTS: Thank you. Other comments?

We're coming right up on 12 o'clock. I'm suspecting that -- I'm looking at Dr. Richards as the lead discussant on Number 2. I suspect we're probably going to want a little time to discuss responses for Number 2.

So let's go ahead and break now for lunch. And we can come back replenished and restored at, say, 1 o'clock and take up Question Number 2.

Let's adjourn now, and we'll see everyone at 1 o'clock.

(Thereupon, a luncheon recess was taken.)

DR. ROBERTS: Before we take up Question Number 2, our designated federal official, Mr. Paul Lewis, has an announcement.

MR. LEWIS: Thank you, Dr. Roberts.

The panel were distributed additional comment from Syngenta in reference to clarifying some of their remarks at yesterday's meeting. It is available to the panel and will also be available in the public docket for public review and inspection. Thank you.

DR. ROBERTS: Thank you, Paul.

Dr. Bradbury, let's go ahead and proceed to Question Number 2.

DR. STEEGER: In its evaluation of the existing field studies, the agency has concluded that these investigations are of limited value.

The reasons include, one, the high variability in environmental conditions and uncertainties in the preexisting status and condition of field collected animals.

Two, the spatial and temporal aspects of atrazine exposure, i.e., spatial and temporal variability over the course of the studies and the extent to which such aspects of atrazine exposure were empirically measured or otherwise accounted for.

And three, the possible cooccurence of additional chemicals and/or non chemical stressors.

Question 2 A is, to the extent that the field studies appear to indicate that atrazine may not adversely affect development, please comment on EPA's conclusion that the body of data from the field studies does not provide the means to ascertain whether the lack of a relationship between atrazine exposure and developmental effects is due to the absence of a causal relationship or limitation in study methodologies.

DR. ROBERTS: Dr. Richards is our lead discussant on this question. I will ask Dr. Richards to begin our response.

DR. RICHARDS: Thank you. I'm going to make some general comments on a number of different aspects of this question. I think I will tend to treat A and B together, although, will certainly address both parts of that question.

And I invite the second and third readers on this question to jump in at any point.

In essence, the field studies that have been presented in the literature and the presentations over the last couple days are very good.

In one respect, they provide a great deal of information. I think one of the most important things that they seem to indicate is that the abnormalities, gonadal abnormalities that appeared in some cases in laboratory experiments are seen in the field.

These abnormalities sometimes are seen in very different geographic regions to some degree among different species.

And from that standpoint, I think that's critical, because I think all these questions ultimately relate to an ecological role and a population role. And without looking at sort of natural field type of situations, we can't really see whether some of the things that we're looking at are relevant.

Now, however, the studies do not seem to provide substance to

the nature of why these gonadal abnormalities or other endpoints are related to atrazine.

And that's where it breaks down to problems in experimental design. Often times, the experiments were never really designed to ask such questions. Typically, they have very poor statistical power to address such of a question.

Most of them are essentially descriptional studies. Descriptional studies or observational studies can be very good. They can be powerful. They can give you relationships. But they require that a number of parameters be accounted for in order to derive any use from them.

In the studies that have been set up to use a control or a reference situation, those control and reference situations have usually not been adequately described or they have some fundamental flaws. And the sample sizes used, the end size used is typically totally inadequate given the variability and some of the measures that we're looking at.

The studies that have been descriptional in attempting to use more of aggression based approach have not adequately dealt with the problems of scale.

The studies we have looked at have included scales including

looking at continental scales, looking at the size of a county, comparing a few ponds within a small area.

All of those have very significant things that have to be accounted for in order to account for the variance associated with individual assessment endpoints.

And I will just begin to list a few of those things. But the Number 1 thing that we're dealing with here is aquatic organisms, organisms that have a very significant part, at least one part of their last cycle tied to the aquatic medium.

In order to do a descriptional study or to set up a good field experiment, you have to adequately account for the movement of water in and out of those systems.

A small ditch is very different than a river, is very different than a wetland. It's very different than a backwater on a river.

So it comes back to both describing the environment that the animal lives in, but very much describing in a general way or a specific way the exposure that these animals actually encounter in the field.

And for small experimental studies, that needs to be accounted for and in much detail. But on the larger, more descriptive studies, that can be accounted for in a much better way than has even been

attempted in any of these studies.

There are many tools available that allow us to look at even relatively large geographic scale studies that can quantify and allow you to partition some of that variance in a stratified experimental design.

And basically, that's relating to how does surface water interact with the bodies of water that these experiments are being conducted in or observations are being made, what's a relative contribution of groundwater, what are flow events during a period of exposure, what are the flow events that actually occur amongst seasons, in cases where experiments have been run, have there been flooding events that may influence exposure or may influence the movement of organisms in or out of those experimental situations.

And also related to hydrology is -- to have an adequate depiction of hydrology, you have to know what the watershed is. You have to know what is upstream of the event that you are observing.

And there are some fairly easy, relatively powerful ways to describe watersheds both in a strictly hydrologic sense, but also in terms of what the watershed is composed of.

Things like soil characteristics and hydrology connectivity to other water bodies, these are all things that are fundamental both to

the biology and movement of these creatures, as well as the chemical and physical environments that the animals live in.

Basically, some of the studies have provided that type of information perhaps in an appendix or listing of things they may or may not have measured. Some of them did not provide any of that information.

But essentially, none of the studies attempted to use that as a co-correlate in any way to try to partition variance amongst characters that again provide variation in these populations that have been observed.

So in essence, I think what we're looking at is that statistical designs have not been sufficient to actually address questions specifically related to atrazine.

And I think that that falls into the B part of this question, also, is that there are co-stressors, potential co-stressors. Many of those we can guess, many of those that we could potentially stratify out in an appropriate statistical design or to partition into in the analysis, but that takes a great deal of forethought in terms of development of a design.

In many cases, had we seen as much attention given to sound experimental design as we have at least to date with some of the

laboratory measures, I think we could have been a little bit further forward.

I think I'll ask my co-readers to jump in after that.

DR. ROBERTS: Dr. Gibbs, you are an associate discussant. Is there anything you want to add? Or do you want me to take Dr. Skelly first?

DR. SKELLY: First of all, I would like to point out that that's a single sentence up there, and so it took me a while to figure out what it means, but I think I got a handle on it.

I guess the end of the sentence is the most critical part. So do we think that the absence of causal relationship or limitation and study methodologies is the reason why the EPA's conclusion that field studies don't provide the means to ascertain whether there is something going on here.

I guess in my opinion, I'm going to agree with Dr. Richards and say that the limitation and study methodologies is probably most important here, and comment that the absence of causal relationship has never slowed down any ecologist I have ever met.

We often like to pretend that we know why things happen when we're working in the field, but that shouldn't stop us from moving forward and trying to understand what the associations are.

Because after all, everything that we're doing is keeping our fingers crossed that we get the mechanism right. Even in the finest laboratory, using the best laboratory practices, we can never know for sure that we have the mechanism right.

So specifically, I think the limitations and study methodology that were most important concur largely with what Dr. Richards said. I think the lack of power is an issue, but it is very easy to be critical of that in hindsight.

These studies that have been done can now be used moving forward to come up with better ideas with what needs to happen.

Having said that, the scale of the field studies and I say this from the standpoint of having quite a bit of experience in field sampling amphibian population, these were not large studies in terms of the number of sites that were examined.

There are many more ambitious studies and I think we can look to a lesson from how the UV light on amphibians controversy is being resolved now.

It has taken very large scale studies looking at hundreds of wetlands over very large areas dealing with many of the same issues that we have been talking about here today.

Some of the people in this room have been involved in this

work, so they know what it takes to pull inference out of these sorts of big observational studies.

That leads me to the second point, which is the issue of site selection. I think it is hard to go out and use a map ahead of time or just go out and look at a place and say, there is a cornfield here or there isn't a cornfield here, to say whether this is the right site to be an exposure site or a control site.

And some of the studies that were not considered here and are not necessarily particularly relevant to this issue but where people have taken an alternative approach, they might take a year or two years just to pick their sites.

I'm going to come back to this point when we talk about some of the questions later on. I'll leave that for now.

The final point, the limitation in the study methodologies may be this is not a limitation study methodology, but a significant limitation in the studies that I saw was to me looking at what the EPA is going for here, the assessment endpoints that are talked about are fertility reproduction recruitment.

Ultimately, what we are trying to think about -- something about viability of populations and those people in this room who know about measuring population viability know what a giant task

that is.

Even if we go back to the assessment endpoints here the motivation for doing field studies should be to get at those things. I don't think most of the field studies that have been done to date address that issue. In moving forward that's something that should be tackled very concretely.

In response to B, to the extent that we do see something going on here, is it true that these studies don't provide sufficient information to resolve the potential role of additional cooccuring stressors?

I guess that is always, always going to be true to some extent with the field study, but for reasons that we'll probably talk about later on I still think it is extremely important for much of this work to be done in a context, in a field context.

To that end, as I have harped on before and I will say now, virtually all the field work that is talked about here and if we exclude the mesacosm experiment all of it has been observational.

It is possible even in this difficult kind of context of working with pesticides to do field experiments. Especially -- we've got a registered pesticide here, we can use it in the world out there and see what it does at population scales.

That can help us significantly get at the issue of co-occurring factors because we can choose sites that are sort of tabularasas to begin with and add our stressor that we're interested in.

I'm going to echo -- in finishing, I will echo what Dr. Richards said. That is that, I think, the critical thing here that we can't ignore is we can talk about limitations of any study we want all day long, multiple groups have gone out and looked for gonadal abnormalities in nature and they have found them and these match the morphological characters people are seeing in the lab.

At this point, I think it is fair to say we don't know why, but the fact that those abnormalities exist I think is significant. I think if a bunch of groups had gone out there and had not found them we would be thinking about this differently. I will end there and pass to Dr. Gibbs.

DR. ROBERTS: Dr. Gibbs.

DR. GIBBS: We have spoken extensively as a group, so I don't want to reiterate too many points. I do personally agree with the statement that essentially study designs have precluded really determining whether there is or there is not an association between the occurrence of gonadal abnormalities in the field populations of amphibians in the presence or absence of levels of atrazine.

We don't want to imply that observational studies cannot address these issues. I think they are actually very important for the reason that Dr. Skelly just mentioned.

The risk assessment goal here really does focus on population viability. One needs to go to the field essentially to measure many of these parameters.

I would -- perhaps this is getting away from the question, but there are other modes of studying and tackling these problems which essentially fall in the realm of field experimentation.

We have been discussing some other situations that occur in the field with temporary breeding -- temporary pool breeding amphibians such as the amphibians that use vernal pools.

Vernal pools occur in isolated, small, populations in great numbers in homogenous landscapes that are really quite amenable to experimental manipulations that could get at both -- would yield information both from histological perspective but also from demographic perspective quite relevant to many of the issues that the risk managers -- risk assessment folks have to deal with. I think that's worth exploring at some point. But to get back to the point at hand, I agree broadly with many of the statements that Dr. Richards raised, issues with inappropriate or not outlining sampling frames,

low levels of replication in terms of using wetlands as actually replicates and the amphibians therein.

And really a difficulty in establishing whether there is or is not causal relationship here between what are some fairly high levels of abnormalities being seen in the field, but whether or not there is a relationship with atrazine. In my mind that can't be attributed based on the designs of many of these studies.

DR. ROBERTS: Thank you, Dr. Gibbs. Let me ask other members of the panel if they agree or disagree?

DR. MATSUMURA: I agree with Dr. Skelly and Dr. Richards. I certainly emphasize the importance of the field studies.

I was trying to think what the precedence I could think of, and, of course, like Gillette's work on the alligators and how long did it take, and what started that whole project, and like finding a really contaminated site like Lake Apopka was very important for that study. And comes from field studies like eggshell thinning, it took a long, long time to say it was DDT.

But again, field observation that all those eggs are crashing and that's a problem and the bald eagles not just thinning but by the weight of their mothers just sitting on those eggs are crashed. Those observations really helped.

And finally, I would like to make sure that one aspect to emphasize, that means analysis of burden of other contaminants. I was reading this Reeder's paper reporting this intersex confined to the -- correlated to atrazine. But at the same time they had the PCDD and PCDF.

And so you have to leave some questions always. Knowing what the other stressors and other chemicals are there, that could really help.

DR. ROBERTS: Dr. Green.

DR. GREEN: I agree with your comments. For my own edification, I would like to know from your experience and perspective what denotes a healthy amphibian population observationally in a field study? What implies that the population is sickly and on the decline, absolute head counts and how long does it take to get that kind of information?

DR. GIBBS: Insofar as you linked health to decline, you are talking about a temporal phenomenon. That may indicate the need for repeated sampling to actually detect a change in population size, population structure.

With these particular organisms, some of them have -- with frogs, incredibly short life spans and then generation times and hence

population turnovers.

So one can fairly quickly, unlike working with many other vertebrates, see if there actually are population declines or increases occurring because you need much less time with generation times and sometimes one to two to three years to see these kinds of effects.

Sheer abundance is one good measurement. Dr. Skelly may have other ideas.

DR. SKELLY: I would agree with what Dr. Gibbs just said. I would just add that we have settled on a group here that has notoriously volatile population dynamics and going out in a given year and seeing recruitment failure for some species is actually the norm.

Having total cohort failure is -- if we were talking about grizzly bears in Yellowstone people might get quite upset about that, but most of the wood frog populations I survey every year in most years crash out.

This year they are floating across the roadways and they are doing quite well with all the rain. In most years they dry up with their pond. It definitely is something you have to look at over time.

We're fortunate many of the species that we would be interested in in terms of native amphibians do have short life spans. It does

have to be looked at over time if we're interested in any sort of understanding of population robustness.

DR. ROBERTS: There's that word again. Dr. Delorme, then Dr. Richards.

DR. DELORME: Just following up on Dr. Green's question. Could you give us any indication of some measurement endpoints or what variables you might be looking for to look for effects in populations if you are out there with your rubber boots?

DR. SKELLY: Population --

DR. GREEN: I think that will be addressed in question five. We have some estrogenic biomarkers we put together that --

DR. DELORME: I was asking from a populations perspective, like what population variables might you go out and measure.

DR. ROBERTS: Were you planning on bringing it up later in the context of five or do you want --

DR. SKELLY: I actually think we will address that later.

DR. ROBERTS: Dr. Richards.

DR. RICHARDS: Another context sort of thing is amphibian population is often very variable that is the way they are. That is the nature of the waterbodies they live in, to get back to the hydrology of these situations.

One of the things that we know about amphibians is that they interact over a relatively large geographic scale. You just can't look at a pond the size of the space between our tables here, you have to be cognizant of the larger network of waterbodies that they are connected to.

Because when one local population disappears they are colonized by another population that may be half a kilometer away. Looking at a series of small ditches that are by nature connected is very important as opposed to an isolated pool.

Are you looking at a species like the South African animal that lives entirely, its whole life cycle in water versus something like the rana species that are out moving around?

These are very critical questions. Both designing the experiment and asking questions about is a population healthy in part it is related to a little bit larger scale than we frequently focus on.

DR. ROBERTS: Dr. Skelly has a follow up, then Dr. LeBlanc.

DR. SKELLY: I guess as a follow up to that, we're painting a picture that can seem to get more and more difficult. Actually, the genetic techniques that are being developed now are getting us to the point were we can actually distinguish the boundaries of populations. Up until recently, people just kind of

crossed their fingers and assumed a pond was a population. I think we're moving well beyond that. So we are actually starting to understand in a much more detailed way how much movement there is and over what scales we should be assessing population level responses in order to say whether a population is going to remain there or is it likely to go extinct.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: This question is posed to the discussants. Based on what I just heard, my question is would it be -- due to the volatile nature of the population dynamics of frogs would it be difficult to discern population level effects of environmental contaminants?

DR. RICHARDS: Yes and no. I think sometimes with some of these measures dropping down out of the sky on one occasion and listening for how many frog calls there are you are not going get a real good answer. I think because of the fluctuating dynamics of these populations in their actual environment some manner of looking at more of a prolonged view of the population is needed.

There are some things as genetics that give us a backward view of what is going on there, and that's great, but there is much room for innovation on this. The book is not completely written.

DR. ROBERTS: I think Dr. Skelly wants to respond as well.

DR. SKELLY: I guess, I would say in order to make the picture a little less bleak here, there are very volatile dynamics. But if you follow a set of populations in a defined area over a long period of time, you see this volatility from year to year.

You also see very striking patterns. I will give you an example that is directly relevant to the sort of thing we're considering here.

For many, many years people have been doing experiments in mesacosms in the laboratory that were showing that amphibians undergo interspecific competition and that interspecific competition could even be possible for population extinction.

That was the way -- that was the inference that was coming out of this work. There is excellent laboratory and mesacosm based evidence that there was competition going on, and if we change the words, that's exactly what we have been talking about here, the same sort of thing where people were doing these very focused studies, they were getting mechanistic, they were looking for interference compounds, they were looking for resource based competition, and so forth.

We have been able to show more recently that from everything we can tell even though this is an actor in these experimental context,

it does not seem to be important for the kinds of patterns that the EPA is interested in for assessment endpoints and for their overall criteria.

Instead we have been able to discover starting with field observations, then moving to field experiments, and then into laboratory experiments, that other factors such as hydroperiod and such as the light level in these ponds that influences temperature and food gradients is much more important.

That's been a real lesson for me in how we discover what is really going on, how to parse this out from the perspective of a field scientist so that the lab scientists are working on the right stuff so they are not just going down the garden path.

DR. ROBERTS: Dr. Isom.

DR. ISOM: Since atrazine has been applied to the environment for over 40 years in North America, is there historical population data that perhaps could be looked at to give us some answers to these longer term population issues?

DR. SKELLY: There has been -- because of the overall interest in amphibian declines, everything that existed and much work over the last 12 years or so has been done along those lines.

We probably for the purposes of this panel can parasitize a

much larger database than you might believe. There is much known on that. The problem of course is going to be attributing cause to these historical studies where the other measurements that we are interested in were not made.

I can give you as an example, there were surveys done in the early part of this century in Iowa that describe high abundances of amphibians over many, many counties. When the resurvey was done in 1980's, abundances and species composition over large areas had changed.

Now that's over the same time span as the green revolution. Does it have anything to do with that, does it have anything to do with pesticides? We have absolutely no idea.

In general, the consensus seems to be now that many, many amphibian populations are declining. So that's the state of our knowledge, the state of our sciences that many populations have declined.

When people go out in their back yards -- and I give lots of lectures to the public and the first question I get from the public, which is an excellent one, is I can be deafened sitting on the back porch by the spring peepers. So are amphibians declining?

What I say to them is because this is a temporal phenomenon

maybe you would have gone deaf in one week instead of two, 100 years ago. We don't know what it was -- we don't have enough data to be able to say over very broad scales what amphibian abundances were like.

But Dr. Gibbs maybe can answer this a little bit better than I can. One of the things that has happened over that same time period is that wetlands have disappeared or been modified, often in an agricultural context, in ways that change their suitability for different amphibian species over the same time span.

There has been talk about multiple co-occurring factors. It is there. I think the most interesting historical data that we could get are in the animals. So this issue of evolved resistance could be really important in showing or in evaluating whether there have been broad scale evolutionary responses to pesticides.

DR. ROBERTS: Dr. Gibbs, did you want to respond also?

DR. GIBBS: Just quickly, amphibian monitoring in a systematic and large scale fashion only has begun since 1990 or so with various initiatives such as the USGS and other groups have put together. There really is not a lot of data, even that effort is limping along.

Unfortunately, we are constrained and we will remain so for

quite a few more decades before we can get something like the US -- the breeding bird survey on line for amphibians, that's the goal. We really don't have any good solid baselines over large areas. And a decade of data on a few spots is about as good as we can do.

This underlies a lot of the controversies and debates over amphibian decline this lack of monitoring data. Unfortunately, that is the state of the science on amphibian monitoring.

DR. ROBERTS: Dr. Richards.

DR. RICHARDS: I would like to hopefully dispel a little bit of the gloom here that I myself have been raising.

At one level amphibians are no different than any other aquatic organisms that are dealt with by EPA and aquatic ecologists for a long time.

They still reproduce, they still have fecundity, they still recruit to a population, there is predation, there is density and nondensity dependent aspects to their population sizes, and that makes them particularly amenable to experimentation. That doesn't mean we can't experiment with these animals and can't derive some creative ways of examining population parameters.

They do things just like when you are chasing little fish along the margins of a stream. If you have ever done that you know you can

go there one time and they will be there, next time they won't.

That doesn't keep us from having a great body of regulatory approaches to dealing with toxic antieffects on fish.

DR. ROBERTS: Dr. Delorme, did you want to respond to that as well? I have a couple of other folks who have questions.

DR. DELORME: I wanted to make a comment on an observation. I can wait.

DR. ROBERTS: Let me go to Dr. Denver, then Dr. Heeringa, and then you.

DR. DENVER: I just wanted to comment on something that Dr. Skelly said. It has to do with single species experiments versus competitive interactions, and at the risk of making this even more complicated I think it is important to realize the importance of the competitive interactions. Most of the studies that we have reviewed have been studies of single species. We published a study actually last year that showed that exposure to PCBs can actually change the competitive interactions among species.

These were studying the northern leopard frog and also wood frogs. That's another layer of complexity that needs to be considered.

DR. ROBERTS: Dr. Heeringa.

DR. HEERINGA: With much more expertise, Dr. Denver

anticipated my question. Mine is much more direct and I deal a little bit with waterfowl populations.

Is there evidence, either from zoological samples in museums, et cetera, or other studies, on sort of either a lack of increased hybridization or competition in these natural populations?

Are there species that are invading historically, turf for other species that have been a concern to ecologists, is that practice, it could happen naturally, but do you see that happening and are these populations still relatively isolated and nonhybridized?

DR. GIBBS: Certainly, and the bullfrog is a classic example of invasions, but very much associated with the activities of people and moving them around.

I'm trying to think of an analogous situation with a hybrid zone shifting or one native species, a sympatric species, one moving into the other's range. I'm failing to --

DR. SKELLY: The example that Dr. Hayes talked about that was the first one that I had seen evidence for.

DR. ROBERTS: We've got Dr. Delorme, then Dr. Matsumura.

DR. DELORME: I was sitting here listening to Dr. Skelly talk in response to Gary Isom's question and it occurred to me that one of the things that EPA has asked us to look at is the assessment

endpoints and they put forth an assessment endpoint of the population.

You guys can shoot me down if you want but some of the measures that you are talking about, species diversity and relative species abundance, perhaps maybe an alternate assessment endpoint is also communities, in terms of their composition.

