FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

JUNE 19, 2003

DAY 3 OF 3

Located at: Crown Plaza Hotel
1489 Jefferson Davis Highway
Arlington, VA 22202

Reported by: Frances M. Freeman

1 **C O N T E N T S**

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3 Proceedings.....Page 3

1 DR. ROBERTS: Good morning. I want to take the opportunity
2 now to briefly reintroduce the panel. And let me as we have done in
3 days previously begin with Dr. LeBlanc and ask each member of the
4 panel going around the table to state their name, their affiliation and
5 their expertise.
6 Dr. LeBlanc.
7 DR. LEBLANC: Good morning. My name is Gerry LeBlanc.
8 And I'm a professor in the department of environmental and molecular
9 toxicology at North Carolina State University. And my area of
10 expertise is endocrine toxicology.
11 DR. KELLEY: I'm Darcy Kelley. I'm a professor of biological
12 sciences on the faculty for the center for environmental research and
13 conversation and a member of the Earth Institute at Columbia
14 University.
15 And my area of expertise is sexual differentiation of the
16 amphibian xenopus laevis.
17 DR. KLOAS: My name is Werner Kloas. I'm professor for
18 endocrinology at University of Berlin.
19 I'm also heading the department of inland fisheries at the
20 Leibniz Institute of Freshwater Ecology and Inland Fisheries. And
21 my expertise is endocrine disruptors acting on sexual differentiation

1 and thyroid system in amphibians.

2 DR. GREEN: My name is Sherril Green. I'm an associate

3 professor in the department of comparative medicine at Stanford

4 University. My special interest and expertise is in the care and

5 husbandry of laboratory amphibians, xenopus laevis and other

6 species.

7 DR. COATS: My name is Joel Coats. I'm in the department of

8 entomology, chair of the department, and professor of entomology and

9 toxicology at.

10 DR. ISOM: State University.

11 My expertise is environmental toxicology, environmental

12 chemistry of pesticides.

13 DR. DENVER: I'm Robert Denver. I'm from the department of

14 molecular cellular developmental biology of the University of

15 Michigan at Ann Arbor. And my expertise is developmental

16 neuroendocrinology of amphibians.

17 DR. GIBBS: My name is James Gibbs. I'm an associate

18 professor of conversation biology at the State University of New

19 York's College of Environmental Science and Forestry. And my area

20 of expertise is amphibian demography.

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

1 biology at the University of Minnesota Duluth, and I'm director of the
2 Minnesota Sea Grant College Program. My expertise is aquatic
3 ecologist and landscape ecology.
4 DR. DELORME: My name is Peter Delorme. I'm a senior
5 environmental risk assessor with the Canadian Government, Pest
6 Management Regulatory Agency. My area of expertise is aquatic
7 ecology and risk assessment methods.
8 DR. SKELLY: My name is David Skelly. I'm an associate
9 professor of ecology at Yale University. And my area of expertise is
10 population and community ecology of amphibians.
11 DR. MATSUMURA: My name is Fumio Matsumura. I'm a
12 professor of environmental toxicology. I also serve as the director of
13 our Center for Environmental Health Sciences. My area of expertise
14 are molecular toxicology. And I also study some neural effect of the
15 pesticides.
16 DR. THRALL: I'm Mary Anna Thrall. I am a professor of
17 veterinary pathology at Colorado State University. And my area of
18 expertise is veterinary clinical pathology.
19 DR. ISOM: I'm Gary Isom, professor of toxicology at Purdue
20 University. And my area of expertise is neurotoxicology.
21 DR. HEERINGA: I'm Steve Heeringa, senior research scientist

1 with the Institute for Social Research at the University of Michigan.
2 I'm a statistician. My specialization is in the design of research and
3 in specialist research relating to human and animal populations.
4 DR. ROBERTS: And I'm Steve Roberts. I'm a professor at
5 University of Florida with joint appointments in the College of
6 veterinary medicine in the College of Medicine, and serve also there
7 as director for the Center for Environmental and Human Toxicology.
8 My areas of expertise are toxicology and risk assessment.
9 Before we proceed with the public comments this morning, at
10 the close of yesterday, there was a little bit of confusion about the
11 status of some research reports that were -- from research performed
12 by Dr. Hayes while at Ecorisk.
13 Dr. Sielken has offered to perhaps clarify the situation on that.
14 I would like Dr. Sielken to do that at this point for the panel.
15 DR. SIELKEN: Thank you, Mr. Chairman, and good morning to
16 the panel.
17 Just to be as clear as possible, I did print out a copy of the
18 clarification statement that I would like to make. And this is a
19 clarification of the materials that I submitted yesterday to the record
20 and to you all. That's my Texan coming through, you all.
21 On the morning of Wednesday, yesterday, Dr. Hayes made

1 reference to his study number 99XLATZ2. At the beginning of the
2 afternoon session, I submitted four items to the EPA or SAP related to
3 my review of the statistics in Dr. Hayes' report for that study.
4 These four items were as follows: And they are numbered here.
5 I won't read the titles. But the first one was a document that was
6 prepared for a meeting with EPA in June of 2002. And that document
7 was briefly summarizing the comments that I had made in my
8 presentation to Dr. Hayes on September 19th of 2000 at Berkeley.
9 There is no change in that. I'm just clarifying that that was the
10 first thing that I gave you yesterday.
11 DR. HAYES: We didn't have a meeting in September --
12 DR. SIELKEN: That is the date that was on the front page of
13 the presentation that I gave. It was at the meeting in Berkeley,
14 whatever date it was.
15 DR. HAYES: It was January or February. It wasn't September.
16 DR. SIELKEN: I have been corrected. It was January. Boy.
17 This is what happens when you go to bed at quarter to 4 in the
18 morning.
19 It was at the one and only meeting that I attended in Berkeley
20 with Dr. Hayes.
21 The second item was the overheads that I presented at that

1 meeting, whenever it was. It expressed my professional concerns as
2 a statistician about characteristics of the study, including what Dr.
3 Hayes described as "haphazardly selecting a sample to analyze for
4 laryngeal size" instead of taking a random sample, the need to
5 explicitly provide more of the numerical data and errors I found in a
6 spot-check of the numerical accuracy of the report.
7 These overheads also contain the results of what I believe to be
8 more appropriate statistical analyses of the data on laryngeal
9 cross-sectional area. And the details of my findings are written out
10 there for you.
11 Basically, what I found was that there was not a substantial
12 reduction in laryngeal size due to atrazine. This was the same
13 conclusion that Dr. Hayes had noted in the body of his report, but did
14 not note in his summary.
15 The third thing that I handed out, which was probably pretty
16 obscure to you, was the copies of the 24 transparencies that I prepared
17 to show examples of some of the errors and inconsistencies I found in
18 Dr. Hayes' Excel spreadsheets, worksheets, and which I had
19 presented to Dr. Hayes at that meeting in Berkeley. Correct the date.
20 I just listed there some idea of what those errors and
21 inconsistencies were. I had copied the transparencies that I used to

1 identify those things and had provided those to you yesterday.

2 Now, where the confusion really probably arises is with respect

3 to Number 4. And the version of the final report signed by Nigel

4 Noriega on June 23rd, '00, was the copy that I submitted to you.

5 The first three of the items that I briefly discussed above were

6 reproduced from original copies that I had prepared and brought with

7 me to this meeting.

8 I did not think that I needed to bring my copy of the final report

9 that I reviewed. I didn't think I needed to bring those here since I

10 assumed that there would be plenty of copies here and I didn't need to

11 carry it in my luggage.

12 Therefore, I asked Ecorisk staff to provide a copy of the final

13 report for me to submit. The Ecorisk staff provided the only final

14 report that was signed. And I assumed that that was the same report

15 that I had been looking at.

16 Unfortunately, the only version of the -- and unfortunately, the

17 only version that the final report that was on the CD that was

18 provided to the SAP members.

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

20 report.

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

1 me, then that's what they did. They reproduced that signed report.
2 And that's the same report that is on your CD.
3 That's the version of the report that was readily available
4 yesterday since Dr. Hayes' statement therein, that there were no
5 abnormal, undifferentiated or intersexual gonads were observed in
6 any of the treatments or controls had been discussed over lunch.
7 So that was why that copy was just out and ready and ready to
8 go.
9 During Dr. Hayes' presentation, he had shown a figure showing
10 the distributions of laryngeal sizes in male frogs. I understood him to
11 say that this figure referred to later studies, that is, later than this
12 99XLATZ2.
13 I remember that figure since I had referred to it extensively in
14 my presentation at Berkeley.
15 Since I had given all the copies of the final report to the SAP
16 staff, I left the SAP meeting during the last public commentor's
17 comments to check that this figure was indeed in the copies of the
18 final report I had submitted to SAP.
19 To my chagrin (embarrassment), I realized that the copy of the
20 final report I had submitted could not have been the copy that I had
21 reviewed for that Berkeley meeting since the figure of interest was

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

2 I understand that after I left the meeting, Dr. Hayes stated that

3 the copy of the final report I had submitted was not the final version.

4 It seems that the signed final report that I copied and gave to

5 the panel yesterday was not the final version. In fact, there is no final

6 version. And there is only one signed and dated version. And that's

7 the one I gave you.

8 Furthermore, all other versions of the final report I could find

9 said that they were final reports. All the cover pages are the same.

10 And none were dated except for that one signed copy that I gave you.

11 The only version of the final report that contained the figure of

12 interest was an unsigned version of the final report with a handwritten

13 note on the cover page stating that, "Cathy, here is a copy of the

14 report that was sent to Ecorisk several months ago.

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

16 Here the first Cathy is Cathy Benz, who is the Ecorisk quality

17 assurance person. And the second Kathy with a K is Katherine Kim

18 who is CEO of Sokoke, Incorporated, and also Dr. Hayes' wife.

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

20 I checked this version of the final report. And it did contain the

21 figure as I recalled it. Furthermore, this version contained on Page 41

1 the erroneous correlation coefficient of minus 2.61 that I had
2 remembered commenting on, since a minus 2 is impossible.
3 Therefore, I went to Kinkos last night, and skipped dinner, and
4 had 26 copies of this version of the final report made from the CD that
5 had been provided to EPA.
6 So there was a copy of that signed version in the miscellaneous
7 materials that Syngenta had submitted to EPA.
8 Although this version contains the statement that there are no
9 abnormal, undifferentiated or intersexual gonads were observed in
10 any of the treatments animals and the figure as I recalled it and the
11 erroneous correlation coefficient that I remembered, a closer
12 examination of the figure, about 11 o'clock last night, revealed that
13 the figure labels were slightly different.
14 The dots and the figure were still the same. But the axis label
15 had changed. And there was a line in there for where the female mean
16 was.
17 I said, well, this can't be the report that I reviewed either.
18 So of the reports that were available and that Syngenta had
19 provided to EPA and they had provided only the final or what they
20 thought to be the final to the panel members, it meant that there had to
21 be another version somewhere.

1 I recall that at the time that I did my review, Keith Solomon,
2 Dr. Solomon, had received an electronic copy of the version of the
3 final report and of the Excel spreadsheet that Dr. Hayes had provided
4 and that I had reviewed.
5 Therefore, this morning, instead of waking at my office in the
6 middle of the night, I asked Dr. Solomon to print a copy of that
7 version of the final report.
8 And I will be distributing copies of that to the SAP members as
9 well as to Dr. Hayes as soon as they come off the printer downstairs.
10 This version of the report also contains the statement about no
11 abnormal, undifferentiated or intersexual gonads were observed in
12 any of the treatments or controls.
13 Let me make a personal apology for my unintentional mistake
14 of submitting the only signed version of the final report -- or the only
15 version of the final report that was signed by the study director to
16 SAP, since, apparently, from Dr. Hayes' statement, it is not the final
17 report.
18 I do apologize for any confusion. I have made in that box 26
19 copies of the version that Kathy sent to Cathy. And as soon as they
20 come off the printer downstairs, I will give you the version that I
21 received prior to the Berkeley meeting and that I reviewed at the

1 Berkeley meeting.

2 Just for your own edification, there is a not a great deal of

3 difference in these reports, but there are some differences. You are

4 welcome to review them at your pleasure and make your own

5 decisions.

6 DR. ROBERTS: Thank you, Dr. Sielken, and thank you for

7 your efforts to clarify that.

8 Dr. Hayes, I see you raising your hand.

9 DR. HAYES: I'll be brief.

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

11 Let me just explain that what the panel is trying to do is obtain

12 copies of reports of your earlier work with Ecorisk. And we're trying

13 to get what represents the best -- a best representation of that

14 research. And apparently, there are multiple versions going around.

15 And if you could assist us by identifying which version you consider

16 to be the most accurate representation of the research, that would be

17 very helpful.

18 DR. HAYES: Yes. In terms of the gonadal abnormalities, I've

19 already addressed that. I started out showing how we discovered the

20 abnormalities. It was during that experiment where we were trying to

21 measure steroids in animals. And we were sexing the animals without

1 buens (ph). And that's when it was discovered. And I reported that to
2 Ecorisk, not to Syngenta, to Ecorisk in November of 2000.
3 And that's when we reanalyzed the data. So that is correct. I
4 already addressed that.
5 The other thing I wanted to say, I think Bob Sielken has already
6 said better than I can, you see the sort of level of confusion and the
7 round and round they go about, there is never a final report because
8 there is always we need to do this, you do that. And things never get
9 signed.
10 That's exactly why I left the panel. We started reporting
11 adverse effects as early as 1999. And because there was never a final
12 report and things never got signed on and they would ask to change
13 this and that and this version and that -- I think you see exactly why I
14 left the panel.
15 So I won't comment anymore. It was exactly what was just
16 described to you.
17 DR. ROBERTS: Okay. But we will now have several versions
18 of that report. And if to the extent to which -- before we close public
19 comment, if you could look at those and identify which one you think
20 is the most accurate --
21 DR. HAYES: I can identify the last final report.

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

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

6 DR. ROBERTS: Dr. Thrall has a question.

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

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

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

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

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

1 appreciate your efforts to clarify this for the panel.

2 Let us proceed, then, with public comments. And as I have done

3 at the opening of each of the public comment sessions that we have

4 had, let me remind the public commentors and the panel that we are

5 here focused on some specific issues related to potential

6 developmental effects of atrazine on amphibians.

7 So we're focused on scientific issues because we are the

8 Scientific Advisory Panel. We're not here to consider policy issues,

9 legal issues and so forth.

10 So we could confine comments and our discussion to the

11 scientific issues, I think that will be best. Let me see. Our first

12 commentor that we have signed up for this morning is Dr. Jennifer

13 Sass on behalf of Natural Resource Defense Council.

14 And she will be followed by Dr. Diana Post, just as a heads up,

15 Dr. Post. She may not be here.

16 DR. SASS: Should I start? I was just going to introduce myself

17 while this is --

18 DR. ROBERTS: Good morning, welcome.

19 DR. SASS: Thank you. I'm Jennifer Sass. I'm a Ph.D. with the

20 Public Health Program of the Natural Resources Defense Council. It

21 is an environmental nonprofit group. We're an advocacy group for

1 public health here in the U.S.

2 And we have been following atrazine very closely. In fact, it is

3 because of a consent decree with NRDC that EPA is reviewing

4 atrazine.

5 I want to first thank you all for giving your time to this

6 extremely important issue. I know that you are all extremely busy.

7 And I know that this is a tremendous amount of time, both in

8 attending the meeting and in previewing prior to coming to the

9 meeting. And I want you to know that we really appreciate your

10 attention to this important matter.

11 I also want to thank the EPA for preparing the white paper. As

12 you know, most of the studies, in fact, all of the Syngenta submitted

13 studies were not published and, therefore, not available for peer

14 review or public scrutiny. So we rely on the EPA scientists to review

15 boxes and boxes of data that we would never even want to look at

16 were it publicly available because of the time and the energy

17 required.

18 So I do really appreciate the excellent review that the EPA has

19 done in the white paper. Thank you.

20 I'm going to make my comments very brief. First of all, I want

21 to point out that the charge questions to the EPA in the white paper I

1 thought were unusually, unprecedently vague and ambiguous.

2 And that's possibly because the charge questions were in an

3 unprecedented interest of the White House -- actually passed through

4 the White House for review prior to getting put into the white paper.

5 And I don't think they reflect the white paper in terms of the scientific

6 assessment.

7 So I would like to start out by redirecting a little bit. What I

8 think that the key questions are that the EPA really needs to address

9 in its legal obligations with the NRDC in reviewing atrazine -- in fact,

10 the reason that this SAP is here today is because NRDC negotiated

11 with EPA to send this data to a scientific advisory panel for review

12 and since at the time the EPA was not going to look at the data.

13 And the key questions in that agreement were, does atrazine

14 threaten wildlife amphibian populations. And does atrazine act as an

15 endocrine disrupter.

16 Those questions are not in the charge questions anywhere. But

17 those are the key questions that the EPA needs to answer to fulfill its

18 legal obligations and to complete its assessments.

19 And those really are the questions that this scientific advisory

20 panel can provide input and advice on because of the expertise that is

21 here around this table.

1 I also want to point out that those are the key questions that are
2 data driven. In other words, those are the questions that are going to
3 be answered by a review of the science of the data that is available
4 and the full body of literature. And from the answers to those
5 questions, the next question will be, can atrazine be used safely.
6 At that point, the question that follows, which is not a question
7 for the SAP, is at what concentration or dose or under what conditions
8 can atrazine be considered to be safe. That's presuming that it can be
9 used safely. Presuming there is some answer to that middle question.
10 At what concentrations or dose can it be used safe. In other
11 words, from a policy point of view, we talk about a baseline or a no
12 effect level or NOEL or reference dose and for human consumption.
13 Those are policy driven questions. Those are not appropriate
14 questions for a scientific advisory panel. And in that light, I don't
15 think that the white paper should be criticizing or critiquing or
16 undermining the published literature because it doesn't provide that
17 kind of an answer.
18 Those aren't answers that can be derived directly from the kinds
19 of studies that the published literature were designed to answer, to
20 tackle all kinds of questions.
21 So I would like to focus on the data driven questions and point

1 out that it is not a fair critique to say that a study doesn't come up
2 with a dose response, therefore, the study somehow is insufficient to
3 include in the review.
4 The NRDC asks that the scientific advisory panel provide a fair
5 and complete review of the available literature with greatest
6 consideration given to those data from robust and well designed
7 studies published in the peer reviewed and scientific literature.
8 We ask that the amphibian data be evaluated as to its
9 consistency with the whole body of available data, including
10 mammalian, aquatic and mechanistic studies.
11 We ask that the scientific advisory panel provide its expert
12 scientific opinion as to the effect of atrazine on amphibian health and
13 to make recommendations as to whether or not it can be used in a
14 manner that will not harm amphibian populations.
15 We believe based on the published literature and reviews
16 therein that there is compelling evidence that atrazine is a multi-site,
17 multi-species endocrine disrupter.
18 Despite variability between study designs, there are data for
19 mutually consistent studies with sufficient statistical power published
20 in the peer reviewed literature demonstrating that atrazine acts in
21 mammals, amphibians and aquatic organisms through at least two

1 mechanisms of action to disrupt hormonal pathways critical to
2 reproductive development and function.
3 These findings compel a determination that atrazine is an
4 endocrine disrupter on wildlife and that its use should be banned or
5 severely restricted. That's the position of the NRDC.
6 I've handed out my comments yesterday or the day before, how
7 ever long we have been sitting here, on paper looking something like
8 this. I handed out 25 copies. I hope you have them.
9 It provides more of a comprehensive literature review. In the
10 back all references are there for everything that I'm talking about
11 here. What I'm going to do is very quickly breeze through that. I'm
12 not going to go into any detail on any study. They are not my studies.
13 I can't defend or answer to them in any detail.
14 I just want to put the question of amphibian risk in the larger
15 context of the published literature on atrazine.
16 What I want to point out in this graph, which, again, you have
17 in the hand out is that there are a number of studies in rats, and there
18 are a number of different studies that show tumor formation. But
19 there are strained specific responses or rather differences between
20 how strains of rats respond to atrazine.
21 So for instance, in one single study, a full litter resorption was

1 seen at low doses in the F344 strain, but not in the Long Evans and
2 the Sprague Dawley strain, for example. And that was seen at 50
3 milligrams per kilogram when treated through gestational day 6 to 10.
4 There has been tumors seen in some strains of rats and not in
5 other strains of rats and at different doses.
6 And those tumors have been reproductive organ associated
7 tumors such as mammary and different reproductive organs.
8 There has been a suppression of luteinizing hormone and
9 prolactin seen in Long Evans rats, but Sprague Dawley rats did not
10 respond the same way.
11 Wistar rats, both males and females, showed delayed puberty.
12 Females were less sensitive. They showed effects at 50 milligrams
13 per kilogram when treated during the period of critical development
14 of those organs, whereas males showed effects at 12.5. Much less.
15 I want to point out that I think that the published studies
16 demonstrate taken together that atrazine acts as an endocrine
17 disrupter in rats, but the different strains respond differently.
18 Strains differ in their response to different concentrations and
19 also with different measured endpoints. This does not represent
20 disagreement in the published literature, but, rather, demonstrates the
21 complex action of atrazine like all endocrine disruptors on the

1 complex web of hormonal regulation. Don't think of these pathways as
2 linear.
3 All results summarized below in this table are of statistical
4 significance. I only picked what the authors themselves picked as
5 their conclusions or results.
6 I think that in addition there are some multiple mechanisms that
7 have been demonstrated. I don't think any of these have been
8 demonstrated in many studies, but they have been demonstrated at
9 least to be indicative or suggestive of wider implications in the
10 luteinizing hormone and prolactin levels.
11 There has been some studies in whole animals. These have
12 been done by EPA scientists. And in fact, it's some of these studies
13 that the EPA is now using to set a no effect level for atrazine.
14 As well there has been some demonstration of aromatase
15 activity. You have heard about that ad nauseam in this meeting.
16 I want to point out, though, that the Sanderson study that was
17 referred to, the first publication actually had one of the Ecorisk
18 people on that as authorship, John Giesy. And then later in the
19 subsequent follow-up publication, his name was removed -- or he did
20 not participate in the second follow-up study.
21 But he seemed to be in agreement with those findings, at least

1 the first time around. And there have been follow-up studies. So
2 again, I don't think that that data is tight, but I think it is indicative.
3 Atrazine disrupts hormonal pathways in multiple species. In
4 rats, there is evidence that prostatitis has resulted in the suckling rat
5 pups when the mothers were treated with atrazine.
6 These are interesting studies because the atrazine does not seem
7 to have come through the milk to the pup. It actually affected the
8 mother, the dam, and then the pups were subsequently affected by
9 alterations in the dam hormonal responses to atrazine. And this was
10 by EPA scientists.
11 There has also been demonstration of reduced testosterone,
12 reduced sperm motility and the delayed puberty in males and females
13 in the Wistar rats. Again, this was in the EPA study.
14 In pigs, there is one. Delayed estrus after oral feeding of
15 atrazine-laced feed.
16 And in alligators, you have already heard, there is some
17 induced aromatase activity.
18 There is one paper I found in tiger salamanders that I thought
19 was interesting because it showed that the salamanders were
20 responding differently at different concentrations, that at lower
21 concentrations development was delayed, but size and weight weren't

1 affected, whereas at the higher concentrations development
2 progressed normally, but size and weight were reduced.
3 Again, I don't think that this represents disagreement. I think it
4 accurately represents the complex reactions of biological systems to
5 hormonal disruptors.
6 I think that the published literature is consistent in
7 demonstrating that atrazine may disrupt hormonal pathways resulting
8 in disruption of reproductive hormones and reproductive cycles.
9 While the Ecorisk people were digging up data last night, I was
10 actually on the phone late last night with someone named Shana Swan
11 (ph) who has just published yesterday in EHP on line a new study that
12 where she collected, her group collected semen and urine samples
13 from fertile men, about 200 each from Minnesota and Missouri, and
14 shown that the risk of poor semen quality was elevated with several
15 different pesticides. But with atrazine it was elevated 12 fold. And
16 those are very statistically significant.
17 The populations are relatively small, but then again, finding a
18 needle in a haystack. If this is able to be seen in such a small
19 population, I think it is worth a follow-up. And so does she. This
20 data will be followed up. But as of today, this is, I think, consistent
21 with the body of literature that we're reviewing.

1 In frogs, there has been demonstrations in multiple species both
2 in the wild and under controlled laboratory conditions.
3 The xenopus laevis, of course, is what the lab is addressing in
4 large part. Also, in rana pipiens, lab and field work. Also, in bufo
5 marinus, in field work.
6 The interesting thing about the bufo marinus work, and again, it
7 is not publicly available, so I have not been able to review that, any
8 published studies in any way, but my understanding from discussions
9 with the author is that there is a built-in concentration gradient
10 because of the frogs closer to the cane fields had more effects.
11 In other words, more females, skin coloration, than the frogs
12 living farther away from the field. But obviously, I'm not the one to
13 discuss that work.
14 I think that the ecological risks from atrazine are unacceptably
15 high and that there is no relief in site as the EPA assessment now
16 stands.
17 And I will quote from EPA environmental scientists, the risk
18 quotients exceeded the levels of concern for chronic effects on
19 mammals, birds, fish, aquatic invertebrates and non-target plants.
20 The risks are possible at maximum and in some cases typical use
21 rates.

1 So the EPA ecological assessors are extremely aware that the
2 atrazine at current use rates under current use patterns is posing an
3 unacceptably high threat to wildlife populations. And some of these
4 wild populations include endangered species.
5 There are concerns, again, I'm quoting from the EPA scientists,
6 for adverse toxicological effects on freshwater and estuarine plants
7 and their communities as well as indirect adverse effects on aquatic
8 invertebrates and fish populations at monitored atrazine levels in
9 surface waters.
10 So based on real readings of real atrazine in the real world,
11 there is real cause for concern according to the EPA ecological
12 scientists.
13 The ongoing use of atrazine jeopardizes endangered species and
14 their critical habitats. The exposure of aquatic communities to
15 atrazine levels at 10 to 20 part per billion, this is based on an EPA
16 assessment, can result in community level and population level
17 effects.
18 This is significantly below the EPA's current proposal in its
19 current assessment, which is to allow a seasonal average or 90 day
20 average up to 37.5 part per billion before any action is triggered.
21 Up until 37.5 part per billion, the registrant, Syngenta, will

1 voluntarily do more intensive monitoring.

2 So other than monitoring, there will be no change in the use or

3 use rate or use patterns of atrazine unless the 37.5 part per billion

4 trigger level is exceeded for a 90 day average.

5 The U.S. Fish and Wildlife submitted comments on this. They

6 claim that EPA's assessment underestimates the ecological impacts of

7 atrazine in part because it does not consider sublethal effects on

8 reproductive ability.

9 So to end and to thank you for your time, I want to point out

10 that there are some very serious and more scientific questions that the

11 EPA needs to address. And those questions are, does atrazine

12 threaten wild amphibian populations and does atrazine act as an

13 endocrine disrupter in wildlife populations.

14 These are data driven questions. And if the answer is yes, then

15 the onus falls on the EPA to answer the policy questions of what

16 doses and under what conditions atrazine can or cannot be used

17 safely.

18 Thank you for your time.

19 DR. ROBERTS: Thank you, Dr. Sass.

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

21 you.

1 Dr. Matsumura.

2 DR. MATSUMURA: Regarding this new report on the human

3 sperms, did those people really measure the level of the atrazine in

4 sperms?

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

6 I spoke with the author last night and have read some of the

7 various different reports of it. And my understanding is they

8 measured in the fluid levels.

9 Atrazine was one of a small handful of chemicals that came to

10 light as associated with this. Alachlor and diazonal metabolites were

11 the others.

12 DR. MATSUMURA: Thank you.

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

14 Sass.

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

16 Dr. Post has requested time to speak. I don't know whether Dr.

17 Post is here this morning yet. If not, we can move to the next person

18 and then come back to her.

19 I have Dr. Stephen Sheffield listed as requesting the

20 opportunity for public comment. Is Dr. Sheffield here?