It gets into sensitivity of different species and whether it is different or not. Just want to know if you guys think this idea has any merit as alternate assessment endpoint?

DR. ROBERTS: You three are on the spot again. Who wants to take it?

DR. RICHARDS: I'll touch a little bit of it.

There are some studies out there that have indicated over relatively large geographic areas that anuran communities can be a fairly useful way of looking at relationship to landscape parameters. Whether they'll be useful for looking at toxic related questions, I don't think has been delineated.

Certainly, communities can be manipulated -- the data from communities can be manipulated into a series of metrics much like we use fish or invertebrates or other things in a way to look at patterns over relatively large geographic scales.

DR. ROBERTS: Dr. Skelly, did you want to add anything?

DR. SKELLY: I guess I would just insert a note of caution that it can be -- if we are trying to link these studies, eventually, back to a mechanism and link them to laboratory studies, logistically and pragmatically, it may be challenging enough to do on a single species level.

I'm very interested in communities. I think that they can sometimes show responses that may give us more information than single species.

But on the idea that we need to walk before we run, I guess I would say that we should try to get out into the field more working with the single species before we look at multiple species simultaneously.

DR. ROBERTS: Dr. Matsumura. DR. MATSUMURA: I'm not doing that, but I'm collaborating with the good herbatologist in the pit studies, on the study on the yellow legged mountain frogs, rana muscosa.

As far as that particular species is concerned, it is declining, everybody agrees, at least, in California. It has a long larval life span that it spends in such a cold place for three to almost four years in the water, and they are disappearing.

When we made a survey, we found that there are still some ponds and lakes teamed with the yellow legged frog but they all on the eastern slope. On the western slope, there are only about three sites that we found.

There is no question that you can talk to those rangers. They say, well, I used to know that particular pond had the muscosa, lots of them and they are disappearing in the last year 10 years. They are species that you can really count on not just by virtue of long life in that particular case. You can really see that's how they are disappearing.

So, it is not related to atrazine.

But by studying those, we start finding also, hyla regilla, pacific tree frogs, are also disappearing from the same western slope. That's the reason why we are suspecting air pollution and particularly PCBs.

In the lowland area also the red legged frogs also disappearing, but they are being displaced by bullfrogs. So that is the same example that you are citing.

I get the feeling that just looking at those mountain frogs endangered species which we cannot study well, that there could be some combination of right kind of species, very sensitive ones that

you could pick on and try to see whether those are the ones who are effected the most by this kind of pollution.

So some field biologists can really look for, they may find a good combination. I just wanted to add.

DR. ROBERTS: Thank you.

I haven't heard much in the way of disagreement in this.

Let me ask the panel, is there anything that they disagree with that has been stated or are there any other important points that have been left out of the discussion that perhaps should be included in the response?

Dr. Kelley.

DR. KELLEY: I don't disagree with the conclusion as stated based on the available data. I just simply wish to make a comment about the field studies in South Africa, which is that if the EPA decides that it wants to adopt xenopus as some sort of model system for studying a sex environmental effects, including atrazine, but there are others as well for which it's being the considered, inclusion of field studies in gathering field data in xenopus will be extremely useful.

It will enable you to take the best studied laboratory anuran and correlate those results with the field. I don't think it is possible for

any other animal. We have neglected in our literature survey the data on xenopus which exists in the South African literature.

I would, if that route is taken, I think -- going forward more carefully designed and better powered field studies in xenopus will be useful in trying to relate laboratory results to potential effects in the field.

Now they won't tell you anything about our North American anurans, necessarily, but they will tell you something about the limitations of the transform.

DR. ROBERTS: Thank you. Dr. Bradbury, it looks like there is strong endorsement for the value of field studies and the panel has a number of comments about what is required to really get good information from those studies.

Are there any follow up questions that you have for the panel on this topic?

DR. BRADBURY: Just for the record, we have question 2 B.

DR. ROBERTS: I sort of sense that our discussion has covered both 2 A and 2 B. I will defer to the discussants on that.

DR. BRADBURY: I feel comfortable that we got the picture between the two aspects but if there is something left in your laptop.

DR. ROBERTS: Dr. Richards, is there anything left in your

laptop?

DR. RICHARDS: My fingers are fingered out, but I think in my own comments I was implying that it was very difficult to examine the effects of costressers given the studies we have seen so far.

DR. BRADBURY: I think we're in good shape on our end.

DR. ROBERTS: Are there any other points that need to be made or anything we need to resolve before we move onto the next question? If not, let's move on to question three.

DR. STEEGER: In evaluation of the existing laboratory based studies the agency concluded that there was sufficient information to establish a hypothesis that atrazine could cause adverse gonadal developmental effects.

However, due to the different experimental designs and variability in the nature and extent of experimental conditions, i.e., level of excessive mortality, delayed developmental and untreated organisms, lack of response to positive controls, it was not possible to adequately assess the hypothesis that atrazine causes developmental effects.

It was further concluded that the current body of information did not prove the means to characterize the nature of any associated dose response relationships.

Please comment on EPA's determination that the laboratory studies provide a plausible basis for the means to establish hypothesis concerning the potential for atrazine to cause development effects.

Also, please comment on whether the overall body of available data are adequate to demonstrate whether or not atrazine causes developmental effects under the conditions described in these studies.

DR. ROBERTS: An important question Dr. LeBlanc, what do you think?

DR. LEBLANC: I concur with the EPA, their determination that laboratory studies on the affects of atrazine on anuran gonadal development are sufficient to establish the hypothesis that atrazine interferes with normal gonad development.

Clearly, the available data are limited. However, the existing data as related specifically to the laboratory investigation, I believe, support the hypothesis of the eight laboratory studies that we were provided with that evaluating effects of atrazine on gonad development.

Five of these studies detected such abnormalities from three different laboratories. Certainly, in my laboratory, if a student comes to me with an observation, be it induction of enzyme, suppression of protein, or a message RNA level, the first thing I tell them is go do it

again.

They come back and I tell them to go do it again and perhaps after six, seven, or eight times I will accept that data, which doesn't mean that perhaps two of those times they weren't able to reproduce the effect.

But if the preponderance of the evidence consistently shows the effect that they initially observed and that we were pursuing, I wouldn't discount the data.

There certainly were differences observed among the various studies that we examined as related to the effects that were observed and many attributes were ascribed that could have contributed to these differences, including species differences, differences in exposure design, and specific endpoints that were selected in the individual evaluations.

Significant data gaps exist in our understanding of the affects of atrazine on anuran development. These include a lack of understanding of the mechanism by which atrazine elicits its developmental toxicity, the nature of the concentration response relationship, definition of susceptible windows of exposure, and identification of a threshold concentration.

However, it is certainly my view, that the existing data does

support acceptance of the hypothesis that atrazine interferes with normal anuran gonadal development.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I agree a great deal with what Gerald already told you. I think one of the points why we find so many differences, and the big difference should be that we have no standardized protocols in various studies.

I think one of the goals for the future in future studies is, first, to design more or less a standardized protocol to work on amphibians, especially on xenopus laevis, but also if you use ranids.

So, we are comparing apples with pears if you don't go ahead with a really standardized protocol. That's one comment on it.

DR. ROBERTS: Dr. Denver, do you have something to add?

DR. DENVER: I just agree that we have sufficient data to generate a hypothesis but insufficient data to test the hypothesis.

DR. ROBERTS: Other members of the panel? Dr. Matsumura.

DR. MATSUMURA: I agree with Dr. LeBlanc's statement so long as it is limited to gonadal abnormalities. I'm not convinced about the whole scale developmental effect even including laryngeal effect.

But regarding the abnormalities, I feel that the having Dr.

Carr's independent experiment as well as John Giesy's, I feel that qualitative there is some clear cut independent verification of the phenomenon. But others, I would not stick my neck. So that's just my personal feeling.

That's why I asked Dr. Carr to just tell me that he can really stand behind the data. He told me that. So, I'm satisfied by independent verification. But other parameters I would not agree that there is sufficient base.

DR. ROBERTS: Dr. Matsumura raised the point -- you know the question asked about developmental effects. I guess, we need to be careful how we define those in terms of our conclusions.

I would also like to point out that I want to be clear, when the question asks whether or not the overall body of available data is adequate to demonstrate whether or not atrazine causes developmental effects.

I thought I heard two different answers to that question among panel members. I want to sort of see what people think.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: I think the question is whether the data is sufficient to entertain the hypothesis that atrazine produces developmental effects.

The answer was a narrowly focused one. It said that the data on gonadal development coming from two very different frog species, one totally aquatic and one at least partially terrestrial, terrestrial during most of the lifespan, did indicate an effect on gonadal development.

So for that reason, entertaining the hypothesis was regarded as an appropriate thing to do.

DR. ROBERTS: So, not to put words in your mouth, but your opinion is that the data are sufficient to support the hypothesis or to justify the hypothesis, but not necessarily to demonstrate which I would interpret as prove the hypothesis.

DR. KELLEY: It was very well put by the EPA. You could look at the data and say the data is such that we could reject this hypothesis. I do not believe the panel would support that conclusion although we haven't heard from everybody. I do not believe that we could reject the hypothesis. So, what that means is the hypothesis is still viable. Is the hypothesis more than a hypothesis, do we actually believe that atrazine will, at some dose that we don't know now and some range of species that we don't know now, reliably result in gonadal abnormalities?

I don't think nor do I believe the panel thinks that the data are

sufficient to accept that hypothesis. But the data are certainly sufficient not to rule it out and to continue to entertain it as a model for looking further.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: I'm going to push you a little bit more though. I'm just going to reread the statement just to make sure we answer the question as asked. The statement -- the question ends with essentially available data -- EPA is questioning whether available data is adequate to demonstrate whether or not atrazine causes developmental affects. So, you're saying no.

DR. KELLEY: Read the last phrase.

DR. LEBLANC: Under conditions described in these studies.

DR. KELLEY: So we have data. We have data from a reasonably large number of studies that indicate that at least one dose there was a gonadal defect in a varying proportion of animals.

I have a where there is smoke there is fire reaction to that. I think there is something going on, that is my bottom line, but I don't know there is something going on.

I believe it is worthwhile investigating the hypothesis further, but I wouldn't believe that it was proven at this point. It certainly raises concerns.

DR. ROBERTS: Whenever we're not sure exactly what the question is asking, we sometimes ask the agency to clarify for us.

Is this question asking whether or not the data provided by the existing studies is sufficient to demonstrate that affect or is it more along the lines of what Dr. Kelley is talking about?

Is it justify pursuit of the hypothesis but not necessarily demonstrate the phenomenon which is what I, sort of, read into the white paper. If you can clarify that for us.

DR. BRADBURY: It's amazing how one can wordsmith, and work and work, and think it's clear, and then you need to work on it.

The second phrase is commenting on whether the overall body of available data is adequate to demonstrate in the describe studies.

I think that's an important phrase. Based on the body of information is there some sense, given the quality of the studies and the characteristics of the studies, is there something going on, is there some smoke?

That is sort of in the context in is there enough smoke to say, I think it's reasonable to formulate a hypothesis to look to see if there is fire associated with the smoke. That's the context of the question, intended meaning of the question.

I apologize if you're confused.

DR. ROBERTS: With that clarification, then, both of you have answered in the affirmative then?

DR. LEBLANC: I have.

DR. ROBERTS: Dr. Kelley?

DR. KELLEY: I have as well.

DR. ROBERTS: Dr. Denver?

DR. DENVER: Yes.

DR. ROBERTS: Is there anyone else that would like to weigh in on this?

Dr. Kloas you were a discussant did you want to indicate one way or the other on this? Dr. Coats?

DR. COATS: Yes, I will weigh in. I think there is enough data to pursue forward movement to test the hypothesis.

DR. DELORME: I would concur as well. I think one of the things we saw is there are a number of factors with the husbandry and whatnot that might have resulted in something going on. So, it is not really, really, clear that atrazine is the root cause. So, we need to pursue the hypothesis.

DR. ROBERTS: Would anyone else like to weigh in on this before we move on to B?

DR. SKELLY: I will concur broadly. I think the way that Dr.

LeBlanc and the others responded reflects my feelings as well.

DR. ROBERTS: Dr. Green?

DR. GREEN: I concur as well.

DR. ROBERTS: Good. Dr. Richards?

DR. RICHARDSON: I'll join the party.

DR. ROBERTS: Then let's go ahead and take part B?

DR. STEEGER: Please comment on EPA's conclusion that given the variability in the available dose response data across the studies, e.g., approximately 250-fold difference in reported thresholds for observed developmental effects as well as reports of monotonic and nonmonotonic dose response curves, it is not possible to ascertain the relationship, if any, of atrazine exposure to developmental effects in amphibians.

DR. ROBERTS: Dr. LeBlanc, what do you think?

DR. LEBLANC: The short answer is I agree wholeheartedly, and I think I only need to expand on that a little bit.

There is clearly major deficiencies that exist in the data as related to describing the relationship between atrazine exposure and gonadal toxicities that have been reported.

We can extract, I think, a small amount of data as related to that relationship that presumed relationship, at this point in time, although

the conclusions are very, very limited.

For example, I think, I hope, we're all in agreement that atrazine at a concentration of 0.01 microgram per liter appears to have no effect on anuran gonadal development.

The data seems to suggest that exposure to frog larvae to atrazine concentrations greater than or equal to .1 microgram per liter can elicit developmental abnormalities, not does but can.

There is limited data available to us to make any judgments as to whether or not concentrations in the range of .1 to 10 micrograms per liter truly elicit adverse effects.

However, when we consider concentrations in the vicinity of 25 micrograms per liter, there seems to be some reasonably good concordance that this concentration does elicit gonadal effects.

Clearly, more data is required to define the concentration response relationship between atrazine and gonadal development of anuran larvae.

At this time, I would have confidence only in concluding that the threshold concentration for this material exists between .01 and 25 micrograms per liter.

DR. ROBERTS: Thoughts from other panel members? I will start with the lead discussants, Dr. Kloas, did you want to add

anything or comment on this?

DR. KLOAS: Concur.

DR. ROBERTS: Dr. Denver?

DR. DENVER: I would just concur with the statement that we put together last night.

DR. ROBERTS: Other comments from other members of the panel?

Does anyone feel that the data are adequate to describe the dose potential dose response relationships?

Dr. Matsumura?

DR. MATSUMURA: I don't think it is adequately describing, all we're saying is that the threshold concept can be applied and that some experiments indicate that there is such a thing.

Real threshold experiments must be done rather carefully. It is not that easy. How many years it took to have any agreement on the cancer dose and the effect relationships, particularly with the hormones, there are feedbacks and -- you know, Jere.

So, I would vote for the statement that the threshold is a good way to go about it and there are some indications, incipient indications.

DR. ROBERTS: In the spirit of Dr. LeBlanc's earlier comment

which I concurred that these were actually concentration response relationships and not dose response relationships. So, I'm going to correct myself in agreement with that.

DR. LEBLANC: I think we need to recognize that the great majority of the studies that we have reviewed, I can think of one exception, were not designed to evaluate the concentration response relationship. Certainly, future studies need to be designed appropriately so that the response relationship can be appropriately evaluated. I made some attempts to drive some general understanding of what this relationship might be based upon all the information that was provided to us.

The best that I could come up with, and I don't have a lot of confidence in it, is that .01 micrograms per liter has no effect and all concentrations evaluated greater than that had an effect on average.

And on average, that effect regardless of the concentration seemed to have been around 20 percent incident of effect whatever the investigators may have been monitoring.

Now, I don't know what that means. It could mean that the maximum response that we should anticipate is 20 percent. And that the concentration response is going to occur between .01 and .1, the threshold will be between there. I don't know that that's the case. It

is just a suggestion.

And there are precedents for observing partial effects where perhaps we would be anticipating greater effects? What comes to mind are my own studies looking at intersex in snails as related to tributal 10. In laboratory exposures where we try and replicate what we see in the environment in 10 contaminated environments where the incidence is 100 percent intersex, the best we can generate is about 30 percent. We're not alone, other labs have had similar success in generating intersex in only about 30 percent of the animals. We really don't know why. We assume it is a deficiency in our experimental design.

We know the animals are capable of -- the population is capable of totally responding, but in the lab they simply don't. I don't think we need to go into these experiments with the anticipation that we're going to see 100 percent response in a population if 20 percent is truly the maximum that we can anticipate, we should accept that and live with it.

But we should still be able to define that relationship between a threshold concentration and that maximum effect whatever it might be.

DR. ROBERTS: Okay. Are there other comments you want to

express regarding this?

I'll say for myself personally, I agree that I don't think the data are adequate to describe the dose response relationship.

I have not gone through the analysis you have. I have no idea where an apparent threshold might be. Speaking personally, I have no idea what the shape of the dose response curve might be. But I do agree there is not enough data to establish that.

Anyone else? Let's go ahead and move on to the next.

DR. STEEGER: Many of the available studies proposed that aromatase induction results in elevated estrogen levels that lead to feminization as characterized by ovotestes, intersex, and hermaphroditism in genetically male amphibians.

Please comment on EPA's conclusion that to date aromatase induction by atrazine has not been demonstrated in any anuran in controlled laboratory investigations.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: First of all, I would like to say that it is not the only hypothesis which could raise such phenomenons, there are feminizational demasculization phenomenon.

We would have two ways how to obtain them estrogenic and anti-androgenic ones, that's the first remark I want to make.

Answering question 4 A, of course it is correct. There are no data showing up any aromatase induction or stimulation caused by atrazine. But however, I think we have already discussed there is no approach done which would be appropriate to demonstrate it.

So, I think that experimental designs done up to now, they don't support this hypothesis but they cannot support such a hypothesis for aromatase stimulatory effects.

Would you like to add something?

DR. ROBERTS: Thank you. So in your opinion it is still an open question?

DR. KLOAS: Yes.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: I would agree. I would like to point out that the aromatase gene or at least one of them, they probably have two, the spene laevis (ph) has been cloned in xenopus laevis. It will be possible to study the expression of the aromatase gene in the developing gonad in the presence and the absence of agents that are thought to affect it.

While the MRNA expression is not definitive with respect to protein expression or the activity, the enzymatic activity of that protein, on the other hand, you do have to have the gene expressed to

have it there. So we now have available the tools to test this hypothesis in a rigorous fashion. It should be possible to test that.

I do concur absolutely that although this is a popular hypothesis, it is not the only hypothesis that could provide a mechanism of action for effects that have been reported.

And so to focus on this hypothesis to the exclusion of other kinds of ideas like changes in hormones within the animal's body and steroid hormones changes in the hypothalamic, pituitary, gonadal axis is almost certainly a mistake. There are bound to be other mechanisms that are involved. DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I would also like to add one comment. It could be also due that there is a direct interaction with the enzyme aromatase. So I think if you could invitro assay, you should add atrazine at different concentrations to show up if there is any stimulatory effect which could be done very easily because already aromatase was assessed biochemically in several labs. And it is a routine assay.

I think that's second possibility. Not only looking on gene expression, but also on direct interference with the enzyme as a second possibility to rule out if there is any interference with aromatase and the output for estrogens.

DR. ROBERTS: Thank you.

Dr. Denver.

DR. DENVER: The data to at least generate the hypothesis are based on studies in human cell lines and a limited analysis in alligators. The hypothesis has not been directly tested in amphibia.

Just one point I would add, to test it, not only do we need to take advantage of the molecular tools that are available, but also do it at the appropriate developmental stage at least in one that is hypothesized to be sensitivity to atrazine.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: First, I would like to just agree with everything that has been said. But I would like to make the point strongly that in agreeing that aromatase induction has not been demonstrated, it should not be construed to mean that it's not induced from my perspective.

It simply means that the appropriate experiments haven't been done to demonstrate whether or not induction occurs.

And certainly, a point that I feel extremely strongly about in future studies looking at aromatase induction is that the experiments need to be conducted in the right life stage, not in the adult, but in the larvae that we're interested in.

DR. ROBERTS: Dr. Kelley, you are nodding. Agreed?

DR. KELLEY: Yes. To demonstrate that you have an effect of an agent in an adult, say, for example, hormone levels, and then to infer that that same effect with an unknown mechanism should have happened at developmental stage is taking the hypothesis quite far down the line without evidence.

So really, you have to go back to the stage when the effects are thought to occur in order for the mechanism to be established or not established.

DR. ROBERTS: So the comments so far I think have expressed the opinion that it has not been demonstrated that aromatase is induced. However, the experiments have not necessarily been appropriate design to adequately test that.

There have been some suggestions about how the best to approach that test as well as a caution from the panel that about focusing specifically on aromatase as a possible mode of action that there are other endocrine mechanisms that need to be considered as well.

Does everybody sort of agree with that summarization? Are there any other points or is there disagreement?

Dr. Matsumura.

DR. MATSUMURA: Just I want to make sure that -- yes, our chairman really summarized right. We should not really lock into that. Dr. Kloas also suggested some different approaches such as the antagonist of the testosterone.

This is not the typical estrogenic response. It doesn't happen in the females. It doesn't look like any effects. So should not be locked in. I agree with Dr. Kelley's statement that there are other possibilities that we have to keep our minds open.

DR. ROBERTS: Any other thoughts or comments about A? Is the feedback clear? Great.

Let's go to B.

DR. STEEGER: The variability associated with plasma sex steroid concentrations and aromatase activities is high. Is this variability normal.

Please comment on any readily apparent or available methodological improvements, for example, changes in sampling design, analytical techniques that could efficiently address this variability in future studies.

DR. ROBERTS: Dr. Kloas and Dr. Kelley I believe during some earlier discussions you made some points about the sex steroid measurements.

What is your response to this question?

DR. KLOAS: First of all, I think all of us being aware of this kind of determinations agree that there is always in lower vertebrates a high variability and steroid levels in general and also activities in aromatase may vary widely.

I think there should be agreement here. So the variability shown up, until yet measured, seems to be normal, what you will normally get.

Anyhow, we have to commend or we have some remarks about ELISA data, especially for estradiol measurements presented.

I think there are at least one order or two orders of magnitude higher than all the old data measured also in some of our labs that would suggest to be in this level. So they are much higher. So there should be any proof that this really is the right way to assess estradiol in xenopus.

For anything else, I think changes in sampling design -- I would like to show up set series (ph) involvement in steroid or in sexual steroid genesis. I think first of all there should be short term exposures and short term measurements for estradiol as well as for androgens.

I would also refer that there could be also some interference

with ratio of testosterone, dehydro (ph) testosterone. And I would also refer that there should be also some improvements to include five alpha reductase measurements, because this could be also another hypothesis how it works and how it could work in anti-androgenic way (ph).

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: I'm going to read my remarks.

The variability associated, the largest variability, two orders of magnitude, in other words, 1,000 times, in the studies reviewed by the EPA reflects differences in results obtained in studies sponsored by the registrant using ELISA assays and studies in the open literature using radioimmunoassay.

The most likely explanation for this large discrepancy is the method used to measure hormone levels -- so that's the analytical comment up here. Resolving these discrepancies should be straightforward.

Within the open literature, on the other hand, variability is more typical typically in the two times range, a range well within diurnal and seasonal variation.

It should be recognized that species studied extensively in the laboratory, xenopus laevis, may have originated from different

populations in their native habitat.

Populations can differ in seasonality even within the same country. For example, in South Africa, the Cape population breeds in the African winter, and the Johannesburg population in the African summer.

And we would thus expect differences between the two populations in the field and in the laboratory as this species maintains circannual rhythms even after many, many generations in the laboratory.

When wild caught animals are brought into the laboratory for study, they should be characterized genetically to identify the population of origin, this is possible now based on literature, and also to verify species because I'm sorry to tell you every xenopus looks like every other xenopus pretty much, except some are big and some are small. Although, if you listen to their songs, you can tell the difference.

And the same approach must be employed in characterizing groups of animals that are used for laboratory studies. So this is by way of saying that if we're going to standardize the animal as a test species, we have to develop standards that don't just involve the laboratory assays that we do, but that involve the biology of the

animal that we're looking at.

DR. ROBERTS: Dr. Denver.

DR. DENVER: I just want to reiterate the importance of validation of immunoassays, especially assays for estradiol. Estradiol is known to be present at very low concentrations in plasma and is routinely measured at least in the laboratories that are represented here by radioimmunoassays.

And historically, radioimmunoassays have been used to measure plaza sex steroids and tend to be more sensitive than ELISA's, although some of the current generation of ELISA's may be just as sensitive.

But I think it is important to compare the two methods to make sure that they are in concordance and to validate the assay methods for each species under study.

There is a tendency these days to purchase a kit and put things into it. I'm not saying that this is what was necessarily done by the investigators here, but it is really important to evaluate that kit for the species under study and show that you're actually measuring what you think you are measuring and that you're actually recovering from your sample.

In experiments where you add known amounts of cold steroid,

you can actually recover that in the assay.

So those are important things. Because the data that you generate are only as good as the methods that you are using to generate them. There has been a lot of discussion about animal husbandry, which is important, but I think there should be similar discussion of the methods used to analyze steroids, because perhaps much or perhaps some of this variability, at least, could be due to analytical methods.

The fact that one lab can use a kit and get the same result doesn't necessarily mean that result is correct. You can repeat an artifact until you are blue in the face and it doesn't necessarily mean that that is a correct number.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I think also another possibility to overcome such a problem for measuring estradiol could be to measure an estrogenic biomarker. A lot of estrogenic biomarkers are now already available.

The methodology is well-established. For instance, vitellogenin, you can measure it in the plasma, but also as vitellogenin MRNA by RTPCR techniques. That's routine measurement.

You can also maybe in an indirect way demonstrate that there

could be any estrogenic interactions or interference caused by atrazine.

So This is another possibility to have another methodological approach to demonstrate estrogenic pathways.

DR. ROBERTS: Yes. Dr. Kelley raised that issue earlier in discussion with one of the presenters.

If she wants, we could include that as part of our response to this question.

DR. KELLEY: I also want to make the comment that snapshot measurements of hormone levels at a particular point in time do not give a valid picture of the history of exposure of animal to the hormone in question.

What you really want, what I want, are biomarkers that show you in living realtime color what the animal is seeing. We are fortunate with xenopus that those are available now, actually. We will be able to look at flashing green frogs and know what tissue is being exposed to what level of hormone, when and how, which is an unusual situation.

But even without this fancy molecular method, the biomarkers such as have just been described are really very well characterized.

And you know that if you see a male that has any vitellogenin

or further that shows rapid response to estrogen challenge, you know that male has seen a level of vitellogenin that is not present in the literature.

So these are available assays and should be used and added to the armamentarium.

DR. ROBERTS: Dr. Kelley, can we provide some specifics and citations in our minutes?

DR. KELLEY: I have them in my giganto reference list.

DR. ROBERTS: Somehow I knew you would.

Dr. Tietge I believe has a question.

DR. TIETGE: I'm a little confused on one point here. Maybe I didn't catch it quite correctly.

Dr. Kloas said that there is high variability in these measurements and that's somewhat normal.

Dr. Kelley said that the typical range of variability is twofold. Are you in agreement, the two of you? Did I get that right?

DR. KELLEY: The typical range -- would you agree that the typical range of variation is not two orders of magnitude? 1,000 times?

DR. ROBERTS: That's 100.

DR. KELLEY: Sorry.

Well, you know what I mean. It's not 1,000 times?

DR. ROBERTS: I think she meant two orders of magnitude perhaps.

DR. KLOAS: Not twofold.

DR. KELLEY: I'm sorry. I meant two orders of magnitude.

DR. TIETGE: That clarifies it. Thank you.

DR. KLOAS: We agree with each other.

DR. KELLEY: We agree that twofold is normal, and 1,000 times is not normal. I actually wrote that down.

DR. ROBERTS: Two orders of magnitude, yes. Three orders of magnitude, no.

DR. KELLEY: Is twofold two orders of magnitude? No. Twofold is within an order of magnitude. It's within 10 times.

DR. MATSUMURA: I would like to support the Werler's (ph) comment. Really, those steroid levels can change really by the hour.

So I would like to really recommend that the endpoint of those steroid action -- actually, I have plasmid for the vitellogenin on the control by ERE. It comes from xenopus. I got it from Shapiro. And I also have a PS2, which is very sensitive.

It is well constructed. It works. I offer to Joe Giesy, if he wants, and anybody who wants I have it. Of course, I have to write to

Shapiro saying that I will transfer that.

But really, those are sensitive. I was so amazed. I compared that to mammalian construct and the xenopus is better. Much purer ERE. You can detect the PS2 very easily or PGR, progesterone receptor.

Those are well, well accepted. You can run that easily. I support that. Because that is more stable a way of measuring.

Some time ago hormone was up, but its effect is still here.

DR. ROBERTS: Any other comments on this before we move on to the next one?

Clarification?

DR. BRADBURY: I'm sure you all do it. If you can get all this written down, some of the dialogue. That will be very helpful.

Based on the discussions over the last few days, still within the realm of this question, but one of the hypotheses was it was -- the increase in estradiol, that the estradiol doesn't get into the plasma, it stays within the tissue.

Are there techniques available to measure changes in estradiol concentration within the tissues?

Could you comment on that?

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I think it is easy to measure also from tissue's cystorrheic (ph) levels. You can extract them easily. But, however, I think it is a completely lipophilic compound, how to keep it -- it has to be really completely resorped by receptors at the same time.

Normally, there should be any leakage to the circulation. I wouldn't expect that could be stored, of course. So should be a change.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: As Dr. Kelley said also, it is really a snapshot that we're taking be it in the serum or be it in a given tissue.

And that snapshot tends to reflect very well the level of synthesis that's taking place in that tissue at that point in time.

So another alternative would be rather than looking at tissue levels at a given hormone, if we're working with gonads would be perhaps to remove the gonads and in tissue culture look at the production steroid synthesis.

And another strength to that approach too is you could be at the same time looking at production of multiple steroids, androgens and estrogens.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: Measheta (ph) and coworkers in Japan have

routinely cultured the Stage 51 gonad. In the presence of aromatase inhibitors and also in the presence of estrogen, are able to observe sexual differentiation invitro and are able to observe effects of the agents that they add.

That assay is very available.

Also, I want to point out, I know I pointed this out before, but the liver is not that close to the gonad. Typically, if you have evidence of vitellogenin -- let me explain for the public what vitellogenin is. It is the yolk proteins. Birds have it. We don't have so much yolk. But the frogs have a lot of it. A mature egg has to yolk up in order for it to be oviposited, ovulated and oviposited.

If you see vitellogenin in the liver, it had to have arrived there, I believe there is agreement, via the circulation. So it had to have circulated at some point. That would mean that it got out of the organ.

It is worth pointing out, it has been pointed out, for example, females use testosterone as a precursor for estrogen. Dr. Hayes was right, that he didn't get the yolk up except in rana.

But xenopus uses testosterone as a precursor for estrogen. You can measure really high levels of testosterone in females when their ovaries are activated.

So circulating levels can be useful and shouldn't be denied, but there are other tools available to us.

DR. ROBERTS: Let me ask Dr. LeBlanc and Dr. Kelley a question of clarification for my edification. Not for the agency's.

The experiments in gonadal culture that you described, are those -- are you envisioning an exvivo experiment where they are exposed, you are removing gonads from exposed animals and culturing them and then measuring synthesis rate?

DR. LEBLANC: Yes.

DR. ROBERTS: So the exposure would occur in the animal, but not while in consult (ph) culture.

DR. LEBLANC: That's correct.

DR. ROBERTS: And you would still expect to see a continuing effect of the atrazine even though the gonads in culture would not be exposed to atrazine?

DR. LEBLANC: It certainly depends on the mechanism of the effect. But if it were induction of the enzyme, then in the short term, yes, you would expect to see --

DR. ROBERTS: Short term until it declines, okay.

DR. KELLEY: Just to echo a point made here about short term exposure, it is a tremendous advantage that you can get sexual

differentiation over a 48 hour period in xenopus.

Because of that, you can do it in culture. You don't have to worry about the rundown of the cells so much. You don't have to worry about the compensatory mechanisms within the entire animal. And that's a big experimental advantage.

DR. ROBERTS: Are there any follow-up questions from the agency on this? Any other comments?

Let's then move on to the next question.

DR. STEEGER: Please comment on whether there are additional data, other than those summarized in the white paper, that suggest late exposure of amphibians, i.e., juveniles or adults to estrogens or estrogenic chemicals can induce ovotestes formation.

DR. KLOAS: As far as we are aware, not in xenopus, at least.

I cannot give you the citation, but there is some Japanese people talking about ranids where you could reverse probably in late stages of rana rugosa. You can induce sex reversal. But not for xenopus. It is not reported, at least.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: In ranids, it has long been known that aging animals will show gonadal changes spontaneously. Whether those are associated with environmental agents has never known.

I remember reading an old report where old female ranids will shed sperm. In xenopus, this has never been reported, to my knowledge.

So I don't know of any data on this, but there may be people who know better than we do.

DR. ROBERTS: Dr. Denver.

DR. DENVER: No, I don't have any additional data to add.

DR. ROBERTS: Anybody else on the panel?

DR. ROBERTS: Let's go ahead on to the next one.

DR. STEEGER: Please comment on whether there are additional data other than those summarized in the white paper that suggest alternative mechanisms that could explain the apparent feminization of genetically male amphibians.

DR. ROBERTS: I suspect Dr. Kloas has an idea or two on this. Go ahead.

DR. KLOAS: I think we don't have really completely new data and not been included in the white paper. But from the last days and from some presentations we saw, we feel that you can create quite a lot of hypothesis more or less which could account for these findings presented by different groups.

I just would like to summarize a little bit which possibilities

could happen.

First, aromatase induction or effect -- change in activity of aromatase could be one. Still one of the hypotheses which could work. The next one, we could also have anti-androgenic effects. A third one would be as already mentioned yesterday, influence by the hypothalamus pituitary gonad axis. So more than (inaudible) endocrine pathway.

First possibility would be inhibition of steroidogenesis, especially on sex reverse steroids.

We have shown very easily that if you expose animals to atrazine, at least testosterone levels went down and really pronounced.

And I think furthermore there is not completely evidence for me that you can exclude any interference with the thyroid system. There is still some data available also from the public comments. Dr. Sheffield also he reminds me again to one paper on larval salamanders that shows the inhibition or delay of metamorphosis. I think still from the data we got and because of conduction arose (ph) from the husbandry, it's not completely clear that there couldn't be any interference with the thyroid system.

Next week we're going to present some data that sometimes you

will not -- you cannot see it really for developmental stages, but you will still have counter-regulation if you have inhibitory effects on the thyroid system.

This could be counter-regulated by more pronounced TSH production. This way, with this pathway we couldn't exclude also.

I'm making up everything. I'm sorry for that. But the data presented here still suggests that we have so many facilities and possibilities which could be one of the pathways or several pathways how it could work.

DR. ROBERTS: Dr. Kelley, do you have anything to add?

DR. KELLEY: Let me comment a little bit on the thyroid hormone question. Metamorphosis in xenopus is completely dependent upon thyroid hormone and requires as has been shown in a number of studies the expression of thyroid hormone receptor, which there are two which exist in a variety of isoforms.

I was not able to detect thyroid hormone receptor alpha in developing gonads. So I do not know if the developing gonad needs expression for formation, for what I call sex determination.

In fact, in that study in which thyroid hormone was blocked, the gonad proceeded to develop to the point of having spermatids. Although, of course, frank spermatid gonia did not develop because --

this is the other side of the equation, you cannot get effects of steroid hormones in xenopus unless the animal has first been exposed to thyroid hormone.

And some effects require thyroid hormone induction of prolactin.

So it is clear in xenopus that there is this strong interaction in terms of steroid hormone effects for many tissues between pituitary hormones, thyroid hormones, this is extending now to the axis, the pituitary hypothalamic gonadal axis now to the thyroid and the steroid hormones.

While this may sound like a nightmare like the ecological studies in point effect, it's an exquisitely regulated developmental system which provides then a very good assay system for a variety of endocrine perturbations.

So I agree that we can't at this point completely exclude thyroid hormone effects. I don't know about the gonads. But if there are any other effects of the agents that are being studied, we should take this into account.

DR. ROBERTS: Dr. Denver.

DR. DENVER: I don't know of any additional data that would support any alternative mechanisms, but I think it is important to at

least entertain the hypothesis that if there are gonadal effects, that they could be mediated by nonendocrine mechanisms, that is, that there could be direct effects on genes that are important for gonadal determination that may have nothing to do with hormones.

I don't think that should necessarily be ruled out.

DR. ROBERTS: It seems we didn't have a lot of specific data to offer, but the panel members did have suggestions for a number of alternative mechanisms and seemed to strongly think that those ought to be considered as well.

Are there any other comments?

Dr. Matsumura.

DR. MATSUMURA: I was intrigued at least by the fact that the HCG reversed some of those actions, but not all the way.

I would like to suggest at least that area should be followed up to see what it is.

It remind me of the Precosin (ph) studies, insect studies. This really is part of capsulatum (ph).

In that case, you can reverse it except that it doesn't come back to the same level because part of the function of the capsulatum is gone. It looks like that, but again, just one experiment. I'm quite sure Dr. Hayes is intrigued by that too. So we'll see what happens

then.

DR. ROBERTS: Any other comments?

Dr. LeBlanc.

DR. LEBLANC: I just want to make a point that in relation to all the possible effects that Dr. Kloas talked about with terminal hormones, the androgens, the estrogens, thyroid, hormone, that the effects need not be self exclusive, that there could be effects at the level of the hypothalamus, the pituitary that results in several effects on these terminal hormones resulting in decreased testosterone, increased estradiol and some effect on thyroid hormone perhaps.

So they don't have to be self exclusive. There could be a common target upstream that is affecting many hormones.

DR. ROBERTS: Anything else?

Any follow-up questions or clarifications needed?

DR. ROBERTS: I would like to go ahead and take question 5 and then do a break.

DR. STEEGER: With regard to specific endpoints, the agency does not have currently have sufficient information to quantitatively relate gonadal laryngeal effects to reproductive outcomes.

A major underlying uncertainty is the ecological relevance of ovotestes occurrence to the maintenance of anuran populations.

Can the panel provide sources of data on background rates of ovotestes occurrence in amphibian species and any associated considerations for interpreting this information in the context of the reviewed studies?

DR. ROBERTS: Our lead discussant on this is Dr. Green.

DR. GREEN: Witchie reported sporadic cases of hermaphroditism early -- in the late 1950s.

And there is at least one study in which the prevalence of ovotestes in a controlled population of laboratory frogs has been recently described. And this is a paper by Dr. Kloas which is in press right now.

To our knowledge, however, the background rates of ovotestes in any amphibian population have not been reported.

The panel members believe the frequency of occurrence of ovotestes in normal healthy populations of amphibians is probably very low.

This is of course based on the rare occurrence and our observations of ovotestes in our own laboratory animal populations.

So without information on the background rates of ovotestes, it is not possible to assess the impact, if any, of the presence of ovotestes on anuran populations.

DR. ROBERTS: Comments by other discussants?

Dr. LeBlanc.

DR. LEBLANC: No comments. I agree.

DR. KLOAS: I agree.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: Yes, I think this falls into the category of data that we had described by the EPA panel before, which was that in some cases you will only have anecdotal data.

None of us ever thought it was important to document how many ovotestes you saw in opening up thousands of frogs. There are no published data on those.

But if you will take the collective wisdom of people who have opened up thousands of frogs will tell you that Witschi reports only one case, actually, in the 50s by somebody else. And I actually have never seen one in the laboratory.

DR. ROBERTS: Is this an issue for which we need -- is there reasonably clear understanding about what you mean by ovotestes?

DR. KELLEY: I have made a PDF file of the Witschi paper. It's actually a chapter from a book. It is has a very nice picture of a mature gonad with clear testicular tissue and very well yolked up eggs.

I submit that that was what I would call an ovotestis. And everything else I regard as kind of subpar and up for grabs. But when you have an ovary that has a testis and eggs, which are pretty unmistakable, that's what I would call it. And this is an important issue, the terminology issue is very important.

The question of whether the occasional oocyte in a testis is normal or not, Witschi himself in this -- for you guys who don't know who Witschi is -- was he German or Swiss? I believe he was Swiss.

He was Swiss, and he started working on xenopus. He brought them in a tea kettle to Bozzle (ph). He performed almost all the early experiments with Chang and McComo (ph) on sexual differentiation in xenopus and was a very gifted biologist, I believe.

He has a very nice picture of this in this chapter summarizing many, many years of work.

Anyway, I have given this to the EPA. I have the paper with me. And the book should be available in the libraries. It will be in the bibliography.

DR. ROBERTS: Perhaps it would be useful since I believe we're probably going to comment subsequently on the problems created by lack of terminology that we clarify in our response what we mean by ovotestes.

DR. KELLEY: That's a very good suggestion.

DR. ROBERTS: Any other comments to add on this one?

Let's go to B then.

DR. STEEGER: Can the panel characterize any evidence that suggests that the presence of ovotestes in male anurans results in reproductive impairment via reductions in fertility?

DR. GREEN: To our knowledge, there are no studies that show that the presence of ovotestes in male anurans results in reproductive impairment. However, the panel recommends that feminized males be included in grow-out studies for the purposes of using them in breeding experiments to test this hypothesis.

DR. ROBERTS: Other responses? Other panel members?

And by no evidence, you mean that there is no evidence, but is there any evidence that they do not? There is just no evidence?

DR. GREEN: There is no evidence either way.

DR. ROBERTS: Either way. We should make that clear in our response.

Dr. Skelly.

DR. SKELLY: This may be the most appropriate point to make this comment. I was glad to hear that Dr. Green mentioned reproductive behavior. Because I think fertility is only part of the

story.

I think it is going to be very important if we're interested in ecological relevancy to see what these genetically male and phenotypically, whatever they are, how they behave when they are given the opportunity to mate.

And I see three broad categories of possibilities at the population level. One is that there may very well be no effect. And I think someone else mentioned this earlier. If you remove a few males from a population of frogs out in nature -- I mean, ecologists make jokes about how males are superficial all the time.

DR. KELLEY: We make jokes about that too.

DR. GREEN: I left those out.

DR. SKELLY: Thank you. It may not take very many many males to keep a population going.

This is a group of organisms for which minority of males in many populations may routinely do most of the matings.

However, it may be possible if these animals that have developmental abnormalities, if they actually behave as males but are not fertile and they convince females that they are fertilizing their eggs and leave a bunch of rotting unfertilized eggs around, that could be a serious population level effect.

On the other hand, if these developmentally abnormal genotypic males actually function and act as females, you could increase the population size. You could increase viability of populations.

I don't know how that's going to work genetically. Maybe Dr. Kelley can comment on that.

But I see those three broad categories. And I don't see any way of figuring out how to get towards the broader goals of ecological relevancy that the EPA set out without looking at this in a field context on native species.

DR. ROBERTS: Perhaps our response could include that caution.

Anything else, Dr. Gibbs and then Dr. Matsumura.

DR. GIBBS: Just another body of evidence that pertains to this particular question is that interspecies comparisons have shown that testes size and sperm production are positively correlated.

So it stands to reason that within the individual, any structure such as -- over that reduce the size of testes would reduce the sperm production.

I don't know if that's too much of an extrapolation to make. But there is that correlation there.

DR. ROBERTS: Perhaps we should mention that, then, with

some appropriate citation in our response.

DR. MATSUMURA: I think it is pretty important to at least test in the lab that those individuals with the abnormality would indeed be reproductively successful or not.

But I was just thinking how would you do that. You can't do any invasive method to say which one was, really had discontinuous ovotestes.

How would you design, Dr. Green or Dr. Kelley?

DR. ROBERTS: Dr. Green.

DR. GREEN: I have not actually tested this myself, but I have been to amphibian workshops where I have seen this done.

Some amphibians are small enough that as you know you can see through them. If you hold them up to the light, you can see internal organs.

One method would be to ultrasound the animal's abdomen because there are ultrasound probes now that have been miniaturized for the purpose of use in mice. So that's a possibility that, if you are good enough, you might be able to detect gonadal structures with an ultrasound probe.

Another possibility would be minimally invasive endoscopic techniques. There are endoscopes now that have been miniaturized

for mice, again, that would be a small puncture. You could go in and look and suture the animal up. Very similar to harvesting oocytes. They would probably recover just fine and go on. So those are two methods.

And then, of course, fancier methods would be MRI and that sort of thing, and which they now make coils that are small enough for mice, so I'm sure they would easily accommodate xenopus laevis, which would not require sacrificing the animal, any of those three test methods.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: I'm going to make my pitch, although not entirely appropriate now, for using known ZZ individuals.

If you ran this experiment in animals where the offspring of sex converted females that had been phenotypically female because they have been grown up in estrogen, and males, all of their offspring are male, so then you know that you have nice sibs to compare to, and then you can actually run a very well-controlled experiment under those conditions.