21 Come forth, please, and identify yourself.

1 DR. SHEFFIELD: Thank you, Mr. Chair. My name is Steve
2 Sheffield. And first of all, I greatly appreciate the opportunity to
3 address the SAP today.
4 In my mind, this is a very important issue. I would echo the
5 comments of the previous speaker in acknowledging the amount of
6 time and your willingness to serve on this panel. It is a very
7 important issue. And I greatly appreciate all the efforts you are going
8 to put into this over the next couple of days and the efforts you
9 already put into it.
10 I should also indicate that I am an affiliate professor in the
11 department of environmental science and policy at George Mason
12 University, and that I'm actually providing these comments to the SAP
13 as a professional wildlife toxicologist and private citizen, and that
14 these comments shouldn't be construed as an official position of the
15 university.
16 I should probably spend just a second giving you information
17 on my pertinent background and experience. I have a Ph.D. in
18 environmental toxicology from Oklahoma State University. I was on
19 the faculty of environmental toxicology at Clemson University for
20 four years. And I have been at George Mason for four years.
21 I have experience with both laboratory and field experiments

1 examining exposure and possible effects of pesticides on amphibians,
2 including both frogs, rana species, hyla species and xenopus as well
3 as salamanders, ambystoma species, and have coauthored a book
4 chapter on multiple chemical stressors and amphibians that will be
5 published by SETAC press this summer.
6 I have been following with great interest the atrazine amphibian
7 issue as it has unfolded over the last several years. As a result, I was
8 wanting to share with you my perspective on the subject. I will
9 include some thoughts on atrazine in general, some comments on the
10 studies highlighted in the white paper, some comments based on the
11 peer reviewed literature and some recommendations and thoughts on
12 the subject.
13 As a wildlife toxicologist, one of the things that catches my
14 attention is the high volume pesticides. That's not to say that all of
15 them are bad. I'm just saying that when I go about looking at things,
16 looking at exposure and effects of pesticides on wildlife species, it
17 the high volume ones that generally catch my attention.
18 In the United States, from the estimate of the literature and
19 other sources I found, are roughly 75 to 150 million pounds of active
20 ingredient annually over 40 years on up to 100 million acres of
21 atrazine is applied or has been applied. It is ubiquitous in the

1 environment. It's found in all environmental media, including surface
2 water, ground water, soil, sediment, air, including fog, and biota and
3 it's atmospherically transported.
4 I think that this is an incredible volume of atrazine use and it's
5 very difficult to comprehend that sheer volume of amount of use and
6 the amount in the environment that I kind of liken it to the idea of
7 trying to comprehend how much 100 million dollars is. I know I
8 certainly can't do that. I have a hard time comprehending that
9 number. That's why I'm saying that this is a very hard number to
10 comprehend because it is so high.
11 Therefore, as Dr. Hayes made mention yesterday, control sites
12 that are free of atrazine contamination are most difficult to find.
13 Further, peak application coincides closely with peak
14 amphibian reproductive seasons. I see this as a scenario for potential
15 trouble for amphibians, not just from atrazine, but from the various
16 chemical mixture in which they are exposed.
17 Dr. Hayes' presentation yesterday, he had several different
18 slides showing atrazine levels found in the environment. I don't need
19 to repeat that. The levels that he has looked at and that some others
20 have looked at are environmentally relevant, which is important.
21 I like to tend to discount some of the ones that look at really

1 high unrealistic levels and look at the ones that are just -- focus on
2 the ones that are just environmentally relevant.
3 One of the things that I like to focus on a lot is exposure routes.
4 And as far as exposure routes, I see amphibians getting exposed
5 through oral, dermal and inhalation routes for atrazine. I don't know
6 if there is any data on maternal deposition into eggs or not. But I
7 imagine that that is possible. It does happen for other compounds.
8 It also needs to be considered the persistence of atrazine in the
9 environment. The estimates range very widely. But it's generally
10 relatively persistent. In aquatic systems, it can vary quite a bit.
11 In some of the experimental microcosms and mesocosms it does
12 not have a very long persistence. But in some natural farm ponds and
13 natural lakes it can persist almost a year. That's significant.
14 In terrestrial systems, one citation I found recently, the Talbert
15 and Fletchall paper, 1964, atrazine persisted for 17 months in the soil
16 at a two-pound active ingredient per acre application rate.
17 Now I'm going to move to a couple specific areas that I'm
18 concerned with. One is bioaccumulation. At least one person so far
19 in this panel -- in this proceedings has mentioned bioaccumulation.
20 The study by Allran and Karasov of 2000 found levels of
21 atrazine in tadpoles that were six times higher than the concentration

1 in the test water.

2 That may or may not be a significant finding, but it raises a flag

3 with me. It could possibly result in more continuous exposure of the

4 target organs.

5 And amphibians are the only vertebrate taxa that I'm aware of

6 that also accumulate organophosphate insecticides.

7 Another finding that could have a bearing on amphibians, given

8 the suggestion that atrazine effects may potentiate or even synergize

9 organophosphate effects.

10 As far as possible fitness effects of atrazine on amphibians,

11 there is a couple different studies that have indicated possible fitness

12 effects. And I'm very concerned about that as well.

13 The Allran and Karasov 2001 paper used exposure rates of 0 to

14 20 micrograms per letter. And frogs at the highest level stopped

15 eating immediately upon introduction of atrazine and did not eat

16 during the entire 14 day experiment, an anorexic like effect that could

17 have implications for fitness in amphibians.

18 The Brown-Sullivan and Spence 2003 paper, which was a study

19 that looked at atrazine and nitrate in combination, this particular

20 study found that at 40 micrograms per letter of atrazine and 37

21 milligrams per litter of nitrate using an additive model, the snout vent

1 length at metamorphosis significantly decreased. They saw reduced
2 growth and delayed metamorphosis.
3 This possible selective disadvantage for these frogs could
4 result in fitness effects.
5 And finally, the Diana, et al., 2000 paper that examined effects
6 of atrazine on amphibian growth and survival in artificial aquatic
7 communities, they looked at hyla versicolor, which are the gray tree
8 frogs, and atrazine concentrations of 0, 20, 200 and 2000 micrograms
9 per liter.
10 They found frogs from two high dose groups were five percent
11 shorter and 10 percent lower body mass at metamorphosis than those
12 of the control and low atrazine groups.
13 Decrease in amphibian length and weight at metamorphosis can
14 indicate a reduction in fitness in wild populations of anurans exposed
15 to atrazine at these levels, although, I do admit that these levels, the
16 200 to 2000 micrograms per liter are not common, usually, except
17 during the peak times when the runoff accumulates.
18 Now, moving to the studies that have been performed and
19 published by Hayes, et al., first, I have to say that I was greatly
20 impressed with the presentation given by Dr. Hayes yesterday. He
21 was very thorough in scope and covered the contentious areas to my

1 satisfaction.

2 Overall, I would characterize these studies as very carefully

3 conducted and analyzed, statistical sound and believe that the

4 conclusions reached were reasonable given the data.

5 Do I find fault with some of his methodology? Yes. But not

6 enough to discount the findings of the study.

7 Regarding the methodology, there is a few things that I would

8 do differently. As an example, I would use glass containers instead of

9 plastic. That's what I have used in any previous studies.

10 I would also use an atrazine formulation instead of technical.

11 That's an personal choice of mine. Just as examples.

12 I don't understand the criticisms of their work because they

13 suggested a mode of action for the effects seen or for failing to find

14 an acceptable dose response curve.

15 Although they speculate on a mode of action of a particular

16 fact, that's a normal part of a discussion section of any peer reviewed

17 scientific publication.

18 The high incidence of males with gonadal abnormalities,

19 whether it be testicular oocytes, intersex or hermaphroditic, how ever

20 you want to say it, is of great concern to me. Particularly, if it's

21 shown that these individuals are not fertile or otherwise

1 reproductively impaired, which has not yet been looked at.

2 From what I heard from the group on Tuesday, the intersex

3 individuals do not get that way by themselves. It apparently takes

4 exposure to an endocrine disrupting compound during gonadal

5 development to make this happen.

6 In regard to the registrant studies, there was a relatively large

7 number of studies that were funded by the registrant over the past

8 year or two. Just as a personal comment or a personal observation, it

9 was apparent to me that the studies were highly reactionary in nature.

10 And reactionary not only to the results of Dr. Hayes, but also for

11 inclusion in the atrazine IRED document.

12 Therefore, I see that they are put together and conducted

13 rapidly. They contain many design and other flaws that limited their

14 use and rendered them largely uninformative.

15 Further, only one of these studies is peer reviewed, the Carr, et

16 al., paper. And I really hope that I get the ultra rapid turnaround this

17 paper was afforded when I submit my next paper to Environmental

18 Toxicology and Chemistry.

19 As far as field studies, Dr. Hayes beat me to the punch on this

20 one, but I'm going to expand on his comments from yesterday.

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

1 completely written off as a study with nonsignificant data. However,
2 a point that seems to be overlooked on their work is the fact that
3 although they found significant correlation between PCB and PCDFs
4 and sex ratio reversal, they state that there was an association
5 approaching significance, the P value of 0.07 between the detection of
6 atrazine and intersex individuals.
7 The failure of this study to find a significant correlation for
8 this was pointed out in subsequent studies on this topic, including in
9 the Carr, et al., 2003 paper. However, I have to point out that I'm
10 very comfortable in assigning a significance level of P equal of 0.1
11 for field studies such as this due to the large inherent variability and
12 my willingness to accept a slightly higher error in these cases.
13 In fact, this is a common statistical practice for field studies,
14 including the set of field studies conducted at the EPA Corvallis
15 mesocosm facility over the past 10 years, of which I have peer
16 reviewed many of them for journals.
17 Therefore, I would ask the panel to consider the use of P equal
18 0.1 significance level in field studies when appropriate.
19 I'm not saying it's always appropriate. I'm saying that it's very
20 commonly used in field studies because the variability is so high that
21 you are willing to accept a little bit more error to try to tease out what

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

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

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

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

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

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

1 collected at most of their contaminated study sites.

2 There has been one mention so far in proceedings about

3 immunotoxicology. I'm very interested in that area. The Christin, et

4 al, paper, 2003, that was published in the most recent issue of ET&C,

5 Environmental Toxicology and Chemistry, exposed juvenile rana

6 pipiens for 21 days to a mixture of six pesticides, including atrazine,

7 and then challenged with a parasitic nematode.

8 They found that pesticide mixtures caused diminished

9 phagocytosis and splenocyte numbers, thereby suggesting a

10 compromised immune system in these frogs.

11 I have three other considerations that I want to mention. One is

12 an interaction consideration. Both potentiation and synergy. I

13 alluded to this study earlier, the Belden and Lydy study, 2000.

14 Exposure to nontoxic concentrations of atrazine cause potentiated

15 toxicity of OPs, of organophosphate insecticides in amphibians.

16 In a previous study by Lydy in 1997 showed the toxicity of

17 atrazine and organophosphates was synergistic to invertebrates. The

18 ubiquity of atrazine in the environment makes me wonder how much

19 additional damage of the OPs are causing amphibians due to an

20 interaction such as this.

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

1 alachlor mixture that was more toxic than either of the two
2 compounds separately.
3 As far as formulation, this is a personal bias of mine, but I
4 would submit that there is no such thing as technical grade atrazine in
5 the real word.
6 The real world, which is the crops and other plants in which
7 atrazine is applied, receives various formulations of atrazine. I
8 believe that it should be formulations of atrazine that we should be
9 examining and not technical grade.
10 I'm fully aware of the arguments for using technical grade
11 pesticides in experiments, but in my mind, that has little practical use
12 if it's not the exact chemical in which the organisms are being
13 exposed in the field.
14 And my other area of consideration is atrazine metabolites and
15 degradates. These compounds such as hydroxyatrazine and
16 de-ethylatrazine and others should not be ignored in any amphibian
17 studies. These compounds are also ubiquitous in the environment and
18 may be exerting some effect.
19 So the way I see it, the major studies that need to be done, are
20 intersex frogs fertile? To me, that's the Number 1 thing from the two
21 days and part of a third day that I have seen so far. That's the

1 question I think needs to be addressed immediately. Are these
2 intersexed frogs fertile.
3 If yes, is there a problem. If no, you have identified a big
4 problem. And then where do we go from there.
5 Number 2, as I alluded to already, the HPI axis in amphibians is
6 something that is in bad need of being examined.
7 Number 3, I strongly suggest including a salamander species in
8 the testing that will be done.
9 A study of mine that will be published later this year found that
10 salamanders are on the order of two to four times more sensitive to an
11 organophosphate insecticide than three species of anuran frogs
12 including xenopus.
13 In this study, I used the marbled salamander, ambystoma
14 opacum, and found it to be an ideal test species for studying possible
15 effects of contaminants. It is an autumn breeder with relatively large
16 clutch sizes, larvae that are relatively easy to raise in the lab and eggs
17 whose development can be started on demand.
18 Number 4, the use of xenopus as a primary test species. I would
19 point out to the panel that there are inherent strengths and weaknesses
20 to going this route.
21 The strengths are well-known. But in addition to being a

1 non-native species and only related to our native species at the order
2 level, there is an important life history difference between xenopus
3 and native anurans. The adult xenopus are 100 percent aquatic,
4 whereas native anurans can spend significant time on land. Thus,
5 differential exposures to atrazine can occur.
6 Native species leave the water and feed on terrestrial pray
7 items, move through sprayed vegetation and can pick up both dermal
8 and inhalation exposure that could be more significant or at least
9 different than in water.
10 For example, a frog moving through a freshly sprayed field
11 could be exposed to atrazine through oral, dermal and inhalation
12 routes where the atrazine would not be diluted as it would be in water.
13 This is the major point. These two differences can make
14 extrapolation between xenopus and native anurans less reliable.
15 Number 5 is the tolerance resistance question. I noticed with
16 interest that this has been touched on already in these deliberations.
17 It is important to address this issue because this might be contributing
18 what is apparently being seen in the field, the populations of frogs in
19 heavy atrazine use areas are still there.
20 In areas where heavy atrazine use has gone on for 40 years,
21 such as some of the areas we have heard about already, that is more

1 than enough time to have had enough generations in which to evolve
2 genetic resistance.
3 Other vertebrates have done it. Mainly, muroid rodents and
4 gambusia, mosquito fish. But I submit that the tolerance resistance
5 question is very important to this. Particularly, in looking at why all
6 these frogs are still there after 40 years.
7 And Number 6, population level effects. Certainly, this could
8 be argued to be the ultimate question to examine. However, I firmly
9 believe it may take many years to possibly see an effect at this level.
10 If some males are losing reproductive function, other males
11 may hop right in and be more than happy to increase their
12 contribution to the gene pool. If fertility is negatively impacted in
13 intersex individuals, intersex males die and are lost to the gene pool
14 without contributing.
15 As the frequency of intersex individuals increases in the
16 population, the effective population size should decrease as fewer
17 individuals are contributing to the subsequent generations.
18 Also, as frogs die out of a particular area, other frogs may come
19 in and colonize, recolonize the area, making it likely very difficult to
20 detect a population crash.
21 So if this scenario could be maintained for a relatively long

1 period of time, a population level effect may take many, many years
2 to be detected.
3 My final thoughts. I have a couple final thoughts here and I'll
4 end. The static renewal verse continuous flow-through tests. I have
5 modified this -- I've added this since the deliberations have started.
6 I strongly agree with Dr. Hayes and others on this. I believe for
7 these tests it is imperative to mimic as closely as possible the natural
8 conditions of the test organisms.
9 Native anurans and xenopus tend to be found in pools, ponds,
10 vernal pools and ephemeral standing water such as puddles and
11 ditches, not flowing waters like streams and rivers. And if they are
12 found in streams, which I have found some in streams, they are likely
13 found mainly in the intermittent pools within streams and not in the
14 moving water.
15 Also, cost is a major factor in this. As being a researcher at a
16 university, static renewal is relatively inexpensive and continuous
17 flow-through is so expensive as to be prohibitive to most researchers.
18 I do agree that the flow-through systems successfully deal with
19 the water quality issue, but it's probably not worth the trade-off.
20 Second, I'm not going to spend any time talking about this other
21 than just mentioning it because it is getting away from things a bit.

1 But it is proposing that the EPA consider some sort of action the
2 auspices of the Clean Water Act such as the National Water Quality
3 Criteria for Amphibians.
4 Something like this was proposed previously in the 1990s, but
5 no water quality criteria exists for amphibians in the United States.
6 And it's automatically assumed that criteria for fish and human health
7 are adequate to protect all aquatic species, when, in fact, this
8 assumption has yet to be tested.
9 Finally, regarding the white paper and the charge to the panel.
10 I was impressed with all the work done by Dr. Tom Steeger and his
11 colleagues for this white paper. And I commend Tom and his
12 colleagues for their effort.
13 However, when I got to the eight questions at the back of the
14 white paper, that was a different story. To say that I was sorely
15 disappointed with them is an understatement.
16 In my opinion, they were poorly written and off base from
17 where I think this SAP should be headed. I say this in part because
18 they failed to address one of the most fundamental questions one
19 could ask regarding atrazine and amphibians. Is it an endocrine
20 disrupter in amphibians.
21 Why is there no question that states, does the panel think, given

1 the data available from the 17 studies, atrazine is causing some level
2 of endocrine disruption in amphibian populations. And does this
3 effect on the endocrine system have the potential to translate into
4 population level effects.
5 That concludes my comments. Thank you very much. And I
6 wish you the best of luck in the next two days in your deliberations.
7 DR. ROBERTS: Thank you, Dr. Sheffield.
8 Are there questions from the panel? Dr. Green, then Dr. Thrall.
9 DR. GREEN: I have two short questions. You refer to the
10 paper by Belden and Lydy of 2000, exposure to nontoxic
11 concentrations of atrazine cause potentiated toxicity of OPs in
12 amphibians.
13 Do you recall just ballpark what these nontoxic concentrations
14 of atrazine were?
15 DR. SHEFFIELD: I don't, but I believe I have -- no, I don't
16 have the paper with me. I don't know what the compounds were. But
17 I know that the two OPs tested were chlorpyrifos and diazinon. Those
18 effects were potentiated with the atrazine exposure.
19 DR. GREEN: The second question I have is could you clarify
20 the difference between technical grade atrazine and other
21 formulations of atrazine?

1 DR. SHEFFIELD: Yes, absolutely.

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

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

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

13 For example, diazinon, a compound that I have used quite a bit.
14 50 percent, an emulsifiable concentrate of 50 percent is used. And
15 the other 50 percent is inert ingredients. So they will put other
16 compounds in there to dissolve the diazinon and other things needed
17 to make that formulation go on that particular crop or whatever
18 application is being used.

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

1 in the real world. And For atrazine, there is many different kinds of
2 formulations.
3 I don't know exactly what the active ingredient percent is in
4 these formulations. Offhand, I don't know what it is. But it is some
5 percentage. So what there is, there's other compounds, inert
6 compounds in with the atrazine.
7 And they may have an effect on their own. Some of the
8 compounds in with diazinon that's mixed in as an emulsifiable
9 concentrate are known teratogens and other things like that.
10 So you are moving away from getting a look at a pure
11 compound, but you're looking at something that is environmentally
12 realistic because that's what the amphibians are getting exposed to.
13 Does that help?
14 DR. ROBERTS: Dr. Thrall, then Dr. LeBlanc.
15 DR. THRALL: I was particularly interested in the
16 immunosuppression aspect of atrazine that we haven't touched on very
17 much here. And I was wondering if you -- I'm not familiar with the
18 Christin 2003 paper you alluded to. I wondered if you could give me
19 a little bit more detail on that, the pathophysiology of the nematode
20 and how the decreased spleen function interacted with that
21 pathophysiology.

1 DR. SHEFFIELD: That's a really good question. I have the
2 paper with me. I don't remember all the details of it because it just
3 came out. But I will tell you I noticed, because I have done studies
4 like this before, what they did, they used the pesticides as a -- they
5 exposed the organism to the pesticides. And they used a parasite as a
6 challenge to the immune system and tested it that way.
7 And they used a mixture which included atrazine. Like I said, I
8 have the paper with me. I can provide you a copy of that paper if you
9 want to look at it. I don't remember all the details offhand because,
10 like I said, it just came out.
11 DR. ROBERTS: Dr. LeBlanc and then Dr. -- Dr. Green, do you
12 want to follow up to that question?
13 DR. GREEN: I want to follow up.
14 I also have that paper, Dr. Thrall. I think relevant to this
15 discussion about atrazine, it was used in the mixture to treat these
16 rana pipiens or various rana species. And then these species were
17 inoculated with lung worms.
18 The conclusion by the authors, and there were two papers, the
19 first paper just stated that it appeared that in the presence of atrazine
20 in particular, the virulence of the pathogen was enhanced, and that it
21 accelerated their life cycle such that they matured and were present in

1 greater numbers in the atrazine treated frogs. And they speculated
2 that it might have something to do with immune suppression.
3 The follow-up paper by the second group came back, repeated
4 the same experiments and did T lymphocyte function tests. They
5 show lymphocyte suppression, and they propose -- I don't believe they
6 followed up with this, that there might be B cell suppression and
7 immunosuppression of immunoglobulins that would protect against
8 parasites.
9 But neither of those two papers had any data that documented
10 that the frogs were compromised in growth or that they died earlier
11 because of it.
12 So they didn't extend to make any speculation about what this
13 might do to an amphibian, a wild population that was exposed to
14 atrazine.
15 But I think we have both of those papers here if you would like
16 to look at them.
17 DR. SHEFFIELD: Thank you, Dr. Green.
18 The one other comment I make on that is that I think another
19 thing that would be useful instead of using the parasite, and it's
20 something that I've done in the past, is use a pathogenic challenge.
21 That way your endpoint is mortality.

1 So you get to see immediately what the effect is. So you don't
2 have to drag it out and look if the parasite is going to kill the animal
3 down the line or not.
4 DR. ROBERTS: Dr. LeBlanc and then Dr. DeLorme.
5 DR. LEBLANC: You noted that atrazine has a bioconcentration
6 factor of six in, I think, frog tadpoles.
7 In looking at that number, my interpretation would be, well, at
8 least one thing we don't have to worry about is atrazine's ability of
9 propensity to bioaccumulate in these animals.
10 But it raises a flag in your mind. And I just wondered if you
11 could expand on that a little bit. Is there something unique to
12 amphibians and bioconcentration that we should be aware of? Or is
13 six really a significant number?
14 DR. SHEFFIELD: I don't know. It just struck me as something
15 that I thought was -- I guess maybe it was because of the fact that I
16 had the other paper in mind and I was thinking about the Lydy work
17 that showed the potentiation and synergy of atrazine with the OPs.
18 The organophosphate insecticides are also very ubiquitous in
19 the environment. That's getting away from the purview of this panel,
20 but for me, anyway, it has implications.
21 If there is potentiation and synergy effects from atrazine

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

2 That's what it was -- I don't think the six is alarmingly high or

3 anything. It indicates that they do accumulate it and because I know

4 that they are the only taxa that accumulates OPs also.

5 So there is something unique about amphibians that the other

6 vertebrate taxa don't have.

7 DR. LEBLANC: Atrazine is pretty water soluble, and six is

8 pretty nonsignificant. So I think in that respect, at least, the

9 amphibians are probably responding like everybody else and they are

10 not accumulating the material.

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

12 DR. DELORME: With respect to your concerns about

13 formulation, are you aware of -- versus technical product, are you

14 aware of any studies that show concentrations of the formulants that

15 are mixed with atrazine in the end-use products and their

16 concentrations in water?

17 Because, obviously, atrazine isn't applied directly to water. It

18 is applied on fields. There is going to be differential degradations

19 and the ratios may change.

20 Are you aware of any data that would indicate what of the

21 formulants get into aquatic systems?

1 DR. SHEFFIELD: Not offhand I'm not. I'm not sure exactly
2 what they use to make the atrazine formulations either. Sometimes --
3 I don't know with atrazine, but sometimes a lot of chemical companies
4 are very sensitive as to what they put in there. And that's not public
5 information. I don't know if that's the case with atrazine or not.
6 So I don't know what that is. And I don't know how it
7 differentially divides. Once it is applied and it starts its fate in the
8 environment, I don't know how it divides up or not. All I'm
9 maintaining is that I have seen many studies that have shown, that
10 have looked at technical grade pesticides and other studies that have
11 looked at the same pesticide with formulations and found very
12 different results.
13 So I'm saying as a real world type situation, I think the
14 formulations are important to look at regardless of how they divide
15 out. The chemical pool is still going to be there in the amphibian's
16 habitat regardless of how they divide out. The pool is still going to
17 be there of all the different chemicals that are in the formulations.
18 Whether some of those chemicals disappear faster than others
19 -- they probably do. They have different fates because they are
20 different chemicals.
21 I don't know. I don't really know what else to say on that one.

1 DR. ROBERTS: Dr. Skelly?

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

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

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

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

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

1 Plus, you can do some things that Dr. Haye's group and others
2 have done with just looking at -- going out to field sites that are
3 contaminated.
4 They didn't -- they picked their sites based on atrazine sales
5 and then went there and looked at various sites. You can do a little
6 bit more as far as preselecting sites or actually setting up
7 experiments, experimental ecosystems, for example.
8 There has been some work on that done. I would like to see
9 some more on that with this particular question, setting up
10 experimental ecosystems. At that point, you have a lot more control
11 over your system and it is in the field.
12 I would say that the field studies are definitely harder to deal
13 with. They are harder -- there just is a lot more things that can go
14 wrong. But they are very useful.
15 And I my emphasis on that may have been simply because of the
16 fact that I have a slight bias towards -- and a lot of enthusiasm,
17 towards field studies.
18 They are a group package. They have to go together. And just
19 doing one at a time is not necessarily anything. I mean, they can
20 overlap. There is no written rules for this.
21 Basically, it's adaptive management. You are going along. You

1 find something. You adapt. You figure out what to look at next. If
2 that question could be best asked in the field, then that's where you
3 go. And if not, then you do more lab work.
4 So it is, like I said -- in some of these areas in wildlife
5 toxicology, you are going down a road that has not been paved yet. It
6 is adaptive management.
7 But the field studies are important. But like I said, there is a
8 lot more inherent variability. You can possibly accept a little bit
9 more error because of that inherent variability. They are harder to do,
10 so they haven't been done as much. But they are valuable.
11 DR. SKELLY: Thank you.
12 DR. ROBERTS: Thank you, Dr. Sheffield. We appreciate you
13 coming in and sharing your comments and thoughts with us this
14 morning.
15 DR. SHEFFIELD: Thank you very much. Good look to all of
16 you in the next two days.
17 DR. ROBERTS: Next public commenter is Mr. James Tozi (ph)
18 on behalf of -- I'm not sure what the note says. But I'm sure he will
19 tell us.
20 And he will be followed by Mr. John Hall.
21 MR. TOZI: Thank you, Mr. Chairman. Good morning. I'm Jim

1 Tozi with the National Business Services. And with a title like that,
2 given the chairman's remarks that you want scientific remarks, you
3 may say there is somewhat of a divergence there, but I assure you
4 there won't be.
5 One of our main roles is to ventilate to the public the scientific
6 basis or bases for regulatory decisions.
7 And the question is how do we do that. We do that by the use of
8 a web site called Cyberactivist.US. You might not have heard of that
9 site, but if you go to your web master, it is probably by traffic
10 gradings the highest, if not one of the most highly trafficked, web
11 sites in the world on the very specialized area of regulatory policy.
12 And it is used throughout the world.
13 Now, questions given that, why am I here. I think this panel,
14 and it just happens to be that you drew this card, that subsequent to
15 the passage of a very important act, the Data Quality Act, and we look
16 throughout the entire government for proceedings, this proceeding is
17 the first major proceeding that's going to be subject to the Data
18 Quality Act when the agency goes to use it.
19 And I haven't been here all the day, so you might have heard
20 some of this before.
21 Now, keeping with the chairman's charge, I'm not going to get

1 into law because I don't think the law is any different than which most
2 scientists do anyway.
3 The basic two important portions of the law says the data used
4 by the government and regulatory decisions first must be reproducible
5 and second it must be transparent.
6 And many scientists when a law passes, big deal, we do that all
7 the time. Well, some of the regulators thought it was a big deal. The
8 scientists did not.
9 So I just want to say that those two standards, reproducible and
10 transparency, are going to be judged on anything that comes out of
11 this whole proceeding.
12 What does that mean? That means any third party such as I, on
13 National Business Services, or anyone that testifies, including this
14 committee, you can write and say anything you want. I can write and
15 say anything I want. It need not be reproducible and it need not be
16 transparent.
17 However, if the agency is going to use anything that I give in
18 this proceeding, anything any other third party gave or whether they
19 are going to use your report, it is going to have to meet those two
20 standards.
21 If it does not, any third party can move to strike that

1 information from decisionmaking in the government.

2 Now, a point that I wanted to emphasize here in terms of what

3 we're going to do to advise this committee, we are very interested in

4 this proceeding because of the data quality aspects.

5 But I think you can't look at data quality unless you have data.

6 And so, we are going to put on this cyber activist web site Monday

7 evening a request that petitioners have made from EPA on the data on

8 this proceeding.

9 I haven't looked at all the data, so don't ask me about all the

10 details, but I'm advised that -- and now would I want to compete with

11 you on what the data means. But I'm advised that there's petitioners

12 that have asked EPA for the data and it comes out in these kind of

13 categories.

14 There were seven data sets, I understand, but this will all be on

15 the web site, five of which were given to people, some of which were

16 encryptive, some of which was withheld.

17 We're not going to take a position on these matters on the site.

18 That complete data set will be put up on Cyberactivist.US, and it will

19 be out for public comment.

20 And the form, you are familiar with forms, it is not a web site.

21 It is a form and it is live so any person in the world, anyone in this

1 panel, anyone in this room can look at that data set and make a
2 comment on it in realtime, which we'll be expecting a lot of
3 comments.
4 And I will tell you when we put it up, we're going to urge
5 people to ask three questions when they look at this data set. First, on
6 the data received, was it reproducible. Second, how does use of
7 encrypted data fit into regulatory proceedings. And third, when the
8 federal regulatory agencies withhold data, how does FICA (ph)
9 committees and how do people handle this.
10 We think by putting it on Cyberactivist.US and the charge of
11 this committee as well as the composition of this committee on the
12 web site, we think we'll be able to ventilate way beyond this
13 proceeding an importance how this type of information is used by
14 influential bodies such as yours.
15 We urge you to look at that site and the comments we'll receive.
16 Some of them, we've received thousands of comments. Thank you
17 very much.
18 DR. ROBERTS: Thank you. I think there is a question from
19 Dr. Heeringa and then Dr. Green.
20 DR. HEERINGA: Mr. Tozi, as a scientist operating in a
21 university environment, I believe in full and open disclosure on this

1 data, obviously, there are proprietary interests, so and I commend you
2 on that.
3 Just a comment too that data alone aren't sufficient. You need
4 documentation to accompany it. I presume that part of your posting
5 of these data will be to post the relevant documents that describe the
6 study designs and protocols used to collect the data too. Because as
7 you open that up to analysts, it is going to be very, very critical. And
8 I think particularly in the case of the data sets we're looking at here,
9 that those documentations of procedures and assessments be
10 available simultaneously too.
11 MR. TOZI: Let me ask you one question on this. I think that's
12 very good. What I would encourage when we post these that anyone
13 that has something that they want, and it is very easy, you just press,
14 click on a button and you put an attachment and it goes right up, we
15 will put some of that up. The initial things are going to be what was
16 response to the FOIA.
17 But I agree with you. There are going to have to be additional
18 postings to interpret that. I have looked at some of the data. It is not
19 my technical expertise to say what is there, but I agree with you.
20 We would encourage anyone that is involved in this proceeding,
21 whether around this table or in the room, that have relevant

1 information, because I will tell you that a lot of other agencies way
2 beyond EPA -- which, by the way, EPA on third party data and the
3 Data Quality Act is probably one of the leading agencies.
4 You can criticize some stuff they have done. And I know the
5 agency from when they were born, they are doing a very good job. I
6 agree with you. And to the extent I can get that kind of information
7 and people want to put it on, we welcome them.
8 DR. HEERINGA: One more additional comment. Just a
9 technical question for people who would access this. What data
10 storage format will it be in. Will it be in Excel spreadsheets? SPSS
11 Sass? Raw data?
12 MR. TOZI: I can tell you how you do it now. You register on
13 the site. You type something in. You press a button. I think it comes
14 all out on HTML. There is attachment. The question is how do you
15 get that attachment on there.
16 Generally, the people that put the attachments on now either
17 have it on their web site and they cut and paste it over or they send it
18 to us and we'll scan it in.
19 We prefer you have it on your web site because of the amount of
20 traffic.
21 DR. HEERINGA: Thank you.

1 DR. ROBERTS: Dr. Green had a question.

2 DR. GREEN: Yesterday and today several presenters have

3 alluded to encrypted data, which I'm not clear what data people are

4 talking about that has been encrypted.

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

6 MR. TOZI: Thank you. One of the people told me I was

7 supposed to say that. Having worked in OMB for years, some things I

8 don't address, maybe because I don't want to.

9 They asked me this, yes. From the FOIA requests that I

10 understand that people filed out of these seven -- I just say I haven't

11 gone through all the data, five data sets came back, four of which are

12 data.

13 When I say encrypted, I understood, but others in the room

14 would know more about this than I, the term encrypted means it was

15 in some machine code that you couldn't interpret.

16 So when they put that up, I want to ask them -- they will

17 identify which one was encrypted. What it meant. But I got from the

18 conversations that unless you had some transition or translation

19 codes, you could not interpret the data. You needed some other kind

20 of information to turn it into meaningful data.

21 DR. ROBERTS: Are there any other questions?

1 If not, thanks very much for coming in and your comments and
2 letting us know about that.
3 Mr. Hall has requested the opportunity to speak to the panel.
4 As a heads up, he will be followed by Mr. Botts.
5 MR. HALL: Good morning, Mr. Chairman, members of the
6 panel.
7 My name is John Hall. I'm with Hall and Associates. I'm here
8 today representing the Kentucky Corn Growers Association.
9 By way of background, I'm an environmental engineer and an
10 attorney. I suppose maybe from this panel's perspective that puts two
11 strikes against me since I'm not a scientist per se.
12 In general, I have spent the last 20 years of my career dealing
13 with water quality related issues under the Clean Water Quality act,
14 development of water quality standards and the like.
15 The information that has been presented over the last two days,
16 as you are aware, not only has an effect on the reregistration of
17 atrazine, but also affects what EPA is doing in terms of its water
18 quality standards development for atrazine.
19 I come from that perspective in terms of my review as to the
20 information presented. In the water quality standards issue area, they
21 generally look at impacts in terms of what I will call the big picture.

1 They don't typically parse out mechanistically why an impact
2 occurs, what the exact cause of the effect is, but they look at whether
3 or not there is a significant effect on growth, reproduction or
4 survival, the three main endpoints that are often the, if you will, the
5 end result of most research to decide what the real world impacts are.
6 I have just a couple comments with regard to the information
7 presented and some comments regarding an earlier presentation to this
8 panel.
9 Dr. Hayes' presentation in my mind raised at least one issue. I
10 guess others have thought it raised several. His analysis focused on
11 abnormalities in gonadal reproduction.
12 And without looking at the environmental significance of that,
13 the question I had was that these abnormalities were classified as such
14 because apparently they weren't seen in certain controls during the
15 study. But that in possibly later studies that were conducted, did find
16 some of these abnormalities in the controls.
17 And therefore, I guess there is at least a question in my mind as
18 to if the later studies exhibited some of these abnormalities, should
19 the results of the earlier studies that classified certain things as
20 abnormalities kind of gone back and corrected those charts. If that, in
21 fact, was the case.

1 The other thing I would note is that in terms of at least
2 statistically, by classifying abnormalities as something you don't see
3 in controls, you automatically get a zero abnormality in your control
4 group.
5 And then when you do your statistics and comparison to that,
6 very often you can get anything being mathematically significant in
7 terms of a difference compared to a zero effect on the control.
8 I would note, though, that at least out of all the studies that I
9 have heard about today and in the prior days, that the environmental
10 significance of any of these effects that have been noted is not
11 demonstrated.
12 I have not seen any information presented that these effects
13 actually cause impacts on frog populations, which in the end is I think
14 what we're really concerned about.
15 Let me just move on to a couple other points. In the NRDC
16 presentation, they indicated that the charge to this panel was
17 misplaced. I could not disagree more.
18 The charge to the panel is not misplaced. What we have is
19 different agendas occurring on from different parties that I believe
20 are a little misplaced.
21 NRDC's view is that endocrine disruption equals ban. In other

1 words, any level of impact you find regardless of its significance to
2 the real world triggers a ban.
3 And that trigger on endocrine disruption shouldn't be ecological
4 significance. It is simply statistical significance.
5 That is not the standard. That is applicable under any federal
6 law I am aware of.
7 The Clean Water Act requires you to avoid significant adverse
8 impacts on aquatic life. And the endpoints that EPA has used for
9 three decades are growth, reproduction and survival.
10 In other words, does this have a real world effect in the real
11 world. You may find an effect, a histopathological effect in the
12 organism. But the question is does that cause any significant adverse
13 effect later on such as populations are affected -- unless you don't
14 classify something as a substantial adverse effect and regulate it.
15 OPP, the pesticides office, uses a no unreasonable adverse
16 environment effect endpoint. Again, it is a similar type of legal
17 standard.
18 All these programs in summary look at population-based
19 impacts. They require documentation of those impacts and not merely
20 speculation that the impacts occur.
21 And that, of course, is the charge to this panel. And I think

1 you will serve us all greatly by answering the question of, whatever
2 impacts these studies have shown, whatever they are, is there any
3 information that shows these impacts are demonstrated to impact
4 these organisms at a population level?
5 If the answer is no, then you don't regulate the pollutant -- the
6 constituent more stringently at this time. You may research it some
7 more if there are questions that you have, but you don't decide that
8 you are going to cancel a registration over something that is not
9 connected to a real impact.
10 I will tell you one thing that was said in the earlier presentation
11 that is clearly misleading and incorrect.
12 NRD stated that EPA's report confirmed that there were real
13 readings and real world concerns showing significant risks. It is
14 simply not true.
15 What EPA does when they do these risk assessments, I call it
16 triage, I guess is the best way to look at it, you go through doing a
17 very gross level of review and decide, my god, if you can pass that
18 test, you are clearly not an effect.
19 That's what they call a Tier 1 assessment. And they make
20 multiple, multiple worse case assumptions. And if you can pass all
21 those worst case assumptions, you are out the door. We leave you

1 alone.

2 If you can't pass the series of worst case assumptions, they go

3 to a higher level review. They don't declare that they have

4 determined something that is causing significant real world effects.

5 You move on forward.

6 One example, and NRDC relied on it in notifying the EPA, they

7 intended to sue them under the Endangered Species Act on this issue.

8 EPA in the report took data from what they called estuarine

9 areas. The entire Chesapeake Bay. They took data from areas that

10 were even 30, 50 miles upstream in non-tidal waters, clearly

11 freshwater systems. And in their report, they classified that as

12 estuarine data.

13 You would have thought that the Chesapeake Bay had 30 parts

14 per billion of atrazine occurring in it. In fact, it does not.

15 Well, if you read the EPA report, and not very carefully, what

16 you think is the Chesapeake Bay has these enormous high

17 concentrations of atrazine.

18 When you look at the underlying database, you find the

19 concentrations of atrazine in the Chesapeake Bay proper, around the

20 order of 10ths and 100ths of a PPB unmeasurable levels.

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

1 the data before you decide that what is in that EPA report has
2 demonstrated there is real readings, real world concerns before you
3 make claims that leather back turtles are adversely affected in
4 Chesapeake Bay.
5 I guess I would like to end with I applaud what this panel is
6 doing. I think you will do us all a great service by clarifying what the
7 real effects are, what the information really does show and whether
8 and how we should move on to do further evaluations.
9 I think it would be extremely helpful if this panel confirms
10 what I believe is a correct assessment on EPA's part that no one has
11 demonstrated a real world connection to population-based impacts
12 from these endocrine disrupter endpoints.
13 That is not to say no one ever will. That is just to say that it
14 has certainly not been demonstrated to date.
15 Thank you very much. If there's any questions, I'll be happy to
16 take them.
17 DR. ROBERTS: Thank you, Mr. Hall. Let me ask the panel if
18 they have questions.
19 I don't see any. Thanks very much for coming in and sharing
20 your comments and thoughts with us.
21 MR. HALL: You are welcome.

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

2 MR. BOTTS: Thank you, sir. My name is Dan Botts. I'm the

3 Director of the Environmental and Pest Management Division of

4 Florida Food and Vegetable Association.

5 And as such, we're a voluntary trade association that represents

6 the producers of about 60 different commodities in the state of

7 Florida, including sugar cane, sweet corn and sod, all of which use

8 atrazine.

9 But the reason I'm here today is because I have been following

10 this issue for at least 10 years, even before the special review was

11 issued in 1994. And have looked forward to some resolution at some

12 point down the road in determining if, in fact, atrazine is safe, but

13 more importantly, that triazines as a class of compounds. Larger

14 acreage use of triazines in the state of Florida is simazine use on

15 citrus.

16 And because of that, your deliberations here today pave a

17 pathway of how this whole class of compounds will be dealt with.

18 And not only this class of compounds, but others as we move into the

19 new process of determining endocrine disruption impacts and how to

20 regulate those impacts for the office of pesticides programs.

21 I look forward to your deliberations, and looking extremely

1 forward to your final report, having sat through many SAPs over the
2 past since 1997 after FQPA passed on all kind of issues, everything
3 from modeling for OP, cumulative exposure to OPs and dose response
4 curves for other issues.
5 I'm extremely encouraged by the discourse that has already
6 occurred among the panel members and between the panel members
7 and the people presenting. It is a much more engaged panel than a lot
8 of SAP panels have been in the past.
9 And I will keep this extremely brief for that respect. I know
10 you are already a half a day behind schedule. It's going to be
11 extremely time consuming to go through the questions that have been
12 presented. I would first applaud the agency for taking a very
13 controversial set of data and the information and compiling it and
14 looking at it in a manner to put forward the review that they did in
15 their white paper and framing the issue to go forward.
16 And I'll be honest with you. I didn't read the charge of the
17 questions until the plane ride up here, so I don't have as great a
18 familiarity with the intent or tone of the questions as some of the
19 other previous speakers have.
20 But after listening to the comments and conversations, I am
21 sure that any issue that might be floated out in the vagueness or the

1 generalities associated with those questions will come forward as this
2 committee deliberates.
3 I would draw one thing to your attention, though. In the
4 presentation yesterday afternoon, there was one slide that was put up
5 by Dr. Hayes relative to several epidemiology studies relating to the
6 human health effects and potential carcigenocity of atrazine.
7 This committee can be informed by the deliberations of the SAP
8 panel which was held in June of 2000. I'm sure that report is up on
9 their web site, and would be available for you.
10 Most of the studies that were referenced in his presentation
11 yesterday were discussed at length in that document. Whether they
12 are relevant or not to deliberations of this panel I would suggest are
13 probably not the case.
14 Some of the mechanistic issues that might have come up in the
15 previous part on determining the cancer risk associated with atrazine
16 probably are.
17 There will also be an SAP panel later this summer to deal with
18 epidemiological information relative to cancer, and those discussions
19 would probably be more appropriate at that point.
20 With that, I encourage you to move forward since you don't
21 have to listen to us in the public anymore and can deliberate among

1 yourselves. And I look forward to your discussions over the next day
2 and a half.
3 DR. ROBERTS: Thank you, Mr. Botts. We appreciate you
4 coming in and sharing your thoughts and comments. Let me ask the
5 panel very quickly if they have any questions for you.
6 I don't see any. Again, thanks very much for your thoughts.
7 Dr. Post had requested the opportunity to speak. I asked if she
8 was here earlier. Is she here now?
9 This concludes the list of people who have asked previously to
10 speak. Let me ask now if there is anyone in the audience who has not
11 had an opportunity as yet to address the panel and would like to do so.
12 This will be your last opportunity to make a public comment.
13 Because when the public comment session closes, the next item of
14 business will be for the panel to begin their deliberations.
15 So final call to the audience. Is there anyone who has not yet
16 had the opportunity to address the panel that would like to speak?
17 Seeing none, this closes, then, the public comment portion of
18 the meeting. Let's take a break for about 15 minutes and reconvene,
19 and the panel will then begin their deliberation of the questions.
20 (Thereupon, a brief recess was taken.)
21 DR. ROBERTS: Before we begin deliberation of the questions

1 posed by the agency, our designated federal official, Mr. Paul Lewis,
2 has a few announcements about keeping the docket squared away,
3 some announcements about submission of documents.
4 MR. LEWIS: Thank you, Dr. Roberts. Just briefly, during the
5 break we distributed to the panel two pieces of information. One is a
6 CD that was provided by the EPA Office of Pesticides Programs of
7 additional data for the panel to review, to consider. In addition,
8 additional data, additional clarification provided by Syngenta based
9 on comments they made yesterday.
10 The material is available to the panel now. In addition, we will
11 make it available in the public docket.
12 DR. KELLEY: Do you know where this came from?
13 MR. LEWIS: Let me give this back to the chair.
14 DR. ROBERTS: The question came from Dr. Kelley regarding a
15 CD that we were distributed, and she wanted to know the source. And
16 it is the one that says Hayes data sets.
17 DR. STEEGER: The data sets on that CD are all of the data that
18 Dr. Hayes has provided to support his report to Syngenta, the report
19 that was distributed earlier today.
20 It also contains data to support the standard operating
21 procedures that he developed in his lab to determine feeding rates.

1 There is an additional file on there that provides the password
2 for the one password protected data set that is contained among the
3 seven.
4 DR. ROBERTS: Thank you, Dr. Steeger.
5 We have a follow-up question by Dr. Kelley.
6 DR. KELLEY: For clarification, so the feeding data which
7 were alluded to yesterday, does this just include the methodology for
8 gathering them and not the results? Or does this include the results of
9 different feeding regimens?
10 DR. STEEGER: My understanding is they are the results of
11 different feeding regimens to determine what would be the ideal
12 feeding rate for xenopus in Dr. Hayes' lab.
13 DR. KELLEY: Thank you.
14 DR. ROBERTS: And just as a general comment, the panel very
15 much appreciates the data and the reports that were submitted during
16 the course of the meeting and the comments.
17 We will try and consider and utilize those in our deliberations
18 as best we can. But time is very short for us to be able to consider
19 those. So we'll do the best we can with it.
20 Yes, Dr. Steeger.
21 DR. STEEGER: I want to make one more comment about the

1 encryption of the data.

2 As Dr. Hayes indicated in his presentation, his treatments are

3 color coded. One of the data sets is codes dot X L S. It's an Excel

4 spreadsheet. It provides the way of associating the colors with the

5 actual treatment levels.

6 DR. ROBERTS: Thank you, Dr. Steeger.

7 Dr. Bradbury, the panel has received a great deal of public

8 comment over the last couple of days, a lot of information.

9 We appreciated the opportunity to hear from the investigators

10 and asked them questions. We now turn to deliberation of the

11 questions posed by the agency. And I wondered if you had some

12 introductory remarks or comments you wanted to make to help us

13 make sure we have the right focus as we begin to that.

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

15 opportunity.

16 What I would like to do is just spend a few minutes to recapping

17 where we have been over the last few days and perhaps help set the

18 stage for moving through the questions.

19 What I would like to do is sort of again get us back into the

20 context of the science and the risk assessment issue that we're facing

21 and to put this question in the context of the agency's ecological risk

1 assessment guidelines, the process whereby the science is evaluated
2 to help inform the regulatory decisions that the agency needs to make.
3 We talked about this on Tuesday morning, that one of the
4 important phases of an ecological risk assessment is the problem
5 formulation stage. It provides the context, it provides the foundation
6 for proceeding further in the actual risk assessment.
7 The risk assessment, of course, is designed to help inform the
8 risk management decision, science for a purpose. The science for this
9 purpose is to help inform decisions about the potential risk of atrazine
10 to amphibians.
11 As we discussed on Tuesday morning, ecological risk
12 assessments tend to be an iterative process. And some of the speakers
13 over the last couple days noted the risk assessment that has already
14 been completed in terms of the potential effects of atrazine on aquatic
15 community structure and function.
16 As part of that it iterative process, questions came up from the
17 public, from NRDC and from others. And in the context of interacting
18 with our agency's risk managers, it formulates another question.
19 The question being whether or not atrazine can cause
20 developmental effects on amphibians, and, if so, what could be the
21 consequences if that effect occurs.

1 As one moves in theory from problem formulation into the
2 analysis phase, that's where one blends the exposure information with
3 the effects information and takes that into risk characterization
4 where, in fact, we attempt to provide an estimate of the magnitude and
5 likelihood of potential adverse effects. If you will, try to establish
6 the probabilities that certain events can happen at different atrazine
7 exposure concentrations.
8 The goal of the risk assessment is to provide that exposure
9 response profile and articulate what the ecological significance of
10 that exposure response profile is. And then communicate that to the
11 risk manager.
12 And as we have heard over the last few days, there is a number
13 of issues that come into play in making a decision about the
14 registration and reregistration of a pesticide. Science is only one
15 aspect of the overall decision. There are many other factors that go
16 into making a regulatory decision.
17 The challenge for the scientific community, not just the
18 scientists in the office of pesticide programs and the office of
19 research and development, but the scientific community that spans
20 academia industry and public groups, is to ensure that the science that
21 goes into making these decisions is clear and transparent and provides

1 the risk managers an objective understanding of what we know and
2 what we don't know so that certainties in their decisionmaking is clear
3 to all concerned.
4 So that's the context. We're inside the box. We're inside the
5 box of science, but it is science for a purpose and it is science that has
6 urgency. Decisions have to be made. Making no decision is, in fact,
7 a decision. And it is a decision that is made in the context of
8 whatever scientific uncertainty or certainties we have at the time.
9 So with that in mind, let us just walk through again a little bit
10 of context on the road map that we're working through. Let's take a
11 look at problem formulation again. And that's really where we are
12 right now.
13 The white paper is, in fact, a problem formulation. Problem
14 formulation is where we integrate available information to try to
15 establish some sense of risk assessment endpoints in the context of
16 environmental management goals and start to articulate what those
17 measures of effects could be to make estimates about those risk
18 assessment endpoints.
19 Based on the integration of available information, we focus on
20 the formation of risk hypotheses and try to develop a conceptual
21 model that could be used, a working hypothesis, if you will, to relate

1 the information that we do have in terms of exposures and effects to
2 then set up the analysis plan for actually undertaking the risk
3 assessment.
4 It is very critical in a risk assessment that has a lot of attention
5 and has a lot of implications to use this problem formulation step to
6 its utmost, to have it be rigorous, to gain the input from the scientific
7 peers, to ensure that we've thought this thing through the way it needs
8 to be thought through.
9 At the end of the analysis phase, there are sort of three broad
10 paths one could imagine going down. One possibility is that through
11 the analysis of the available information and dialogue with the risk
12 managers it could be concluded that there is no need to do a risk
13 assessment, that there is sufficient certainty in the information, in the
14 context of the certainty that is required to make a regulatory decision
15 that there is no need to do a risk assessment. That's one possible
16 outcome of problem formulation.
17 Another outcome, possible outcome of problem formulation
18 would be that, in fact, there is sufficient information to formulate the
19 working hypotheses in the conceptual model. The analysis plan lays
20 out how to use the available information to proceed with the risk
21 assessment. Acknowledgment of potential uncertainties are

1 recognized in the analyses plan, but the decision is that one could go
2 ahead and actually start doing the risk assessment to move into the
3 analysis phase and begin ultimately to characterize risk.
4 A third outcome of problem formulation could be that there is
5 sufficient information to formulate a reasonable conceptual model to
6 formulate reasonable risk hypotheses, to formulate a reasonable
7 working hypothesis and to develop an analysis plan that outlines the
8 amount of information that is available and outline the data gaps that
9 are facing the ability to proceed with the risk assessment with varying
10 levels of certainty.
11 That's sort of where we're at right now in taking a look at the
12 white paper, the problem formulation and gaining your insight and
13 input and wisdom on the paths, the possible paths to go forward after
14 problem formulation.
15 Is there sufficient information to proceed with the risk
16 assessment and quantify to varying degrees the probability of adverse
17 effects to amphibians in terms of development based on varying
18 atrazine exposures?
19 Is there sufficient information to say there is no need to go
20 forward; there is no plausible risk hypothesis for the potential of
21 atrazine to cause adverse effects on amphibian development or are we

1 somewhere in between. That's the ultimate question before us. And a
2 series of questions to help us get to where we need to get.
3 One thing I want to point out is that in the process of going
4 through problem formulation and ultimately getting to risk
5 characterization, we're talking about uncertainties, we're talking
6 about what we know, we're talking about what we don't know and
7 we're pulling that information together.
8 There has been some discussion that it is a weight of evidence
9 approach. In fact, it is not a weight of evidence approach. In fact,
10 the agency's ecological risk assessment guidelines are very clear that
11 it is not a weight of evidence approach.
12 In fact, it is termed the lines of evidence approach. And to
13 quote from the agency guidelines, the phrase, lines of evidence, is
14 used to deemphasize the balance of opposing factors based on
15 assignment of quantitative values to reach a conclusion about a
16 "weight." in favor of a more inclusive approach, which evaluates all
17 available information even evidence that may be qualitative in nature.
18 So the point is we're not balancing pounds of information.
19 We're looking at lines of evidence. If I could be so bold as to change
20 the analogy a bit from the risk assessment guidelines, you may want
21 to think about it as pieces of evidence, pieces of information and

1 think about how we might try to build a puzzle. Think about building
2 a jigsaw puzzle with lots of pieces on the table, all different shapes,
3 all different sizes, all different contours. Some with many edges,
4 some with a few edges.
5 Are all those pieces of information on the table even part of the
6 same jigsaw puzzle? If they are, how many of those pieces on the
7 table can one start to put together to start to build the picture? Is
8 there enough pieces on the table that are starting to connect to each
9 other to actually see what the picture is and to talk about the picture
10 and describe the picture in great detail?
11 Or is it apparent that the pieces on the table don't even allow
12 one to start to put the pieces together? Is it even possible that the
13 pieces don't even belong in the same puzzle? Or are there enough
14 pieces starting to come together that one can start to imagine what
15 that puzzle could look like if one could get more pieces and can start
16 to see what kind of pieces would be the most critical to start building
17 the picture, to start painting the picture.
18 I think the other idea in terms of not using the term weight but
19 using the term lines or pieces of evidence is that some pieces in this
20 puzzle may be very, very small, but have lots of edges and they
21 connect lots of pieces that are on the table. They may be very small,

1 but they may be very critical.

2 I think another aspect of thinking about this in terms of

3 painting a picture or putting together a puzzle is that as we have

4 certain pieces come together, there may be some gaps between some

5 of the pieces. But depending upon the shape of the pieces and how

6 those pieces are coming together, it may be very relatively easy to

7 imagine what that missing piece would look like, i.e., we might be

8 able to extrapolate to what that missing piece would look like, a

9 reasonable level of confidence.

10 In some cases the edges of the pieces may lead us out into parts

11 of the universe we haven't been before, and that may require actually

12 getting some pieces to help put the picture together.

13 So it is an inclusive process. There is no right or wrong. There

14 are no winners or losers in this process. The only winners are the

15 people of the United States getting the kind of information it takes so

16 that the risk management decisions can be informed.

17 The only winners in this operation, in this endeavor is a

18 science, and the science being blended together to maximize all the

19 information possible to make the most informed decision that we can

20 make.

21 In going through problem formulation, one sort of gets the

1 impression it's a linear process. It is not. You sort of take different
2 information and you work it through. Let's just recap real briefly sort
3 of where we are coming from in the white paper.
4 We initially laid out on Tuesday morning the statement of the
5 risk management goal, the environmental management goal as well as
6 the risk assessment endpoints, and those being the reproductive and
7 recruitment capability of native anurans. I don't think I need to go
8 into great detail on that.
9 But what I would like to do is sit back and think about what that
10 means in terms of being able to make some estimates about
11 reproduction and recruitment of native anurans. That's a big question.
12 That's a question that brings in all sorts of fields of biology,
13 landscape ecology.
14 Problem formulation helps us get started on the process of
15 putting this information together to answer that question.
16 When the agency has to take on chemical risk assessments in
17 the ecological realm, we take on a challenge that, with all due respect
18 to the human health risk assessors of the agency, I think we have a
19 bigger challenge and a more exciting challenge, because we have to
20 work across many layers of biological organization.
21 And the examples of the levels of biological organization on

1 that slide don't even capture all the levels of biological organization
2 as Carl Richards and others on the panel know, but to at least get it all
3 to fit on one slide, I think I can get the point across.
4 This concept on this slide has been published by many folks in
5 many different venues. But I think it illustrates very nicely some of
6 the challenges in the integration of information that is required as one
7 goes forward in a risk assessment.
8 One could enter our examination of a question at any level of
9 biological organization, but the concept is that in general as one goes
10 down levels of biological organization, one creates greater
11 understanding of the potential or actual interaction of a chemical with
12 a biological system.
13 But certainly as one goes down in levels of biological
14 organization, our understanding of the relevancy of those events at
15 the population or community or landscape level become less and less
16 certain.
17 So, in fact, doing an ecological risk assessment is a blending of
18 many levels of biological organizations to inform, to help us
19 understand what is going on and to help us understand the relevancy
20 of the ecological risk assessment.
21 I think this panel is a nice example of the kinds of skills and

1 disciplines and professions that need to come together to understand
2 in the problem formulation context of where we are and where we
3 need to head.
4 So if I could try one more analogy. We talked about pieces of a
5 puzzle in terms of creating that puzzle and creating the picture to
6 understand where we are and what the world looks like. We also
7 could think about it in terms of threads and how threads get woven
8 together to create a tapestry, a tapestry that has texture, a tapestry
9 that has a lot of vibrant aspects to it.
10 And it means we're blending in the skills and talents of folks
11 that are experts in molecular biology and interactions of chemicals
12 with receptors or enzymes as well as interacting with folks that are
13 experts in landscape ecology and understanding how spatial and
14 explicit descriptions of habitat are critical for understanding the
15 population demographics of amphibians or other species.
16 So our challenge is how to weave these threads together to
17 create a coherent picture that blends understanding and relevancy.
18 So that as a context, I would like just a couple more minutes if I
19 can, Mr. Chairman, for Tom, Tom Steeger, and I to just touch base
20 again on the integration of available information for this specific risk
21 assessment, problem formulation for atrazine, touch on the conceptual

1 model again just to touch base and kind of get us all reequilibrated
2 and then to just touch base on the first aspect of our analysis blend for
3 the problem formulation.
4 DR. STEEGER: Over the past two days, panel members have
5 received range of input from researchers engaged in studying the
6 effects of atrazine on amphibian development.
7 Panel members have been provided copies of each of the studies
8 discussed and EPA's assessment of the studies.
9 To a large extent, the presentations have focused on the results
10 generated from research efforts. As has been pointed out by a member
11 of the SAP, it isn't sufficient, though, to look exclusively at the data.
12 But rather, you have to also consider the study design and study
13 conditions in which the data were collected.
14 As Steve Bradbury, Joe Tietge and I have pointed out, the
15 agency follows a process for conducting ecological risk assessment.
16 The initial stage of ecological risk assessment is problem formulation
17 where risk assessors work with risk managers to integrate available
18 information on a chemical to define potential hazards, their impact on
19 assessment endpoints and the uncertainties associated with the
20 measurement endpoints used to identify potential hazards.
21 Typically, the agency relies on guideline studies to assess

1 risks. However, open literature and nonguideline studies are also
2 considered.
3 Consistent with the agency's process for evaluating studies,
4 studies were evaluated using the following criteria: Experimental
5 design, study protocols, protocols and quality assurance, the strength
6 of the cause effect relationship, whether there was a dose response,
7 whether the observed effects have plausible mechanism of action that
8 is consistent with what is known about the chemical, and, finally,
9 whether the measured effects are ecologically relevant.
10 Did we know what to expect? No. While the agency routinely
11 receives studies that have a very standardized protocol and
12 established databases on which to gauge the conduct and outcome of
13 the studies, the studies under current review on amphibian effects
14 represented a new area of information.
15 Did the agency have expectations on whether the data were
16 realistic. As Dr. Giesy testified, the data are what they are. Agency
17 reviewers, though, examine how the data were collected and analyzed
18 to determine whether they would have come to the same conclusions
19 as the study authors.
20 The agency, however, is looking for input from this panel on
21 whether the data provided from the current suite of studies are

1 reasonable for gauging the effects of atrazine on amphibian
2 development.
3 Also, there was some discussion about the agency's unrealistic
4 expectation of a monotonic dose response curve. The agency is not
5 focussed on the shape of the curve as much as on the consistency of
6 the dose response.
7 We do not insist that a chemical exhibit a monotonic dose
8 response, but simply that we are able to understand and project what
9 type of response might be expected in a consistent fashion from
10 exposure to particular levels of atrazine.
11 By now, the panel is painfully aware that the agency has
12 reviewed seventeen studies that were received as a February 28th
13 consent decree deadline. 12 of the studies were submitted by the
14 registrant. Five were drawn from open literature. Seven studies were
15 conducted in the laboratory and ten were conducted in the field.
16 While the presentations by researchers over the past two days
17 have focused on study results, the agency has a number of evaluation
18 criteria that extend beyond the data and examine the conditions under
19 which the data were collected.
20 As I indicated, the data evaluation records for the 17 studies
21 focused primarily on methodological inconsistencies that were

1 considered to have potentially critical impacts on the study.