And you can study not only testicular development invasively and noninvasively, but you can also study clasping, which is the major reproductive behavior related to fertilization, and also

courtship song, whose importance in xenopus for successful reproduction is not clear, but is a very good marker for sexual differentiation.

DR. ROBERTS: I think what I have heard is our panel's response that there is no definitive information one way or another regarding ovotestes' effects as an impairment, perhaps some basis to speculate that it might, and some discussion of challenges associated with running those kinds of tests to provide the answer to the question and some suggestions as to approaches and some discussion about difficulties in setting up that kind of test and interpreting it.

Any follow-up questions or clarifications needed? Let's go to the next one.

DR. STEEGER: Reduction in laryngeal muscle area suggests diminished testosterone in males. If this is found to be a valid observation and if estrogen concentrations do increase as testosterone concentrations decrease, what other endpoints, for example, secondary sexual characteristics and reproductive behavior would likely be affected?

DR. ROBERTS: Dr. Green again.

DR. GREEN: The panel came up with a list of ten additional estrogenic biomarkers. The first five that I have here are not invasive

and would not require sacrificing the animal and are easy to identify phenotypically.

Obviously, the measurement of snout to vent length and body weight in feminized males should be bigger than the control males.

As Dr. Hayes pointed out yesterday, nuptial pads, the presence or absence or diminishment of nuptial pads and enlargement of the ventral folds of the cloaca strengthen the pattern of the male calling signal.

Dr. Kelley has some really nice recordings if anybody maybe wants to hear them during the break. We were impressed with how reproducible and how subjectively these might be measured.

We also were impressed by the fact that if you want to correlate loss of function, potentially the shrinking of the larynx muscles, with the morphological findings, then this would be a good way to show that not only is the larynx small, it is not functioning. You could do that by recording their calling.

Last, of course, like Dr. Kelley just mentioned, clasping.

There were some additional biogenic or biomarkers that people suggested. One of them we have talked about at length already. I won't go into it. But it is a time course examining the synthesis or the presence of vitellogenin in response to an estrogen challenge. And

then, of course, checking oviduct development.

There are proteins expressed in xenopus laevis in the harderian glands around the eyes. Three different proteins are expressed by those animals that are uniquely female and just one protein by the males.

Also, the number and the size of muscle fibers in the larynx and myosin expression in the larynx muscle.

And last, we have had some additional discussions on this one, but it seems like seminal, I'm not sure seminal fluid analysis is the appropriate term in amphibian, but analysis of the sperm in some of these feminized males would be important to look at.

For example, are they morphologically normal when they mature. Do they have normal motility. Is the fluid they are found in normal.

So there would be an additional assessment of the fertility in some of these feminized males.

DR. ROBERTS: Comments by other panels members?

Dr. Heeringa.

DR. HEERINGA: I would like to make a statistician's comment on the use of the laryngeal muscle or some other sort of continuous measure of masculinity as distinct from other endpoints such as the

gonadal abnormalities.

Looking at the data, I think -- and secondarily to these other measures, I think having a continuous endpoint or continuous measure on a characteristic that appears to be and demonstrated to be related to testosterone levels in the male frog, I think would be important as sort of a secondary confirmation.

It may actually get a little further ahead in looking at mode of action than just at whether there is an effect. But I think to the extent that this was not costly or disruptive to measure when these animals are sacrificed, I think that it would add value to the experimental data.

DR. ROBERTS: Other comments. Dr. Kelley.

DR. KELLEY: As I have pointed out before, the measurement of cross-sectional area of laryngeal muscle is a function of two properties, the size of the muscle fibers and the number of the muscle fibers.

Now, both reflect the history of exposure to androgen. So the two of them together are some kind of indication of the history of exposure to androgen.

However, let me just point out that while the size of the muscle cross-sectional area is not very well-documented developmentally in

the literature, the number of muscle fibers is very well-documented. And becomes statistically significantly different between males and females at the stage which we call PM 1, which we have also characterized.

I want to point out that one of the advantages of xenopus are the very well-characterized metamorphic stages, which allow standardization of experiments, so that when we talk about stage 56 animal, we know what we're talking about.

We carried out a similar set of study for post metamorphic development. Because the development of the larynx is largely post metamorphic. The brain is premetamorphic.

So we have those standardized data for numbers of muscle fibers. You can do it in the paraffin section and you can replicate previous studies.

So that will provide -- it is a continuous variable because a number of fibers is distributed. But the data are very clear on when those are expected to become different and at what time. And it should provide a sensitive marker.

DR. ROBERTS: Dr. Heeringa and then Dr. Richards.

DR. HEERINGA: Thank you very much, Dr. Kelley. I yield to the experts on the exact nature of the continuous measurement. I

think that's an excellent contribution.

My point is I think we would like to add this continuous measurement, something that is differentiated in terms of its outcome potentially at least in terms of observation from the abnormal gonadal development.

DR. KELLEY: The point I forgot to make was that in the model that EPA wants to develop for xenopus, you are going to terminate your experiments at stage 66. And there is variability in the results that we have been presented here as to whether there is a sex difference in laryngeal cross-sectional area at stage 66.

We don't get a difference in weight. I never measured a cross-sectional area. Dr. Hayes does get a difference in cross-sectional area. Other people don't.

If you are going to do a grow out, the shortest period you should grow out is three months when the animals are PM 1. I refer to Tobias, et al., 1991 A, for a description of those experiments and these stages.

DR. ROBERTS: '91 A?

DR. KELLEY: The first was the stages. The second one was when during those stages the processes are hormone sensitive. Developmental biology.

DR. ROBERTS: Great. Dr. Richards.

DR. RICHARDS: I wanted to echo that the development of some of these measures, particularly Dr. Kelley mentioning the vocalization, the sounds related to the laryngeal muscles and clasping behavior, these would be really strong links to then begin looking at real population level studies.

If there is some concrete analyses and relationships that can be developed there, that would be particularly strong for the next step of studies.

DR. ROBERTS: Dr. Gibbs.

DR. GIBBS: One quick comment. I'm a little bothered with what seems to be an implicit assumption that bigger is better when it comes to laryngeal muscle area. Because females are cuing in all sorts of qualities to male calls. Not simply volume and repetition.

I just don't think it is necessarily that simpler a relationship that larger laryngeal muscle area corresponds to greater mating success via the effects on the vocalizations of these males.

DR. KELLEY: That's certainly true in rana, which are highly discriminating animals.

In xenopus, how can I put it, in xenopus, unless you have a male number of muscle fibers, you don't call at all. So if you make a

genetic female have a male number of muscle fibers and have a male size of muscle fibers and she has circulating androgen, this is a testicular transplant study, she will call.

There is very good -- I can tell you how much circulating androgen you need to have in a male for him to call. It is a central effect. Once his larynx is masculinized, it's masculinized forever.

But what I can't tell you and what nobody knows in xenopus is whether the calling male gets the females.

This is well-known in rana where you can do phono taxas experiments in the ponds in South Africa. Finding out whether the calling male gets the female has not yet been established.

So unfortunately, the tractable experimental prep is not the prep for which we have those same kinds of data that you have in rana, which is -- I'm not going to call them a more sensitive system, but in some ways a more subtle system.

DR. GIBBS: But laryngeal area may not well pertain to being a success in rana.

DR. KELLEY: That's perfectly possible. And the other thing is of course in rana, you guys don't talk about female calls, but in xenopus, females have two calls. They have an acoustic aphrodisiac call that drives a male nuts, and they have a turn-off call.

So they have a very highly developed vocal system which they use in their social behaviors beyond sex -- centered around sex, but getting a little bit further out.

So these are actually rather different vocal systems. The xenopus one is much more complex in terms of call number than the rana one. And you are absolutely right about ranae. We don't know the relationship.

DR. ROBERTS: If you think it is important, Dr. Gibbs, we could perhaps include the caution about extrapolation of the findings to reproductive success.

Dr. Skelly, did you want to add?

DR. SKELLY: No. That's what I --

DR. ROBERTS: We can certainly put that in our report. Anything else to add on this one? If not, let's go ahead and take a break. It's 3 o'clock. Let's reconvene at about 3:15. We are miraculously ahead of schedule.

(Thereupon, a brief recess was taken.)

DR. ROBERTS: Let's go ahead and get started. Before we take the next question, just before lunch Dr. Kelley promised to assemble a bibliography. This was in response to Question 1, I believe, and some literature that we could perhaps recommend to the agency.

She has put that together. That has been distributed to the panel. I would just ask each panel member to go over the list to see whether or not you agree with it. If you have papers that you might want to add to it and get back with Dr. Kelley on that.

DR. KELLEY: I apologize. They are slightly out of order and jumbled, but I didn't have very much time. If there are papers that aren't on it, just put the papers on it and I'll add them. If there are papers you want me to take off, I'll take them off, except if they are my papers, in which case that's nonnegotiable. They stay on.

DR. ROBERTS: Let's go ahead to Question 6.

DR. STEEGER: While some of the available data suggests that there may be an association between atrazine exposure and developmental effects in amphibians, the agency's evaluation of the existing body of laboratory and field studies has determined that there is not sufficient scientific evidence to indicate that atrazine consistently produces effects across the range of amphibian species examined.

However, the current body of knowledge has deficiencies and uncertainties that limit its usefulness in assessing potential developmental atrazine effects and the extent of any associated cause effect in dose response relationships.

Consequently, the agency has determined that there are not sufficient data to reject the hypothesis that atrazine can cause adverse developmental effects in amphibians.

Does the SAP concur with these conclusions? If not, what lines of evidence would lead to an alternative conclusion?

DR. ROBERTS: Dr. Delorme, do you concur with that conclusion?

DR. DELORME: What I'm going to do is I'm going to actually go through the conclusions and break it down. I tried to put it through chromatic, and my computer crashed.

The first conclusion that I pull out is that there is not sufficient scientific evidence to indicate that atrazine consistently produces effects across the range of amphibian species examined. I had to agree with the conclusion.

We as a panel, I think, already concluded that atrazine could produce or might produce effects on gonadal development. However, the consistency of the response across a species studied was difficult to asses because of the problems identified with respect to the design and conduct of both the laboratory and the field study. That confounds their interpretation.

The second conclusion is the current body of knowledge has

deficiencies and uncertainties that limit its usefulness in assessing potential developmental atrazine effects. I interpreted this as a risk assessor as meaning limits its usefulness in a risk assessment contact.

Certainly, I don't think I would want to conduct a risk assessment with the data that's been presented. So I agree with that conclusion.

Further, EPA needs to have results from studies done where other factors can be ruled out as a cause in either the presence or the absence of effects. I think that's one of the key things that you guys have brought out.

You need studies that are done where it's unequivocal that atrazine is the route cause of the effect. For example, you need good husbandry in the lab studies, good design for both the field and lab studies and some of the other factors that have been discussed in the other questions. I think that's key in gaining the data you need to do the risk assessment.

Another conclusion, it was stated as, and the extent of any associated cause effect and concentration response relationship. I think we had already agreed that from the data that has been presented, we can't say anything about the exact nature of the response, either the shape of the dose response function or thresholds

or whatnot.

We just can't characterize it at this point from the data that has been presented. It's recognized -- this is a necessary element to conduct a risk assessment. You're going to need to have some sort of idea, whether it's a threshold response, if it's a dose response. What is the nature of the function of the dose response in order to conduct a risk assessment.

Consequently, the agency -- this is the next conclusion. Consequently, the agency has determined that there are not sufficient data to reject the hypothesis that atrazine can cause adverse developmental effects in amphibians. Agreed.

We agree that with the available -- we agree with EPA that the available data does suggest that atrazine can affect amphibian gonadal development. However, the available data does not allow for a proper characterization of the nature and magnitude of the response, nor does it offer sufficient support for the identification of a plausible mechanism.

I guess in the end, if you add it all up, we agree with the conclusions, or at least I do and Joel. We wrote this together.

Joel, did you have anything to add?

DR. ROBERTS: That sort of answers my next question, but I'll

let Dr. Coats respond.

DR. COATS: I concur with the opinion there since it includes some of my ideas.

One other comment. We have spent over this time period and through 17 or so studies an awful large amount of time picking them apart and looking at every detail.

On the other hand, they do really constitute the body of what we do know about this. There is a lot we don't know yet. And I wanted to make the point they all have made contributions toward the progress here and the understanding of, is it a problem, is it not, or how big a problem is it or not.

That's one thing we should acknowledge.

DR. ROBERTS: Dr. Richards, did you have anything to add?

DR. RICHARDS: That I concur with the statements that the two previous speakers have made. I have nothing more to add.

DR. ROBERTS: I think Dr. Isom had a question.

DR. ISOM: I have a question for clarification. What do you mean by adverse developmental effects as opposed to just developmental effects, which we have been talking about previously?

DR. BRADBURY: Good question. And it, I think, ties back to the some of the previous questions in terms of the responses that have

been described in the literature thus far in the context of the risk assessment endpoint, which is getting at issues of reproductive fitness.

So again, had we probably polished the question a little better, it would have been clearer, but in the context of these endpoints, measures of effects in the context of the risk assessment endpoint that we laid out in the problem formulation.

So to the extent these, for example, gonadal abnormalities could be related to male fertility or reproductive fitness measures.

DR. ISOM: With that definition, would that change then the discussion we just had?

DR. ROBERTS: Does anyone feel that that would change the response based on -- change the panel's answer based on the response by Dr. Bradbury?

Dr. Green.

DR. GREEN: Would you repeat that one more time?

DR. BRADBURY: I should pull out the problem formulation. I think we're all tracking, but we should make sure.

That abnormalities that have been described in the literature for an ecological risk assessment then need to be connected to the measures of effects, the risk assessment endpoint in the

environmental management goal. The environmental management goal was maintain or viability of anuran populations, the risk assessment endpoint being connected to reproduction and fitness of populations, and the measures of effects being connected to those developmental processes that go on in amphibians that are related to their ability to reproduce successfully and then hence maintain populations.

So it is trying to maintain that causal chain. Does this toxicological effect move up the levels of biological organization? If you go back to that slide I had at the beginning of today, this midmorning, sort of the connection between the effects at different levels of biological organization connecting to the risk assessment endpoint.

DR. GREEN: I guess in my mind the question too implies the difference between whether or not the effect that we see is going to have an adverse effect.

For most of these parameters we have been looking at, we don't know yet because they haven't been carried out in grow-out studies far enough.

So at this point, any developmental effect would be just a developmental effect. And maybe we should strike the word adverse

because we don't know if it is adverse yet or not unless it is associated with mortality.

DR. BRADBURY: Right.

DR. GREEN: Is that fair?

DR. ROBERTS: We can clarify that in our response that we interpreted this as being developmental effects and as we have stated earlier at least with respect to some of these effects that we don't know yet the consequences of these observations.

DR. BRADBURY: Right.

If we go back to some of the earlier questions, you all were discussing sort of the connections between changes in the larynx to potential calling or other kind of secondary characteristics, which is part of that discussion of what is the causal link, what is the toxicological, the ecological pathway that we're addressing.

DR. ROBERTS: We can put that, draft that, put the caveat in there.

DR. DELORME: The way it's written now is actually kind of broad. Because what I said is the available data does not allow proper characterization of the nature and magnitude of the response.

What we're saying is we don't know how far it is going, but I can amend that to say at the organism or population level, if that's

agreeable.

DR. ROBERTS: Sure. Does anyone disagree with the statements that have been made so far?

I don't see any. Any clarifications or follow-up questions from the agency?

Let me clarify one thing for the audience. From time to time now as the panel have given their responses, they have indicated that they have worked with somebody else on the panel in terms of writing something up.

Under the Federal Advisory Committee Act, individual panel members can talk to other panel member is they have responsibility on a same topic, and sort of discuss the issues.

But I want to make very clear that the panel has not met and undergone any deliberations other than in this room in open session.

There have been discussions among panel members during breaks and in the evening, that sort of thing. As individuals, that's allowed, but there has not been any closed session of this panel to deliberate any of these issues.

Let's go Question 7.

DR. STEEGER: Assuming the agency determined an ecological risk assessment with a greater degree of certainty concerning

developmental effects of atrazine on amphibians were needed, please comment on EPA's conclusion that additional information is required to evaluate potential causal relationships between atrazine exposure and gonadal development.

Please also comment on the added utility, if any, of additional information to interpret the shape of dose response curves for potential developmental endpoints and the extent to which threshold or nonthreshhold response relationships can be quantified.

DR. ROBERTS: Dr. Delorme.

DR. DELORME: I'm going to handle the first part of this question and let Dr. Coats handle the part about the utility of interpreting the shape of the dose response curve.

I think we agree with that, the statement that -- or EPA's conclusion that additional information is required to evaluate the potential causal relationship between atrazine exposure and gonadal development.

The relationship is there, we think. There is tantalizing evidence that something is going on. But it needs to be confirmed. Or what is being suggested needs to be confirmed. And we need to characterize the nature of the dose response function.

One of the tenets of the scientific methods is the repeatability

of experiments. Certainly, we have had attempts made to repeat this that have been submitted to EPA and we have looked at.

But definitely, we need to firm up the existence of the causal relationship, and, if it is there, if it does exist, we need to characterize the nature of the dose response function.

There is also a need to identify a plausible mechanism. I think one thing we have to recognize is the identification of the mechanism is important because it can in part aid in the extrapolation of the results from the surrogate test species, the species of concern in the environment.

DR. ROBERTS: Dr. Coats.

DR. COATS: I have a few things to add.

The plausible mechanism explanation needs to be put forth with some data.

Secondly, there needs to be some similarity of data or patterns or trends from several research groups to show repeatability of the experiments.

The dose response curves are extremely important to the question of any detrimental effect of any toxicant on an organism, regardless of whether the relationships demonstrate a typical or atypical concentration response curve for a given endpoint.

It should be possible to ascertain the shapes of the curve given enough concentrations, enough replications and controlled conditions.

Repeatability in other labs should also be feasible if the same species, stage, water concentrations and timing are utilized.

Another point is that studies on quantitative structure activity relationships can often provide information about the mechanism of action as well or provide rationale for the data is generated from comparative testing.

Experiments that use a series of closely related compounds, atrazine, cyanazine, propazine, simazine, terbutyl azine (ph) et cetera, could elucidate patterns that can help explain the interaction between the molecule and the putative receptor addressing the causal relationship.

This approach seems to be lacking so far and could be valuable in the invivo test for gonadal development as well as enzyme induction or MRNA expression.

DR. ROBERTS: Thank you, Dr. Coats.

Dr. Kelley, did you have anything to add?

DR. KELLEY: No. I completely concur.

DR. ROBERTS: Anyone else have any comments?

DR. GREEN: I also concur.

DR. ROBERTS: Thank you, Dr. Green. Anyone else like to weigh in on this one?

Does anyone disagree, I guess I should ask that. I don't hear any disagreement.

Any follow-ups or any clarification needed? Let's then go on to Question 8, which I will point out is a seven-part question.

Dr. Richards has got that one.

DR. RICHARDS: Being the nonexpert that I am on almost every part of this --

DR. ROBERTS: Let's let the agency go ahead and pose the question to us.

DR. STEEGER: The agency has developed a conceptual model from which to develop a set of study of protocols for evaluating the potential effects of atrazine on gonadal development on amphibians.

The agency has proposed a research approach using focused empirical laboratory studies based on initial investigations with xenopus laevis followed by selective confirmatory studies with frog species native to North America.

This is a proposal. It is not set in stone. We have -- as has been indicated in a number of follow-up questions. Please comment on the proposed sequence of the study objectives.

DR. ROBERTS: Dr. Richards, we'll go through these I guess one at a time. Do you want to take the first one?

DR. RICHARDS: Yes. I'm going to invite the persons listed and not listed on this question to jump in on an open discussion here.

But on the please comment on the proposed study of sequence objectives, I think Dr. Kelley had already and several others have mentioned about the potential and importance of some types of field oriented studies.

That was the one comment that I had on this also, and not to preclude them in a parallel track with some of the other laboratory based studies.

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

DR. SKELLY: Just to support what Dr. Richards just said, I think it is going to be important to get the field component of any evaluation undergoing as soon as any of this happens in part because field studies for lots of reasons take a long time to set up and get going.

They are not necessarily as money intensive as some of the laboratory studies, but observational studies and experimental studies take time to find locations as we have seen in evaluating some of the past data. That can make a lot of difference in how useful these

studies will be when it comes to interpreting them.

I guess I just can't emphasize enough that I think it is going to be critical to get that side of things moving in order to meet the objectives laid out in the conceptual model by the EPA.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: Could you remind me again, what is the first thing that you plan to do?

DR. BRADBURY: Phase one in the white paper.

DR. KELLEY: Oh, yes.

DR. DELORME: Test for apical gonadal effects of --

DR. KELLEY: So I concur with that. I would argue that we should attempt to strictly replicate some of the studies that show an effect, especially a low dose effect.

I accept the fact that in a replication one will often want to add groups and so on. But we have a real discrepancy in the threshold for an effect if in fact one exists.

I would argue for a strict replication, perhaps a replication of the high dose and a replication of the study that did the low dose.

I think it is worthwhile knowing how reliable and repeatable the initial observations are before we go forward.

Now, there has been -- anyway, I could go through it. But I

think it is very important to track down the sources of variability and the results before going forward. Because suppose you were never able to repeat any of them or you always repeated them and in a much lower dose.

It would affect so strongly the next steps in the sequence that I would argue very strongly for starting with that.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I concur with the comments already made.

I would also point out maybe it is time saving and saving money if you could start again already in parallel with doing some mechanistic studies.

For instance, there was some claim there is no interference with estrogen receptors, with androgen receptors.

There are some experience which could be very easily maybe to say there is nothing going on. Especially, I have some concern maybe if there is only a small effect on thyroid system, you will not see it on morphological -- looking just on morphological stages.

There could also be one biomarker, for instance, TSH, to show up any inhibitory effect in addition which could be done in parallel for doing such a developmental study.

DR. ROBERTS: I'm going to jump in and make a comment too.

I think that if we do additional mechanistic studies, if there are variable aspects, I think we -- as Dr. Kelley pointed out, I think we have to get a handle on those first because we have to know when animals respond and when they don't respond.

Because we want to be sure we're looking in animals that are responding to do, to see whether or not the mechanisms we think might be operating are, in fact, in place.

I don't disagree that mechanistic studies are important, but I think getting a real solid handle on the phenomenon and being able to reproducebly observe that, I agree with Dr. Kelley, is a first priority.