2 While Dr. Sielken was correct in pointing out that collapsing

3 replicates can potentially increase the likelihood of a type one error,

4 that is, drawing a conclusion that there is a statistical difference when

5 one does not actually exist, agency reviewers conducted statistical

6 analyses based on both the original study design and on collapsing the

7 data sets in order to explore whether statistical relevancy could be

8 extracted from the highly variable data sets.

9 However, it is important to keep in mind that regardless of what

10 approach was used to view the data, confounding effects across all of

11 the studies limited, if not precluded, the utility of the data regardless

12 of how they were analyzed.

13 Thus, atrazine contamination in the controls, poor water

14 quality, poor growth and development and survival, high variability

15 in endpoint measurements, lack of reproduceability and unresponsive

16 positive controls were recurrent themes that were considered critical.

17 One question that was posed on Tuesday was whether panel

18 members should use any of these studies in determining whether there

19 are sufficient data to gauge whether atrazine is impacting gonadal

20 development.

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

1 amphibians' endocrinology, plus extensive laboratory and field
2 research coupled with the understanding of the science of risk
3 assessment to provide feedback to the agency on that very question.
4 The agency views that the studies do have utility in helping to
5 identify potential effects on amphibian development. Additionally,
6 the studies provide insight on the sources of variability and they
7 provide insight on the appropriate test species and study conditions
8 that may be utilized in future studies.
9 If the risk managers wish to reduce the current uncertainties
10 regarding the potential effects of atrazine on amphibians, the agency
11 recommends that additional studies be initiated. These studies should
12 build on the current body of information.
13 I suspect that it is often times frustrating for the public to
14 recognize that the agency is not omniscient in its understanding of the
15 available research on a particular subject.
16 While certain background information would be particular
17 relevant to the measurement endpoints under consideration, the
18 agency may simply not be aware of its existence.
19 Additionally, because of research limitations, the agency has
20 come to rely on the testing of surrogate species to be representative of
21 the effects of a very broad range of organisms. Thus, while it might

1 be ideal to test actual species that may be likely to be exposed to
2 pesticides, these data are not typically available.
3 Additionally, surrogate test species are not selected based on
4 their sensitivities to chemicals, but rather on their ability to survive
5 under laboratory conditions.
6 Also, while laboratory test conditions may not be deemed as
7 ecologically relevant, they are intended to provide sufficient control
8 over environmental conditions to permit better elucidation of
9 potential treatment effects.
10 Steve will now recap on the approach that the agency is going
11 to recommend to address the current uncertainties.
12 DR. BRADBURY: Following up on what Tom just indicated, a
13 written description of the risk hypothesis and, as the guidelines
14 recommend to the extent possible, try to visualize what the risk
15 hypothesis is in a conceptual model. And the white paper provided an
16 image similar to this.
17 Before we get started on talking about this conceptual model,
18 I'm reminded of a quote, I think it is by George Fox that says, all
19 models are wrong, but some are useful. And I'm really used to that
20 phrase. My research is more on quantitative structure activity
21 relationships. And we're always dealing with the fact that you're

1 trying to predict what you don't know.

2 So all models don't capture reality. But if used in the proper

3 context, they provide insight into how to move forward.

4 I think another way to view it and perhaps a less harsh way

5 would be to say that all models have limitations, but all can be useful

6 if placed in the proper context.

7 I think a lot of what we heard over the last few days and we'll

8 be discussing over the next day and a half will be the context of

9 models, be they biological models, be they experimental models, be

10 they representations of the field to try to capture what all of the

11 ecosystems in the country do, they are models. And they are models

12 used to try to help us find a path to move forward.

13 So in that spirit, a conceptual model is a working hypothesis,

14 but you have to start somewhere. You have to start somewhere.

15 So in our conceptual model, we're focusing on that top, for me,

16 the top left-hand corner of our conceptual model. That's the focus and

17 we're going to move forward. We have talked about the idea of

18 linking the potential molecular effects to the effects in the working

19 hypothesis in elevated E 2 (ph) to the concentration on or the focus

20 initially on gonadal effects in males which would then lead to the

21 issue of impaired fertility, reproductive and success and then to our

1 risk assessment endpoint.

2 As we discussed in the white paper, the analysis plan of moving

3 forward is a phased approach. Again, the idea that one doesn't have to

4 know everything to know something to then moved forward in a risk

5 management criteria.

6 This then lays out the first part of our analysis plan, which gets

7 at taking a look at the apical effects of gonadal development in

8 amphibians, provide the logical point to break through that logic train

9 that's in the conceptual model. Again, to go back to the earlier slide,

10 understanding combined with ecological relevancy.

11 This working hypothesis starts off with sort of posing the

12 question on ourselves, can we establish with greater confidence the

13 potential of atrazine to cause developmental effects, start to get a

14 handle on what the stressor response profile is for that effect as a

15 launching pad to either simultaneously look for ecological relevancy

16 as well as mechanistic.

17 That's what we'll be looking forward to hear, the panel's

18 discussion on the pathway of moving forward.

19 The phase one aspect of the analysis plan we feel is the most

20 important part or at least would hope that the panel would spend a fair

21 amount of time on what we're proposing as phase one. Or if you feel

1 there is a different phase that we should start on, of course we'll be
2 very happy to entertain that.
3 But at least from our perspective as a working hypothesis for
4 you all to test, our phase one component then is focusing on whether
5 or not atrazine exposure results in gonadal effects in males and
6 perhaps females, and again, to try to determine what the dose
7 response relationship is.
8 We have no preconceived notion of what a "right" dose
9 response relationship is. That's not the issue. The issue is what is the
10 dose response relationship and what kind of confidence do we have in
11 quantifying that dose response relationship.
12 In the context of the phase one studies, we indicated on
13 Tuesday morning a number of issues in terms of getting started. We
14 started a little bit of discussion on Tuesday morning about the choice
15 of the biological model to get started on. There probably will be a
16 number of biological models we can consider.
17 Again, all models have limitations, but all can be useful if
18 placed in the proper context, the context of understanding chemical
19 toxicity, the context of studying ecological relevancy.
20 There is probably not one model that can answer all our
21 questions. But what are the types of models we should use for the

1 different types of questions at hand.

2 We spent a lot of time as Tom summarized in looking at the

3 available information in terms of some standards that exist in the

4 scientific literature as a function of scientific bodies, in terms of

5 some of the basic conditions that are required to do aquatic

6 toxicology testing.

7 The history of aquatic toxicology is about 35 year old now.

8 Over 35 years there has been a lot of advancements in the technology

9 of how to do aquatic toxicology testing. There has been a lot of work

10 to describe what the conditions of organisms should be expected and

11 the terms of doing those tests.

12 And in this context, the American Society for Testing Material

13 has established standards, expectations of quality in terms of

14 undertaking an aquatic toxicology test, including test with

15 amphibians. We feel that's an important criteria to take into account

16 in terms of doing aquatic toxicology.

17 This will be one of the exciting parts of the discussion, because

18 the methodologies used in aquatic toxicology may not be the same

19 methodologies that are used in developmental biology and they may

20 not be the same methodologies that are used in field biology

21 protocols. We're going to have to weave that tapestry, weave those

1 threads together to try to create a vibrant textured tapestry.

2 Obviously, you know where we're coming from in terms of how

3 we think there is a way to approach maintaining appropriate quality in

4 the bioassays and combined with keeping track at the end that's going

5 to be required to test some of these hypotheses.

6 We obviously have a proposal on the table in terms of using a

7 flow-through technology to meet those ASTM standards. That doesn't

8 mean that all tests that could be done to answer these questions have

9 to be done with flow through. In our opinion, the most important

10 criteria is going to be whatever method is used, it has to meet ASTM

11 standards.

12 Whatever is needed then to answer the statistical power issues,

13 let it be what it will be, but ASTM standards we feel are important

14 because they are based on 35 years of aquatic technology and we're

15 somewhat nervous in backing away from what people have been

16 developing over the years of aquatic toxicology, to not belabor the

17 point, some of the data quality indicators that we discussed

18 previously and which I'm highlighting just in the last few minutes.

19 So with that, we'll wrap it up. Again, just to bring it back home

20 to the risk assessment issue at hand, is there sufficient information

21 available to describe the certainties? What are the certainties? What

1 are the uncertainties?

2 Risk managers can use that information to decide if we should

3 go ahead and do a quantitative risk assessment or if no risk

4 assessment needs to be done. Under the assumption there may be a

5 request for greater certainty in performing a risk assessment for

6 atrazine, we laid out an analysis plan to describe what we considered

7 would be important information to reduce the uncertainty.

8 We look forward to the dialogue with the panel.

9 DR. ROBERTS: Thank you, Dr. Bradbury. I think your

10 presentation and your comments by Dr. Steeger are very important to

11 help us refocus as we begin our deliberation of the questions posed by

12 the agency.

13 A considerable amount of effort has been taken to put the

14 available information regarding potential effects of atrazine on

15 amphibians before the panel. It is now time for the panel to go to

16 work and offer our best scientific evaluation and recommendations.

17 I would like to go ahead and take the first question and ask

18 either Dr. Bradbury or Dr. Steeger if they would pose the first

19 question to the panel.

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

21 DR. STEEGER: First question.

1 In reviewing the available laboratory and field studies, the
2 agency used a number of criteria to evaluate individual
3 investigations. Criteria such as experimental design, test protocols
4 and quality assurance information were used to ascertain the
5 reliability of the generated data in terms of its ability to adequately
6 assess the hypothesis that atrazine elicits developmental effects in
7 amphibians, and, if so, the nature and strength of associated dose
8 response relationships.
9 Then part one of a two-part question.
10 Does the SAP have any comments and recommendations
11 regarding the EPA's approach and criteria used to evaluate the
12 studies?
13 And secondly, given the evaluation criteria employed by the
14 agency, please comment on EPA's overall characterization of the
15 currently available studies.
16 DR. ROBERTS: Dr. Kelley, as lead discussant, I will ask you
17 to start out on this. I will leave it to you. Do you want to take both
18 parts together or do you want to have discussion on Part A and then
19 discuss Part B?
20 DR. KELLEY: What I thought I would do is to read a response
21 to the question that I have written after discussion with several but

1 not all panel members, and it includes both parts. And then ask the
2 panel for comments on what I have written so that it can be revised to
3 more accurately reflect the viewpoint of the panel.
4 Let me ask the panel to begin with whether that's an acceptable
5 procedure to them.
6 DR. ROBERTS: Sure.
7 DR. KELLEY: And let me just tell you. Feel free to disagree
8 violently. Not a problem.
9 DR. ROBERTS: I don't want them to disagree violently.
10 DR. KELLEY: Violently in the academic sense. That is to say
11 how could you possibly say that. That would be violent for an
12 academic.
13 DR. ROBERTS: Proceed then.
14 DR. KELLEY: Bear in mind we may amend this. All right?
15 We felt that the review was thorough and the conclusions were
16 appropriate, given the data reviewed by the EPA. And here I'm
17 referring to the seventeen studies.
18 We agreed that additional studies are warranted. There are
19 several studies supported by the registrant or by other agencies that
20 indicate that atrazine can cause developmental abnormalities. On the
21 other hand, a range of abnormalities are reported and they are not

1 consistent from study to study (bearing in mind that the literature
2 includes several anuran species.)
3 Further, though not considered in the white paper, the findings
4 are consistent with studies in other vertebrate, both aquatic (e.g. fish)
5 and terrestrial (some rodents.) Given the conservation of many basic
6 pathways for endocrine regulation, these studies are relevant to this
7 white paper focused on anurans.
8 Comments from the panel?
9 DR. ROBERTS: I have one and then I'll open it up to other
10 commenters. A asks regarding the approach in the criteria used by the
11 EPA to evaluate those studies.
12 And maybe I missed it. But did you basically agree with their
13 criteria?
14 DR. KELLEY: I thought -- yes. The review was thorough. I
15 think that's implicit. So let me add a phrase saying that the criteria
16 were appropriate.
17 DR. ROBERTS: Yes. If you believe so. I think we should
18 make that explicit in the response.
19 DR. KELLEY: I'll add that.
20 DR. ROBERTS: And then Dr. Coats. You had a comment.
21 DR. COATS: Yes. I'm a secondary discussant on this. I would

1 like to read my opinion which is certainly consistent with the
2 opinions expressed already by Dr. Kelley. The agency's
3 approach on criteria are valid and address a number of very important
4 points from the exposure side of the risk assessment, which is where
5 some of my concerns are.
6 Number 1, the data evaluation reports could include a
7 description of the analytical methods, that be ELISA or
8 chromatographic methods to enhance the completeness of the reports
9 and their interpretation.
10 Measured concentrations which are extremely important of the
11 chemical in the water need to be obtained and any potentially
12 bioactive metabolite should also be quantified as mentioned in the
13 white paper.
14 In some research papers, the exposure concentrations are
15 nominal with no measured concentrations provided or recovery
16 confirmed given, but no values.
17 The importance of having measured concentrations could be
18 addressed more directly in weighing the validity of the work.
19 Thirdly, the significance of the exposure method was pointed
20 out, including some of the shortcomings on the static renewal
21 systems. The ASTM standards for flow-through systems should be

1 followed and the method strongly encouraged.

2 Fourth, body burdens must be measured in the organisms to

3 reflect the degree of exposure and help explain the mode of action.

4 Once again, bioactive metabolites also should be included.

5 Determination of residues in specific tissues would also be very

6 valuable, but at the very least the whole body residues could confirm

7 the exposure.

8 On the hazard side of the risk assessment equation, it is critical

9 that the dose response relationships be shown as pointed out by the

10 agency. Even if it is an atypical inverted U shape, nonmonotonic

11 response, a dose response curve can be generated if appropriate

12 concentrations are tested.

13 This of course would help delineate safe versus unsafe

14 concentrations, but also could assist in elucidating mechanism of

15 action.

16 DR. ROBERTS: Dr. Coats, I think I heard three different kinds

17 of comments there. One was perhaps some deficiencies in reporting

18 among the existing studies.

19 Some were aspects, experimental aspects that perhaps should be

20 addressed in other studies. But the question really asks about the

21 EPA's characterization of currently available studies. And I think

1 some of your comments touched on that.

2 But I want to be sure that when we respond to this question that

3 we sort of don't mix other things, other points that we want to make in

4 with it. So I think it is going to be important that we give it as clear a

5 response as we can to be about things that you feel and other panel

6 members feel that any deficiencies in their characterization where

7 there are things that they didn't describe or that they inaccurately

8 described that perhaps could be tweaked in the document.

9 Dr. Kelley?

10 DR. KELLEY: I actually did in my written comments separate

11 out A and B, I see here. And so I did have an additional comment,

12 which is that the design of the experiments reviewed by the EPA and

13 the analysis of the data were flawed in many instances, which was the

14 EPA's conclusion.

15 And then this relates to the dose response -- if there is a

16 threshold effect for atrazine, that threshold is not firmly established.

17 With respect to dose response, any requirement that functions

18 be monotonic are clearly inappropriate. And as we've heard, I think

19 the EPA would agree with that.

20 So I think these were in discussions of the panel members

21 among the most important issues that came to mind, the threshold

1 dose for observing an effect if, in fact, an effect can be observed
2 strikes us as a very important piece of information to be gathered.
3 DR. ROBERTS: So you think that perhaps the report as the
4 EPA said should clarify that in their criteria to evaluate the studies
5 there was not an insistence on a monotonic dose response
6 relationship. Is that correct?
7 DR. KELLEY: And I think actually that it is just a wording
8 matter. I think use of the word threshold is useful here, unless it has
9 some technical meaning that escapes me. So threshold is the question
10 here.
11 And then of course it will have a shape, and we can't predict in
12 advance what shape that will be.
13 DR. ROBERTS: Dr. Green.
14 DR. GREEN: I'm the third discussant on that question. And
15 following up on what Dr. Coats just read to you, I think what we were
16 trying to convey is that we would like for the EPA to include in their
17 approach and criteria for evaluating the study the fact that so few
18 studies, if any, evaluated water levels and tissue levels in the animals.
19 And that should be emphasized in an EPA report describing
20 their approach and criteria and noting that so few studies did that with
21 any. That would be quite helpful.

1 DR. ROBERTS: Thank you.

2 Comments from other panel members?

3 Dr. Matsumura?

4 DR. MATSUMURA: I really appreciate what Dr. Kelley said

5 about the comparative aspect in comparing the action of atrazine to

6 other organisms, particularly in the vertebrates, so that we formulate

7 some idea what happens in other organisms. I think this document

8 could have more information on the other organisms. Some people

9 have pointed out too.

10 And regarding the question that Joel Coats raised, you are using

11 the water concentration of atrazine. In some setting, it is just crazy.

12 As Dr. Hayes pointed out, the next day when you come a

13 concentration may be quite different if it has rained the next day or

14 dried up.

15 So criterion that one should use really is the residue levels in

16 the frogs themselves, including metabolites.

17 So I know our chairman don't want to mix up with later

18 questions, but still other criterion we should include whether residues

19 can be found.

20 In the Reed study from Illinois, they were measuring the

21 residues. And really using the water concentration is not a really

1 good method. We have to identify the residues, including the
2 metabolites. Even if it is sort of ephemeral, still we have to do that.
3 DR. ROBERTS: Dr. Richards?
4 DR. RICHARDS: I believe that in a general way that the
5 agency's review of these is pretty much in line, particularly with
6 respect to the field studies. I think that they have done a fairly
7 reasonably qualitative look at availability of the studies and the data
8 and information that was provided.
9 DR. ROBERTS: Are there other criteria that you would like to
10 see added or articulated?
11 DR. RICHARDS: I think that we will address that in other
12 questions, specifically.
13 DR. ROBERTS: Okay. Dr. DeLorme?
14 DR. DELORME: I just wanted to concur that for the most part
15 the criteria they used were reasonable and that they are reflecting the
16 need for sound science and consistency in the science used to conduct
17 ecological risk assessments. That's their job. And they want to make
18 sure that the science that they are using and the studies that they are
19 using are sound.
20 With respect to the overall characterization of the studies, I
21 found it to be reasonable. Certainly, there were some minor points

1 that we might disagree with on specific studies, but the overall
2 characterization, I think they did a good job. They have identified
3 their concerns with the studies from a risk assessment and from a
4 scientific perspective.
5 They have also identified any conclusions or information or
6 contributions that they felt the study made. And they have also
7 identified uncertainties that result from the studies. And I think they
8 have done a reasonable job in doing that.
9 DR. ROBERTS: Dr. Skelly.
10 DR. SKELLY: Overall, I think the EPA did a good job of
11 characterizing the studies. And I just want to make a comment about
12 how studies were divided into categories.
13 The distinction between laboratory and field studies was made.
14 And in a couple places in the report, I don't know whether it is
15 referring to all of the studies that were submitted, but they were
16 characterized as experiments.
17 I just would like to point out that the field studies that are
18 included here include something called a microcosm experiment,
19 which, in my experience, is actually a mesocosm experiment. That's
20 probably just technical.
21 But mesocosm experiments are not field experiments. I think

1 that's an important distinction when we're thinking about this. And
2 most of the field studies were observational, which isn't a criticism,
3 but I think the criteria for evaluating a field observational study, a
4 field experiment, of which from what I can tell there are none here,
5 and a mesocosm experiment might be distinct.
6 DR. ROBERTS: So some of the terminology may need to be
7 tweaked a little bit in terms of how they are described or classified, is
8 that your recommendation?
9 DR. DELORME: So that the terminology might be tweaked and
10 also the criteria for evaluating things like the distinction between a
11 field observational study and a field experiment in terms of what sort
12 of expectations you have for variation and environmental conditions
13 could be different.
14 And I think we're going to talk about that a bit more when we
15 get to the next question.
16 DR. ROBERTS: Thank you. Just a heads up for Dr. Kelley --
17 DR. KELLEY: Would you like me to put that wording into this
18 first thing? I'll put it in. You can check it later.
19 DR. ROBERTS: Just as a heads up for Dr. Kelley as the lead
20 discussant, when we finish our discussion, I'm going to ask you to
21 sort of give me your sense of the capsule summary of this.

1 DR. KELLEY: Thank you.

2 DR. ROBERTS: Dr. Denver.

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

9 DR. ROBERTS: Dr. LeBlanc.

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

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

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

21 Concentrations, aqueous concentrations are important because

1 the bottom line is that's probably how the regulatory agency is going
2 to make decisions, based upon exposure concentrations and not true
3 doses that the animals get.
4 Throughout the document, there is discussion of dose response
5 relationships, and I would just advise that consideration be given
6 where appropriate to use the term concentration response relationship
7 and use dose response relationship where appropriate, but don't
8 interuse the two.
9 DR. ROBERTS: I agree. Any other comments? Dr. Delorme.
10 DR. DELORME: I just wanted to add something. Dr.
11 Matsumura and I believe Dr. Kelley indicated that one of the criteria
12 that they felt might have been addressed was inclusion of data on
13 other vertebrates.
14 I think that was -- especially as goes to ecological relevance, it
15 might be -- had some consideration.
16 DR. ROBERTS: Other comments?
17 I'm thinking about, trying to think about whether or not I agree.
18 I think their intent was to at least, I think what this statement is, to
19 summarize studies on a particular topic.
20 I think at some point you have to try and perhaps put that in
21 context, in broader context. I think at that point you would look for

1 analogy from other species.

2 But I'm not sure I would criticize this summary for not going --

3 making that broader look. I think it had a very specific purpose, not

4 to say those other comparisons might not be important in terms of

5 understanding potential effects, but I thought it was very well

6 focused.

7 Personally speaking, I was satisfied with it.

8 Any other comments? Dr. Gibbs.

9 DR. GIBBS: Just one quick question in terms of the

10 case-by-case treatment of all the studies. I felt that on the possibility

11 for type 2 statistical errors to be committed in all of the studies wasn't

12 very frequently considered.

13 I think the focus was on the statistical significance reported,

14 but I didn't -- and clearly EPA is aware of the problems with sample

15 sizes. But I felt many of the studies had serious issues with not being

16 able to detect effects should they have occurred. And I just felt

17 across studies that that consideration of type 2 errors wasn't

18 particularly prevalent.

19 DR. ROBERTS: Dr. Heeringa.

20 DR. HEERINGA: I just add my support to that comment. I

21 think -- as I reviewed these studies and the EPA's assessment and

1 review of them, I generally agree with the conclusions, but I also
2 support the last comment, too, that I think if we look at these and the
3 other contributing sources of error we have talked about, that many of
4 these studies are underpowered to detect the type of effects that we
5 are measuring.
6 In spite of some of the identified measurement, contamination
7 or quality problems, all of these contribute potentially to biases in
8 results, but there are other sources of variable errors that I think
9 really would lead us to assume that even given nominal sample sizes
10 that a lot of these studies are underpowered.
11 And a recommendation I'll make later on is that, if anything,
12 when we start into new studies, if they are conducted, that they be
13 overpowered to start with simply so we don't find ourself in this
14 quandary of being right on the edge of type 1 versus type 2 error
15 problems.
16 DR. ROBERTS: Any other comments or responses to 1 A and 1
17 B?
18 Dr. Kelley, I know you have been taking notes. To the extent
19 possible, can you try and summarize your discussion?
20 DR. KELLEY: Yes. I'm not totally up to speed on Dr. Skelly's
21 comments. But I will try to summarize them.

1 So in general, the panel felt that both the evaluation criteria
2 and the thoroughness of the EPA's white paper were appropriate.
3 The panel will get to the hypothesis in a moment. But the panel
4 did support the hypothesis that there was enough data out in the
5 literature given this evaluation to proceed with evaluating the
6 hypothesis that atrazine may contribute to developmental
7 abnormalities in amphibia.
8 Some concerns were raised by the panel with regard to the
9 evaluation, areas of emphasis that the panel felt should have been
10 weighed in more heavily than other areas. That includes possibility
11 of type 2 errors, which is, for the public, also my stat students always
12 get this confused, but the inability to detect effects that are really
13 there for whatever reason. And some of the possible reasons were
14 outlined here. So that was one area of concern.
15 Another area of concern was the ability to compare across
16 studies between nominal concentrations of application of atrazine and
17 actual tissue concentrations of atrazine.
18 And the third was that the characterization of the field data in
19 terms of what was actually obtained was felt by panel members to be,
20 how can I say, somewhat superficial, I think would probably be the
21 best.

1 DR. GREEN: Observational.

2 DR. KELLEY: Observational. Thank you.

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

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

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

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

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

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

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

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

1 DR. ROBERTS: Would anyone like to edit or make suggestions
2 regarding that?
3 Dr. Bradbury, is our response reasonably clear?
4 DR. BRADBURY: One question of clarification.
5 In interpreting or evaluating future studies, am I to understand
6 the consensus of the panel that future studies should have measured
7 aqueous concentrations of atrazine at a minimum and, depending upon
8 the nature of the hypothesis being tested in a given study, perhaps
9 tissue concentrations as well and perhaps activated metabolites?
10 DR. KELLEY: I think actually the panel might feel a bit more
11 strongly than that. It might feel that measured tissue concentrations
12 of atrazine is not a perhaps but should be a requirement going
13 forward.
14 But I don't want to speak for the panel as a whole.
15 DR. ROBERTS: Dr. Green.
16 DR. GREEN: I still think in the laboratory environment that
17 water concentrations of atrazine are important to know through the
18 experiment.
19 DR. ROBERTS: Others want to weigh in on this? Dr. Coats
20 and then Dr. LeBlanc.
21 DR. COATS: I want to reemphasize. I believe that the tissue

1 concentrations would be very helpful and water absolutely needs to be
2 measured.
3 DR. ROBERTS: Dr. LeBlanc.
4 DR. LEBLANC: It is my opinion that measuring aqueous
5 concentrations is mandatory and measuring tissue levels is desirable
6 based upon the questions being asked in a given experiment.
7 DR. ROBERTS: And speaking for myself, I would concur with
8 that as well.
9 Anyone else want to weigh in?
10 Are there any other follow-ups or clarifications?
11 Dr. Steeger.
12 DR. STEEGER: I would like to make one additional comment
13 for the benefit of many of the researchers.
14 Dr. Heeringa is correct that type 2 errors are a critical
15 consideration in the design of studies. Many of the researchers did
16 attempt to design their studies with that in mind. The data evaluation
17 records that were prepared didn't fully capture those efforts, but I
18 think that a genuine effort was made.
19 Unfortunately, as things progressed in the labs and things didn't
20 turn out as well as they had hoped, the study designs didn't support
21 the data in terms of controlling some of the variability that really got

1 out of hand.

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

3 that.

4 DR. HEERINGA: Thank you very much. I agree completely. I

5 didn't intend to say that, you know, forehand that there was

6 inappropriate planning in terms -- I think across these studies that

7 adequate planning went in.

8 But as you say, during the course of the studies as we learn

9 more about some of the measurement problems and some of the other

10 issues that came in, that clearly this caused those sort of a priori

11 expectations to be modified and in a way that my recommendation is

12 really that for the future that we can anticipate these things will

13 reoccur to varying degrees, and we may have even other unmeasured

14 sources of error in the experimental process.

15 And instead of sort of delaying the result of this, let's sort of

16 design with a margin of error on these type 2 error problems so that

17 we can accommodate them as they arise in laboratory and testing

18 situations.

19 DR. ROBERTS: Dr. LeBlanc.

20 DR. LEBLANC: I would just like to revisit aqueous

21 concentrations again for a moment. Certainly, in many of the studies

1 that I have read, it appeared to me that concentrations of atrazine
2 were measured only in solutions that were freshly prepared.
3 And certainly depending upon the delivery system that's
4 selected and the design of the experiments, ultimately, I think
5 consideration should be given to make sure that concentrations are
6 measured at times at which you would expect high levels of atrazine,
7 but also the lower levels that might be resulting in these treatments.
8 DR. ROBERTS: Any other comments or points to add on 1 A
9 and 1 B? We also had a 1 C.
10 DR. BRADBURY: We have a 1 C and a 1 D.
11 DR. ROBERTS: I don't have a D on mine.
12 DR. STEEGER: Actually, it's pretty much like 1 C.
13 1 C is, Please comment on the availability, as of February 28th,
14 2003, of additional relevant studies in the open literature that were
15 not addressed in the white paper.
16 And 1 D is, Since February 28th, 2003, is the panel aware of
17 any studies that would be relevant?
18 DR. ROBERTS: We have both of those. The second one, at
19 least on mine, was not separately marked as D.
20 Let me then go back to Dr. Kelley.
21 DR. KELLEY: Yes. So the review of the panel, while very

1 thorough and complete, was narrow in its focus. So the question is
2 what kind of guidance would the EPA like from us about what we
3 consider to be the relevant literature.
4 Let me tell you what I have in mind. So omitted from this were
5 several studies that I found in the open literature on effects of
6 atrazine, usually at very high doses on early mortality, say, in
7 xenopus, tadpoles and some more recent effects on slightly later
8 animals. So those were omitted.
9 The EPA analysis raised issues of consistency in the data and
10 consistency of things like the stages of sexual differentiation. Yet,
11 of course, there were none of the background papers on sexual
12 differentiation in that panel. So those are two areas.
13 And the third is of course the issue that the panel has raised
14 that are relevant to things like the mechanism of action of atrazine if
15 it, in fact, has an effect that might be addressed productively by
16 references that included work on other than amphibians.
17 So I'm in the midst of collecting a series of papers of that sort.
18 But what I would like to know -- but I have to say I didn't find a paper
19 that you guys had left out on effect on gonadal development in
20 amphibia due to atrazine. I haven't found such a thing yet.
21 So I do believe that in terms of the focus of review that you

1 didn't omit any obvious and glaring paper. But in view of the panels's
2 desire to provide scientific guidance to the process going forward, I
3 think that there are probably a number of studies that we would like to
4 bring to your attention.
5 DR. ROBERTS: Other panel members?
6 Dr. Matsumura.
7 DR. MATSUMURA: I would like to ask the panel to consider
8 to add the paper by Bevan appeared in the EHS regarding action of
9 alkylphenols. Of course it is not the atrazine, but this particular
10 paper really shows what the estrogenic effect of the alkylphenol
11 really look like.
12 It is summarizing their work on some of the early stages of
13 development, and the curvature that develop in the tadpoles are very
14 clear on the melanocyte site formation. You can see what real
15 estrogenic compounds could look like.
16 This is the one endpoint studied in the xenopus. And it is a
17 very clear cut effect that you expect.
18 And knowing that alkylphenols, particularly in nonylphenol can
19 be found in many detergents, including the triatonix 100 and all those
20 agents that you use in the lab washing your cages as well as in the
21 formulation in some of the pesticides.

1 We should really show that this is a kind of thing, if it is a
2 direct effect on the estrogen receptor, this is what you expect. So I
3 would like to suggest that.
4 I also would like to suggest that the Christin's papers that Dr.
5 Green mentioned too and, yes, we are looking very narrow way and
6 there are other effects of the atrazine on the immuno competency. So
7 that I would like to suggest.
8 DR. ROBERTS: Dr. Isom.
9 DR. ISOM: I would just like to point out for the record that the
10 Hayes paper in EHP came out in April 2003. It has been referred to
11 several times throughout the proceedings. And that is actually a
12 detailed publication of the nature paper that appeared this past year.
13 DR. ROBERTS: Dr. Steeger.
14 DR. STEEGER: The requirement for the review is that studies
15 had to be submitted by February 28th. You are correct that the
16 published hard copy of that report came out in EHP in April.
17 However, the online version was indeed published or available
18 in October. And that was the version that was reviewed for the white
19 paper.
20 DR. ROBERTS: I think Dr. Isom's response is of 1 D.
21 DR. STEEGER: That April publication is identical to the

1 October one.

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

3 What sorts of guidance would you like to have? Clearly, we can

4 give guidance going forward. I still see no -- within the very narrow

5 focus of what you reviewed, I don't think you missed anything, but we

6 have compiled a bibliography that we thought would be helpful.

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

8 DR. BRADBURY: This is sort of an awkward question to

9 answer because it presumes the path the panel may or may not go

10 down.

11 Hypothetical. If the panel is thinking about a pathway about

12 gathering additional information, my sense is that some of the

13 information you are talking about would be very instructive in

14 thinking about the design and nature and aspects of other information

15 that would provide greater insight either to do a risk assessment today

16 or insights into what kind of information and pathways to gather new

17 information would be helpful.

18 So my feeling is that, yes, it would be very helpful is more in

19 the context of the proceedings --

20 DR. KELLEY: But what about my question about the omission

21 of acute toxic effects of atrazine on very early tadpoles from the

1 bibliography?

2 DR. BRADBURY: I'll turn it over to Tom some, but in looking

3 at gonadal developmental effects and I think a sense of what those

4 exposure concentrations were sort of kept us focused in that context.

5 Putting it all into the context of full dose response and full effects

6 endpoints, that could be helpful in the white paper now because it

7 provides some founding in terms of effects of exposures.

8 DR. STEEGER: From my perspective as a scientist, I would

9 very much appreciate to have access to that information to improve

10 our understanding and representation of the effects of atrazine.

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

12 DR. ROBERTS: Right.

13 DR. KELLEY: I bring this up because, of course, mortality was

14 an endpoint that was studied in all these studies.

15 And even though the doses in these acute studies were much

16 higher and the mortality was much faster, still, in all, that's the

17 compound that you are interested in and the species that you are

18 interested in.

19 So their exclusion seems to me inappropriate even if they are

20 not directly relevant to gonadal development because they die before

21 they had any gonad.

1 DR. ROBERTS: Perhaps the panel could recommend that they
2 be included if for no other reason to help provide a perspective for the
3 doses at which developmental effects may or may not occur.
4 For suggestions on other papers, I think we should clearly
5 identify that if the agency is interested in studies that do not relate
6 specifically to gonadal development but may in fact inform -- by
7 analogy help understand whether or not a potential phenomenon
8 occurred, here are some examples that we can point out so that it's
9 very clear that we don't necessarily consider them omissions for the
10 focused purpose of this paper, but they may be useful for the
11 ecological risk assessment.
12 DR. KELLEY: Let me tell you how I would like to proceed.
13 What I would like to do is to complete my list of additional sources of
14 information that would be helpful categorizing the papers as we have
15 just discussed under different categories, studies and other species,
16 acute effects on toxicity, studies that might be useful if a risk
17 assessment goes forward and why they might be useful.
18 And I will compile that list over lunch, and I will get it printed
19 up and I will distribute it, and then I will allow -- of course I will
20 allow -- the panel will make me make sure that we haven't missed
21 studies that individual panel members have discovered. And then at

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

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

3 DR. ROBERTS: Thank you. Other comments?

4 We're coming right up on 12 o'clock. I'm suspecting that -- I'm

5 looking at Dr. Richards as the lead discussant on Number 2. I suspect

6 we're probably going to want a little time to discuss responses for

7 Number 2.

8 So let's go ahead and break now for lunch. And we can come

9 back replenished and restored at, say, 1 o'clock and take up Question

10 Number 2.

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

12 (Thereupon, a luncheon recess was taken.)

13 DR. ROBERTS: Before we take up Question Number 2, our

14 designated federal official, Mr. Paul Lewis, has an announcement.

15 MR. LEWIS: Thank you, Dr. Roberts.

16 The panel were distributed additional comment from Syngenta

17 in reference to clarifying some of their remarks at yesterday's

18 meeting. It is available to the panel and will also be available in the

19 public docket for public review and inspection. Thank you.

20 DR. ROBERTS: Thank you, Paul.

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

1 DR. STEEGER: In its evaluation of the existing field studies,
2 the agency has concluded that these investigations are of limited
3 value.
4 The reasons include, one, the high variability in environmental
5 conditions and uncertainties in the preexisting status and condition of
6 field collected animals.
7 Two, the spatial and temporal aspects of atrazine exposure, i.e.,
8 spatial and temporal variability over the course of the studies and the
9 extent to which such aspects of atrazine exposure were empirically
10 measured or otherwise accounted for.
11 And three, the possible cooccurence of additional chemicals
12 and/or non chemical stressors.
13 Question 2 A is, to the extent that the field studies appear to
14 indicate that atrazine may not adversely affect development, please
15 comment on EPA's conclusion that the body of data from the field
16 studies does not provide the means to ascertain whether the lack of a
17 relationship between atrazine exposure and developmental effects is
18 due to the absence of a causal relationship or limitation in study
19 methodologies.
20 DR. ROBERTS: Dr. Richards is our lead discussant on this
21 question. I will ask Dr. Richards to begin our response.

1 DR. RICHARDS: Thank you. I'm going to make some general
2 comments on a number of different aspects of this question. I think I
3 will tend to treat A and B together, although, will certainly address
4 both parts of that question.
5 And I invite the second and third readers on this question to
6 jump in at any point.
7 In essence, the field studies that have been presented in the
8 literature and the presentations over the last couple days are very
9 good.
10 In one respect, they provide a great deal of information. I think
11 one of the most important things that they seem to indicate is that the
12 abnormalities, gonadal abnormalities that appeared in some cases in
13 laboratory experiments are seen in the field.
14 These abnormalities sometimes are seen in very different
15 geographic regions to some degree among different species.
16 And from that standpoint, I think that's critical, because I think
17 all these questions ultimately relate to an ecological role and a
18 population role. And without looking at sort of natural field type of
19 situations, we can't really see whether some of the things that we're
20 looking at are relevant.
21 Now, however, the studies do not seem to provide substance to

1 the nature of why these gonadal abnormalities or other endpoints are
2 related to atrazine.
3 And that's where it breaks down to problems in experimental
4 design. Often times, the experiments were never really designed to
5 ask such questions. Typically, they have very poor statistical power to
6 address such of a question.
7 Most of them are essentially descriptional studies.
8 Descriptional studies or observational studies can be very good. They
9 can be powerful. They can give you relationships. But they require
10 that a number of parameters be accounted for in order to derive any
11 use from them.
12 In the studies that have been set up to use a control or a
13 reference situation, those control and reference situations have
14 usually not been adequately described or they have some fundamental
15 flaws. And the sample sizes used, the end size used is typically
16 totally inadequate given the variability and some of the measures that
17 we're looking at.
18 The studies that have been descriptional in attempting to use
19 more of aggression based approach have not adequately dealt with the
20 problems of scale.
21 The studies we have looked at have included scales including

1 looking at continental scales, looking at the size of a county,
2 comparing a few ponds within a small area.
3 All of those have very significant things that have to be
4 accounted for in order to account for the variance associated with
5 individual assessment endpoints.
6 And I will just begin to list a few of those things. But the
7 Number 1 thing that we're dealing with here is aquatic organisms,
8 organisms that have a very significant part, at least one part of their
9 last cycle tied to the aquatic medium.
10 In order to do a descriptional study or to set up a good field
11 experiment, you have to adequately account for the movement of
12 water in and out of those systems.
13 A small ditch is very different than a river, is very different
14 than a wetland. It's very different than a backwater on a river.
15 So it comes back to both describing the environment that the
16 animal lives in, but very much describing in a general way or a
17 specific way the exposure that these animals actually encounter in the
18 field.
19 And for small experimental studies, that needs to be accounted
20 for and in much detail. But on the larger, more descriptive studies,
21 that can be accounted for in a much better way than has even been

1 attempted in any of these studies.

2 There are many tools available that allow us to look at even

3 relatively large geographic scale studies that can quantify and allow

4 you to partition some of that variance in a stratified experimental

5 design.

6 And basically, that's relating to how does surface water interact

7 with the bodies of water that these experiments are being conducted in

8 or observations are being made, what's a relative contribution of

9 groundwater, what are flow events during a period of exposure, what

10 are the flow events that actually occur amongst seasons, in cases

11 where experiments have been run, have there been flooding events

12 that may influence exposure or may influence the movement of

13 organisms in or out of those experimental situations.

14 And also related to hydrology is -- to have an adequate

15 depiction of hydrology, you have to know what the watershed is. You

16 have to know what is upstream of the event that you are observing.

17 And there are some fairly easy, relatively powerful ways to

18 describe watersheds both in a strictly hydrologic sense, but also in

19 terms of what the watershed is composed of.

20 Things like soil characteristics and hydrology connectivity to

21 other water bodies, these are all things that are fundamental both to

1 the biology and movement of these creatures, as well as the chemical
2 and physical environments that the animals live in.
3 Basically, some of the studies have provided that type of
4 information perhaps in an appendix or listing of things they may or
5 may not have measured. Some of them did not provide any of that
6 information.
7 But essentially, none of the studies attempted to use that as a
8 co-correlate in any way to try to partition variance amongst characters
9 that again provide variation in these populations that have been
10 observed.
11 So in essence, I think what we're looking at is that statistical
12 designs have not been sufficient to actually address questions
13 specifically related to atrazine.
14 And I think that that falls into the B part of this question, also,
15 is that there are co-stressors, potential co-stressors. Many of those
16 we can guess, many of those that we could potentially stratify out in
17 an appropriate statistical design or to partition into in the analysis,
18 but that takes a great deal of forethought in terms of development of a
19 design.
20 In many cases, had we seen as much attention given to sound
21 experimental design as we have at least to date with some of the

1 laboratory measures, I think we could have been a little bit further
2 forward.
3 I think I'll ask my co-readers to jump in after that.
4 DR. ROBERTS: Dr. Gibbs, you are an associate discussant. Is
5 there anything you want to add? Or do you want me to take Dr. Skelly
6 first?
7 DR. SKELLY: First of all, I would like to point out that that's a
8 single sentence up there, and so it took me a while to figure out what
9 it means, but I think I got a handle on it.
10 I guess the end of the sentence is the most critical part. So do
11 we think that the absence of causal relationship or limitation and
12 study methodologies is the reason why the EPA's conclusion that field
13 studies don't provide the means to ascertain whether there is
14 something going on here.
15 I guess in my opinion, I'm going to agree with Dr. Richards and
16 say that the limitation and study methodologies is probably most
17 important here, and comment that the absence of causal relationship
18 has never slowed down any ecologist I have ever met.
19 We often like to pretend that we know why things happen when
20 we're working in the field, but that shouldn't stop us from moving
21 forward and trying to understand what the associations are.

1 Because after all, everything that we're doing is keeping our
2 fingers crossed that we get the mechanism right. Even in the finest
3 laboratory, using the best laboratory practices, we can never know for
4 sure that we have the mechanism right.
5 So specifically, I think the limitations and study methodology
6 that were most important concur largely with what Dr. Richards said.
7 I think the lack of power is an issue, but it is very easy to be critical
8 of that in hindsight.
9 These studies that have been done can now be used moving
10 forward to come up with better ideas with what needs to happen.
11 Having said that, the scale of the field studies and I say this
12 from the standpoint of having quite a bit of experience in field
13 sampling amphibian population, these were not large studies in terms
14 of the number of sites that were examined.
15 There are many more ambitious studies and I think we can look
16 to a lesson from how the UV light on amphibians controversy is being
17 resolved now.
18 It has taken very large scale studies looking at hundreds of
19 wetlands over very large areas dealing with many of the same issues
20 that we have been talking about here today.
21 Some of the people in this room have been involved in this

1 work, so they know what it takes to pull inference out of these sorts
2 of big observational studies.
3 That leads me to the second point, which is the issue of site
4 selection. I think it is hard to go out and use a map ahead of time or
5 just go out and look at a place and say, there is a cornfield here or
6 there isn't a cornfield here, to say whether this is the right site to be
7 an exposure site or a control site.
8 And some of the studies that were not considered here and are
9 not necessarily particularly relevant to this issue but where people
10 have taken an alternative approach, they might take a year or two
11 years just to pick their sites.
12 I'm going to come back to this point when we talk about some
13 of the questions later on. I'll leave that for now.
14 The final point, the limitation in the study methodologies may
15 be this is not a limitation study methodology, but a significant
16 limitation in the studies that I saw was to me looking at what the EPA
17 is going for here, the assessment endpoints that are talked about are
18 fertility reproduction recruitment.
19 Ultimately, what we are trying to think about -- something
20 about viability of populations and those people in this room who
21 know about measuring population viability know what a giant task

1 that is.

2 Even if we go back to the assessment endpoints here the

3 motivation for doing field studies should be to get at those things. I

4 don't think most of the field studies that have been done to date

5 address that issue. In moving forward that's something that should be

6 tackled very concretely.

7 In response to B, to the extent that we do see something going

8 on here, is it true that these studies don't provide sufficient

9 information to resolve the potential role of additional cooccuring

10 stressors?

11 I guess that is always, always going to be true to some extent

12 with the field study, but for reasons that we'll probably talk about

13 later on I still think it is extremely important for much of this work to

14 be done in a context, in a field context.

15 To that end, as I have harped on before and I will say now,

16 virtually all the field work that is talked about here and if we exclude

17 the mesacosm experiment all of it has been observational.

18 It is possible even in this difficult kind of context of working

19 with pesticides to do field experiments. Especially -- we've got a

20 registered pesticide here, we can use it in the world out there and see

21 what it does at population scales.

1 That can help us significantly get at the issue of co-occurring
2 factors because we can choose sites that are sort of tabularasas to
3 begin with and add our stressor that we're interested in.
4 I'm going to echo -- in finishing, I will echo what Dr. Richards
5 said. That is that, I think, the critical thing here that we can't ignore
6 is we can talk about limitations of any study we want all day long,
7 multiple groups have gone out and looked for gonadal abnormalities
8 in nature and they have found them and these match the morphological
9 characters people are seeing in the lab.
10 At this point, I think it is fair to say we don't know why, but the
11 fact that those abnormalities exist I think is significant. I think if a
12 bunch of groups had gone out there and had not found them we would
13 be thinking about this differently. I will end there and pass to Dr.
14 Gibbs.
15 DR. ROBERTS: Dr. Gibbs.
16 DR. GIBBS: We have spoken extensively as a group, so I don't
17 want to reiterate too many points. I do personally agree with the
18 statement that essentially study designs have precluded really
19 determining whether there is or there is not an association between
20 the occurrence of gonadal abnormalities in the field populations of
21 amphibians in the presence or absence of levels of atrazine.

1 We don't want to imply that observational studies cannot
2 address these issues. I think they are actually very important for the
3 reason that Dr. Skelly just mentioned.
4 The risk assessment goal here really does focus on population
5 viability. One needs to go to the field essentially to measure many of
6 these parameters.
7 I would -- perhaps this is getting away from the question, but
8 there are other modes of studying and tackling these problems which
9 essentially fall in the realm of field experimentation.
10 We have been discussing some other situations that occur in the
11 field with temporary breeding -- temporary pool breeding amphibians
12 such as the amphibians that use vernal pools.
13 Vernal pools occur in isolated, small, populations in great
14 numbers in homogenous landscapes that are really quite amenable to
15 experimental manipulations that could get at both -- would yield
16 information both from histological perspective but also from
17 demographic perspective quite relevant to many of the issues that the
18 risk managers -- risk assessment folks have to deal with. I
19 think that's worth exploring at some point. But to get back to the point
20 at hand, I agree broadly with many of the statements that Dr. Richards
21 raised, issues with inappropriate or not outlining sampling frames,

1 low levels of replication in terms of using wetlands as actually
2 replicates and the amphibians therein.
3 And really a difficulty in establishing whether there is or is not
4 causal relationship here between what are some fairly high levels of
5 abnormalities being seen in the field, but whether or not there is a
6 relationship with atrazine. In my mind that can't be attributed based
7 on the designs of many of these studies.
8 DR. ROBERTS: Thank you, Dr. Gibbs. Let me ask other
9 members of the panel if they agree or disagree?
10 DR. MATSUMURA: I agree with Dr. Skelly and Dr. Richards.
11 I certainly emphasize the importance of the field studies.
12 I was trying to think what the precedence I could think of, and,
13 of course, like Gillette's work on the alligators and how long did it
14 take, and what started that whole project, and like finding a really
15 contaminated site like Lake Apopka was very important for that study.
16 And comes from field studies like eggshell thinning, it took a long,
17 long time to say it was DDT.
18 But again, field observation that all those eggs are crashing and
19 that's a problem and the bald eagles not just thinning but by the
20 weight of their mothers just sitting on those eggs are crashed. Those
21 observations really helped.

1 And finally, I would like to make sure that one aspect to
2 emphasize, that means analysis of burden of other contaminants. I
3 was reading this Reeder's paper reporting this intersex confined to
4 the -- correlated to atrazine. But at the same time they had the PCDD
5 and PCDF.
6 And so you have to leave some questions always. Knowing
7 what the other stressors and other chemicals are there, that could
8 really help.
9 DR. ROBERTS: Dr. Green.
10 DR. GREEN: I agree with your comments. For my own
11 edification, I would like to know from your experience and
12 perspective what denotes a healthy amphibian population
13 observationally in a field study? What implies that the
14 population is sickly and on the decline, absolute head counts and how
15 long does it take to get that kind of information?
16 DR. GIBBS: Insofar as you linked health to decline, you are
17 talking about a temporal phenomenon. That may indicate the need for
18 repeated sampling to actually detect a change in population size,
19 population structure.
20 With these particular organisms, some of them have -- with
21 frogs, incredibly short life spans and then generation times and hence

1 population turnovers.

2 So one can fairly quickly, unlike working with many other

3 vertebrates, see if there actually are population declines or increases

4 occurring because you need much less time with generation times and

5 sometimes one to two to three years to see these kinds of effects.

6 Sheer abundance is one good measurement. Dr. Skelly may

7 have other ideas.

8 DR. SKELLY: I would agree with what Dr. Gibbs just said. I

9 would just add that we have settled on a group here that has

10 notoriously volatile population dynamics and going out in a given

11 year and seeing recruitment failure for some species is actually the

12 norm.

13 Having total cohort failure is -- if we were talking about grizzly

14 bears in Yellowstone people might get quite upset about that, but

15 most of the wood frog populations I survey every year in most years

16 crash out.

17 This year they are floating across the roadways and they are

18 doing quite well with all the rain. In most years they dry up with

19 their pond. It definitely is something you have to look at over time.

20 We're fortunate many of the species that we would be interested

21 in in terms of native amphibians do have short life spans. It does

1 have to be looked at over time if we're interested in any sort of
2 understanding of population robustness.
3 DR. ROBERTS: There's that word again. Dr. Delorme, then
4 Dr. Richards.
5 DR. DELORME: Just following up on Dr. Green's question.
6 Could you give us any indication of some measurement endpoints or
7 what variables you might be looking for to look for effects in
8 populations if you are out there with your rubber boots?
9 DR. SKELLY: Population --
10 DR. GREEN: I think that will be addressed in question five.
11 We have some estrogenic biomarkers we put together that --
12 DR. DELORME: I was asking from a populations perspective,
13 like what population variables might you go out and measure.
14 DR. ROBERTS: Were you planning on bringing it up later in
15 the context of five or do you want --
16 DR. SKELLY: I actually think we will address that later.
17 DR. ROBERTS: Dr. Richards.
18 DR. RICHARDS: Another context sort of thing is amphibian
19 population is often very variable that is the way they are. That is the
20 nature of the waterbodies they live in, to get back to the hydrology of
21 these situations.

1 One of the things that we know about amphibians is that they
2 interact over a relatively large geographic scale. You just can't look
3 at a pond the size of the space between our tables here, you have to be
4 cognizant of the larger network of waterbodies that they are
5 connected to.
6 Because when one local population disappears they are
7 colonized by another population that may be half a kilometer away.
8 Looking at a series of small ditches that are by nature connected is
9 very important as opposed to an isolated pool.
10 Are you looking at a species like the South African animal that
11 lives entirely, its whole life cycle in water versus something like the
12 rana species that are out moving around?
13 These are very critical questions. Both designing the
14 experiment and asking questions about is a population healthy in part
15 it is related to a little bit larger scale than we frequently focus on.
16 DR. ROBERTS: Dr. Skelly has a follow up, then Dr. LeBlanc.
17 DR. SKELLY: I guess as a follow up to that, we're painting a
18 picture that can seem to get more and more difficult.
19 Actually, the genetic techniques that are being developed now are
20 getting us to the point were we can actually distinguish the
21 boundaries of populations. Up until recently, people just kind of

1 crossed their fingers and assumed a pond was a population. I
2 think we're moving well beyond that. So we are actually starting to
3 understand in a much more detailed way how much movement there is
4 and over what scales we should be assessing population level
5 responses in order to say whether a population is going to remain
6 there or is it likely to go extinct.
7 DR. ROBERTS: Dr. LeBlanc.
8 DR. LEBLANC: This question is posed to the discussants.
9 Based on what I just heard, my question is would it be -- due to the
10 volatile nature of the population dynamics of frogs would it be
11 difficult to discern population level effects of environmental
12 contaminants?
13 DR. RICHARDS: Yes and no. I think sometimes with some of
14 these measures dropping down out of the sky on one occasion and
15 listening for how many frog calls there are you are not going get a
16 real good answer. I think because of the fluctuating dynamics
17 of these populations in their actual environment some manner of
18 looking at more of a prolonged view of the population is needed.
19 There are some things as genetics that give us a backward view
20 of what is going on there, and that's great, but there is much room for
21 innovation on this. The book is not completely written.

1 DR. ROBERTS: I think Dr. Skelly wants to respond as well.

2 DR. SKELLY: I guess, I would say in order to make the picture
3 a little less bleak here, there are very volatile dynamics. But if you
4 follow a set of populations in a defined area over a long period of
5 time, you see this volatility from year to year.

6 You also see very striking patterns. I will give you an example
7 that is directly relevant to the sort of thing we're considering here.

8 For many, many years people have been doing experiments in
9 mesacosms in the laboratory that were showing that amphibians
10 undergo interspecific competition and that interspecific competition
11 could even be possible for population extinction.

12 That was the way -- that was the inference that was coming out
13 of this work. There is excellent laboratory and mesacosm based
14 evidence that there was competition going on, and if we change the
15 words, that's exactly what we have been talking about here, the same
16 sort of thing where people were doing these very focused studies, they
17 were getting mechanistic, they were looking for interference
18 compounds, they were looking for resource based competition, and so
19 forth.

20 We have been able to show more recently that from everything
21 we can tell even though this is an actor in these experimental context,

1 it does not seem to be important for the kinds of patterns that the EPA
2 is interested in for assessment endpoints and for their overall criteria.
3
4 Instead we have been able to discover starting with field
5 observations, then moving to field experiments, and then into
6 laboratory experiments, that other factors such as hydroperiod and
7 such as the light level in these ponds that influences temperature and
8 food gradients is much more important.
9 That's been a real lesson for me in how we discover what is
10 really going on, how to parse this out from the perspective of a field
11 scientist so that the lab scientists are working on the right stuff so
12 they are not just going down the garden path.
13 DR. ROBERTS: Dr. Isom.
14 DR. ISOM: Since atrazine has been applied to the environment
15 for over 40 years in North America, is there historical population data
16 that perhaps could be looked at to give us some answers to these
17 longer term population issues?
18 DR. SKELLY: There has been -- because of the overall interest
19 in amphibian declines, everything that existed and much work over
20 the last 12 years or so has been done along those lines.
21 We probably for the purposes of this panel can parasitize a

1 much larger database than you might believe. There is much known
2 on that. The problem of course is going to be attributing cause to
3 these historical studies where the other measurements that we are
4 interested in were not made.
5 I can give you as an example, there were surveys done in the
6 early part of this century in Iowa that describe high abundances of
7 amphibians over many, many counties. When the resurvey was done
8 in 1980's, abundances and species composition over large areas had
9 changed.
10 Now that's over the same time span as the green revolution.
11 Does it have anything to do with that, does it have anything to do with
12 pesticides? We have absolutely no idea.
13 In general, the consensus seems to be now that many, many
14 amphibian populations are declining. So that's the state of our
15 knowledge, the state of our sciences that many populations have
16 declined.
17 When people go out in their back yards -- and I give lots of
18 lectures to the public and the first question I get from the public,
19 which is an excellent one, is I can be deafened sitting on the back
20 porch by the spring peepers. So are amphibians declining?
21 What I say to them is because this is a temporal phenomenon

1 maybe you would have gone deaf in one week instead of two, 100
2 years ago. We don't know what it was -- we don't have enough data to
3 be able to say over very broad scales what amphibian abundances
4 were like.
5 But Dr. Gibbs maybe can answer this a little bit better than I
6 can. One of the things that has happened over that same time period
7 is that wetlands have disappeared or been modified, often in an
8 agricultural context, in ways that change their suitability for different
9 amphibian species over the same time span.
10 There has been talk about multiple co-occurring factors. It is
11 there. I think the most interesting historical data that we could get
12 are in the animals. So this issue of evolved resistance could be really
13 important in showing or in evaluating whether there have been broad
14 scale evolutionary responses to pesticides.
15 DR. ROBERTS: Dr. Gibbs, did you want to respond also?
16 DR. GIBBS: Just quickly, amphibian monitoring in a
17 systematic and large scale fashion only has begun since 1990 or so
18 with various initiatives such as the USGS and other groups have put
19 together. There really is not a lot of data, even that effort is limping
20 along.
21 Unfortunately, we are constrained and we will remain so for

1 quite a few more decades before we can get something like the US --
2 the breeding bird survey on line for amphibians, that's the goal. We
3 really don't have any good solid baselines over large areas. And a
4 decade of data on a few spots is about as good as we can do.
5 This underlies a lot of the controversies and debates over
6 amphibian decline this lack of monitoring data. Unfortunately, that is
7 the state of the science on amphibian monitoring.
8 DR. ROBERTS: Dr. Richards.
9 DR. RICHARDS: I would like to hopefully dispel a little bit of
10 the gloom here that I myself have been raising.
11 At one level amphibians are no different than any other aquatic
12 organisms that are dealt with by EPA and aquatic ecologists for a long
13 time.
14 They still reproduce, they still have fecundity, they still recruit
15 to a population, there is predation, there is density and nondensity
16 dependent aspects to their population sizes, and that makes them
17 particularly amenable to experimentation. That doesn't mean
18 we can't experiment with these animals and can't derive some creative
19 ways of examining population parameters.
20 They do things just like when you are chasing little fish along
21 the margins of a stream. If you have ever done that you know you can

1 go there one time and they will be there, next time they won't.
2 That doesn't keep us from having a great body of regulatory
3 approaches to dealing with toxic antieffects on fish.
4 DR. ROBERTS: Dr. Delorme, did you want to respond to that
5 as well? I have a couple of other folks who have questions.
6 DR. DELORME: I wanted to make a comment on an
7 observation. I can wait.
8 DR. ROBERTS: Let me go to Dr. Denver, then Dr. Heeringa,
9 and then you.
10 DR. DENVER: I just wanted to comment on something that Dr.
11 Skelly said. It has to do with single species experiments versus
12 competitive interactions, and at the risk of making this even more
13 complicated I think it is important to realize the importance of the
14 competitive interactions. Most of the studies that we have
15 reviewed have been studies of single species. We published a study
16 actually last year that showed that exposure to PCBs can actually
17 change the competitive interactions among species.
18 These were studying the northern leopard frog and also wood
19 frogs. That's another layer of complexity that needs to be considered.
20 DR. ROBERTS: Dr. Heeringa.
21 DR. HEERINGA: With much more expertise, Dr. Denver

1 anticipated my question. Mine is much more direct and I deal a little
2 bit with waterfowl populations.
3 Is there evidence, either from zoological samples in museums,
4 et cetera, or other studies, on sort of either a lack of increased
5 hybridization or competition in these natural populations?
6 Are there species that are invading historically, turf for other
7 species that have been a concern to ecologists, is that practice, it
8 could happen naturally, but do you see that happening and are these
9 populations still relatively isolated and nonhybridized?
10 DR. GIBBS: Certainly, and the bullfrog is a classic example of
11 invasions, but very much associated with the activities of people and
12 moving them around.
13 I'm trying to think of an analogous situation with a hybrid zone
14 shifting or one native species, a sympatric species, one moving into
15 the other's range. I'm failing to --
16 DR. SKELLY: The example that Dr. Hayes talked about that
17 was the first one that I had seen evidence for.
18 DR. ROBERTS: We've got Dr. Delorme, then Dr. Matsumura.
19 DR. DELORME: I was sitting here listening to Dr. Skelly talk
20 in response to Gary Isom's question and it occurred to me that one of
21 the things that EPA has asked us to look at is the assessment

1 endpoints and they put forth an assessment endpoint of the
2 population.
3 You guys can shoot me down if you want but some of the
4 measures that you are talking about, species diversity and relative
5 species abundance, perhaps maybe an alternate assessment endpoint is
6 also communities, in terms of their composition.
7 It gets into sensitivity of different species and whether it is
8 different or not. Just want to know if you guys think this idea has any
9 merit as alternate assessment endpoint?
10 DR. ROBERTS: You three are on the spot again. Who wants to
11 take it?
12 DR. RICHARDS: I'll touch a little bit of it.
13 There are some studies out there that have indicated over
14 relatively large geographic areas that anuran communities can be a
15 fairly useful way of looking at relationship to landscape parameters.
16 Whether they'll be useful for looking at toxic related questions, I
17 don't think has been delineated.
18 Certainly, communities can be manipulated -- the data from
19 communities can be manipulated into a series of metrics much like we
20 use fish or invertebrates or other things in a way to look at patterns
21 over relatively large geographic scales.