Dr. DeLorme and Dr. LeBlanc and Dr. Denver.

DR. DELORME: I just wanted to concur with Dr. Kelley. I think that what has been proposed is quite a logical sequence. The first thing you really need to do is confirm whether or not there is any relationship between atrazine and the effect.

And certainly what Dr. Kelley had proposed was repeating the experiments to see if you can find that, but do it under controlled conditions where the husbandry and whatnot is -- any factors that might affect it are controlled for.

Followed by looking in possibly native anurans from North America to see whether or not you are going to get the same kinds of

effect. I think that's another good logical step to take.

DR. ROBERTS: Dr. LeBlanc and then Dr. Denver.

DR. LEBLANC: From my perspective, the further you go up in that cascade of complexity that Dr. Bradbury had up there earlier on, you are reducing your chances of seeing the effect that you are trying to confirm upfront and sort of give you some evidence that you should proceed.

Certainly, I wouldn't do preliminary studies or initial studies at the population level. I think gonad development is the critical endpoint of interest, but I'm not sure I would do it at that level.

I think that perhaps the first experiments that I would do would be looking at some cellular response that I'm more likely to see. And once I have identified doses at which that response occurs and I'm comfortable that that response is occurring, I think I would build upon that.

And then I would look at tissue level effects in terms of gonadal development and ultimately effects of the individual and the populations, is my perspective.

DR. ROBERTS: Dr. Denver.

DR. DENVER: What we really want to know here, we want to know whether there are population level effects ultimately. Is that

correct?

And that's something that is going to take presumably a while to sort out.

The next level, I assume, is to understand what might be fitness effects at the individual level.

And I wonder -- this actually is a more general question that goes beyond just simply atrazine. But I wonder if we can begin to get a handle on those fitness, those individual fitness effects by looking at individuals or individuals that are presumably intersex or have gonadal abnormalities in the field, which I think we agree has been documented, and find a way to identify those individuals which we discussed a bit earlier perhaps using some MRI approaches or something of that sort. And do the grow-out experiments and determine if there are really fitness consequences of having these intersex gonads.

Is that something that we should be doing now? As I said, it goes to a larger issue, larger than just simply atrazine.

And these intersex individuals may result from contaminants other than atrazine. But is that something that we want to consider sooner rather than later?

DR. ROBERTS: We're getting some interesting differing views

on sequence of or highest priorities.

Any other panel members want to weigh in on this?

Dr. Delorme.

DR. DELORME: That is actually an interesting thought. I never thought of that before.

But if there was some chemical that you could come up with to induce intersex that you knew for search was going to the happen, that might answer some of the questions with respect to fertility and possibility of effects at higher levels of biological organization. Instead of waiting to find out what is actually going to go on and identify --

You could do it in parallel with atrazine. Because certainly as we go through -- as risk assessor, I'm talking, as we go through other chemicals, there certainly are going to be cases where these kinds of effects arise.

It is always a question. What is the ecological relevance of ovotestes. I know in the fish community it is something they are grappling with now. Because in a lot of the rivers in Britain and in the states, they have identified fish populations where ovotestes type effects do occur. The so what question is still there. Well, do they contribute to the population or does it have a population level effect.

So that's actually an interesting take on things.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: So there is good news. There is a chemical that does it. In xenopus, estrogen does it. It is very well worked out. The critical period is worked out. How much ovotestes. I'm using that word in the sense in which I used it before, and I refer you to the figure in the Witschi paper. It is very well worked out.

The animals that were in that paper were adult. They persisted until adulthood. It looked like it wasn't resorbed. I would suggest that there is no necessity to run this as a serial experiment. I'm totally opposed to that.

You run it in parallel. You run it in parallel also with beginning your ecological assessments, because I'm in total agreement. Field work takes forever. Getting it published takes forever. Anyway, just doing the work takes forever.

So I would start the field studies and I would start a replication of the previous studies on the atrazine effects. I would do it in ZZ animals. You know what genotype you were dealing with.

I would do it in a well-characterized population of animals and I would run a group along with it with estrogen at various time periods during the defined critical window.

And then you have to grow those animals out. It's a male. That's the good news. It's only six months. And then you run those animals in behavioral and fertility tests. And then you know if animals with 66 percent ovaries and 33 percent testes or something produce fewer offspring than animals with a different ratio.

Those studies are actually fairly easy to do in a well-defined controlled way. You can either use natural behaviors or you can kill the animals and mush up their testes and fertilize eggs with them.

You don't have to kill the animals. You can just take their testes out.

DR. ROBERTS: Other thoughts?

Lots of different ideas, Dr. Richards, unfortunately for you to capture.

DR. RICHARDS: I'm sure many of those words will be on to a text file and given to me.

DR. KELLEY: Yes, after dinner.

DR. ROBERTS: Before we move on to B, let me make sure. Is there any follow-up question or is it as clear as it can be given the fact that there are some differences of opinion about sequences?

DR. BRADBURY: I think it would be helpful for the dialogue to continue a bit. I'm approaching this carefully.

And with all due respect to all members of the panel, in the context of science, for a purpose, which is trying to incrementally improve our ability to reduce the uncertainties on the risk assessment and how that marries up with and is complimentary to advancing knowledge, because it is important to advance knowledge, and to the extent the panel has any feel of, for lack of a better word, where you could get the biggest bang for your buck to start either sequence how you would stage information gathering or a sense of what information we start to clarify or reduce some of the uncertainties in the current ability to go through the risk assessment process.

It is sort of -- the dialogue has been interesting. I think Jere in thinking about the endocrine disrupter screening assays and some of the other descriptions about using QSAR and then going from the field down sort of to illustrate very legitimate differences of opinion in terms of how you blend mechanistic understanding with ecological relevancy and where are you in that continuum, it seems like we're getting advice to do everything all at once.

And while maybe that's the only way we can solve this problem, if that's the conclusion, then that's the conclusion.

I think it would be helpful to hear about what some of the trade-offs would be at least in picking different places to start in the

phased approach that we provided.

It may mean you may all feel that you should start all five phases all at once, and that's cool. But it would be helpful to at least get input from the panel in terms of the trade-offs if one couldn't do all five phases at the same time.

DR. ROBERTS: Let's ask the panel. What if you couldn't do them all at once, where would you start? Dr. Kelley and then Dr. Green.

DR. KELLEY: One in five.

DR. ROBERTS: Beg your pardon?

DR. KELLEY: That was it.

DR. GREEN: Testing the working hypothesis phase experiment Number 1, the test for apical gonad effects and Number 5, ecological relevancy of the study. I concur with Dr. Kelley's comment.

But since you have solicited additional dialogue, I have a few more things that I think are imperative to standardizing the conditions. And this should be done in Phase 1. So right from the get-go we can do the best we can.

One of the things that struck me yesterday about the housing and the husbandry conditions was the extreme variability and the fact that one of the things we were measuring was growth rate and trying

to make a connection between the effect of atrazine on growth rate and then on the growth rate in the control tank when between labs and within the lab there were specific issues with water quality and feeding and other things that could account for variability in growth rate alone whether atrazine was there or not.

So I'm going to take the opportunity to make a plea. And part of this bleeds into Question Number 4, which I was actually waiting to answer, about two issues.

And the first one is with water quality. This is not pertaining to measuring levels of atrazine. But I think we should make some attempt to define the stocking density first off for both embryos and adults and juveniles for xenopus laevis.

Unfortunately, that hasn't been standardized in Laboratory Animal Medicine even yet, but there are some recommendations.

The proceedings for the National Academy of Sciences has recommended one to two liters for adults. And there are various textbooks and original papers for tadpole stocking and density. I think we talked about that a little bit already. So that would be one thing.

One thing that was lacking in the descriptions of many of the papers was the kind of water that was used. I couldn't tell. Was this

deionized or R O treated or reconstituted salt water. Was it well water from a source. Was it chlorinated or chloraminated, potable tap water that had been filtered.

And I think all those water sources are perfectly suitable. But it needs to be stated and, if possible, standardized so that everybody who runs the test on atrazine uses the same kind of water.

The other thing would be water quality testing. For this purpose, because there is evidence in the literature that atrazine and nitrites interact and that they have some by-product of ammonium metabolism, when the ammonia levels get high in tanks, it may falsely elevate the detrimental effects, if any, that any other chemical has in there. It could be chlorine. It could be atrazine.

So water quality should be measured on a regular basis. Depending on the duration of the experiment and the stocking density and the water turnover, I would say once a day. It could be done with a quick dip stick that is a relatively inexpensive test. It doesn't have to be the Hawk (ph) analytical test every day, but some notation of water quality parameters on a regular basis.

That would include pH, conductivity, water temperature, ammonia, nitrate, nitrite. And because we want to know what kind of water is used, we should be measuring for chlorine and chloramine

and the heavy metals that we know interfere with tadpole development like copper and some of the other things.

So I would like to see that in most of these reports at least have been part of the quality control.

So water quality is one issue in Phase 1 that I think if we could standardize. And you can't go to the literature and find this.

There is a paper that was published in the Laboratory Animal Medicine Journal that reported a survey across the nation of what most xenopus users are doing right now that you can use as a guide.

I have the reference here that I'll add to the list for Dr. Kelley on all these parameters.

The second issue that I wanted to bring up was feeding. I'm sure Dr. Kelley will bring this up as well.

But Dr. Hayes pointed out that he felt that maybe some of the animals in the Syngenta studies had been underfed. And I looked at that -- I went back and looked at that paper and looked at some of his publications on what he was feeding.

It struck me that he is feeding rabbit chow. I find this a little bit disconcerting because adult xenopus are strict carnivores. And that is an herbivore diet.

DR. HAYES: The adult xenopus were fed trout chow, not rabbit

chow.

DR. GREEN: Dr. Hayes in the back said his adult xenopus were fed trout chow. But if we continue these studies into grow-out experiments where juveniles and adults are used, then we should probably feed them a diet that's at least 14 percent protein, which is more appropriate for a carnivore.

There are references. Kevin Wright has published in another textbook, Amphibian Medicine and Husbandry, a chapter. In that, he recommends that we not feed adult and juvenile xenopus laevis, anyway, diets for omnivorous fish and turtles or for herbivores.

So you can go to a feed company, there are several reputable ones, that will make xenopus chow for you or you can buy it from the big distributors. Nasco and Xenopus 1 have their own that they sell.

I think in an attempt to standardize across labs, that's probably the appropriate food for that age.

Now, tadpole feeding -- those companies also sell food, if I'm correct, for younger metamorphs and juveniles as well. And they eat phytoplankton.

I'm not so certain how important it is that they have a high protein diet to maximize growth and health under these conditions.

But if you are going to make those comparisons in growth rates

and fecundity, then probably standardizing to a carnivorous amphibian diet would be the best thing to do.

The companies also have based those diets and the amount of feed on growth rate curves that they have developed. I believe they will share them with you. I have asked for them and they have been very helpful in advising me.

They also make recommendations on how much to feed per animal. That's based on known anuran kilocaloric requirements at different temperatures.

So if we hold tanks for some of the juvenile and adults at 19 degrees versus 25, we should feed them accordingly. There are ways to do that.

If you look to the fish literature, as young fish grow, they are regularly weighed once a month on mass. The tank feed is adjusted accordingly.

I know that it is not standard practice right now to do that for xenopus. Usually, you have about, whatever, 5, 50 or 100 in a tank and you throw so much food and make sure they eat it all.

But in terms of water quality level, what is not eaten and the amount they excrete will affect the amount of ammonia, which in turn affects the amount of atrazine that is or isn't available or potentiated.

Those are two conditions, at least, the water quality and the feeding that I think this Phase 1 should be spelled out as best we can. And the temperature that we all agree would be the right place to start with in conducting these experiments.

DR. ROBERTS: I think it will be important for us to convey Dr. Green's recommendations in our report. I don't think it necessarily belongs as part of 8 A, but we'll get it in there. I think it is important to pass that information along to the agency.

With regard to the question posed by Dr. Bradbury, if you couldn't do them all at once, what would you do?

Dr. Delorme.

DR. DELORME: I would concur with Dr. Kelley that probably the first thing you want to do is -- if your goal is to move the atrazine risk assessment forward, then I think you have to confirm the causal relationship. That's the first thing you need to do. That would be the first priority to do.

DR. ROBERTS: Dr. Coats.

DR. COATS: I think that the laboratory approach as described in Phase 1 is probably the most obvious and least risky from a benefit -- from the point of time invested and money invested.

And so that's where I think we should start.

DR. ROBERTS: Dr. Skelly.

DR. SKELLY: At the risk of boring everyone, I'm going to reiterate that I think pushing ecological relevancy off to Phase 5 could be a mistake.

We're testing for apical gonadal effects in Phase 1. Then Phase 2 is sex steroid measurements. Phase 3 is aromatase activity measurements. Phase 4 is aromatase inhibitor study. Phase 5 is ecological relevance.

I think there could be a giant woops there if we get to the end and find out that the population level effects aren't what we're all being concerned about here.

DR. ROBERTS: Dr. Delorme.

DR. DELORME: Just as a point. If you look at figure one in the white paper, it actually shows after the test for apical gonadal effects and a yes being found, there is a line off to the side with Number 5.

Can EPA comment on what was intended with that? It wasn't clear whether or not you intended the ecological relevance test to start at that point.

DR. SKELLY: Is ecology fifth level priority or not?

DR. TIETGE: It just so happens that we used numbers. We

could have used colors I guess to indicate what order to do them in.

Clearly, as Dr. Delorme pointed out, we had that split in the sequence to suggest that if you get effects at the individual level, then it may be more prudent from a risk assessment point of view to conduct some field work.

I wouldn't read into the ordinal numbers here very much.

DR. ROBERTS: Does that help, Dr. Skelly?

DR. SKELLY: Yes. If I can follow up.

I'm fine with that with one important caveat. That is, we're going to get to this a little bit farther down. But if the panel feels that there are significant concerns about context dependence between species, and at least in my case I think we have seen some evidence that that could be the case, then I don't see that a no on apical gonadal effects in xenopus necessarily means that we shouldn't be thinking about an ecological relevance study for native species.

DR. ROBERTS: Dr. Bradbury.

DR. BRADBURY: Just to get a clarification, because the white paper talks about using xenopus to get started as a biological model bla, bla, bla, but also talks about other species to also be looked at in the context of phase one style experiments.

I guess the clarification that if xenopus didn't reproduce the

previous results, would the panel feel it would be useful to try it out in rana in the Phase 1 style experiment or to immediately go to a long term reproductive study or go out to the field to look at rana or other native species?

DR. ROBERTS: Expression of preference on that?

Dr. Kelley.

DR. KELLEY: Well, you know, I'm for parallel processing. Let's face it. We don't care here in America about the survival of our xenopus because they are well taken care of in the lab.

But we care about the survival of our native frog species. You are just going to have to start the field studies right away. But I do agree that it would be useful to see if we could run some rana experiments in the lab.

Of course the problem is rana is a lot harder to run in the lab. And rana is about a zillion times more difficult to work on than xenopus and so forth.

But that isn't to say that it shouldn't be attempted, because it will be very informative. There are folks that are good at rana. I'm not one of them. But I know there are people that are.

It would be worthwhile to try in a limited way to see if we could get that to go. But it is going to be a lot harder. The

background literature that is available for xenopus is just not available for rana.

It will be somewhat more difficult to interpret the results. But those are the results we care about. Because these are our frogs, our North American frogs. We care a lot about them.

We should study them along with the indicator species, which has many, many advantages, but is not native to our country.

DR. ROBERTS: Other thoughts?

Dr. Richards.

DR. RICHARDS: I concur with what Dr. Kelley has brought up here. I think that ultimately the agency is going to have to go there no matter what answer we get from xenopus. And it could lead us down some very good trails.

Ultimately, the question is going to come to is what does that mean to ranid species. And if we don't start now in trying to further hone or develop methods that are appropriate for them, it is just going to prolong the whole process.

DR. ROBERTS: Dr. Tietge.

DR. TIETGE: I think what we have to keep in mind is that using the rana species or the intent of using the rana species is to do the confirmatory work.

But you do the hard work I think in xenopus because it is a more useful model at this point in time. So I don't think we meant one or the other.

I think -- I agree with what I'm hearing here that you probably will end up doing it in both species at least in that Phase 1 study.

But the intent of the xenopus is to go faster and farther to understand, for example, mechanistic pathways that you would then go back and in a more focused approach look at with the rana species.

DR. BRADBURY: Just to help clarify because you've been getting some understandably different kinds of views.

At one point we heard we should try taking account of variability associated with some of the current studies to try to see if we could replicate what had happened in the past.

And all there is a ranid study. There are several xenopus studies. That would imply get started with xenopus to see if you can get it to happen in xenopus again. Start with ranid.

Again, I know you are probably thinking this through as we go. But as the panel deliberates, it will be helpful to get, if not definitive answers, at least some thoughts of the cost benefit, that's not the right word, but the ups and downs, the trade-offs associated with some of the different choices in the pathway.

DR. ROBERTS: Dr. Denver and then Dr. Green.

DR. DENVER: Let me sort of echo the comments of Dr. Richards and play devil's advocate.

What if you are unable able to replicate the xenopus results and you are unable to show in fact any effects of atrazine? What decision would you then make?

Would you then decide to go on to ask the question in North American ranid species or would you conclude that, in fact, there is no basis for concern?

DR. BRADBURY: I think some of that dialogue -- some of the issues that one would face in making that decision are some of the latter questions we have in the series of question eight which gets at some of the issues about toxico dynamic and toxico kinetic differences among frogs.

And to not dodge the question, I think I would benefit greatly from the panel's dialogue on those issues that could drive interspecies extrapolation.

DR. ROBERTS: Fair enough.

We've had a lot of dialogue. I hope Dr. Richards has been keeping up.

Let me see if we can decide whether or not the -- is there some

sort of consensus in terms of priority or are there still differences of opinion?

Dr. Richards, what is your sense?

DR. RICHARDS: I'm hearing that most of the panel seems to feel comfortable that some of the -- the Phase 1 experiments need to be replicated, techniques cleaned up. That work needs to go forward. I'm hearing that from most people.

I have heard from a little bit smaller number that some aspects of Number 5 need to go forward in terms of grow out or some of the other basic ecological studies. Not necessarily full ecological studies.

DR. ROBERTS: I sort of heard unanimity in the desirability for the Phase 1 studies to proceed. And some support for, if possible, beginning the Number 5 ecological studies, because recognizing that that -- demonstrating that relevance would be very important for the risk assessment.

Does somebody else have a different take?

Dr. Gibbs.

DR. GIBBS: I would concur. I thought Dr. Kelley had outlined a sequence that is really quite doable in a fairly short time frame in terms of looking at the fitness consequences with intersex in normal

males and then you could actually put those in a natural environment and look at the consequences and mating with maybe even molecular genetic markers getting the tadpoles or metamorphs.

But I don't think until there is -- rather than a full-blown ecological study, 100 wetlands, et cetera, we're not talking about that necessarily for Phase 5. I think just a shorter term study like Dr. Kelley outlined I think is quite doable.

DR. ROBERTS: Does that sound reasonable to the panel? All right. Good. Then let's try and have the minutes the reflect that recommendation.

Any follow-up questions from the agency? Dr. Green and then Dr. Isom.

DR. GREEN: I wasn't clear now. Was the decision that ranA and xenopus experiment should be run in parallel, if possible?

DR. ROBERTS: I think we're going to take up the species --

DR. GREEN: Later on?

DR. ROBERTS: Yes. As we go a little bit further down. Hopefully, after we have that discussion, then we'll be able to clarify our recommendation for that aspect.

Dr. Isom.

DR. ISOM: I wasn't clear also with regards to the first study.

A lot of data, mechanistic data, can be obtained, related mechanistic data can be obtained from that study. Are we just advocating looking at, say, anatomical gross abnormalities in these animals, the survivability, or are we also saying we should be looking at blood levels of hormones and other associated effects in those animals?

If you have those tissues of those animals, why not get the mileage out of them?

DR. BRADBURY: 8 B starts to get at some of the very issues you are bringing up.

DR. ROBERTS: I guess my initial response, Gary, would be to encourage doing superimposed mechanistic studies while they are doing these.

DR. ISOM: It wasn't clear.

DR. BRADBURY: In fact, Question Eight B charged the panel, very explicitly asks the panel for advice and counsel on the attributes of these studies.

DR. ROBERTS: Then let's go to Eight B.

DR. STEEGER: Please comment on whether the agency's first set of proposed studies has accounted for the major sources of uncertainty associated with the potential effects of atrazine on anuran

sexual differentiation.

In addition to the time to metamorphosis, gonadal abnormalities and sex ratios in the proposed Phase 1 assays, please comment on any other endpoints that should be considered in this initial phase.

DR. ROBERTS: Dr. Richards.

DR. RICHARDS: No. The first part I think we pretty much elaborated on, it seems to me, in several of the other questions. No, we haven't accounted for the major sources of uncertainty associated with potential effects.

And I'll let the discussion open up in terms of the other measures as Dr. Isom and others have initially brought up.

DR. ROBERTS: Dr. Green would like to comment on that.

DR. GREEN: I think I already commented basically on the husbandry issues. But in terms of in addition to time to metamorphosis gonadal abnormalities and sex ratios, please comment on any other endpoints, and we addressed that with the 10 different estrogenic biomarkers.

I'm not clear if the goal here is to get these studies done quickly and in what level of detail we want to look at these things. Because all 10 of them would be a lot of work. The first four or five, which

are observational and easy to see, I wouldn't suggest would be the place to start.

DR. BRADBURY: I think that kind of a discussion would be helpful. What would be the experimental investments, time, effort associated with adding the different endpoints in, what is the kind of information that is gained as one gets that information.

Some of the logic in the different phases, probably isn't the right word, but the components or the trying to sequence things is sort of an approach to if we get the frank apical effects, then -- part of this question is getting at are there ways to maybe blend some of the concepts that are in the analysis plan and try to link some things up more quickly. That's the question.

Getting some insights on what some of the efforts would be and some thought about what would this experimental design start to look like I think is important.

DR. ROBERTS: Go ahead and follow up, Dr. Green.

DR. GREEN: We're trying to decide here -- in Phase 1 let's say we're going to start with xenopus laevis. And in Phase 1 we're going to grow them out to three months. That was something Dr. Kelley suggested. And I concur that would be the minimum because then we could see some of the secondary sexual characteristics start to emerge

or not.

And we would look at different stages and ages along the way out to three months. Is that correct?

DR. KELLEY: Let me just talk about a moment the sex ratio.