1 DR. ROBERTS: Dr. Skelly, did you want to add anything?

2 DR. SKELLY: I guess I would just insert a note of caution that

3 it can be -- if we are trying to link these studies, eventually, back to a

4 mechanism and link them to laboratory studies, logistically and

5 pragmatically, it may be challenging enough to do on a single species

6 level.

7 I'm very interested in communities. I think that they can

8 sometimes show responses that may give us more information than

9 single species.

10 But on the idea that we need to walk before we run, I guess I

11 would say that we should try to get out into the field more working

12 with the single species before we look at multiple species

13 simultaneously.

14 DR. ROBERTS: Dr. Matsumura. DR.

15 MATSUMURA: I'm not doing that, but I'm collaborating with the

16 good herbatologist in the pit studies, on the study on the yellow

17 legged mountain frogs, rana muscosa.

18 As far as that particular species is concerned, it is declining,

19 everybody agrees, at least, in California. It has a long larval life span

20 that it spends in such a cold place for three to almost four years in the

21 water, and they are disappearing.

1 When we made a survey, we found that there are still some
2 ponds and lakes teamed with the yellow legged frog but they all on
3 the eastern slope. On the western slope, there are only about three
4 sites that we found.
5 There is no question that you can talk to those rangers. They
6 say, well, I used to know that particular pond had the muscosa, lots of
7 them and they are disappearing in the last year 10 years.
8 They are species that you can really count on not just by virtue of
9 long life in that particular case. You can really see that's how they
10 are disappearing.
11 So, it is not related to atrazine.
12 But by studying those, we start finding also, hyla regilla,
13 pacific tree frogs, are also disappearing from the same western slope.
14 That's the reason why we are suspecting air pollution and particularly
15 PCBs.
16 In the lowland area also the red legged frogs also disappearing,
17 but they are being displaced by bullfrogs. So that is the same
18 example that you are citing.
19 I get the feeling that just looking at those mountain frogs
20 endangered species which we cannot study well, that there could be
21 some combination of right kind of species, very sensitive ones that

1 you could pick on and try to see whether those are the ones who are
2 effected the most by this kind of pollution.
3 So some field biologists can really look for, they may find a
4 good combination. I just wanted to add.
5 DR. ROBERTS: Thank you.
6 I haven't heard much in the way of disagreement in this.
7 Let me ask the panel, is there anything that they disagree with
8 that has been stated or are there any other important points that have
9 been left out of the discussion that perhaps should be included in the
10 response?
11 Dr. Kelley.
12 DR. KELLEY: I don't disagree with the conclusion as stated
13 based on the available data. I just simply wish to make a comment
14 about the field studies in South Africa, which is that if the EPA
15 decides that it wants to adopt xenopus as some sort of model system
16 for studying a sex environmental effects, including atrazine, but there
17 are others as well for which it's being the considered, inclusion of
18 field studies in gathering field data in xenopus will be extremely
19 useful.
20 It will enable you to take the best studied laboratory anuran and
21 correlate those results with the field. I don't think it is possible for

1 any other animal. We have neglected in our literature survey the data
2 on xenopus which exists in the South African literature.
3 I would, if that route is taken, I think -- going forward more
4 carefully designed and better powered field studies in xenopus will be
5 useful in trying to relate laboratory results to potential effects in the
6 field.
7 Now they won't tell you anything about our North American
8 anurans, necessarily, but they will tell you something about the
9 limitations of the transform.
10 DR. ROBERTS: Thank you. Dr. Bradbury, it looks like there is
11 strong endorsement for the value of field studies and the panel has a
12 number of comments about what is required to really get good
13 information from those studies.
14 Are there any follow up questions that you have for the panel
15 on this topic?
16 DR. BRADBURY: Just for the record, we have question 2 B.
17 DR. ROBERTS: I sort of sense that our discussion has covered
18 both 2 A and 2 B. I will defer to the discussants on that.
19 DR. BRADBURY: I feel comfortable that we got the picture
20 between the two aspects but if there is something left in your laptop.
21 DR. ROBERTS: Dr. Richards, is there anything left in your

1 laptop?

2 DR. RICHARDS: My fingers are fingered out, but I think in my

3 own comments I was implying that it was very difficult to examine the

4 effects of costressers given the studies we have seen so far.

5 DR. BRADBURY: I think we're in good shape on our end.

6 DR. ROBERTS: Are there any other points that need to be

7 made or anything we need to resolve before we move onto the next

8 question? If not, let's move on to question three.

9 DR. STEEGER: In evaluation of the existing laboratory based

10 studies the agency concluded that there was sufficient information to

11 establish a hypothesis that atrazine could cause adverse gonadal

12 developmental effects.

13 However, due to the different experimental designs and

14 variability in the nature and extent of experimental conditions, i.e.,

15 level of excessive mortality, delayed developmental and untreated

16 organisms, lack of response to positive controls, it was not possible to

17 adequately assess the hypothesis that atrazine causes developmental

18 effects.

19 It was further concluded that the current body of information

20 did not prove the means to characterize the nature of any associated

21 dose response relationships.

1 Please comment on EPA's determination that the laboratory
2 studies provide a plausible basis for the means to establish hypothesis
3 concerning the potential for atrazine to cause development effects.
4 Also, please comment on whether the overall body of available
5 data are adequate to demonstrate whether or not atrazine causes
6 developmental effects under the conditions described in these studies.
7 DR. ROBERTS: An important question Dr. LeBlanc, what do
8 you think?
9 DR. LEBLANC: I concur with the EPA, their determination
10 that laboratory studies on the affects of atrazine on anuran gonadal
11 development are sufficient to establish the hypothesis that atrazine
12 interferes with normal gonad development.
13 Clearly, the available data are limited. However, the existing
14 data as related specifically to the laboratory investigation, I believe,
15 support the hypothesis of the eight laboratory studies that we were
16 provided with that evaluating effects of atrazine on gonad
17 development.
18 Five of these studies detected such abnormalities from three
19 different laboratories. Certainly, in my laboratory, if a student comes
20 to me with an observation, be it induction of enzyme, suppression of
21 protein, or a message RNA level, the first thing I tell them is go do it

1 again.

2 They come back and I tell them to go do it again and perhaps

3 after six, seven, or eight times I will accept that data, which doesn't

4 mean that perhaps two of those times they weren't able to reproduce

5 the effect.

6 But if the preponderance of the evidence consistently shows the

7 effect that they initially observed and that we were pursuing, I

8 wouldn't discount the data.

9 There certainly were differences observed among the various

10 studies that we examined as related to the effects that were observed

11 and many attributes were ascribed that could have contributed to

12 these differences, including species differences, differences in

13 exposure design, and specific endpoints that were selected in the

14 individual evaluations.

15 Significant data gaps exist in our understanding of the affects

16 of atrazine on anuran development. These include a lack of

17 understanding of the mechanism by which atrazine elicits its

18 developmental toxicity, the nature of the concentration response

19 relationship, definition of susceptible windows of exposure, and

20 identification of a threshold concentration.

21 However, it is certainly my view, that the existing data does

1 support acceptance of the hypothesis that atrazine interferes with
2 normal anuran gonadal development.
3 DR. ROBERTS: Dr. Kloas.
4 DR. KLOAS: I agree a great deal with what Gerald already told
5 you. I think one of the points why we find so many differences, and
6 the big difference should be that we have no standardized protocols in
7 various studies.
8 I think one of the goals for the future in future studies is, first,
9 to design more or less a standardized protocol to work on amphibians,
10 especially on xenopus laevis, but also if you use ranids.
11 So, we are comparing apples with pears if you don't go ahead
12 with a really standardized protocol. That's one comment on it.
13 DR. ROBERTS: Dr. Denver, do you have something to add?
14 DR. DENVER: I just agree that we have sufficient data to
15 generate a hypothesis but insufficient data to test the hypothesis.
16 DR. ROBERTS: Other members of the panel? Dr. Matsumura.
17 DR. MATSUMURA: I agree with Dr. LeBlanc's statement so
18 long as it is limited to gonadal abnormalities. I'm not convinced
19 about the whole scale developmental effect even including laryngeal
20 effect.
21 But regarding the abnormalities, I feel that the having Dr.

1 Carr's independent experiment as well as John Giesy's, I feel that
2 qualitative there is some clear cut independent verification of the
3 phenomenon. But others, I would not stick my neck. So that's just my
4 personal feeling.
5 That's why I asked Dr. Carr to just tell me that he can really
6 stand behind the data. He told me that. So, I'm satisfied by
7 independent verification. But other parameters I would not agree that
8 there is sufficient base.
9 DR. ROBERTS: Dr. Matsumura raised the point -- you know
10 the question asked about developmental effects. I guess, we need to
11 be careful how we define those in terms of our conclusions.
12 I would also like to point out that I want to be clear, when the
13 question asks whether or not the overall body of available data is
14 adequate to demonstrate whether or not atrazine causes developmental
15 effects.
16 I thought I heard two different answers to that question among
17 panel members. I want to sort of see what people think.
18 DR. ROBERTS: Dr. Kelley.
19 DR. KELLEY: I think the question is whether the data is
20 sufficient to entertain the hypothesis that atrazine produces
21 developmental effects.

1 The answer was a narrowly focused one. It said that the data on
2 gonadal development coming from two very different frog species,
3 one totally aquatic and one at least partially terrestrial, terrestrial
4 during most of the lifespan, did indicate an effect on gonadal
5 development.
6 So for that reason, entertaining the hypothesis was regarded as
7 an appropriate thing to do.
8 DR. ROBERTS: So, not to put words in your mouth, but your
9 opinion is that the data are sufficient to support the hypothesis or to
10 justify the hypothesis, but not necessarily to demonstrate which I
11 would interpret as prove the hypothesis.
12 DR. KELLEY: It was very well put by the EPA. You could
13 look at the data and say the data is such that we could reject this
14 hypothesis. I do not believe the panel would support that conclusion
15 although we haven't heard from everybody. I do not believe that we
16 could reject the hypothesis. So, what that means is the
17 hypothesis is still viable. Is the hypothesis more than a hypothesis,
18 do we actually believe that atrazine will, at some dose that we don't
19 know now and some range of species that we don't know now, reliably
20 result in gonadal abnormalities?
21 I don't think nor do I believe the panel thinks that the data are

1 sufficient to accept that hypothesis. But the data are certainly
2 sufficient not to rule it out and to continue to entertain it as a model
3 for looking further.
4 DR. ROBERTS: Dr. LeBlanc.
5 DR. LEBLANC: I'm going to push you a little bit more though.
6 I'm just going to reread the statement just to make sure we answer the
7 question as asked. The statement -- the question ends with essentially
8 available data -- EPA is questioning whether available data is
9 adequate to demonstrate whether or not atrazine causes developmental
10 affects. So, you're saying no.
11 DR. KELLEY: Read the last phrase.
12 DR. LEBLANC: Under conditions described in these studies.
13 DR. KELLEY: So we have data. We have data from a
14 reasonably large number of studies that indicate that at least one dose
15 there was a gonadal defect in a varying proportion of animals.
16 I have a where there is smoke there is fire reaction to that. I
17 think there is something going on, that is my bottom line, but I don't
18 know there is something going on.
19 I believe it is worthwhile investigating the hypothesis further,
20 but I wouldn't believe that it was proven at this point. It certainly
21 raises concerns.

1 DR. ROBERTS: Whenever we're not sure exactly what the
2 question is asking, we sometimes ask the agency to clarify for us.
3 Is this question asking whether or not the data provided by the
4 existing studies is sufficient to demonstrate that affect or is it more
5 along the lines of what Dr. Kelley is talking about?
6 Is it justify pursuit of the hypothesis but not necessarily
7 demonstrate the phenomenon which is what I, sort of, read into the
8 white paper. If you can clarify that for us.
9 DR. BRADBURY: It's amazing how one can wordsmith, and
10 work and work, and think it's clear, and then you need to work on it.
11 The second phrase is commenting on whether the overall body
12 of available data is adequate to demonstrate in the describe studies.
13 I think that's an important phrase. Based on the body of
14 information is there some sense, given the quality of the studies and
15 the characteristics of the studies, is there something going on, is there
16 some smoke?
17 That is sort of in the context in is there enough smoke to say, I
18 think it's reasonable to formulate a hypothesis to look to see if there
19 is fire associated with the smoke. That's the context of the question,
20 intended meaning of the question.
21 I apologize if you're confused.

1 DR. ROBERTS: With that clarification, then, both of you have
2 answered in the affirmative then?
3 DR. LEBLANC: I have.
4 DR. ROBERTS: Dr. Kelley?
5 DR. KELLEY: I have as well.
6 DR. ROBERTS: Dr. Denver?
7 DR. DENVER: Yes.
8 DR. ROBERTS: Is there anyone else that would like to weigh
9 in on this?
10 Dr. Kloas you were a discussant did you want to indicate one
11 way or the other on this? Dr. Coats?
12 DR. COATS: Yes, I will weigh in. I think there is enough data
13 to pursue forward movement to test the hypothesis.
14 DR. DELORME: I would concur as well. I think one of the
15 things we saw is there are a number of factors with the husbandry and
16 whatnot that might have resulted in something going on. So, it is not
17 really, really, clear that atrazine is the root cause. So, we need to
18 pursue the hypothesis.
19 DR. ROBERTS: Would anyone else like to weigh in on this
20 before we move on to B?
21 DR. SKELLY: I will concur broadly. I think the way that Dr.

1 LeBlanc and the others responded reflects my feelings as well.

2 DR. ROBERTS: Dr. Green?

3 DR. GREEN: I concur as well.

4 DR. ROBERTS: Good. Dr. Richards?

5 DR. RICHARDSON: I'll join the party.

6 DR. ROBERTS: Then let's go ahead and take part B?

7 DR. STEEGER: Please comment on EPA's conclusion that

8 given the variability in the available dose response data across the

9 studies, e.g., approximately 250-fold difference in reported

10 thresholds for observed developmental effects as well as reports of

11 monotonic and nonmonotonic dose response curves, it is not possible

12 to ascertain the relationship, if any, of atrazine exposure to

13 developmental effects in amphibians.

14 DR. ROBERTS: Dr. LeBlanc, what do you think?

15 DR. LEBLANC: The short answer is I agree wholeheartedly,

16 and I think I only need to expand on that a little bit.

17 There is clearly major deficiencies that exist in the data as

18 related to describing the relationship between atrazine exposure and

19 gonadal toxicities that have been reported.

20 We can extract, I think, a small amount of data as related to that

21 relationship that presumed relationship, at this point in time, although

1 the conclusions are very, very limited.

2 For example, I think, I hope, we're all in agreement that

3 atrazine at a concentration of 0.01 microgram per liter appears to

4 have no effect on anuran gonadal development.

5 The data seems to suggest that exposure to frog larvae to

6 atrazine concentrations greater than or equal to .1 microgram per liter

7 can elicit developmental abnormalities, not does but can.

8 There is limited data available to us to make any judgments as

9 to whether or not concentrations in the range of .1 to 10 micrograms

10 per liter truly elicit adverse effects.

11 However, when we consider concentrations in the vicinity of 25

12 micrograms per liter, there seems to be some reasonably good

13 concordance that this concentration does elicit gonadal effects.

14 Clearly, more data is required to define the concentration

15 response relationship between atrazine and gonadal development of

16 anuran larvae.

17 At this time, I would have confidence only in concluding that

18 the threshold concentration for this material exists between .01 and

19 25 micrograms per liter.

20 DR. ROBERTS: Thoughts from other panel members? I will

21 start with the lead discussants, Dr. Kloas, did you want to add

1 anything or comment on this?

2 DR. KLOAS: Concur.

3 DR. ROBERTS: Dr. Denver?

4 DR. DENVER: I would just concur with the statement that we

5 put together last night.

6 DR. ROBERTS: Other comments from other members of the

7 panel?

8 Does anyone feel that the data are adequate to describe the dose

9 potential dose response relationships?

10 Dr. Matsumura?

11 DR. MATSUMURA: I don't think it is adequately describing,

12 all we're saying is that the threshold concept can be applied and that

13 some experiments indicate that there is such a thing.

14 Real threshold experiments must be done rather carefully. It is

15 not that easy. How many years it took to have any agreement on the

16 cancer dose and the effect relationships, particularly with the

17 hormones, there are feedbacks and -- you know, Jere.

18 So, I would vote for the statement that the threshold is a good

19 way to go about it and there are some indications, incipient

20 indications.

21 DR. ROBERTS: In the spirit of Dr. LeBlanc's earlier comment

1 which I concurred that these were actually concentration response
2 relationships and not dose response relationships. So, I'm going to
3 correct myself in agreement with that.
4 DR. LEBLANC: I think we need to recognize that the great
5 majority of the studies that we have reviewed, I can think of one
6 exception, were not designed to evaluate the concentration response
7 relationship. Certainly, future studies need to be designed
8 appropriately so that the response relationship can be appropriately
9 evaluated. I made some attempts to drive some general understanding
10 of what this relationship might be based upon all the information that
11 was provided to us.
12 The best that I could come up with, and I don't have a lot of
13 confidence in it, is that .01 micrograms per liter has no effect and all
14 concentrations evaluated greater than that had an effect on average.
15 And on average, that effect regardless of the concentration
16 seemed to have been around 20 percent incident of effect whatever the
17 investigators may have been monitoring.
18 Now, I don't know what that means. It could mean that the
19 maximum response that we should anticipate is 20 percent. And that
20 the concentration response is going to occur between .01 and .1, the
21 threshold will be between there. I don't know that that's the case. It

1 is just a suggestion.

2 And there are precedents for observing partial effects where

3 perhaps we would be anticipating greater effects? What comes to

4 mind are my own studies looking at intersex in snails as related to

5 tributal 10. In laboratory exposures where we try and replicate

6 what we see in the environment in 10 contaminated environments

7 where the incidence is 100 percent intersex, the best we can generate

8 is about 30 percent. We're not alone, other labs have had

9 similar success in generating intersex in only about 30 percent of the

10 animals. We really don't know why. We assume it is a deficiency in

11 our experimental design.

12 We know the animals are capable of -- the population is capable

13 of totally responding, but in the lab they simply don't. I don't think

14 we need to go into these experiments with the anticipation that we're

15 going to see 100 percent response in a population if 20 percent is

16 truly the maximum that we can anticipate, we should accept that and

17 live with it.

18 But we should still be able to define that relationship between a

19 threshold concentration and that maximum effect whatever it might

20 be.

21 DR. ROBERTS: Okay. Are there other comments you want to

1 express regarding this?

2 I'll say for myself personally, I agree that I don't think the data

3 are adequate to describe the dose response relationship.

4 I have not gone through the analysis you have. I have no idea

5 where an apparent threshold might be. Speaking personally, I have no

6 idea what the shape of the dose response curve might be. But I do

7 agree there is not enough data to establish that.

8 Anyone else? Let's go ahead and move on to the next.

9 DR. STEEGER: Many of the available studies proposed that

10 aromatase induction results in elevated estrogen levels that lead to

11 feminization as characterized by ovotestes, intersex, and

12 hermaphroditism in genetically male amphibians.

13 Please comment on EPA's conclusion that to date aromatase

14 induction by atrazine has not been demonstrated in any anuran in

15 controlled laboratory investigations.

16 DR. ROBERTS: Dr. Kloas.

17 DR. KLOAS: First of all, I would like to say that it is not the

18 only hypothesis which could raise such phenomenons, there are

19 feminizational demasculization phenomenon.

20 We would have two ways how to obtain them estrogenic and

21 anti-androgenic ones, that's the first remark I want to make.

1 Answering question 4 A, of course it is correct. There are no
2 data showing up any aromatase induction or stimulation caused by
3 atrazine. But however, I think we have already discussed there is no
4 approach done which would be appropriate to demonstrate it.
5 So, I think that experimental designs done up to now, they don't
6 support this hypothesis but they cannot support such a hypothesis for
7 aromatase stimulatory effects.
8 Would you like to add something?
9 DR. ROBERTS: Thank you. So in your opinion it is still an
10 open question?
11 DR. KLOAS: Yes.
12 DR. ROBERTS: Dr. Kelley.
13 DR. KELLEY: I would agree. I would like to point out that the
14 aromatase gene or at least one of them, they probably have two, the
15 spene laevis (ph) has been cloned in xenopus laevis. It will be
16 possible to study the expression of the aromatase gene in the
17 developing gonad in the presence and the absence of agents that are
18 thought to affect it.
19 While the MRNA expression is not definitive with respect to
20 protein expression or the activity, the enzymatic activity of that
21 protein, on the other hand, you do have to have the gene expressed to

1 have it there. So we now have available the tools to test this
2 hypothesis in a rigorous fashion. It should be possible to test that.
3 I do concur absolutely that although this is a popular
4 hypothesis, it is not the only hypothesis that could provide a
5 mechanism of action for effects that have been reported.
6 And so to focus on this hypothesis to the exclusion of other
7 kinds of ideas like changes in hormones within the animal's body and
8 steroid hormones changes in the hypothalamic, pituitary, gonadal axis
9 is almost certainly a mistake. There are bound to be other
10 mechanisms that are involved. DR. ROBERTS: Dr. Kloas.
11 DR. KLOAS: I would also like to add one comment. It could
12 be also due that there is a direct interaction with the enzyme
13 aromatase. So I think if you could invitro assay, you should add
14 atrazine at different concentrations to show up if there is any
15 stimulatory effect which could be done very easily because already
16 aromatase was assessed biochemically in several labs. And it is a
17 routine assay.
18 I think that's second possibility. Not only looking on gene
19 expression, but also on direct interference with the enzyme as a
20 second possibility to rule out if there is any interference with
21 aromatase and the output for estrogens.

1 DR. ROBERTS: Thank you.

2 Dr. Denver.

3 DR. DENVER: The data to at least generate the hypothesis are

4 based on studies in human cell lines and a limited analysis in

5 alligators. The hypothesis has not been directly tested in amphibia.

6 Just one point I would add, to test it, not only do we need to

7 take advantage of the molecular tools that are available, but also do it

8 at the appropriate developmental stage at least in one that is

9 hypothesized to be sensitivity to atrazine.

10 DR. ROBERTS: Dr. LeBlanc.

11 DR. LEBLANC: First, I would like to just agree with

12 everything that has been said. But I would like to make the point

13 strongly that in agreeing that aromatase induction has not been

14 demonstrated, it should not be construed to mean that it's not induced

15 from my perspective.

16 It simply means that the appropriate experiments haven't been

17 done to demonstrate whether or not induction occurs.

18 And certainly, a point that I feel extremely strongly about in

19 future studies looking at aromatase induction is that the experiments

20 need to be conducted in the right life stage, not in the adult, but in the

21 larvae that we're interested in.

1 DR. ROBERTS: Dr. Kelley, you are nodding. Agreed?

2 DR. KELLEY: Yes. To demonstrate that you have an effect of
3 an agent in an adult, say, for example, hormone levels, and then to
4 infer that that same effect with an unknown mechanism should have
5 happened at developmental stage is taking the hypothesis quite far
6 down the line without evidence.

7 So really, you have to go back to the stage when the effects are
8 thought to occur in order for the mechanism to be established or not
9 established.

10 DR. ROBERTS: So the comments so far I think have expressed
11 the opinion that it has not been demonstrated that aromatase is
12 induced. However, the experiments have not necessarily been
13 appropriate design to adequately test that.

14 There have been some suggestions about how the best to
15 approach that test as well as a caution from the panel that about
16 focusing specifically on aromatase as a possible mode of action that
17 there are other endocrine mechanisms that need to be considered as
18 well.

19 Does everybody sort of agree with that summarization? Are
20 there any other points or is there disagreement?

21 Dr. Matsumura.

1 DR. MATSUMURA: Just I want to make sure that -- yes, our
2 chairman really summarized right. We should not really lock into
3 that. Dr. Kloas also suggested some different approaches such as the
4 antagonist of the testosterone.
5 This is not the typical estrogenic response. It doesn't happen in
6 the females. It doesn't look like any effects. So should not be locked
7 in. I agree with Dr. Kelley's statement that there are other
8 possibilities that we have to keep our minds open.
9 DR. ROBERTS: Any other thoughts or comments about A? Is
10 the feedback clear? Great.
11 Let's go to B.
12 DR. STEEGER: The variability associated with plasma sex
13 steroid concentrations and aromatase activities is high. Is this
14 variability normal.
15 Please comment on any readily apparent or available
16 methodological improvements, for example, changes in sampling
17 design, analytical techniques that could efficiently address this
18 variability in future studies.
19 DR. ROBERTS: Dr. Kloas and Dr. Kelley I believe during
20 some earlier discussions you made some points about the sex steroid
21 measurements.

1 What is your response to this question?

2 DR. KLOAS: First of all, I think all of us being aware of this
3 kind of determinations agree that there is always in lower vertebrates
4 a high variability and steroid levels in general and also activities in
5 aromatase may vary widely.

6 I think there should be agreement here. So the variability
7 shown up, until yet measured, seems to be normal, what you will
8 normally get.

9 Anyhow, we have to commend or we have some remarks about
10 ELISA data, especially for estradiol measurements presented.

11 I think there are at least one order or two orders of magnitude
12 higher than all the old data measured also in some of our labs that
13 would suggest to be in this level. So they are much higher. So there
14 should be any proof that this really is the right way to assess estradiol
15 in xenopus.

16 For anything else, I think changes in sampling design -- I
17 would like to show up set series (ph) involvement in steroid or in
18 sexual steroid genesis. I think first of all there should be short term
19 exposures and short term measurements for estradiol as well as for
20 androgens.

21 I would also refer that there could be also some interference

1 with ratio of testosterone, dehydro (ph) testosterone. And I would
2 also refer that there should be also some improvements to include five
3 alpha reductase measurements, because this could be also another
4 hypothesis how it works and how it could work in anti-androgenic
5 way (ph).
6 DR. ROBERTS: Dr. Kelley.
7 DR. KELLEY: I'm going to read my remarks.
8 The variability associated, the largest variability, two orders of
9 magnitude, in other words, 1,000 times, in the studies reviewed by the
10 EPA reflects differences in results obtained in studies sponsored by
11 the registrant using ELISA assays and studies in the open literature
12 using radioimmunoassay.
13 The most likely explanation for this large discrepancy is the
14 method used to measure hormone levels -- so that's the analytical
15 comment up here. Resolving these discrepancies should be
16 straightforward.
17 Within the open literature, on the other hand, variability is
18 more typical typically in the two times range, a range well within
19 diurnal and seasonal variation.
20 It should be recognized that species studied extensively in the
21 laboratory, xenopus laevis, may have originated from different

1 populations in their native habitat.