You are not going to be able to interpret the sex ratio unless you have a uniform genotype. Here is what I would do. I would get Nasco or Kelley Evans at Xenopus 1 to establish for me a stock colony for researchers to draw their animals from.

They will be willing to do this. I would like to point out to the audience that these places are agricultural facilities in their home states. They support the greater agricultural goods. Xenopus is a farm product.

Regulate is a farm product in many states. We're growing frogs here. It's not corn, but frogs.

Anyway, so you have to start with ZZ animals, otherwise the sex ratio becomes very difficult to interpret without long breeding experiments. It takes two years to get a female to breed. You don't want that. It will really slow you down.

How long will it take to get ZZ animals. If you get donated ZZ animals that people have, you would have to confirm they really are ZZ by mating them to normal males and having all male offspring.

That would take three months.

If you have to make them, that will take six months. Three months minimum, six months maximum.

Then you would run your experiments in the presence and absence of atrazine and various other things, and you should be able to get data on that in, I would say, six months. That's a year.

Nine months to a year minimum. With things going wrong, that's 12 months basically for sure and maybe 18 months.

So 18 months to replicate with a known population under defined growing conditions. That's what that would take in a lab that was up and functional.

In a lab that had never dealt with frogs before, though, I would like to point out that it will take longer. If you contract this out or something to people who have never raised xenopus, it will take longer because there is a learning curve, learning how to keep the animals and keep them happy and so forth. Just catching them. Learning to put lids on so they don't hop. These are major things.

Is that the sort of thing you want? That's how long it is going to take just with xenopus.

DR. BRADBURY: In the white paper we laid out attributes that may or may not be consistent. Some may be, some probably not

consistent with what you just described.

On the screen, we have laid out some of the details of that.

DR. KELLEY: I disagree deeply with some of the details.

DR. BRADBURY: Again, it was a plan to get this kind of dialogue going. I'm trying to figure a way see if I can get the chairman to help us sort of systematically explore some of these.

DR. ROBERTS: I think there is lots of issues. In CS for range and spacing and number of concentration, some of those things are up here -- you are looking for feedback on what should be done in these experiments. And I think we can go sort of go through those aspects.

I think your first question was in B is what endpoints should we look for. And so let's answer that question. And then we can go on to provide you with feedback on other aspects of the experimental design.

You asked for a little bit of dialogue for us in terms of if you did this, it adds this, but it costs this kind of thing. Not only in terms of dollars, but just to give you some sort of feedback in terms of what you get for what extra effort and resources are invested in terms of adding different endpoints.

If I understood you correctly, Dr. Bradbury, that's the kind of advice they are seeking from the panel right now as part of this

particular question.

So if we have some suggestions for them in addition to the endpoints that are identified here, some information about what else, pros and cons.

DR. KELLEY: Since you are concerned about fertility, you have to add a rate of fertilization of eggs as an endpoint. And there are a number of different ways to do that.

Some less variable than others. But that's an important endpoint. And that will require growing animals out longer. Six months.

So that will add to the cost of it. But it is an important endpoint for your goal. I would argue the most important endpoint for your goal.

Do you need more detail on how to do that? There are 42 different ways.

DR. ROBERTS: I don't think at this point. But I think that's exactly what -- provide you with this information which is important, but it means the experiment has to go longer.

DR. KELLEY: Right. But it is not very fancy. You don't have to learn to do a vitellogenin induction assay.

You get the testes and mush it up and fertilize the eggs, you

know.

DR. ROBERTS: Other suggestions? Dr. Isom and then Dr. Skelly.

DR. ISOM: We have talked around this and some of our speakers have mentioned this. I think perhaps this accounts for some of the variability we have seen among studies.

That is, clear definition of the endpoints. We really -- even to define sex ratios, how are we doing that? There is all kinds of variability in the terminology, and I would really encourage somebody to sit down and come up with some standardized terminology and endpoints and how you are going to evaluate those before these studies are conducted, at least this phase one.

DR. ROBERTS: I think that's something that everyone on the panel will concur with, is that the agency, as you begin this effort, is going to have to standardize the terminology for the endpoints by convening a workshop or whatever mechanism is the best way to do it.

But there has to be some terminology that everybody is using and agrees upon. We clearly saw the issues as the agency has. This is not something new. You are not surprised by this recommendation. But I think that's something we'll make in our minutes.

Other endpoints? Dr. Skelly.

DR. SKELLY: Just to build on what Dr. Kelley suggested, if you are going to grow animals out and add that extra investment, I think it is going to be important to beyond castrating the males and mashing up their testes to figure out, and I don't know enough about xenopus to know how this could work, but in the species I work with, it would be possible to measure reproductive behavior and reproductive endpoints of whole living animals.

That would measure their reproductive function. Because what Dr. Kelley suggested is taking a look at some index of male fertility. But we don't know what is going on in this animal's brain and whether it is going to act like a male or female or what it is going to do. I think that's critical to getting beyond the kind of physiology to the behavior in the ecology.

DR. ROBERTS: So you are suggesting another possibility is to add reproductive tests that involve behavioral component, but the downside is that there is -- is the methodology relatively standard or this something they would have to work through?

Dr. Kelley is -- I'll let you respond to this.

DR. SKELLY: Before Dr. Kelley builds on something that I say, just let me say that I don't know that the methods are standardized. They probably vary quite a bit from species to species,

because you are turning on frogs. They all have their own little things they like.

But it's certainly possible to do. And I think it adds significantly too, and then you can mash their testes up.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: So that's what we study, the reproductive behavior of male frogs and female frogs. You can do it. And we have standardized assays for doing it.

There are uncertainties associated with this. I find in my lab that the more things you try to get out of a single animal, the greater the possibility that you will compromise one of the measurements.

I don't like killing animals. I always try to get everything possible from the animal if it is going to give its life in the name of science. But there are problems with running that kind of experiment where you end up confounding it.

If you have a protocol for stimulating the testes that is very reliable and then you take that same animal and test it behaviorally, I think you could do that. And you could test clasping for which there is normative data. The papers are on the list. You could test the vocal behavior quite easily.

So you could do that. It would add to the study. It will be, to

be honest with you, behavior is hard and more variable than numbers of fertilized eggs. It will add a level of uncertainty and a level of interlab variability that will not be trivial.

But it is doable. I agree with you that it is important.

On the other hand, you have to say to yourself, hey, suppose you could never get these male xenopus to clasp. Is that going to be something that you are going to regard as a valid endpoint or not.

I'm a little bit worried about that, particularly since it won't be very easy to transpose that same paradigm to rana where the hormonal requirements for male reproductive behavior are rather different. Very different.

DR. ROBERTS: Dr. Green, did I see your hand up? And then Dr. Denver.

DR. GREEN: I was just thinking along the lines you brought up, Dr. Skelly, that an endpoint might be to just see if these animals can naturally mate in a laboratory. It is a simple easy way to do. It doesn't require castration or anything.

And the evaluation of that would be the number of fertilized eggs at the end of the time they have been in the bucket together or something simple like that. There is a lot of variability in that.

I know. I have seen that. And the females will eat the eggs

sometimes and that kind of thing. But one way to assess their behavior, and I'm sure you can elaborate on this, Darcy, would be if you put them together after hormonal priming, can they -- do they mate like controlled animals do.

DR. KELLEY: So again, I stress that you need a very well-defined stock to do that experiment with. You need to make sure that your controls and your experimentally treated animals are equivalent in establishing that group that everybody agrees are going to be the -- whatever those mice are, C J B 57 or whatever, you know, of the xenopus world is going to take a little bit of work.

DR. ROBERTS: I think as we make some comments, it is important to give the agency some advice about how straightforward these assays are. Let's be honest, some things may be possible. But if there is one laboratory in the world that can do it because they have enough experience, that's an important practical consideration for the agency as opposed to other techniques that are more straightforward that depending upon who happens to do it, they are likely to get decent results as opposed to something that is trickier.

I think it's maybe important to make it clear to the agency as we discuss these things, give them some sort of sense about that.

DR. KELLEY: I can give you a protocol for watching mating. I

have this long boring paper if you want to go read it from my dissertation on hormone levels, amount of time spent in amplexus, number of clasp attempts and controlling for females and so forth.

It is spelled out very clearly. I believe it could be replicated, although I noticed nobody has ever wanted to do it. I think it is possible to do.

In my experience, killing the animal and mushing up the testes is far and away the easiest way to get a first, easy measurement.

I think you would have to take eggs from a variety of females and you will have to have a good control and you will have to make sure it is done double blind bla, bla, bla.

But you will end up with if you fertilize X number of eggs, you get X number of offspring. You will get a quantifiable measure that can be subjected to robust statistical procedures as opposed to nonparametrics requirements, which are required for these noncontinously distributed variables like percentage of males clasping, which is what I used in my original studies.

I'm happy to teach anybody how to watch frogs clasp each other and score it. I think it's pretty easy to do. I'm just telling you that it would be about a zillion times easier, even for me, to do a fertility assay first.

I'm not against studying reproductive behavior. It is what I do. I'm just telling you it is a more variable endpoint in a field in which the endpoints are already very variable.

DR. ROBERTS: My point in raising this is I'm not trying to discourage any particular kind of assay. I just want to disclose as best we can the advice -- take advantage of the experience of you individuals and experts who have done these kinds of things to let them know how easy or how hard they are to do.

Dr. Richards, Dr. Green and then Dr. Delorme.

DR. DENVER: I want to say that getting an estimate of reproductive output when the putative species is the male is going to be really difficult.

On the face of it, it sounds fairly straightforward to do invitro fertilization with oocytes, mash up the testes. But you don't need a lot of sperm to get a reasonably efficient rate of fertilization.

Also, it depends on the quality of the oocytes, as you know. So standardizing something like that I think would be a nightmare. I think -- if the target species were a female, it would be a lot easier to quantify, say, yolk deposition or other aspects of female fecundity.

But I think that given it is a male, we may need to think about other ways to do that. Maybe the behavioral tests might be more

informative or more capable of standardizing the --

DR. KELLEY: I have the behavior paper with me. You guys can read it and see what you think.

DR. DENVER: But what do you think about that, Darcy? You mentioned the invitro fertilization as a way to measure --

DR. KELLEY: You are absolutely right. It is a problem. The other way to do it is simply to do histology on the gonads and look at the spermatozoa. You can stage spermatozoa. It is not as well worked at as mice, but you could stage it.

If you get a big effect, it is never a problem. If you have a marginal problem, it is always a problem. Right? What I would like to do is, if you wouldn't mind, to go back and reread my dissertation paper from 1975 and see what the error bars are like, basically. See how variable the measure is for looking at clasping.

Calling is not going to do you any good. Either they call or they don't. That's it. You could look at amount of time calling at various levels, but that reflects internal androgen. And calling is really difficult. To get robust callers at certain times of year is really hard. But clasping is much less difficult, the amplectant (ph) position.

Let me go back and look at the clasping data and get back to

you.

DR. ROBERTS: Dr. Richards, Dr. Green, Dr. Delorme.

DR. RICHARDS: I just want to point out I have never worked with behavior in amphibians, but I have done a little bit with fish and invertebrates.

Doing behavioral experiments in the lab are problematic. They are very complex. They are difficult to anticipate outcomes often times.

And I think behavior is best viewed in the field. I think that some of the mashing and counting things might be better measurements at this point in the game.

Plus, I'm not sure that federal employees are allowed to watch clasping behaviors.

DR. ROBERTS: Good point.

Dr. Green.

DR. GREEN: You had asked to indicate how feasible, how easy some of these proposed experiments might be to do.

Just to communicate to you, in our animal facility, natural matings are quite common just for the purpose of collecting the fertilized eggs and studying the eggs.

And usually in the fall, we have the arrival of 150 or so new

graduate students and post docs who have never dealt with frogs before. I'll go through the frog rooms and there may be as many 20 to 50 buckets lined up on the floor where animals are paired. They have been primed hormonally. They are put together and essentially left overnight undisturbed. Some labs have video cameras to watch. I don't know why.

So there is a way to record the behavioral aspects of the mating without disturbing the frogs. And then the students come back the next day hopefully early enough that the females haven't eaten the frogs, but there are ways to prevent that from happening with a mesh grid at the bottom.

And then they take the eggs on upstairs. And as I mentioned, I think that seems relatively straightforward. I know all about the variability in that. You go to the dissecting scope and you look for the ones that are fertilized, and you could carry that one step further to see how many actually become viable tadpoles.

The endpoint could go on and on and on. But that basic part is fairly simple to do.

DR. ROBERTS: Dr. Bradbury.

DR. BRADBURY: I just wanted to do a check-in to see if I'm synthesizing the dialogue properly.

At one point there was a statement that seemed to be accepted by several, at least, that there should be an attempt to try to replicate the studies that have been discussed over the last few days, the lab studies. And that that was an important task at hand.

And that xenopus and/or rana simultaneously or -- we're going to think about that a little bit later, the Phase 1, and again, don't get it too sequenced, was a proposal to get out to the panel that wasn't intended to be a detail by detail replication of the previously published studies in part because we had concerns about growth and water quality and those kind of things.

But in the spirit of many of the discussions over the last few days, that if a response seems to be consistent and is occurring in a reproducible, logical pattern, that study designs don't have to be identical, but there are certain principles they are holding to to see if, in fact, they are getting concordance of the information development.

Phase 1 is designed in that spirit. And seemed -- was sort of our proposal for your consideration in terms of "repeating what had been done in the previous studies."

Am I to interpret that that is one aspect of an experiment or a study that would go on, and some of the other discussion we have been hearing would be in the context of aspects of studies we have

been using as phase five? Or am I to interpret that not only do you want us to come up with an experimental design that can capture "what has been previously studied," but also include all these additional endpoints?

I could imagine an experimental design that would do that. But I would be curious if that's what you mean. Or are we talking about a series of separate studies?

DR. ROBERTS: Responses? Dr. Delorme, actually you were next up anyway. DR. DELORME: Actually, I was going to bring up that point, because I was getting confused as to what is going on.

And I flipped back to the slide on phase one which is, test for apical gonadal effects, and the objective was to determine if atrazine exposure results in gonadal effects in males and females in brackets.

When I had put in my consensus that repeating the experiments, basically, that's what this one is about, and then the side bar with the ecological relevance is sort of the next thing, I mean, bringing it back and wearing my risk assessor hat, because that's what I'm doing, I think we have to recognize that EPA wants to move this risk assessment forward.

And the first step in doing that is determining whether or not

that cause effect between atrazine and gonadal effects is there.

So perhaps maybe we want to consider an experiment which would try and replicate, not replicate, but at least firm up that cause effect.

DR. ROBERTS: Dr. Green.

DR. GREEN: I think the issue was whether or not those gonadal effects that you see morphologically have any physiological consequence. It is hard to answer that question if all you are looking for is gonadal abnormalities.

That would be the endpoint, I guess, and a place to start in phase one. If they do, then maybe go on with other experiments to then test the hypothesis as to whether or not that has any effect on fertility and fecundity.

DR. ROBERTS: Dr. Bradbury.

DR. BRADBURY: The way that discussion was going is good. I think it would be helpful for us to hear you explore is it sort of a sequential -- get this information, check in what to do next or do you try to do it all at once.

And I think you opened up dialogue that would be good to hear some more about.

DR. ROBERTS: Dr. Delorme, then Dr. Skelly and then Dr.

Coats.

DR. DELORME: You may be able to design a study that you could actually phase the results where you get the results of the initial study but you have enough animals left over that, if you need to take it to looking at the physiological consequences of having disrupted or abnormal gonads whether or not that has effect.

I guess that would be possible. But again, I come back to what Dr. Green said earlier. The more things you try and get out of a study, then you risk having uncertainty creep in or losing animals or whatever.

So you are going to have to evaluate the relative merits of what you are going to get out based on the design.

If you want to design a study where you could initially try and replicate or look at the causal effect with the idea that you are going to have enough animals built into it that you can take it beyond that should you find out that there is effect, certainly you could do that.

DR. ROBERTS: Dr. Skelly.

DR. SKELLY: Dr. Delorme said much of what I wanted to say. I guess one way to phrase the recommendation back to EPA would be in the context of doing a power analysis and thinking about how many animals need to be available at each stage. How many of them are

going to have to be sacrificed at each stage.

Some things that we're not doing when we're sitting here talking about it may become obvious one way or another that it makes sense to economize and break those up or it makes sense to economize and put them together.

DR. ROBERTS: Good point. Dr. Coats.

DR. COATS: My thinking is that phase one is presented up there and as described in the book is pretty much where we need to start. But that there has been discussion of maybe adding one more endpoint on to the end of that or two if they are not too complicated.

DR. ROBERTS: Others? Dr. Gibbs.

DR. GIBBS: If I can just add one point. Molecular genetics gets right to the heart of the matter, which would be paternity. There are simple ways without getting into behavior. For male frogs, paternity is the bottom line. However, they get there. And I think you could devise some fairly simple experiments, toe clips from all of your adults and all of the metamorphs and you've -- genotyping them and assigning paternity. And you have got some good data. It gets right at the issue.

DR. ROBERTS: Any other comments? What do you think, Dr. Bradbury, is that -- we have sort of honed in on it a little more?

DR. BRADBURY: Yes. I know you are going to be writing. I now have a feeling that I can imagine some of the dialogue that will be on paper that will be helpful based on this last round of discussions.

DR. ROBERTS: I hope Dr. Richards can also imagine that dialogue. Dr. Coats.

DR. COATS: One point had not been discussed very much was the dose response possibility as described in the tier one, phase one there.

And I think it is an extremely important part of it, and would be very informative to elaborate on the dosing scheme. That may be discussed at a later point.

DR. ROBERTS: I think it comes up in C as I would interpret C.

Have we finished then with B, Dr. Richards? Do you want to poll the panel? Do you have a pretty good feel for where we are on this?

DR. RICHARDS: I don't know if the point about conditions and this thing about the ASTM standards and flow through --

DR. ROBERTS: I think we're going to get to that.

DR. RICHARDS: -- is that going to come later?

DR. ROBERTS: I think so. We're still sort of what components

need to be in that phase one and that sort of thing.

Dr. Delorme.

DR. DELORME: I just want to ask for a clarification. You have replication up there as two. Does that mean two tanks or two species or two xenopus?

DR. TIETGE: It was meant to represent two tanks. I think we have since -- this is an older version of the slide for some reason.

DR. DELORME: Do you want to comment on whether or not you think that's appropriate given the context of the discussion?

DR. TIETGE: No. In the context of the power analysis and the discussion that's been going on, it is probably not sufficient.

It is a commonly used approach in aquatic toxicology, at least two.

DR. ROBERTS: Should we go on to 8 C then?

DR. STEEGER: Please also comment on the range, spacing and number of atrazine concentrations that should be employed in the proposed testing sequence to resolve uncertainties in the shape and nature of the dose response relationships for any observed developmental effects.

DR. ROBERTS: I think for this particular question -- I'm sort of looking ahead. We have some questions -- most of the rest of them

deal rather specifically with the species involved and differences and those kinds of things.

I think this is probably the best question to raise not only issues regarding to spacing and dosing of atrazine concentrations, but perhaps some of these other things about experimental design that we have been sort of eager to bring up.

With that said, let's go to Dr. Richards.

DR. RICHARDS: I'm going to open it up to those first statements on range and spacing. I know some of you had some stronger feelings about that if you want to just jump in here.

DR. ROBERTS: Dr. Heeringa had to leave for the airport. But he did give me typed up comments. With your forbearance, let me read them, because I think that's probably the best way to get them into the record. And I would not dare try and paraphrase his points for fear of not getting them right.

In regard to 8 C, he says, this question must be answered in the context of a

presumed constraint on the cost and effort that can be devoted to a single replication of the study to determine if aqueous concentrations of atrazine bears a relationship to gonadal irregularities and any associated mechanicism for endocrine disruption in frogs.

The scope of the study is determined by the number of concentrations and controls tested, the number of intralab replications, for example, tanks for each control concentration level, and the number of test animals per experimental replication. Range in spacing of the experimental concentration levels is obviously related to the number of feasible experimental points. Range in spacing should also be governed by the specific test hypothesis concerning the potential shape of any underlying concentration response relationship.

Consider the components of this design in the following order. One, selection of controls. Two, range of observations for experimental concentrations. Three, number of independent replicates per treatment. Four, number of test animals per experimental replicate. And five, number in spacing of experimental concentrations.

Number one, selection of controls. The experiment should include untreated control replicates and a positive control under which test animals are exposed to a concentration of estrogen. I support the EPA statement that a positive androgen control group is not needed, although would be beneficial if laryngeal muscle measurement is included as an endpoint.

As a protective factor in the test of hypothesis concerning the dichotomous or polyotomous, (ph) Dr. Hayes' classification, gonadal deformity endpoint, which is based on existing data, suggests a one-sided test. The sample size for the untreated control should be increased beyond the levels that would be assigned to concentration points in the nonzero domain of the test range.

Number two. Range of observations. Setting aside the positive controls, the range of experimental concentration should span zero or untreated atrazine concentration across ecologically relevant concentrations and extend to concentrations that meet and at least one point exceeds the upper percentile bounds that have been measured in natural aquatic environments.

Number three, number of replications for each experimental treatment. For the xenopus, and that's Hayes and Carr studies, and the rana studies, Hayes, estimates of the empirical intrareplication or intratank correlation should be obtainable.

Based on concordance of the values or maximum of estimates if highly variable, the estimated intraclass correlation should be used to determine the number of replicates per treatment arm and the allocation of total sample size to replications and test animals per replication.

This should be based on the best empirical data from the existing studies. Underestimating the intrareplicate correlation and planning the sample size allocation may seriously attenuate the true power of the test of the hypothesis concerning the chosen endpoints and in particular test of hypotheses concerning a dichotomous that is deformity outcome or the differentiated polytomous classification of deformities proposed by Dr. Hayes in his presentation to the panel.

Number 4, number of animals. Subjects per replication having established a working value for the intrareplicate correlation for the class of outcomes of interest and a desired level of statistical power for a specific hypothesis test.

The determination of the optimal number of animal subjects can be determined jointly with the determination of number of treatment replicates.

This allocation is obviously constrained by bio-loading and water quality considerations that are discussed in the EPA white paper.

Finally, Number 5, optimal determination of the number in spacing of treatments as governed by the hypothesized shape of any underlying concentration response relationship.

The panel has determined that data from existing studies lend

support to further testing the hypothesis of a relationship between atrazine concentration and gonadal abnormalities in frogs.