2 Populations can differ in seasonality even within the same

3 country. For example, in South Africa, the Cape population breeds in

4 the African winter, and the Johannesburg population in the African

5 summer.

6 And we would thus expect differences between the two

7 populations in the field and in the laboratory as this species maintains

8 circannual rhythms even after many, many generations in the

9 laboratory.

10 When wild caught animals are brought into the laboratory for

11 study, they should be characterized genetically to identify the

12 population of origin, this is possible now based on literature, and

13 also to verify species because I'm sorry to tell you every xenopus

14 looks like every other xenopus pretty much, except some are big and

15 some are small. Although, if you listen to their songs, you can tell

16 the difference.

17 And the same approach must be employed in characterizing

18 groups of animals that are used for laboratory studies. So this is by

19 way of saying that if we're going to standardize the animal as a test

20 species, we have to develop standards that don't just involve the

21 laboratory assays that we do, but that involve the biology of the

1 animal that we're looking at.

2 DR. ROBERTS: Dr. Denver.

3 DR. DENVER: I just want to reiterate the importance of

4 validation of immunoassays, especially assays for estradiol.

5 Estradiol is known to be present at very low concentrations in plasma

6 and is routinely measured at least in the laboratories that are

7 represented here by radioimmunoassays.

8 And historically, radioimmunoassays have been used to

9 measure plaza sex steroids and tend to be more sensitive than

10 ELISA's, although some of the current generation of ELISA's may be

11 just as sensitive.

12 But I think it is important to compare the two methods to make

13 sure that they are in concordance and to validate the assay methods

14 for each species under study.

15 There is a tendency these days to purchase a kit and put things

16 into it. I'm not saying that this is what was necessarily done by the

17 investigators here, but it is really important to evaluate that kit for

18 the species under study and show that you're actually measuring what

19 you think you are measuring and that you're actually recovering from

20 your sample.

21 In experiments where you add known amounts of cold steroid,

1 you can actually recover that in the assay.

2 So those are important things. Because the data that you

3 generate are only as good as the methods that you are using to

4 generate them. There has been a lot of discussion about animal

5 husbandry, which is important, but I think there should be similar

6 discussion of the methods used to analyze steroids, because perhaps

7 much or perhaps some of this variability, at least, could be due to

8 analytical methods.

9 The fact that one lab can use a kit and get the same result

10 doesn't necessarily mean that result is correct. You can repeat an

11 artifact until you are blue in the face and it doesn't necessarily mean

12 that that is a correct number.

13 DR. ROBERTS: Dr. Kloas.

14 DR. KLOAS: I think also another possibility to overcome such

15 a problem for measuring estradiol could be to measure an estrogenic

16 biomarker. A lot of estrogenic biomarkers are now already available.

17 The methodology is well-established. For instance,

18 vitellogenin, you can measure it in the plasma, but also as

19 vitellogenin MRNA by RTPCR techniques. That's routine

20 measurement.

21 You can also maybe in an indirect way demonstrate that there

1 could be any estrogenic interactions or interference caused by
2 atrazine.
3 So This is another possibility to have another methodological
4 approach to demonstrate estrogenic pathways.
5 DR. ROBERTS: Yes. Dr. Kelley raised that issue earlier in
6 discussion with one of the presenters.
7 If she wants, we could include that as part of our response to
8 this question.
9 DR. KELLEY: I also want to make the comment that snapshot
10 measurements of hormone levels at a particular point in time do not
11 give a valid picture of the history of exposure of animal to the
12 hormone in question.
13 What you really want, what I want, are biomarkers that show
14 you in living realtime color what the animal is seeing. We are
15 fortunate with xenopus that those are available now, actually. We
16 will be able to look at flashing green frogs and know what tissue is
17 being exposed to what level of hormone, when and how, which is an
18 unusual situation.
19 But even without this fancy molecular method, the biomarkers
20 such as have just been described are really very well characterized.
21 And you know that if you see a male that has any vitellogenin

1 or further that shows rapid response to estrogen challenge, you know
2 that male has seen a level of vitellogenin that is not present in the
3 literature.
4 So these are available assays and should be used and added to
5 the armamentarium.
6 DR. ROBERTS: Dr. Kelley, can we provide some specifics and
7 citations in our minutes?
8 DR. KELLEY: I have them in my giganto reference list.
9 DR. ROBERTS: Somehow I knew you would.
10 Dr. Tietge I believe has a question.
11 DR. TIETGE: I'm a little confused on one point here. Maybe I
12 didn't catch it quite correctly.
13 Dr. Kloas said that there is high variability in these
14 measurements and that's somewhat normal.
15 Dr. Kelley said that the typical range of variability is twofold.
16 Are you in agreement, the two of you? Did I get that right?
17 DR. KELLEY: The typical range -- would you agree that the
18 typical range of variation is not two orders of magnitude? 1,000
19 times?
20 DR. ROBERTS: That's 100.
21 DR. KELLEY: Sorry.

1 Well, you know what I mean. It's not 1,000 times?

2 DR. ROBERTS: I think she meant two orders of magnitude

3 perhaps.

4 DR. KLOAS: Not twofold.

5 DR. KELLEY: I'm sorry. I meant two orders of magnitude.

6 DR. TIETGE: That clarifies it. Thank you.

7 DR. KLOAS: We agree with each other.

8 DR. KELLEY: We agree that twofold is normal, and 1,000

9 times is not normal. I actually wrote that down.

10 DR. ROBERTS: Two orders of magnitude, yes. Three orders of

11 magnitude, no.

12 DR. KELLEY: Is twofold two orders of magnitude? No.

13 Twofold is within an order of magnitude. It's within 10 times.

14 DR. MATSUMURA: I would like to support the Werler's (ph)

15 comment. Really, those steroid levels can change really by the hour.

16 So I would like to really recommend that the endpoint of those

17 steroid action -- actually, I have plasmid for the vitellogenin on the

18 control by ERE. It comes from xenopus. I got it from Shapiro. And I

19 also have a PS2, which is very sensitive.

20 It is well constructed. It works. I offer to Joe Giesy, if he

21 wants, and anybody who wants I have it. Of course, I have to write to

1 Shapiro saying that I will transfer that.

2 But really, those are sensitive. I was so amazed. I compared

3 that to mammalian construct and the xenopus is better. Much purer

4 ERE. You can detect the PS2 very easily or PGR, progesterone

5 receptor.

6 Those are well, well accepted. You can run that easily. I

7 support that. Because that is more stable a way of measuring.

8 Some time ago hormone was up, but its effect is still here.

9 DR. ROBERTS: Any other comments on this before we move

10 on to the next one?

11 Clarification?

12 DR. BRADBURY: I'm sure you all do it. If you can get all this

13 written down, some of the dialogue. That will be very helpful.

14 Based on the discussions over the last few days, still within the

15 realm of this question, but one of the hypotheses was it was -- the

16 increase in estradiol, that the estradiol doesn't get into the plasma, it

17 stays within the tissue.

18 Are there techniques available to measure changes in estradiol

19 concentration within the tissues?

20 Could you comment on that?

21 DR. ROBERTS: Dr. Kloas.

1 DR. KLOAS: I think it is easy to measure also from tissue's
2 cystorrheic (ph) levels. You can extract them easily. But, however, I
3 think it is a completely lipophilic compound, how to keep it -- it has
4 to be really completely resorped by receptors at the same time.
5 Normally, there should be any leakage to the circulation. I
6 wouldn't expect that could be stored, of course. So should be a
7 change.
8 DR. ROBERTS: Dr. LeBlanc.
9 DR. LEBLANC: As Dr. Kelley said also, it is really a snapshot
10 that we're taking be it in the serum or be it in a given tissue.
11 And that snapshot tends to reflect very well the level of
12 synthesis that's taking place in that tissue at that point in time.
13 So another alternative would be rather than looking at tissue
14 levels at a given hormone, if we're working with gonads would be
15 perhaps to remove the gonads and in tissue culture look at the
16 production steroid synthesis.
17 And another strength to that approach too is you could be at the
18 same time looking at production of multiple steroids, androgens and
19 estrogens.
20 DR. ROBERTS: Dr. Kelley.
21 DR. KELLEY: Meashita (ph) and coworkers in Japan have

1 routinely cultured the Stage 51 gonad. In the presence of aromatase
2 inhibitors and also in the presence of estrogen, are able to observe
3 sexual differentiation invitro and are able to observe effects of the
4 agents that they add.
5 That assay is very available.
6 Also, I want to point out, I know I pointed this out before, but
7 the liver is not that close to the gonad. Typically, if you have
8 evidence of vitellogenin -- let me explain for the public what
9 vitellogenin is. It is the yolk proteins. Birds have it. We don't have
10 so much yolk. But the frogs have a lot of it. A mature egg has to yolk
11 up in order for it to be oviposited, ovulated and oviposited.
12 If you see vitellogenin in the liver, it had to have arrived there,
13 I believe there is agreement, via the circulation. So it had to have
14 circulated at some point. That would mean that it got out of the
15 organ.
16 It is worth pointing out, it has been pointed out, for example,
17 females use testosterone as a precursor for estrogen. Dr. Hayes was
18 right, that he didn't get the yolk up except in rana.
19 But xenopus uses testosterone as a precursor for estrogen. You
20 can measure really high levels of testosterone in females when their
21 ovaries are activated.

1 So circulating levels can be useful and shouldn't be denied, but
2 there are other tools available to us.
3 DR. ROBERTS: Let me ask Dr. LeBlanc and Dr. Kelley a
4 question of clarification for my edification. Not for the agency's.
5 The experiments in gonadal culture that you described, are
6 those -- are you envisioning an exvivo experiment where they are
7 exposed, you are removing gonads from exposed animals and
8 culturing them and then measuring synthesis rate?
9 DR. LEBLANC: Yes.
10 DR. ROBERTS: So the exposure would occur in the animal, but
11 not while in consult (ph) culture.
12 DR. LEBLANC: That's correct.
13 DR. ROBERTS: And you would still expect to see a continuing
14 effect of the atrazine even though the gonads in culture would not be
15 exposed to atrazine?
16 DR. LEBLANC: It certainly depends on the mechanism of the
17 effect. But if it were induction of the enzyme, then in the short term,
18 yes, you would expect to see --
19 DR. ROBERTS: Short term until it declines, okay.
20 DR. KELLEY: Just to echo a point made here about short term
21 exposure, it is a tremendous advantage that you can get sexual

1 differentiation over a 48 hour period in xenopus.

2 Because of that, you can do it in culture. You don't have to

3 worry about the rundown of the cells so much. You don't have to

4 worry about the compensatory mechanisms within the entire animal.

5 And that's a big experimental advantage.

6 DR. ROBERTS: Are there any follow-up questions from the

7 agency on this? Any other comments?

8 Let's then move on to the next question.

9 DR. STEEGER: Please comment on whether there are

10 additional data, other than those summarized in the white paper, that

11 suggest late exposure of amphibians, i.e., juveniles or adults to

12 estrogens or estrogenic chemicals can induce ovotestes formation.

13 DR. KLOAS: As far as we are aware, not in xenopus, at least.

14 I cannot give you the citation, but there is some Japanese

15 people talking about ranids where you could reverse probably in late

16 stages of rana rugosa. You can induce sex reversal. But not for

17 xenopus. It is not reported, at least.

18 DR. ROBERTS: Dr. Kelley.

19 DR. KELLEY: In ranids, it has long been known that aging

20 animals will show gonadal changes spontaneously. Whether those are

21 associated with environmental agents has never known.

1 I remember reading an old report where old female ranids will
2 shed sperm. In xenopus, this has never been reported, to my
3 knowledge.
4 So I don't know of any data on this, but there may be people
5 who know better than we do.
6 DR. ROBERTS: Dr. Denver.
7 DR. DENVER: No, I don't have any additional data to add.
8 DR. ROBERTS: Anybody else on the panel?
9 DR. ROBERTS: Let's go ahead on to the next one.
10 DR. STEEGER: Please comment on whether there are
11 additional data other than those summarized in the white paper that
12 suggest alternative mechanisms that could explain the apparent
13 feminization of genetically male amphibians.
14 DR. ROBERTS: I suspect Dr. Kloas has an idea or two on this.
15 Go ahead.
16 DR. KLOAS: I think we don't have really completely new data
17 and not been included in the white paper. But from the last days and
18 from some presentations we saw, we feel that you can create quite a
19 lot of hypothesis more or less which could account for these findings
20 presented by different groups.
21 I just would like to summarize a little bit which possibilities

1 could happen.

2 First, aromatase induction or effect -- change in activity of

3 aromatase could be one. Still one of the hypotheses which could

4 work. The next one, we could also have anti-androgenic effects. A

5 third one would be as already mentioned yesterday, influence by the

6 hypothalamus pituitary gonad axis. So more than (inaudible)

7 endocrine pathway.

8 First possibility would be inhibition of steroidogenesis,

9 especially on sex reverse steroids.

10 We have shown very easily that if you expose animals to

11 atrazine, at least testosterone levels went down and really

12 pronounced.

13 And I think furthermore there is not completely evidence for me

14 that you can exclude any interference with the thyroid system. There

15 is still some data available also from the public comments. Dr.

16 Sheffield also he reminds me again to one paper on larval salamanders

17 that shows the inhibition or delay of metamorphosis. I think still

18 from the data we got and because of conduction arose (ph) from the

19 husbandry, it's not completely clear that there couldn't be any

20 interference with the thyroid system.

21 Next week we're going to present some data that sometimes you

1 will not -- you cannot see it really for developmental stages, but you
2 will still have counter-regulation if you have inhibitory effects on the
3 thyroid system.
4 This could be counter-regulated by more pronounced TSH
5 production. This way, with this pathway we couldn't exclude also.
6 I'm making up everything. I'm sorry for that. But the data
7 presented here still suggests that we have so many facilities and
8 possibilities which could be one of the pathways or several pathways
9 how it could work.
10 DR. ROBERTS: Dr. Kelley, do you have anything to add?
11 DR. KELLEY: Let me comment a little bit on the thyroid
12 hormone question. Metamorphosis in xenopus is completely
13 dependent upon thyroid hormone and requires as has been shown in a
14 number of studies the expression of thyroid hormone receptor, which
15 there are two which exist in a variety of isoforms.
16 I was not able to detect thyroid hormone receptor alpha in
17 developing gonads. So I do not know if the developing gonad needs
18 expression for formation, for what I call sex determination.
19 In fact, in that study in which thyroid hormone was blocked, the
20 gonad proceeded to develop to the point of having spermatids.
21 Although, of course, frank spermatid gonia did not develop because --

1 this is the other side of the equation, you cannot get effects of steroid
2 hormones in xenopus unless the animal has first been exposed to
3 thyroid hormone.
4 And some effects require thyroid hormone induction of
5 prolactin.
6 So it is clear in xenopus that there is this strong interaction in
7 terms of steroid hormone effects for many tissues between pituitary
8 hormones, thyroid hormones, this is extending now to the axis, the
9 pituitary hypothalamic gonadal axis now to the thyroid and the steroid
10 hormones.
11 While this may sound like a nightmare like the ecological
12 studies in point effect, it's an exquisitely regulated developmental
13 system which provides then a very good assay system for a variety of
14 endocrine perturbations.
15 So I agree that we can't at this point completely exclude thyroid
16 hormone effects. I don't know about the gonads. But if there are any
17 other effects of the agents that are being studied, we should take this
18 into account.
19 DR. ROBERTS: Dr. Denver.
20 DR. DENVER: I don't know of any additional data that would
21 support any alternative mechanisms, but I think it is important to at

1 least entertain the hypothesis that if there are gonadal effects, that
2 they could be mediated by nonendocrine mechanisms, that is, that
3 there could be direct effects on genes that are important for gonadal
4 determination that may have nothing to do with hormones.
5 I don't think that should necessarily be ruled out.
6 DR. ROBERTS: It seems we didn't have a lot of specific data to
7 offer, but the panel members did have suggestions for a number of
8 alternative mechanisms and seemed to strongly think that those ought
9 to be considered as well.
10 Are there any other comments?
11 Dr. Matsumura.
12 DR. MATSUMURA: I was intrigued at least by the fact that the
13 HCG reversed some of those actions, but not all the way.
14 I would like to suggest at least that area should be followed up
15 to see what it is.
16 It remind me of the Precosin (ph) studies, insect studies. This
17 really is part of capsulatum (ph).
18 In that case, you can reverse it except that it doesn't come back
19 to the same level because part of the function of the capsulatum is
20 gone. It looks like that, but again, just one experiment. I'm quite
21 sure Dr. Hayes is intrigued by that too. So we'll see what happens

1 then.

2 DR. ROBERTS: Any other comments?

3 Dr. LeBlanc.

4 DR. LEBLANC: I just want to make a point that in relation to

5 all the possible effects that Dr. Kloas talked about with terminal

6 hormones, the androgens, the estrogens, thyroid, hormone, that the

7 effects need not be self exclusive, that there could be effects at the

8 level of the hypothalamus, the pituitary that results in several effects

9 on these terminal hormones resulting in decreased testosterone,

10 increased estradiol and some effect on thyroid hormone perhaps.

11 So they don't have to be self exclusive. There could be a

12 common target upstream that is affecting many hormones.

13 DR. ROBERTS: Anything else?

14 Any follow-up questions or clarifications needed?

15 DR. ROBERTS: I would like to go ahead and take question 5

16 and then do a break.

17 DR. STEEGER: With regard to specific endpoints, the agency

18 does not have currently have sufficient information to quantitatively

19 relate gonadal laryngeal effects to reproductive outcomes.

20 A major underlying uncertainty is the ecological relevance of

21 ovotestes occurrence to the maintenance of anuran populations.

1 Can the panel provide sources of data on background rates of
2 ovotestes occurrence in amphibian species and any associated
3 considerations for interpreting this information in the context of the
4 reviewed studies?
5 DR. ROBERTS: Our lead discussant on this is Dr. Green.
6 DR. GREEN: Witchie reported sporadic cases of
7 hermaphroditism early -- in the late 1950s.
8 And there is at least one study in which the prevalence of
9 ovotestes in a controlled population of laboratory frogs has been
10 recently described. And this is a paper by Dr. Kloas which is in press
11 right now.
12 To our knowledge, however, the background rates of ovotestes
13 in any amphibian population have not been reported.
14 The panel members believe the frequency of occurrence of
15 ovotestes in normal healthy populations of amphibians is probably
16 very low.
17 This is of course based on the rare occurrence and our
18 observations of ovotestes in our own laboratory animal populations.
19 So without information on the background rates of ovotestes, it
20 is not possible to assess the impact, if any, of the presence of
21 ovotestes on anuran populations.

1 DR. ROBERTS: Comments by other discussants?

2 Dr. LeBlanc.

3 DR. LEBLANC: No comments. I agree.

4 DR. KLOAS: I agree.

5 DR. ROBERTS: Dr. Kelley.

6 DR. KELLEY: Yes, I think this falls into the category of data

7 that we had described by the EPA panel before, which was that in

8 some cases you will only have anecdotal data.

9 None of us ever thought it was important to document how

10 many ovotestes you saw in opening up thousands of frogs. There are

11 no published data on those.

12 But if you will take the collective wisdom of people who have

13 opened up thousands of frogs will tell you that Witschi reports only

14 one case, actually, in the 50s by somebody else. And I actually have

15 never seen one in the laboratory.

16 DR. ROBERTS: Is this an issue for which we need -- is there

17 reasonably clear understanding about what you mean by ovotestes?

18 DR. KELLEY: I have made a PDF file of the Witschi paper.

19 It's actually a chapter from a book. It is has a very nice picture of a

20 mature gonad with clear testicular tissue and very well yolked up

21 eggs.

1 I submit that that was what I would call an ovotestis. And
2 everything else I regard as kind of subpar and up for grabs. But when
3 you have an ovary that has a testis and eggs, which are pretty
4 unmistakable, that's what I would call it. And this is an important
5 issue, the terminology issue is very important.
6 The question of whether the occasional oocyte in a testis is
7 normal or not, Witschi himself in this -- for you guys who don't know
8 who Witschi is -- was he German or Swiss? I believe he was Swiss.
9 He was Swiss, and he started working on xenopus. He brought
10 them in a tea kettle to Bozzle (ph). He performed almost all the early
11 experiments with Chang and McComo (ph) on sexual differentiation
12 in xenopus and was a very gifted biologist, I believe.
13 He has a very nice picture of this in this chapter summarizing
14 many, many years of work.
15 Anyway, I have given this to the EPA. I have the paper with
16 me. And the book should be available in the libraries. It will be in
17 the bibliography.
18 DR. ROBERTS: Perhaps it would be useful since I believe
19 we're probably going to comment subsequently on the problems
20 created by lack of terminology that we clarify in our response what
21 we mean by ovotestes.

1 DR. KELLEY: That's a very good suggestion.

2 DR. ROBERTS: Any other comments to add on this one?

3 Let's go to B then.

4 DR. STEEGER: Can the panel characterize any evidence that

5 suggests that the presence of ovotestes in male anurans results in

6 reproductive impairment via reductions in fertility?

7 DR. GREEN: To our knowledge, there are no studies that show

8 that the presence of ovotestes in male anurans results in reproductive

9 impairment. However, the panel recommends that feminized males be

10 included in grow-out studies for the purposes of using them in

11 breeding experiments to test this hypothesis.

12 DR. ROBERTS: Other responses? Other panel members?

13 And by no evidence, you mean that there is no evidence, but is

14 there any evidence that they do not? There is just no evidence?

15 DR. GREEN: There is no evidence either way.

16 DR. ROBERTS: Either way. We should make that clear in our

17 response.

18 Dr. Skelly.

19 DR. SKELLY: This may be the most appropriate point to make

20 this comment. I was glad to hear that Dr. Green mentioned

21 reproductive behavior. Because I think fertility is only part of the

1 story.

2 I think it is going to be very important if we're interested in

3 ecological relevancy to see what these genetically male and

4 phenotypically, whatever they are, how they behave when they are

5 given the opportunity to mate.

6 And I see three broad categories of possibilities at the

7 population level. One is that there may very well be no effect. And I

8 think someone else mentioned this earlier. If you remove a few males

9 from a population of frogs out in nature -- I mean, ecologists make

10 jokes about how males are superficial all the time.

11 DR. KELLEY: We make jokes about that too.

12 DR. GREEN: I left those out.

13 DR. SKELLY: Thank you. It may not take very many many

14 males to keep a population going.

15 This is a group of organisms for which minority of males in

16 many populations may routinely do most of the matings.

17 However, it may be possible if these animals that have

18 developmental abnormalities, if they actually behave as males but are

19 not fertile and they convince females that they are fertilizing their

20 eggs and leave a bunch of rotting unfertilized eggs around, that could

21 be a serious population level effect.

1 On the other hand, if these developmentally abnormal genotypic
2 males actually function and act as females, you could increase the
3 population size. You could increase viability of populations.
4 I don't know how that's going to work genetically. Maybe Dr.
5 Kelley can comment on that.
6 But I see those three broad categories. And I don't see any way
7 of figuring out how to get towards the broader goals of ecological
8 relevancy that the EPA set out without looking at this in a field
9 context on native species.
10 DR. ROBERTS: Perhaps our response could include that
11 caution.
12 Anything else, Dr. Gibbs and then Dr. Matsumura.
13 DR. GIBBS: Just another body of evidence that pertains to this
14 particular question is that interspecies comparisons have shown that
15 testes size and sperm production are positively correlated.
16 So it stands to reason that within the individual, any structure
17 such as -- over that reduce the size of testes would reduce the sperm
18 production.
19 I don't know if that's too much of an extrapolation to make. But
20 there is that correlation there.
21 DR. ROBERTS: Perhaps we should mention that, then, with

1 some appropriate citation in our response.

2 DR. MATSUMURA: I think it is pretty important to at least

3 test in the lab that those individuals with the abnormality would

4 indeed be reproductively successful or not.

5 But I was just thinking how would you do that. You can't do

6 any invasive method to say which one was, really had discontinuous

7 ovotestes.

8 How would you design, Dr. Green or Dr. Kelley?

9 DR. ROBERTS: Dr. Green.

10 DR. GREEN: I have not actually tested this myself, but I have

11 been to amphibian workshops where I have seen this done.

12 Some amphibians are small enough that as you know you can

13 see through them. If you hold them up to the light, you can see

14 internal organs.

15 One method would be to ultrasound the animal's abdomen

16 because there are ultrasound probes now that have been miniaturized

17 for the purpose of use in mice. So that's a possibility that, if you are

18 good enough, you might be able to detect gonadal structures with an

19 ultrasound probe.

20 Another possibility would be minimally invasive endoscopic

21 techniques. There are endoscopes now that have been miniaturized

1 for mice, again, that would be a small puncture. You could go in and
2 look and suture the animal up. Very similar to harvesting oocytes.
3 They would probably recover just fine and go on. So those are two
4 methods.
5 And then, of course, fancier methods would be MRI and that
6 sort of thing, and which they now make coils that are small enough for
7 mice, so I'm sure they would easily accommodate xenopus laevis,
8 which would not require sacrificing the animal, any of those three test
9 methods.
10 DR. ROBERTS: Dr. Kelley.
11 DR. KELLEY: I'm going to make my pitch, although not
12 entirely appropriate now, for using known ZZ individuals.
13 If you ran this experiment in animals where the offspring of sex
14 converted females that had been phenotypically female because they
15 have been grown up in estrogen, and males, all of their offspring are
16 male, so then you know that you have nice sibs to compare to, and
17 then you can actually run a very well-controlled experiment under
18 those conditions.
19 And you can study not only testicular development invasively
20 and noninvasively, but you can also study clasping, which is the
21 major reproductive behavior related to fertilization, and also

1 courtship song, whose importance in xenopus for successful
2 reproduction is not clear, but is a very good marker for sexual
3 differentiation.
4 DR. ROBERTS: I think what I have heard is our panel's
5 response that there is no definitive information one way or another
6 regarding ovotestes' effects as an impairment, perhaps some basis to
7 speculate that it might, and some discussion of challenges associated
8 with running those kinds of tests to provide the answer to the question
9 and some suggestions as to approaches and some discussion about
10 difficulties in setting up that kind of test and interpreting it.
11 Any follow-up questions or clarifications needed? Let's go to
12 the next one.
13 DR. STEEGER: Reduction in laryngeal muscle area suggests
14 diminished testosterone in males. If this is found to be a valid
15 observation and if estrogen concentrations do increase as testosterone
16 concentrations decrease, what other endpoints, for example,
17 secondary sexual characteristics and reproductive behavior would
18 likely be affected?
19 DR. ROBERTS: Dr. Green again.
20 DR. GREEN: The panel came up with a list of ten additional
21 estrogenic biomarkers. The first five that I have here are not invasive

1 and would not require sacrificing the animal and are easy to identify
2 phenotypically.
3 Obviously, the measurement of snout to vent length and body
4 weight in feminized males should be bigger than the control males.
5 As Dr. Hayes pointed out yesterday, nuptial pads, the presence
6 or absence or diminishment of nuptial pads and enlargement of the
7 ventral folds of the cloaca strengthen the pattern of the male calling
8 signal.
9 Dr. Kelley has some really nice recordings if anybody maybe
10 wants to hear them during the break. We were impressed with how
11 reproducible and how subjectively these might be measured.
12 We also were impressed by the fact that if you want to correlate
13 loss of function, potentially the shrinking of the larynx muscles, with
14 the morphological findings, then this would be a good way to show
15 that not only is the larynx small, it is not functioning. You could do
16 that by recording their calling.
17 Last, of course, like Dr. Kelley just mentioned, clasping.
18 There were some additional biogenic or biomarkers that people
19 suggested. One of them we have talked about at length already. I
20 won't go into it. But it is a time course examining the synthesis or the
21 presence of vitellogenin in response to an estrogen challenge. And

1 then, of course, checking oviduct development.

2 There are proteins expressed in xenopus laevis in the harderian

3 glands around the eyes. Three different proteins are expressed by

4 those animals that are uniquely female and just one protein by the

5 males.

6 Also, the number and the size of muscle fibers in the larynx and

7 myosin expression in the larynx muscle.

8 And last, we have had some additional discussions on this one,

9 but it seems like seminal, I'm not sure seminal fluid analysis is the

10 appropriate term in amphibian, but analysis of the sperm in some of

11 these feminized males would be important to look at.

12 For example, are they morphologically normal when they

13 mature. Do they have normal motility. Is the fluid they are found in

14 normal.

15 So there would be an additional assessment of the fertility in

16 some of these feminized males.

17 DR. ROBERTS: Comments by other panels members?

18 Dr. Heeringa.

19 DR. HEERINGA: I would like to make a statistician's comment

20 on the use of the laryngeal muscle or some other sort of continuous

21 measure of masculinity as distinct from other endpoints such as the

1 gonadal abnormalities.

2 Looking at the data, I think -- and secondarily to these other

3 measures, I think having a continuous endpoint or continuous measure

4 on a characteristic that appears to be and demonstrated to be related

5 to testosterone levels in the male frog, I think would be important as

6 sort of a secondary confirmation.

7 It may actually get a little further ahead in looking at mode of

8 action than just at whether there is an effect. But I think to the

9 extent that this was not costly or disruptive to measure when these

10 animals are sacrificed, I think that it would add value to the

11 experimental data.

12 DR. ROBERTS: Other comments. Dr. Kelley.

13 DR. KELLEY: As I have pointed out before, the measurement

14 of cross-sectional area of laryngeal muscle is a function of two

15 properties, the size of the muscle fibers and the number of the muscle

16 fibers.