Dr. Hayes provided data and arguments that the relationship is not monotonic potentially in inverted response. But this has not been replicated in other studies.

At this stage, there is insufficient basis to set the spacing of treatments to optimize the experimental design for a functional form for a potential concentration response curve.

A robust design would use multiple concentration points to accommodate the possibility that any effect is monotonic or alternately that there is a simple nonmonotonic or convex relationship.

There was also an advantage to retaining concentration points that have been used in the prior research by Hayes, Carr, Hecker and others, 0, 0.01, 1, 10 and 25 micrograms per liter, and adding an upper concentration level that exceeds the 25 microgram per liter value, at which Dr. Hayes and Carr studies have detected increases in the number of abnormalities, such as basing of concentration treatments should be sufficient to test the hypothesis of an effect and to secondarily test whether any real effect is monotonic or nonmonotonic.

Obviously, these concentration levels would not permit testing threshold responses below the .01 microgram per liter level.

I will give these to Dr. Richards to work into our comments.

Dr. Green, did you want to --

DR. GREEN: I was just going to say I support his recommendations, and I particularly like the dose range that he has proposed in that.

DR. ROBERTS: Other comments. Dr. Coats.

DR. COATS: Yes. I liked some of the comments. I don't think that dose range is going to depict the curve any more clearly than -- concentration response any better than what we have already seen other than one laboratory doing all those concentrations, which Dr. Hayes has mostly done.

I think if there is an unusual response at which point .1 part per billion is a significant concentration, that points near that, above and below ought to help delineate the response curve if it is a curve.

And that if you are going by orders of 10, orders of magnitude, you would miss that.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: I agree completely with Dr. Coats. I think we need to recognize here or at least acknowledge what the intent of the

experiment is and design it appropriately.

We can design the experiment to replicate earlier experiments, that is, determine whether or not atrazine is eliciting the effect, or we can try and characterize the shape of the concentration response curve.

But I don't know that we can do both unless we get lucky. And I would suggest that probably the first time around we follow the recommendations of Dr. Heeringa recognizing that we're probably not going to characterize the concentration response curve, but will have a good design to replicate previous observations and, if we get lucky, perhaps we'll gain information on the concentration response curve as well.

DR. ROBERTS: Dr. Green. Then Dr. Delorme.

DR. GREEN: The aspect I liked about the dose responses or the doses that he has proposed is that they do include the ones that have been looked at previously.

And I agree. They could be added to. And I like the fact that he exceeded the maximum dose that was looked by both labs. I can't recall how many fold, how many times he said higher than the 25.

What was the next value? Did he give one?

DR. ROBERTS: Based on some percentile, I believe, of

observations from the field.

DR. GREEN: That seems important to do.

DR. ROBERTS: Dr. Delorme.

DR. DELORME: I just wanted to ask a clarification of EPA. Were you intending this as an inova (ph) design study which is a hypothesis testing or one which -- a linear design -- or a regression type of design where you actually want to get a dose response?

Because that actually makes a difference on how you space your doses and how many animals you have in your replicates.

This actually goes to some of the other comments that have already been made.

DR. ROBERTS: Dr. Tietge.

DR. TIETGE: To the extent possible, I think dose response, but I think recognizing what Dr. LeBlanc said is you may not be able to hit the proper range of concentrations to achieve that.

DR. ROBERTS: As Dr. LeBlanc said, often times when you do these kinds of studies, especially if there is a sharp inflexion in the curve, then you have to go back and start loading in in that critical range. So it wouldn't be surprising.

But I think at least the recommendations by Dr. Heeringa span the right range of doses.

Any other recommendations or comments about spacing, et cetera? Dr. Delorme.

DR. DELORME: Just out of curiosity, are you worried about the low end of the dose response curve or the upper end? From a regulatory perspective, often times what we're looking for is near to no effects or what we would putatively call acceptable effects.

DR. BRADBURY: I think we're sort of getting into a phase within a phase. I think the first question is can you reproduce the effect and can we use that in dose basings that were recommended I think are reasonable to get some sense of consistency across studies.

Give me a concentration response curve to start work even if it's crude, then we can start talking about those kinds of things. I think it's premature until we get a response relationship.

DR. ROBERTS: Using the chairman's prerogative, I'm going to change my mind about talking about other experimental design aspects at this time. Let's go through the rest of the questions, and then at the end if there are recommendations regarding other aspects of the experimental design or other points, let's go ahead and bring them in at that time.

Are there any other comments, then, on the specific issues raised in this particular question on the range, spacing and number of

atrazine concentrations?

Let's move on, then, to D.

DR. STEEGER: Please comment on the agency's recommendation that xenopus laevis be used as the primary biological model in the proposed studies and whether or not the mechanisms involved in sexual differentiation of the ranid and pipid species are sufficiently similar to predict effects and associated dose response curves for rana and/or to efficiently design rana studies.

DR. ROBERTS: Dr. Richards, do you want to lead off or throw it open for discussion?

DR. RICHARDS: Let me make a comment. I think that the agency and others have indicated there's lots of reasons you use xenopus, for a variety of laboratory tests and quick techniques and so forth.

I think we have also previously identified some need to start initiating a tighter rana procedure in the laboratory.

But I would like to throw it over. I hope Dr. Kelley will respond on the differences in the differentiation.

DR. KELLEY: I don't think we know enough to be able to answer this question. We know a huge amount about xenopus laevis, and we know so much less about rana that I couldn't tell you. I

couldn't answer this question.

DR. ROBERTS: Dr. Skelly.

DR. SKELLY: I guess Dr. Kelley's point suggests to me that -- just to support what EPA has proposed, that rana is used as a corroborating species for these early experiments.

DR. ROBERTS: Any other comments or any comparison of the species or the suitability of one to serve as a model for the other? Dr. Denver, and then Dr. Green.

DR. DENVER: I just want to concur with Dr. Skelly that the ranid species do need to be considered early in the game.

DR. ROBERTS: Dr. Green.

DR. GREEN: I think it is probably obvious, but we could probably all predict that there will be differences between the two species.

DR. ROBERTS: Any other comments on this one? Dr. Gibbs.

DR. GIBBS: Only that the rana maybe should be clarified. It should be a North American rana. There's rana on different continents.

DR. ROBERTS: Then let's let our minutes reflect that clarification.

Let's go ahead and go to E which is sort of the flipside of the

question.

DR. STEEGER: In this regard, are there important differences between the species to conclude that any affected developmental processes observed in xenopus laevis would not occur in rana?

DR. ROBERTS: Dr. Richards.

DR. RICHARDS: I will throw that one open.

DR. ROBERTS: Dr. Kelley.

DR. KELLEY: Every species develops in its own way, but they all develop their gonads, and they differentiated, you know, not too roughly inappropriate times.

There actually are, although I'm not sure how valid, there are tables that relate, the tables of normal development in xenopus to roughly equivalent stages in rana. So it is possible to normalize a little bit in that way.

The developmental biologists believe, although they now mostly study xenopus, in the old days they studied rana, and the developmental biologists believe that the fundamental processes are extremely similar, shpay mons (ph) organizer, induction and so forth.

I don't know of any strong species difference that would lead me to believe that there would be some fundamental reason -- some fundamental difference at the developmental level that would lead

anything affected in xenopus to not necessarily be affected in rana.

So that's a very couched statement, but I believe that the developmental biology community would agree with me.

DR. ROBERTS: Dr. Skelly.

DR. SKELLY: I will support everything Dr. Kelley just said with just an added comment. It seems like timing is important here. And one aspect that xenopus differs from almost all other frogs is in how, say, the onset of reproduction and the development of reproductive morphology differs with respect to the onset of other sort of metamorphic characters in almost all other frogs.

So there are things that just never happen in xenopus. Transitions that either don't take place or take place differently in xenopus than they would in rana and almost all other frogs that undergo metamorphosis.

That suggests that either the genes that control development and some of the developmental pathways, and I'm getting way out on a limb, I'm going back to grad school to remember this stuff, but suggest to me that developmental pathways and timing of things are somewhat different.

And since timing of the exposure to atrazine or whatever can happen at different times relative to developmental sequence, even

though there are these tables that relate xenopus development to rana development, what is going on inside and when genes are turning on and those sorts of things, I don't know that we know that much about that right now.

I guess that's just a note of caution in assuming that these things are going to happen similarly. I will just finish by saying that I think we have seen pictures anyway that suggest that some of the outcomes in gonadal abnormalities seem to manifest themselves somewhat differently in the two species.

DR. ROBERTS: Other points?

Should we go to F which is again related to the same -- different way of sort of tackling the same kind of issue?

DR. STEEGER: Alternatively, are there developmental pathways in rana but not in xenopus laevis that raise concerns about using xenopus laevis as the primary biological model in any future atrazine studies?

DR. ROBERTS: Dr. Richards. You were going to defer to the person who just walked out the door.

DR. RICHARDS: Like a flipside of what we just spoke of here.

DR. SKELLY: I guess I'm getting tired, but I was hoping to make the comment I made for E for F.

You can move that.

(Thereupon, the time was 5 o'clock p.m.)

DR. ROBERTS: I think we could sort of take these up kind of collectively. Because if I understand it correctly, I think the agency is trying to ask in different ways to what extent can we extrapolate data from xenopus to rana, and does it serve as an effective surrogate or is there any reason to be concerned if we used it as our primary model that it would mislead us about what was going on in rana.

Dr. Denver.

DR. DENVER: I don't have any evidence that would suggest that the basic developmental pathways would differ, although, there are distinct differences, obviously, in life history and physiology and we have documented differences in the development of the stress axis when the stress axis becomes responsive, the production of stress hormones in the two species throughout development, things like that.

So the timing of things are different in the two species as Dr. Skelly pointed out. But I don't have any evidence to suggest that the basic mechanisms are that different. So I think the main point there is to realize that the critical periods or the sensitive periods may differ between the two species. So that needs to be considered in any experiments that are designed.

DR. ROBERTS: Other points? Dr. Delorme.

DR. DELORME: Just stepping back and taking the broad picture, I guess what I'm hearing is that you can make the assumption that there may be not too many differences, but you are making an assumption.

And at some point, you are going to have to test that assumption. It may not have to be right away. But certainly, sometime in the future you are going to have to do some work to work on it.

DR. BRADBURY: I think I'm picking up the message that xenopus is a reasonable biological model to further confirm a toxicological signal related to gonadal development and test that with atrazine. That that's a reasonable biological model to get started.

Does that mean that all amphibians will respond exactly the same way as xenopus does? I realize we're not saying that. But just as we do our aquatic toxicology testing, we don't have the luxury to test all the thousands of species of fish in North America. We have to settle on a few surrogate species to give us a sense of the toxicological potential of the chemical and then through other analyses one deals with species extrapolation and other aspects of life history to refine our risk assessments as needed.

So for that matter, we have a handful of mice and rats that we use to go to humans. But at least we have several species to go to one species. Ecological toxicology, we're going from a handful of species out to potentially several or many.

So what I think I'm hearing is that xenopus is a reasonable biological model to get a handle on the consistency of previous studies in terms of the potential for atrazine to initiate these developmental effects.

The proposal, then, is to use a North American species to get some sense of species variability and to be using a species of North America. Does that represent all the North American species? No.

But at this point, I would say that we're sort of in the venue of all of the challenges we have in ecological risk assessment across the board in how to extrapolate across many species when you only have data for a few, unless the panel wants to probe that a bit.

DR. ROBERTS: I think I heard not only just now but discussions earlier during the last couple of days that there are some -- since a primary objective as we just discussed at least initially is to get a handle and do some well controlled studies, that there are a number of advantages in using xenopus to do that because it's well-characterized. There is a lot of experiments and it lends itself to

doing those kinds of experiments that really need to be done initially.

But as you say, we'll also have to do at least some experiments on rana because of uncertainty about the extent to which xenopus would be representative.

Does anybody else want to add anything?

DR. DENVER: I just wanted to add a related point. That came up earlier with regard to measurements of uptake of atrazine and body burdens and that sort of thing.

These two species have very different feeding ecology as tadpoles. And that could translate into differential rates of uptake and different exposure to the compound.

And so I think that at the outset it is important to address those issues. I think as already been -- that point has been made, I don't know that has been made today, but it has been made previously that that be measured and perhaps compared between species and the studies.

DR. ROBERTS: Dr. Denver, can you provide a reference that we can include in our minutes so we can be sure and pass that message?

DR. DENVER: A reference to the previous discussion --

DR. ROBERTS: No, to the different feeding behaviors.

DR. DENVER: Oh, yes, sorry.

Xenopus tadpoles are filter feeders whereas ranids tend to feed on -- try to sit at the bottom of the pond. So they have quite different modes of feeding. And so their rates of uptake of compounds from the environment may be different.

DR. ROBERTS: If you can draft a couple of lines or two and throw in a few citations for a report, that would be very helpful.

Any other comments on this?

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: Maybe if you want to generalize the effects on sexual differentiation on amphibians, I think I would like to mention that we have also another order of amphibians, oradales (ph), which have different androgens.

If you would like to have some comprehensive studies, of course I agree fully that you should go ahead to start out with xenopus. But if you would expect something different, then you should more go to oradales to look in this -- all of amphibians.

Because they have probably also a little bit differences or more pronounced differences concerning sexual differentiations in comparison to xenopus and ranids.

DR. ROBERTS: Dr. Delorme.

DR. DELORME: Just a comment on Dr. Denver's little discourse there on uptake from feeding.

I don't know if there is any data, but it might be worthwhile looking at the relative contribution from food and bioconcentration from the water.

I don't know if one would outweigh the other, but certainly it is a thing to consider, something to consider in the exposure, because we're really treating the water here, not the food.

DR. ROBERTS: Any other points or comments on this, on the appropriateness of the models?

Let's go ahead and take G, then.

DR. STEEGER: Assuming xenopus laevis and rana are sufficiently concordant from a toxico dynamic perspective with regard to potential developmental effects of atrazine, what critical toxico kinetic processes should be considered for extrapolating xenopus laevis dose response relationships to rana and/or for designing subsequent studies with rana.

DR. ROBERTS: Who wants to tackle this one? Dr. Delorme, then Dr. Green, then Dr. Coats.

DR. DELORME: I think we already touched on one of them. That's uptake. The second one would be looking at whether or not the

degradation or the depuration from the animal is similar. I can't tell you.

DR. ROBERTS: Dr. Green.

DR. GREEN: I can't answer this directly, but I know from looking at pharmacokinetic studies again for the purpose of trying to apply veterinary drugs to treat sick animals that there are differences between rana pipiens and mammals. There have been some comparisons in small rodents.

Differences between rana pipiens and X. laevis in pharmacokinetic studies, the comparisons aren't usually directly made. But the margin of safety for many drugs has been proposed to be much lower for X. laevis because it's a fully aquatic species, which means that when they get sick or weak, they can't get up to the surface to gulp air.

So they die from drowning, which I might envision that if at the higher ends of this dose range that we propose for atrazine, the mortality perhaps, maybe not, could be higher in the juvenile and older animals that are fully aquatic at that point, because they will get sick and weak, and not being semiterrestrial like rana pipiens, you might see more of them die earlier on in the study than you would for rana pipiens.

That's speculative on my part. But there is some suggestion in the literature that when it comes to some veterinary drugs, that's what happens to xenopus laevis.

So you can't apply a drug dose for a rana to a xenopus to treat a particular condition without being aware you could kill the xenopus.

DR. ROBERTS: Dr. Coats.

DR. COATS: I agree there is basis to probably expect different pathways of degradation. Most of them probably are detoxifications. There is possibility of some of them not being at detoxification and still resulting in a molecule that would be bioactive. From the uptake perspective, I agree that the water and the food are both important probably and need to be studied.

And certainly absorption through the skin is something relatively unique or at least feasible that would have to be looked at I think in the aquatic forms.

DR. ROBERTS: Just to comment on my part, I think you could do a lot of toxico kinetic studies in terms of absorption and depuration. But an easy thing to do to sort of see what the summation of those processes are is to look at the tissue levels in animals at different concentrations, which really gives you sort of the integration of the intake and outflow.

And we have already talked about the desirability, if possible, of putting that, getting that information as part of the study so you can see whether or not at a particular concentration of atrazine and water you get the same concentrations in xenopus as you do in rana.

On a very simple level, that might be very useful for extrapolations.

Dr. Delorme and then Dr. Kloas.

DR. DELORME: I guess my only concern with this would be that the development of the different organs, especially the liver, which is probably going to be the major detoxification organ, you have to be aware of any differences between the two species and how that can affect what is going on.

I don't know how you are going to do that. I'm not a developmental biologist. It theoretically I guess could have an effect.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: For toxico dynamics I think during the larval developments there shouldn't be a big difference between ranid and xenopus.

But after metamorphosis, the skin of xenopus is relatively impermeable. So there should be a big difference in toxico dynamics, especially if some experiments for the modes of actions might become

designed for juvenile or adults. This should be taken in account that there is severe differences between ranid species versus skin still that keeps on being quite permeable in xenopus.

DR. ROBERTS: Good. Any other comments or suggestions on this point?

Dr. Bradbury, is the input reasonably clear?

DR. BRADBURY: Yes.

DR. ROBERTS: This was the last part of the last question. But during the course of some discussions, I think there have been some aspects of potential experiments that the panel has been anxious to recommend or offer their advice in terms of how some of these studies should be performed. We have already heard some of them. And I think Dr. Green has provided some excellent suggestions on issues in terms of practicalities of doing studies and things that need to be considered.

Maybe if we could go back to the slide which sort of laid out the things in some of the initial phase one studies to sort of, to serve as prompts.

I will ask the panel now as we sort of move beyond the questions posed to use if there are any specific suggestions that they might have that haven't been mentioned so far.

Dr. Green and LeBlanc.

DR. GREEN: I think we talked about water quality issue and nutrition and feeding a little bit. But the issue of what kind of tanks these studies would be performed in, whether they were flow through or static renewal, I don't know if that was resolved.

I was just wondering if the EPA were to conduct these studies in their lab, and I'm not saying that they would, what kind of tanks are routinely used for such studies?

DR. TIETGE: We typically use glass tanks using flow through conditions.

DR. GREEN: What is the water turnover rate in the flow through?

DR. TIETGE: In terms of flow rate, it is 25 mils per minute, which is about 36 liters per day.

DR. GREEN: And the total volume of the tank is how much?

DR. TIETGE: In that particular system, the standing tank volume I believe is four liters and our stocking density is from 20 to 25 organisms.

And if you calculate the maximal, the approximate maximal weight, the total loading approaches the ASTM standard of one gram per liter per day.

DR. GREEN: I don't have a feel for how difficult this would be for labs that might be asked to perform these studies to reproduce.

DR. TIETGE: Well, I kind of got prepared for this. I was anticipating these questions last night while everybody else was awake.

I have a brief little presentation here.

Perceived problems. We hear a lot of different problems brought up. And one is system costs.

Actually, system costs are not necessarily high because there are proportional diluter devices which work on hydrolic principles that really cost probably a few hundred dollars to build.

These have been published. There is published designs for these that date back to the 70s, I believe. One of them is called the Mount Brungs (ph) Deluter. There is a Banoit (ph) Deluter. These kinds of technologies are very old. We have many of them in our laboratory.

I think the notion of cost is often considered an impediment because there are fancier systems that can be computer controlled and can do very complicated exposure or can be used to achieve very complicated exposure designs.

But the basic systems can be very inexpensive and very

reproducible because you are relying on gravity to make them work. They are very low cost systems.

There are concerns about operational costs. For example, waste, chemical costs in terms of the test chemical, which I don't think in this case would be a great impediment, and then costs for water. And I'll get into a few of those a little bit more in a moment.

We often hear that it is suboptimal for anurans. And I guess I'm wondering what the biological basis is for that. I have read anecdotally that it is a suboptimal. All I know is empirically it seems to work pretty well. So if someone has a biological basis for that -- Dr. Kelley mentioned yesterday that stimulation of lateral line could relate to -- could relate to some stress response, and I have seen that in the literature, but I have never seen it actually documented. It seems to be kind of an informal opinion.

Just a quick history about these methods. As I mentioned, some of the technology for doing the flow through methods were developed back in the 1970s at the end of the period of aquatic toxicology where it was called the kill them and count them period where acute lethality in short term static tests were kind of the norm.

As water quality criteria, for example, became more complicated or more, what do I want to say -- well, we started to move

toward more sophisticated approach to water quality criteria. And we began using studies that were more capable of dealing with chronic and subchronic sublethal endpoints.

That's where flow through systems really became developed, was to achieve those ends. And when you get into more subtle effects, such as endocrine disruption, these types of systems provide highly reproducible exposures, which minimizes the variance in the system.

Currently, EPA requires flow through studies in different offices of at least 12 species. We just kind of threw these numbers together last night. And typically, there is 300 studies submitted annually. These are primarily fish studies.

But the point is that there is adequate facilities in the research community to do this. There is the toxicological expertise and, in fact, guidance such as in the ASTM guidelines on how to conduct these study was amphibians as well as fishes.

In general, at our laboratory, we also maintain a very large database for aquatic toxicity. And I can tell you that there is thousands of studies that have been conducted over the last 20 years using flow through methods with at least 30 aquatic species. This is not anything new. That's my point here.

So there is two main lines of reasoning to use flow through methods. The first is the biological rationale. Maintenance of water quality. These issues have been brought up. Temperature, pH, et cetera.

Reduced stress caused by repeated exchanges or handling of organisms during static renewal studies. In a flow through study, you don't need to do that.

And with most species, at least the species that we have worked with, these methods have been demonstrated to promote survival, growth and development.

Now, the toxicological rationale for the flow through methods, first of all, is the maintenance of a stable chemical concentration. Remembering that in this particular case we're not trying to achieve an ecological approach, it is a toxicological approach, to reduce the variables involved so we can, you know, assess the chemical in a tightly controlled system.

It is particularly good for highly hydrophobic chemicals because it eliminates the mass limitations that occur when you are dealing with chemicals, let's say, that are in the 6 to 7 KOW range with labile chemicals which may be -- will degrade due to metabolism, hydrolysis, photolysis, or they might be volatile in a

system.

It also has the added benefit that it reduces or eliminates the accumulation of chemical metabolites. That's of the parent, which was brought up as an issue yesterday. And we get improved dose response or concentration response data from that.

So at our laboratory in Duluth, we have flow through methods that we have used for xenopus laevis. And we have gone through some of these baseline studies that have been mentioned previously.

We have done loading studies to evaluate whether or not we needed to approach the ASTM or utilize the ASTM recommendations. And indeed, when we pushed the loading organism performance based on mostly growth and development, it became problematic. So we have stuck with the ASTM guidelines.

We have done some feeding comparisons. I could get into the detail if anybody is interested. And we have done some baseline developmental studies looking at developmental rate.

One of the added improvements that we see in the flow through is we have better developmental synchrony, which makes the tests a little bit easier to conduct and reduces some variation.

If you use the ASTM standards, you can conduct static tests or you can conduct flow-through tests. And I have listed the biological

loading rates for the two there.

And the reason I was able to pull that number off the top of my head a little bit earlier is because I went through the calculations last night. In our studies, we typically use 25 organisms. Their maximal weights are about one and a half to 1.8 grams. Occasionally, we'll get a 2 gram organism. That usually occurs at about stage 61, 62, I think. So we base our loading on our maximum weights.

And as you can see, if you go through the math, we're at about one gram per liter per day.

One of the things to note here is if you choose the static route and you are going to adhere to this guideline, because we're working in the absence of other validated protocols, then you will see that if you want to run 25 organisms, I did the calculation just for comparison, you would need 75 liters per day for those 25 organisms as opposed to in a flow through condition where you would only need 38 liters per day.

So I think if you make the decision that you are going to stick to the guidance that was developed and first published in 1980 and it had -- it has taken a lot of -- taken advantage of a lot of information that was developed up to that point, then I think it is more efficient to go with the flow through.

At our laboratory, we have conducted approximately 35 toxicological studies. If you are interested, 12 to 15 of those studies are published, and there are several in press. But the thing I want to point out here is that we have worked with xenopus laevis, rana pipiens, clamitans, sylvatica, septentionalis. And all those species are amenable to these methods.

Some are better than others. I know someone was complaining about a doing green frog work. I kind of had to smile because it just takes them forever to develop. So I wouldn't recommend doing green frog or mink frog work with these methods. But they can be done if you are studying larval period.

But if you want to go through metamorphosis, I think the xenopus laevis, rana pipiens and rana sylvatica would be the organisms of choice.

We also hold in culture -- all of our cultures flow through. And we have currently xenopus laevis and xenopus tropicalis in culture. That's all in flow through conditions.

I would be happy to take any questions on that.

DR. ROBERTS: Are there any questions or comments on the flow through versus static issue?

Dr. Green. I believe Dr. LeBlanc had indicated previously you

wanted to -- no? Then Dr. Kloas.

Dr. Green.

DR. GREEN: I'm in full support of using a flow-through system, I think -- for the reasons you just pointed out there. Although, I had many people complain about having to do that seems like once they switched they are overall more satisfied.

And I can't find anything in the literature either except anecdotal reports that it is detrimental to the development of either xenopus laevis or rana pipiens.

One thing I did note was several people felt like, and these are big commercial suppliers of these stock sources for these frogs, said that they feel that laboratory reared and conditioned frogs actually adapt quite well to being in a flow-through system.

You wouldn't want to take wild caught frogs or tads and put them in a system. They probably wouldn't deal with it as well.

The question I had for you was what kind of water is it. Is it reconstituted R O treated water?

DR. TIETGE: No. The water we use is for the most part unmodified Lake Superior water, which is a relatively low conductivity water.

It goes through several treatment -- well, filtration steps and

UV sterilization. But beyond that, it is Lake Superior water.

DR. GREEN: It is potable, chlorinated, filtered water that goes through UV sterilization?

DR. TIETGE: It is not chlorinated.

DR. GREEN: Chloraminated?

DR. TIETGE: It is not chloraminated.

This is taken from the bottom of Lake Superior. We're located right on the lake. And we have an intake that's about a quarter of a mile, I think, from the laboratory out into the lake where there is a specialized filter system that is built into the lake.

And then the water comes in, and then there is further filtration, and then the final step is UV sterilization. There is no chemical additives in the water whatsoever.

DR. GREEN: I know this is being detailed, but if we were to try and reproduce this system or recommend that something like this be used, when you talk about filtered, do you know -- you are filtering particulates. Do you know what size -- the reason being is all the runoff that goes into the lake you want to make sure you get pathogens like --

DR. TIETGE: We have several types of filters involved in our system. First of all, in the lake itself, there is a graded, gravel

sandbag that has fairly crude, I think maybe, 25 micron, I think, is the cutoff range for that. But when it comes into the building, it goes through some five micron filters, at which point it is UV sterilized.

And then prior to introduction to the system, it goes through a more typical laboratory filter that I think is probably two and a half or five microns or in that range.

One thing I meant to mention earlier in terms of efficiency is from experience, I can tell you that these systems require much less labor. And we actually elected to conduct a static renewal study about two years ago for very specific purposes.

And after we got into it, we really regretted it because it was so much work. These systems require very low maintenance.

DR. GREEN: That's what makes me a bigger fan too, low maintenance.

DR. TIETGE: There are numerous universities and contract laboratories that use these. It isn't necessary to use our lake water supply. There is numerous examples of waters.

Of course, I think that, if you wanted to, you could modify a water with additives, salts, whatever using the appropriate technology.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: Did you perform -- not just for the performance of the animals, this is quite convincing. I agree that it would grow and so on.

But do you have any comparative study using positive controls for instance in endocrine disruption, estradiol treatment, something like this, where you can compare static renewal system with flow through and the sensitivity of both exposure regimes?

DR. TIETGE: No, because we don't do static renewal. Our work primarily is focused on thyroid axis disruption. So we've used numerous chemicals to inhibit or to stimulate metamorphosis.

But we have also run a couple of chemicals, nonaromatizable androgen, and we have also -- we did see androgenic effects. We have also run estradiol where we do see -- off the top of my head, I don't remember the concentrations of the study, but I know that we do have feminization in that case.

DR. KLOAS: Because there is always some concern about if it's the same or if you lose sometimes -- maybe you can also lose by permanent loading of humero (ph) compliments, you may lose a little bit sensitivities.

DR. TIETGE: Loss of sensitivity based on what? I'm sorry.

DR. KLOAS: In comparison to static renewal system. I can't

compare. We don't have flow through. But if you compare, some people found -- I'm aware of one or two studies in a lab with a flow through. They use same concentrations of estradiol but are getting much lower sensitivity concerning feminization of xenopus and also ranid species.

DR. TIETGE: I think I'm familiar with at least some of the work you are talking about. I'm not sure if they used -- that was a positive control. I'm not sure that they had analytical verification of that.

We can talk about that. But I'm not sure.

One of the things that also I should mention has to do with the rate of development in some of the studies that we reviewed for the white paper. The developmental rates for xenopus were very long, in our opinion.

And in our laboratory, using the flow through systems, we typically have metamorphosis well underway at seven weeks. About 50 days post fertilization. And it is usually completed within three or four days after that.

Certainly, within 56 days, we are generally done with metamorphosis at that point, unless there is a chemical effect, and then, of course, that is another story. But I'm referring to controls in

that case.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: Joe, I agree with your recommendations for a flow-through system as well as recommending the proportional diluters.

But it seems that proportional diluters scare a lot of people. It must be all those tubes and boxes or something. I was just wondering if there are alternatives that you are aware of in terms of peristaltic pump systems --

DR. TIETGE: Yeah. During the period that those were designed, pump systems weren't quite as reliable as they are today.

There are many, many reliable pump systems that can be used. And some of our newer designs do not use the proportional flow through devices. They have simple dilution or, I should say, solution cells that serve as a stock for a peristaltic pump that might have six or eight lines coming out of it or other pumps.

We have very many systems in our laboratory that represent different design ideas.

I think when you get into a more complex exposure paradigm, then the system has to be more complicated to accomplish it. But pumps work fine, actually.

DR. ROBERTS: Any other comments? Dr. Skelly.

DR. SKELLY: Just real quickly, Dr. Tietge.

We're talking about this I assume because this is going to be what EPA recommends as people go forward or requires.

I think Dr. Bradbury or somebody said before that there doesn't necessarily need to be a requirement that people that want to do experiments that are going to be evaluated and used by EPA to make judgments will use one method or another.

I thought it would be helpful if you could comment on that.

DR. TIETGE: I think in the absence of a validated protocol, one needs to adhere to a standard. And the standard is right now this most appropriate ASTM. There is the flow through recommendation. And there is the static renewal recommendation.

I think if you take a look at the two, it is more efficient to do the flow through. Because for ends that you need to utilize for your statistical design, I think you would be in a lot of trouble with a static test.

DR. ROBERTS: Dr. Skelly I think wanted to respond.

DR. SKELLY: Just to follow up. Maybe the economics of working in a government agency versus a university can be a little bit different where labor can be quite cheap in a university setting,

people getting credits or whatever, whereas equipment costs whatever it costs.

I guess what I'm wondering is will the EPA have an all flowers can bloom sort of attitude towards this? Or are they going to say flow through or nothing.

DR. STEEGER: I think one of the things we're looking to see happen is that the studies that are conducted for regulatory purposes are consistent or as consistent as they can be with our guidelines.

And although we don't have any guidelines right now for amphibian studies, we do have the A 50 guidelines for water quality standards. And we would hope that whatever studies are conducted adhere to those guidelines.

DR. BRADBURY: I think some of the sort of minimal issues in terms of data quality that we're talking about are the ASTM guidelines. They are measured concentrations.

As Dr. LeBlanc mentioned earlier today, if it is a static renewal, you probably need to do more analytical chemistry in terms of numbers of samples over time than one would have to do in a flow through test to ensure that you really know what your concentrations are. Because that's one of the other challenges of a static renewal, can be, depending on the chemical and toxicokinetics, that aspect.

So I think it is more of the, if you will, performance issues, loading, DO, ammonia, measuring the concentrations that are the real important issues to the extent that some studies would be done because people read these documents and think they would be like -- they would like to pursue some research, they will have some ideas on the kinds of quality indicators that would be important for the agency to use -- looking at -- when they look at the open literature to the extent the registrant may be performing some of these studies.

That's sort of a different venue in which some of these studies potentially could be done, if they are done, which gets at what Tom was saying in terms of the kind of guidelines the agency requires for data that's submitted as part of registration or reregistration.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: Concerning atrazine effect, obtained up to now they have been just obtained in static renewal systems. Maybe metabolism of atrazine and so on could also play a role for getting the effect.

If there is really concern about atrazine and -- I agree with several advantages. And from a logical point of view, I agree completely. I would also like to have flow through in my lab to try doing that.

But there is concern about what was done and results obtained up to now. I think to repeat this should contain both at least static renewal and maybe an advance study using the flow through. Because otherwise, we cannot rule out if there is no effect in a flow through anymore.

DR. BRADBURY: One point. I guess we would be curious to hear the panel's response in terms of ASTM loading guidelines, ammonia, D O, those kind of attributes that have to be met in terms of evaluating the quality of the study regardless if it was done flow through or static renewal.

I think the agency in putting out the document to get a response was first understanding your impressions about basic data quality parameters. How you get there. It could be static renewal. It could be flow through.

DR. ROBERTS: Dr. Delorme, then Dr. Green.

DR. DELORME: Dr. Bradbury just answered my question. He said that you had to meet the water quality standards that have been set out.

But just as another note for the panel, as somebody who does risk assessments and gets studies in, typically, when you get a study in on fish or invertebrates, they have to provide information on the

water, which includes the presence of other contaminants, be they heavy metals, pesticides or whatnot.

So the water is characterized typically at the labs that do the studies, whether it be an industry lab or a contracting company.

DR. ROBERTS: Dr. Green.

DR. GREEN: I think Dr. Kloas's point is well taken too. There may be something inherent with the static renewal system that mimics wildlife pond situations closer than the flow through. Particularly, with regards to ammonia and other things in the water at higher levels.

And some of the studies we looked at yesterday clearly had higher levels of ammonia. And that might play a role in interaction with atrazine that if we put them in flow through systems and we adhere to the guidelines, we might not see that.

If it's feasible, it seems a reasonable thing to, if you don't get results in the flow through system that are repeatable, that perhaps a static renewable system would be on a smaller scale something to look at as well.

DR. ROBERTS: Dr. Bradbury.

DR. BRADBURY: I want to get clarification.

If our question at hand is to evaluate the hypothesis that

atrazine can cause developmental effects, sort of putting on my toxicology hat, I would like to try to get a system that gives me the cleanest possible way to interpret whether or not I'm getting a toxicological signal. And then I'll get more complex as I need to.

I guess I would like to get a response to that.

DR. ROBERTS: I think Dr. Green's point is if the answer is if you get a negative response, it is possible that atrazine under conditions, under something less than clean conditions that may be more relevant, in fact, to the environment might give a positive response, you might want to check that before you stop, I guess.

I'm not speaking for Dr. Green. But that's what I think the point is.

DR. GREEN: I think that's right. Otherwise, we'll be open scientifically to the criticism that it wasn't a static and you get negative results on a flow through, and we're back to phase one again.

DR. BRADBURY: Hopefully if some of our discussions about the hill criteria hold up, we'll --

DR. ROBERTS: Dr. Richards.

DR. RICHARDS: This is partly a question, partly response.

I think maybe to step back a bit, some of the people I have heard over the last few days express that the static was more

appropriate because maybe it more mimicked a pond-type situation.

I was trying to think back. I don't know a great deal about the development of the ASTM standards, but when I think of all the species of fish that have been used in the development of those standards, how many of them might you place into the category of being species that might inhabit these mesotrophic or eutrophic sorts of ponds be exposed to relatively high ammonia levels, and that might be considered a normal environment, as would be xenopus and possibly rana pipiens too.

DR. STEEGER: Our test species are chosen again because of their ability to be raised under laboratory conditions.

There are large mouth bass and blue gill sunfish. There are other choices we get. Fathead minnows.

They can live under eutrophic conditions. But those are challenges that can impact the signal that Steve is talking about in terms of just trying to create a situation where we have removed as many of those variables as possible to have as clean a signal as possible, is what the agency uses for regulatory purposes.

Otherwise, it gets to be very difficult to sort out what the real cause of effect was.

DR. RICHARDS: In partial response, am I hearing, then, that,

yes, other organisms like fatheads and so forth that do live often times in mesotrophic or eutrophic situations seem to respond very well and repeatably in flow through conditions.

DR. ROBERTS: I guess in response I would just say that if you find yourself in the situation where you use flow through and you don't get a response, the question will be, and I think I would be sort of compelled to go back and see whether or not the reason you don't get a response has something to do with flow through versus static conditions, and then the question will come up, which is more relevant.

I don't know -- of course, until you understand the basis for the difference between static and flow through, I don't suppose you could answer that question, but we can envision the situation where you would be sort of drawn down that path.

And if you can't duplicate it in static conditions, you can just say, well, we can't duplicate it under static or flow through.

If you get it under static, you don't get it under flow through, then you are going to have to figure out why and figure out which one is more relevant.

I guess we're just saying that.

DR. BRADBURY: Can I ask one more question?

Let's say we go down a path, you do a flow through and you don't get the effect. Maybe that has something to do with the physics of a static renewal is what is important to triggering whatever event occurs.

This is just because I couldn't remember the dialogue. Would it still be the recommendation of the panel that one would try to meet ASTM standards in running that static renewal?

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I think it should be the same conditions whereas people obtained the effect.

I'm completely surprised if I can -- 25 animals and 75 fetus or something. It is hard to handle. But the effect already observed have been used in volumes of four liters or something like that.

I think it wouldn't make sense to use another static renewal system. Really in agreement with ASTM standards. I'm a dirty endocrinologist. I'm sorry. I would like to have a study repeated under the same conditions. Whatever it might make any difference.

I would like to have flow through and to see that there are results. But if you change in another way and doing static renewal under different conditions and you can't repeat again, it is the same question. If you can't repeat it in flow through, then you are again

next step and one step backwards.

DR. BRADBURY: I just have this -- I get a feeling that I'm going down a path that gets us back to some of the white paper's analysis that it was very difficult to interpret the responses from the previous studies because of concern over the ammonia levels, the feeding issues, the DO issues.

Then we're being asked to try to replicate scenarios that -- I'm trying to track the -- I feel like we've started getting into a do-loop.

DR. ROBERTS: I guess Dr. Green can respond.

We got I think some responses by at least a couple different labs. I don't know they all had real high ammonia levels. I don't know that that -- I don't know that you're going to have to really make awful conditions to get --

DR. BRADBURY: I think some guidance on, if not ASTM guidelines, where do you feel a reasonable static renewal in terms of those issues, at least bracket that.

DR. ROBERTS: Sure. Dr. Green.

DR. GREEN: I don't think our intent was to recreate the dirty water with a static renewal system. That wasn't it at all.

We have static renewal systems that house 400 and 500 adult frogs. No matter how hard we work on it, we cannot produce ASTM

clean water to that degree.

I think what is important is the dynamics of the animal sitting and reabsorbing the drug that they are absorbing and excreting for the few hours that they do it in between water changes and for the gradual increase in ammonia.

And certainly they don't have to go up to the toxic lethal levels that one of those papers, I believe, had in it, but that system alone would be something I think -- would be a justifiable attempt to reproduce the results they got in the static system.

Try to keep the water as clean as possible. But you are never going to get it as clean as you would with the flow through system. I would say change the water as they did in the static renewal system, as frequently as you need to to keep the ammonia down and all that stuff.

And it is still going to go up enough that it is only speculative that the interactions in the water chemistry dynamics will be there in a way that they are not there in the flow through system.

DR. ROBERTS: Dr. LeBlanc.

DR. LEBLANC: It just seems like we're getting into the realm of what if, what if we don't -- what if we get a negative result, what do we do.

I just don't know that we should be crossing that bridge. I recognize you need recommendations and you are looking for advice and help. But I think we should proceed with the assumption that we will replicate the results.

And if we don't, at that point we have to look at the new data as compared to the old data and make some judgments as to why perhaps we can't replicate the results. And then design some basic experiments to address those variables. Leave it at that.

DR. ROBERTS: I want to ask if there are there other comments by panel members regarding any of the topics -- this topic and the discussions we have had over the last few days, any points that are important you think that have not been made as yet but that would be important to make and introduce into the minutes.

Dr. LeBlanc.

DR. LEBLANC: Quick technical point. The experimental design that was up there, you had a positive control of estradiol. We have seen some experiments with DHT. I don't see any reason why we would be looking at DHT as a positive control.

But we are interested, I think, in some anti-androgenic effects, not necessarily due to the ability of atrazine to compete at the receptor level, but perhaps to interfere with conversion of

testosterone to DHT or just to overall cripple the synthesis of hormones.

So you might give consideration to a control for anti-androgenic properties. I guess you would have to look at an anti-androgen. And one that I have seen in the literature with frogs and I think Darcy or Sherril mentioned, it was cyproterone acetate. That would be something to consider.

DR. ROBERTS: Dr. Kloas.

DR. KLOAS: I can add three suggestions. You can use cyproterone acetate, which works as an anti-androgen, or you can use para para DDE. Also, vinclozolin would work also.

DR. ROBERTS: Dr. Gibbs.

DR. GIBBS: I still have lingering concerns that far too individuals use the found, the experimental larval populations in some of these laboratory studies.

It is not my area of expertise, but as a population geneticist, it really concerns me when three pairs are used. One onymous individual with three pairs will potentially skew an entire experiment. I think boosting numbers up into the 10s of pairs, I think it seems like a good idea to me.

DR. SKELLY: Just a point that has been touched on a little bit,

but I wanted to make sure it made it clearly into the record.

That is that it may be standard practice in toxicology, but I was struck by the kind of fluctuating use of different levels of statistical units.

In some cases, people were using individuals. In some places, people are using tanks as their units of analysis, even though all the individuals are raised in group tanks.

And in ecology, that's just totally tabu. You don't do that. If things are raised in a tank, they can influence each other. And you don't use individuals as replicates in analysis if they came out of the same tanks.

I don't pretend an SAP can change practice in an entire field. But if that is standard operating practice and if we are going to get into ecological relevance at some point and ecologists are going to be evaluating this stuff, they are going to care about that a whole lot.

That alone will eliminate a paper from peer reviewed literature in ecology.

DR. ROBERTS: Any other suggestions from panel members?

Dr. Bradbury, I think the panel has given you our best advice on this subject. Are there any final clarifications or follow-up questions?

DR. BRADBURY: No. I think it has been a very helpful discussion this afternoon and mid morning. I appreciate all the hard work and input.

DR. ROBERTS: I think as came through in our comments, I think the panel was very impressed with the job that the agency had done on the white paper and your analysis and your thoughts on how to move forward.

I think we thought in general you did a very good job with that. We had some suggestions here and there for things maybe to add and consider. But overall, we thought the agency did an excellent job on a very difficult subject area.

Before we close the meeting, I would also like to thank our public commenters. We had many of them that travelled sometimes a great distance to come and speak to us and share with us their data and their viewpoints on this subject. We always welcome different perspectives and viewpoints on the issues that we face. And we thank the public commenters.

I would also like to thank the hearty soles in the audience who have stayed with us now for a number of days through our presentations and our discussions. I like to thank you.

And of course, always, I would like to thank the SAP staff for

putting this meeting together. They assembled an outstanding panel of experts, got us all here, got us all the documents we needed and have been very supportive.

Finally, I would like to thank the panel members, a very impressive panel. Your expertise was obvious. The fact you came prepared was obvious. We have had excellent discussion. Excellent questions right from the beginning. I think you guys did a terrific job.

Do we have any other announcements before we close the session? Paul, anything you need to say?

MR. LEWIS: Just a few remarks. I want to first thank Dr. Roberts for serving as our chair for the past three days. Did an excellent job in managing the process for this meeting.

Members of the panel, it was a pleasure working with all of you. And looking forward as we work in writing our report that for the public's interest will be available in about four weeks, available on our SAP web site and also in the docket.

And I thank the members of the public for listening to our discussion and for the contributions they made as part of the deliberations that we had in the past three days.

Finally, my colleagues at EPA, both in EFED (ph) and my

colleagues with the SAP staff in working with me, pleasure working with you in getting this meeting off the ground. Thank you.

Dr. Roberts.

DR. ROBERTS: Immediately following the close of this session, I would like the panel to meet in the meeting room for a short closed session just to discuss the logistics of writing up our minutes.

As soon as we're finished here, if we could convene there for a short meeting, that would be great.

If there no other announcements or no other topics to discuss, I would like to now close this session of the FIFRA Scientific Advisory Panel.

(Thereupon, the session concluded at 6 p.m.)

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I, Frances M. Freeman, Stenotype Reporter, do hereby certify that the foregoing proceedings were reported by me in stenotypy, transcribed under my direction and are a verbatim record of the proceedings had.

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