17 Now, both reflect the history of exposure to androgen. So the

18 two of them together are some kind of indication of the history of

19 exposure to androgen.

20 However, let me just point out that while the size of the muscle

21 cross-sectional area is not very well-documented developmentally in

1 the literature, the number of muscle fibers is very well-documented.
2 And becomes statistically significantly different between males and
3 females at the stage which we call PM 1, which we have also
4 characterized.
5 I want to point out that one of the advantages of xenopus are the
6 very well-characterized metamorphic stages, which allow
7 standardization of experiments, so that when we talk about stage 56
8 animal, we know what we're talking about.
9 We carried out a similar set of study for post metamorphic
10 development. Because the development of the larynx is largely post
11 metamorphic. The brain is premetamorphic.
12 So we have those standardized data for numbers of muscle
13 fibers. You can do it in the paraffin section and you can replicate
14 previous studies.
15 So that will provide -- it is a continuous variable because a
16 number of fibers is distributed. But the data are very clear on when
17 those are expected to become different and at what time. And it
18 should provide a sensitive marker.
19 DR. ROBERTS: Dr. Heeringa and then Dr. Richards.
20 DR. HEERINGA: Thank you very much, Dr. Kelley. I yield to
21 the experts on the exact nature of the continuous measurement. I

1 think that's an excellent contribution.

2 My point is I think we would like to add this continuous

3 measurement, something that is differentiated in terms of its outcome

4 potentially at least in terms of observation from the abnormal gonadal

5 development.

6 DR. KELLEY: The point I forgot to make was that in the model

7 that EPA wants to develop for xenopus, you are going to terminate

8 your experiments at stage 66. And there is variability in the results

9 that we have been presented here as to whether there is a sex

10 difference in laryngeal cross-sectional area at stage 66.

11 We don't get a difference in weight. I never measured a

12 cross-sectional area. Dr. Hayes does get a difference in

13 cross-sectional area. Other people don't.

14 If you are going to do a grow out, the shortest period you

15 should grow out is three months when the animals are PM 1. I refer to

16 Tobias, et al., 1991 A, for a description of those experiments and

17 these stages.

18 DR. ROBERTS: '91 A?

19 DR. KELLEY: The first was the stages. The second one was

20 when during those stages the processes are hormone sensitive.

21 Developmental biology.

1 DR. ROBERTS: Great. Dr. Richards.

2 DR. RICHARDS: I wanted to echo that the development of
3 some of these measures, particularly Dr. Kelley mentioning the
4 vocalization, the sounds related to the laryngeal muscles and clasping
5 behavior, these would be really strong links to then begin looking at
6 real population level studies.

7 If there is some concrete analyses and relationships that can be
8 developed there, that would be particularly strong for the next step of
9 studies.

10 DR. ROBERTS: Dr. Gibbs.

11 DR. GIBBS: One quick comment. I'm a little bothered with
12 what seems to be an implicit assumption that bigger is better when it
13 comes to laryngeal muscle area. Because females are cuing in all
14 sorts of qualities to male calls. Not simply volume and repetition.

15 I just don't think it is necessarily that simpler a relationship
16 that larger laryngeal muscle area corresponds to greater mating
17 success via the effects on the vocalizations of these males.

18 DR. KELLEY: That's certainly true in rana, which are highly
19 discriminating animals.

20 In xenopus, how can I put it, in xenopus, unless you have a
21 male number of muscle fibers, you don't call at all. So if you make a

1 genetic female have a male number of muscle fibers and have a male
2 size of muscle fibers and she has circulating androgen, this is a
3 testicular transplant study, she will call.
4 There is very good -- I can tell you how much circulating
5 androgen you need to have in a male for him to call. It is a central
6 effect. Once his larynx is masculinized, it's masculinized forever.
7 But what I can't tell you and what nobody knows in xenopus is
8 whether the calling male gets the females.
9 This is well-known in rana where you can do phono taxas
10 experiments in the ponds in South Africa. Finding out whether the
11 calling male gets the female has not yet been established.
12 So unfortunately, the tractable experimental prep is not the
13 prep for which we have those same kinds of data that you have in
14 rana, which is -- I'm not going to call them a more sensitive system,
15 but in some ways a more subtle system.
16 DR. GIBBS: But laryngeal area may not well pertain to being a
17 success in rana.
18 DR. KELLEY: That's perfectly possible. And the other thing is
19 of course in rana, you guys don't talk about female calls, but in
20 xenopus, females have two calls. They have an acoustic aphrodisiac
21 call that drives a male nuts, and they have a turn-off call.

1 So they have a very highly developed vocal system which they
2 use in their social behaviors beyond sex -- centered around sex, but
3 getting a little bit further out.
4 So these are actually rather different vocal systems. The
5 xenopus one is much more complex in terms of call number than the
6 rana one. And you are absolutely right about ranae. We don't know
7 the relationship.
8 DR. ROBERTS: If you think it is important, Dr. Gibbs, we
9 could perhaps include the caution about extrapolation of the findings
10 to reproductive success.
11 Dr. Skelly, did you want to add?
12 DR. SKELLY: No. That's what I --
13 DR. ROBERTS: We can certainly put that in our report.
14 Anything else to add on this one? If not, let's go ahead and take a
15 break. It's 3 o'clock. Let's reconvene at about 3:15. We are
16 miraculously ahead of schedule.
17 (Thereupon, a brief recess was taken.)
18 DR. ROBERTS: Let's go ahead and get started. Before we take
19 the next question, just before lunch Dr. Kelley promised to assemble a
20 bibliography. This was in response to Question 1, I believe, and some
21 literature that we could perhaps recommend to the agency.

1 She has put that together. That has been distributed to the
2 panel. I would just ask each panel member to go over the list to see
3 whether or not you agree with it. If you have papers that you might
4 want to add to it and get back with Dr. Kelley on that.
5 DR. KELLEY: I apologize. They are slightly out of order and
6 jumbled, but I didn't have very much time. If there are papers that
7 aren't on it, just put the papers on it and I'll add them. If there are
8 papers you want me to take off, I'll take them off, except if they are
9 my papers, in which case that's nonnegotiable. They stay on.
10 DR. ROBERTS: Let's go ahead to Question 6.
11 DR. STEEGER: While some of the available data suggests that
12 there may be an association between atrazine exposure and
13 developmental effects in amphibians, the agency's evaluation of the
14 existing body of laboratory and field studies has determined that there
15 is not sufficient scientific evidence to indicate that atrazine
16 consistently produces effects across the range of amphibian species
17 examined.
18 However, the current body of knowledge has deficiencies and
19 uncertainties that limit its usefulness in assessing potential
20 developmental atrazine effects and the extent of any associated cause
21 effect in dose response relationships.

1 Consequently, the agency has determined that there are not
2 sufficient data to reject the hypothesis that atrazine can cause adverse
3 developmental effects in amphibians.
4 Does the SAP concur with these conclusions? If not, what lines
5 of evidence would lead to an alternative conclusion?
6 DR. ROBERTS: Dr. Delorme, do you concur with that
7 conclusion?
8 DR. DELORME: What I'm going to do is I'm going to actually
9 go through the conclusions and break it down. I tried to put it
10 through chromatic, and my computer crashed.
11 The first conclusion that I pull out is that there is not sufficient
12 scientific evidence to indicate that atrazine consistently produces
13 effects across the range of amphibian species examined. I had to
14 agree with the conclusion.
15 We as a panel, I think, already concluded that atrazine could
16 produce or might produce effects on gonadal development. However,
17 the consistency of the response across a species studied was difficult
18 to asses because of the problems identified with respect to the design
19 and conduct of both the laboratory and the field study. That
20 confounds their interpretation.
21 The second conclusion is the current body of knowledge has

1 deficiencies and uncertainties that limit its usefulness in assessing
2 potential developmental atrazine effects. I interpreted this as a risk
3 assessor as meaning limits its usefulness in a risk assessment contact.
4 Certainly, I don't think I would want to conduct a risk
5 assessment with the data that's been presented. So I agree with that
6 conclusion.
7 Further, EPA needs to have results from studies done where
8 other factors can be ruled out as a cause in either the presence or the
9 absence of effects. I think that's one of the key things that you guys
10 have brought out.
11 You need studies that are done where it's unequivocal that
12 atrazine is the route cause of the effect. For example, you need good
13 husbandry in the lab studies, good design for both the field and lab
14 studies and some of the other factors that have been discussed in the
15 other questions. I think that's key in gaining the data you need to do
16 the risk assessment.
17 Another conclusion, it was stated as, and the extent of any
18 associated cause effect and concentration response relationship. I
19 think we had already agreed that from the data that has been
20 presented, we can't say anything about the exact nature of the
21 response, either the shape of the dose response function or thresholds

1 or whatnot.

2 We just can't characterize it at this point from the data that has

3 been presented. It's recognized -- this is a necessary element to

4 conduct a risk assessment. You're going to need to have some sort of

5 idea, whether it's a threshold response, if it's a dose response. What

6 is the nature of the function of the dose response in order to conduct a

7 risk assessment.

8 Consequently, the agency -- this is the next conclusion.

9 Consequently, the agency has determined that there are not sufficient

10 data to reject the hypothesis that atrazine can cause adverse

11 developmental effects in amphibians. Agreed.

12 We agree that with the available -- we agree with EPA that the

13 available data does suggest that atrazine can affect amphibian gonadal

14 development. However, the available data does not allow for a proper

15 characterization of the nature and magnitude of the response, nor does

16 it offer sufficient support for the identification of a plausible

17 mechanism.

18 I guess in the end, if you add it all up, we agree with the

19 conclusions, or at least I do and Joel. We wrote this together.

20 Joel, did you have anything to add?

21 DR. ROBERTS: That sort of answers my next question, but I'll

1 let Dr. Coats respond.

2 DR. COATS: I concur with the opinion there since it includes

3 some of my ideas.

4 One other comment. We have spent over this time period and

5 through 17 or so studies an awful large amount of time picking them

6 apart and looking at every detail.

7 On the other hand, they do really constitute the body of what we

8 do know about this. There is a lot we don't know yet. And I wanted

9 to make the point they all have made contributions toward the

10 progress here and the understanding of, is it a problem, is it not, or

11 how big a problem is it or not.

12 That's one thing we should acknowledge.

13 DR. ROBERTS: Dr. Richards, did you have anything to add?

14 DR. RICHARDS: That I concur with the statements that the

15 two previous speakers have made. I have nothing more to add.

16 DR. ROBERTS: I think Dr. Isom had a question.

17 DR. ISOM: I have a question for clarification. What do you

18 mean by adverse developmental effects as opposed to just

19 developmental effects, which we have been talking about previously?

20 DR. BRADBURY: Good question. And it, I think, ties back to

21 the some of the previous questions in terms of the responses that have

1 been described in the literature thus far in the context of the risk
2 assessment endpoint, which is getting at issues of reproductive
3 fitness.
4 So again, had we probably polished the question a little better,
5 it would have been clearer, but in the context of these endpoints,
6 measures of effects in the context of the risk assessment endpoint that
7 we laid out in the problem formulation.
8 So to the extent these, for example, gonadal abnormalities
9 could be related to male fertility or reproductive fitness measures.
10 DR. ISOM: With that definition, would that change then the
11 discussion we just had?
12 DR. ROBERTS: Does anyone feel that that would change the
13 response based on -- change the panel's answer based on the response
14 by Dr. Bradbury?
15 Dr. Green.
16 DR. GREEN: Would you repeat that one more time?
17 DR. BRADBURY: I should pull out the problem formulation. I
18 think we're all tracking, but we should make sure.
19 That abnormalities that have been described in the literature for
20 an ecological risk assessment then need to be connected to the
21 measures of effects, the risk assessment endpoint in the

1 environmental management goal. The environmental management
2 goal was maintain or viability of anuran populations, the risk
3 assessment endpoint being connected to reproduction and fitness of
4 populations, and the measures of effects being connected to those
5 developmental processes that go on in amphibians that are related to
6 their ability to reproduce successfully and then hence maintain
7 populations.
8 So it is trying to maintain that causal chain. Does this
9 toxicological effect move up the levels of biological organization? If
10 you go back to that slide I had at the beginning of today, this
11 midmorning, sort of the connection between the effects at different
12 levels of biological organization connecting to the risk assessment
13 endpoint.
14 DR. GREEN: I guess in my mind the question too implies the
15 difference between whether or not the effect that we see is going to
16 have an adverse effect.
17 For most of these parameters we have been looking at, we don't
18 know yet because they haven't been carried out in grow-out studies far
19 enough.
20 So at this point, any developmental effect would be just a
21 developmental effect. And maybe we should strike the word adverse

1 because we don't know if it is adverse yet or not unless it is
2 associated with mortality.
3 DR. BRADBURY: Right.
4 DR. GREEN: Is that fair?
5 DR. ROBERTS: We can clarify that in our response that we
6 interpreted this as being developmental effects and as we have stated
7 earlier at least with respect to some of these effects that we don't
8 know yet the consequences of these observations.
9 DR. BRADBURY: Right.
10 If we go back to some of the earlier questions, you all were
11 discussing sort of the connections between changes in the larynx to
12 potential calling or other kind of secondary characteristics, which is
13 part of that discussion of what is the causal link, what is the
14 toxicological, the ecological pathway that we're addressing.
15 DR. ROBERTS: We can put that, draft that, put the caveat in
16 there.
17 DR. DELORME: The way it's written now is actually kind of
18 broad. Because what I said is the available data does not allow proper
19 characterization of the nature and magnitude of the response.
20 What we're saying is we don't know how far it is going, but I
21 can amend that to say at the organism or population level, if that's

1 agreeable.

2 DR. ROBERTS: Sure. Does anyone disagree with the

3 statements that have been made so far?

4 I don't see any. Any clarifications or follow-up questions from

5 the agency?

6 Let me clarify one thing for the audience. From time to time

7 now as the panel have given their responses, they have indicated that

8 they have worked with somebody else on the panel in terms of writing

9 something up.

10 Under the Federal Advisory Committee Act, individual panel

11 members can talk to other panel member is they have responsibility on

12 a same topic, and sort of discuss the issues.

13 But I want to make very clear that the panel has not met and

14 undergone any deliberations other than in this room in open session.

15 There have been discussions among panel members during

16 breaks and in the evening, that sort of thing. As individuals, that's

17 allowed, but there has not been any closed session of this panel to

18 deliberate any of these issues.

19 Let's go Question 7.

20 DR. STEEGER: Assuming the agency determined an ecological

21 risk assessment with a greater degree of certainty concerning

1 developmental effects of atrazine on amphibians were needed, please
2 comment on EPA's conclusion that additional information is required
3 to evaluate potential causal relationships between atrazine exposure
4 and gonadal development.
5 Please also comment on the added utility, if any, of additional
6 information to interpret the shape of dose response curves for
7 potential developmental endpoints and the extent to which threshold
8 or nonthreshhold response relationships can be quantified.
9 DR. ROBERTS: Dr. Delorme.
10 DR. DELORME: I'm going to handle the first part of this
11 question and let Dr. Coats handle the part about the utility of
12 interpreting the shape of the dose response curve.
13 I think we agree with that, the statement that -- or EPA's
14 conclusion that additional information is required to evaluate the
15 potential causal relationship between atrazine exposure and gonadal
16 development.
17 The relationship is there, we think. There is tantalizing
18 evidence that something is going on. But it needs to be confirmed. Or
19 what is being suggested needs to be confirmed. And we need to
20 characterize the nature of the dose response function.
21 One of the tenets of the scientific methods is the repeatability

1 of experiments. Certainly, we have had attempts made to repeat this
2 that have been submitted to EPA and we have looked at.
3 But definitely, we need to firm up the existence of the causal
4 relationship, and, if it is there, if it does exist, we need to
5 characterize the nature of the dose response function.
6 There is also a need to identify a plausible mechanism. I think
7 one thing we have to recognize is the identification of the mechanism
8 is important because it can in part aid in the extrapolation of the
9 results from the surrogate test species, the species of concern in the
10 environment.
11 DR. ROBERTS: Dr. Coats.
12 DR. COATS: I have a few things to add.
13 The plausible mechanism explanation needs to be put forth with
14 some data.
15 Secondly, there needs to be some similarity of data or patterns
16 or trends from several research groups to show repeatability of the
17 experiments.
18 The dose response curves are extremely important to the
19 question of any detrimental effect of any toxicant on an organism,
20 regardless of whether the relationships demonstrate a typical or
21 atypical concentration response curve for a given endpoint.

1 It should be possible to ascertain the shapes of the curve given
2 enough concentrations, enough replications and controlled conditions.
3 Repeatability in other labs should also be feasible if the same
4 species, stage, water concentrations and timing are utilized.
5 Another point is that studies on quantitative structure activity
6 relationships can often provide information about the mechanism of
7 action as well or provide rationale for the data is generated from
8 comparative testing.
9 Experiments that use a series of closely related compounds,
10 atrazine, cyanazine, propazine, simazine, terbutyl azine (ph) et
11 cetera, could elucidate patterns that can help explain the interaction
12 between the molecule and the putative receptor addressing the causal
13 relationship.
14 This approach seems to be lacking so far and could be valuable
15 in the invivo test for gonadal development as well as enzyme
16 induction or MRNA expression.
17 DR. ROBERTS: Thank you, Dr. Coats.
18 Dr. Kelley, did you have anything to add?
19 DR. KELLEY: No. I completely concur.
20 DR. ROBERTS: Anyone else have any comments?
21 DR. GREEN: I also concur.

1 DR. ROBERTS: Thank you, Dr. Green. Anyone else like to
2 weigh in on this one?
3 Does anyone disagree, I guess I should ask that. I don't hear
4 any disagreement.
5 Any follow-ups or any clarification needed? Let's then go on to
6 Question 8, which I will point out is a seven-part question.
7 Dr. Richards has got that one.
8 DR. RICHARDS: Being the nonexpert that I am on almost
9 every part of this --
10 DR. ROBERTS: Let's let the agency go ahead and pose the
11 question to us.
12 DR. STEEGER: The agency has developed a conceptual model
13 from which to develop a set of study of protocols for evaluating the
14 potential effects of atrazine on gonadal development on amphibians.
15 The agency has proposed a research approach using focused
16 empirical laboratory studies based on initial investigations with
17 xenopus laevis followed by selective confirmatory studies with frog
18 species native to North America.
19 This is a proposal. It is not set in stone. We have -- as has been
20 indicated in a number of follow-up questions. Please comment on the
21 proposed sequence of the study objectives.

1 DR. ROBERTS: Dr. Richards, we'll go through these I guess
2 one at a time. Do you want to take the first one?
3 DR. RICHARDS: Yes. I'm going to invite the persons listed
4 and not listed on this question to jump in on an open discussion here.
5 But on the please comment on the proposed study of sequence
6 objectives, I think Dr. Kelley had already and several others have
7 mentioned about the potential and importance of some types of field
8 oriented studies.
9 That was the one comment that I had on this also, and not to
10 preclude them in a parallel track with some of the other laboratory
11 based studies.
12 DR. ROBERTS: Dr. Skelly and then Dr. Kelley.
13 DR. SKELLY: Just to support what Dr. Richards just said, I
14 think it is going to be important to get the field component of any
15 evaluation undergoing as soon as any of this happens in part because
16 field studies for lots of reasons take a long time to set up and get
17 going.
18 They are not necessarily as money intensive as some of the
19 laboratory studies, but observational studies and experimental studies
20 take time to find locations as we have seen in evaluating some of the
21 past data. That can make a lot of difference in how useful these

1 studies will be when it comes to interpreting them.

2 I guess I just can't emphasize enough that I think it is going to

3 be critical to get that side of things moving in order to meet the

4 objectives laid out in the conceptual model by the EPA.

5 DR. ROBERTS: Dr. Kelley.

6 DR. KELLEY: Could you remind me again, what is the first

7 thing that you plan to do?

8 DR. BRADBURY: Phase one in the white paper.

9 DR. KELLEY: Oh, yes.

10 DR. DELORME: Test for apical gonadal effects of --

11 DR. KELLEY: So I concur with that. I would argue that we

12 should attempt to strictly replicate some of the studies that show an

13 effect, especially a low dose effect.

14 I accept the fact that in a replication one will often want to add

15 groups and so on. But we have a real discrepancy in the threshold for

16 an effect if in fact one exists.

17 I would argue for a strict replication, perhaps a replication of

18 the high dose and a replication of the study that did the low dose.

19 I think it is worthwhile knowing how reliable and repeatable

20 the initial observations are before we go forward.

21 Now, there has been -- anyway, I could go through it. But I

1 think it is very important to track down the sources of variability and
2 the results before going forward. Because suppose you were never
3 able to repeat any of them or you always repeated them and in a much
4 lower dose.
5 It would affect so strongly the next steps in the sequence that I
6 would argue very strongly for starting with that.
7 DR. ROBERTS: Dr. Kloas.
8 DR. KLOAS: I concur with the comments already made.
9 I would also point out maybe it is time saving and saving money
10 if you could start again already in parallel with doing some
11 mechanistic studies.
12 For instance, there was some claim there is no interference with
13 estrogen receptors, with androgen receptors.
14 There are some experience which could be very easily maybe to
15 say there is nothing going on. Especially, I have some concern maybe
16 if there is only a small effect on thyroid system, you will not see it on
17 morphological -- looking just on morphological stages.
18 There could also be one biomarker, for instance, TSH, to show
19 up any inhibitory effect in addition which could be done in parallel
20 for doing such a developmental study.
21 DR. ROBERTS: I'm going to jump in and make a comment too.

1 I think that if we do additional mechanistic studies, if there are
2 variable aspects, I think we -- as Dr. Kelley pointed out, I think we
3 have to get a handle on those first because we have to know when
4 animals respond and when they don't respond.
5 Because we want to be sure we're looking in animals that are
6 responding to do, to see whether or not the mechanisms we think
7 might be operating are, in fact, in place.
8 I don't disagree that mechanistic studies are important, but I
9 think getting a real solid handle on the phenomenon and being able to
10 reproducebly observe that, I agree with Dr. Kelley, is a first priority.
11 Dr. DeLorme and Dr. LeBlanc and Dr. Denver.
12 DR. DELORME: I just wanted to concur with Dr. Kelley. I
13 think that what has been proposed is quite a logical sequence. The
14 first thing you really need to do is confirm whether or not there is any
15 relationship between atrazine and the effect.
16 And certainly what Dr. Kelley had proposed was repeating the
17 experiments to see if you can find that, but do it under controlled
18 conditions where the husbandry and whatnot is -- any factors that
19 might affect it are controlled for.
20 Followed by looking in possibly native anurans from North
21 America to see whether or not you are going to get the same kinds of

1 effect. I think that's another good logical step to take.

2 DR. ROBERTS: Dr. LeBlanc and then Dr. Denver.

3 DR. LEBLANC: From my perspective, the further you go up in

4 that cascade of complexity that Dr. Bradbury had up there earlier on,

5 you are reducing your chances of seeing the effect that you are trying

6 to confirm upfront and sort of give you some evidence that you should

7 proceed.

8 Certainly, I wouldn't do preliminary studies or initial studies at

9 the population level. I think gonad development is the critical

10 endpoint of interest, but I'm not sure I would do it at that level.

11 I think that perhaps the first experiments that I would do would

12 be looking at some cellular response that I'm more likely to see. And

13 once I have identified doses at which that response occurs and I'm

14 comfortable that that response is occurring, I think I would build

15 upon that.

16 And then I would look at tissue level effects in terms of

17 gonadal development and ultimately effects of the individual and the

18 populations, is my perspective.

19 DR. ROBERTS: Dr. Denver.

20 DR. DENVER: What we really want to know here, we want to

21 know whether there are population level effects ultimately. Is that

1 correct?

2 And that's something that is going to take presumably a while to

3 sort out.

4 The next level, I assume, is to understand what might be fitness

5 effects at the individual level.

6 And I wonder -- this actually is a more general question that

7 goes beyond just simply atrazine. But I wonder if we can begin to get

8 a handle on those fitness, those individual fitness effects by looking

9 at individuals or individuals that are presumably intersex or have

10 gonadal abnormalities in the field, which I think we agree has been

11 documented, and find a way to identify those individuals which we

12 discussed a bit earlier perhaps using some MRI approaches or

13 something of that sort. And do the grow-out experiments and

14 determine if there are really fitness consequences of having these

15 intersex gonads.

16 Is that something that we should be doing now? As I said, it

17 goes to a larger issue, larger than just simply atrazine.

18 And these intersex individuals may result from contaminants

19 other than atrazine. But is that something that we want to consider

20 sooner rather than later?

21 DR. ROBERTS: We're getting some interesting differing views

1 on sequence of or highest priorities.

2 Any other panel members want to weigh in on this?

3 Dr. Delorme.

4 DR. DELORME: That is actually an interesting thought. I

5 never thought of that before.

6 But if there was some chemical that you could come up with to

7 induce intersex that you knew for search was going to the happen, that

8 might answer some of the questions with respect to fertility and

9 possibility of effects at higher levels of biological organization.

10 Instead of waiting to find out what is actually going to go on and

11 identify --

12 You could do it in parallel with atrazine. Because certainly as

13 we go through -- as risk assessor, I'm talking, as we go through other

14 chemicals, there certainly are going to be cases where these kinds of

15 effects arise.

16 It is always a question. What is the ecological relevance of

17 ovotestes. I know in the fish community it is something they are

18 grappling with now. Because in a lot of the rivers in Britain and in

19 the states, they have identified fish populations where ovotestes type

20 effects do occur. The so what question is still there. Well, do they

21 contribute to the population or does it have a population level effect.

1 So that's actually an interesting take on things.

2 DR. ROBERTS: Dr. Kelley.

3 DR. KELLEY: So there is good news. There is a chemical that

4 does it. In xenopus, estrogen does it. It is very well worked out. The

5 critical period is worked out. How much ovotestes. I'm using that

6 word in the sense in which I used it before, and I refer you to the

7 figure in the Witschi paper. It is very well worked out.

8 The animals that were in that paper were adult. They persisted

9 until adulthood. It looked like it wasn't resorbed. I would suggest

10 that there is no necessity to run this as a serial experiment. I'm

11 totally opposed to that.

12 You run it in parallel. You run it in parallel also with

13 beginning your ecological assessments, because I'm in total

14 agreement. Field work takes forever. Getting it published takes

15 forever. Anyway, just doing the work takes forever.

16 So I would start the field studies and I would start a replication

17 of the previous studies on the atrazine effects. I would do it in ZZ

18 animals. You know what genotype you were dealing with.

19 I would do it in a well-characterized population of animals and

20 I would run a group along with it with estrogen at various time

21 periods during the defined critical window.

1 And then you have to grow those animals out. It's a male.
2 That's the good news. It's only six months. And then you run those
3 animals in behavioral and fertility tests. And then you know if
4 animals with 66 percent ovaries and 33 percent testes or something
5 produce fewer offspring than animals with a different ratio.
6 Those studies are actually fairly easy to do in a well-defined
7 controlled way. You can either use natural behaviors or you can kill
8 the animals and mush up their testes and fertilize eggs with them.
9 You don't have to kill the animals. You can just take their
10 testes out.
11 DR. ROBERTS: Other thoughts?
12 Lots of different ideas, Dr. Richards, unfortunately for you to
13 capture.
14 DR. RICHARDS: I'm sure many of those words will be on to a
15 text file and given to me.
16 DR. KELLEY: Yes, after dinner.
17 DR. ROBERTS: Before we move on to B, let me make sure. Is
18 there any follow-up question or is it as clear as it can be given the
19 fact that there are some differences of opinion about sequences?
20 DR. BRADBURY: I think it would be helpful for the dialogue
21 to continue a bit. I'm approaching this carefully.

1 And with all due respect to all members of the panel, in the
2 context of science, for a purpose, which is trying to incrementally
3 improve our ability to reduce the uncertainties on the risk assessment
4 and how that marries up with and is complimentary to advancing
5 knowledge, because it is important to advance knowledge, and to the
6 extent the panel has any feel of, for lack of a better word, where you
7 could get the biggest bang for your buck to start either sequence how
8 you would stage information gathering or a sense of what information
9 we start to clarify or reduce some of the uncertainties in the current
10 ability to go through the risk assessment process.
11 It is sort of -- the dialogue has been interesting. I think Jere in
12 thinking about the endocrine disrupter screening assays and some of
13 the other descriptions about using QSAR and then going from the
14 field down sort of to illustrate very legitimate differences of opinion
15 in terms of how you blend mechanistic understanding with ecological
16 relevancy and where are you in that continuum, it seems like we're
17 getting advice to do everything all at once.
18 And while maybe that's the only way we can solve this problem,
19 if that's the conclusion, then that's the conclusion.
20 I think it would be helpful to hear about what some of the
21 trade-offs would be at least in picking different places to start in the

1 phased approach that we provided.

2 It may mean you may all feel that you should start all five

3 phases all at once, and that's cool. But it would be helpful to at least

4 get input from the panel in terms of the trade-offs if one couldn't do

5 all five phases at the same time.

6 DR. ROBERTS: Let's ask the panel. What if you couldn't do

7 them all at once, where would you start? Dr. Kelley and then Dr.

8 Green.

9 DR. KELLEY: One in five.

10 DR. ROBERTS: Beg your pardon?

11 DR. KELLEY: That was it.

12 DR. GREEN: Testing the working hypothesis phase experiment

13 Number 1, the test for apical gonad effects and Number 5, ecological

14 relevancy of the study. I concur with Dr. Kelley's comment.

15 But since you have solicited additional dialogue, I have a few

16 more things that I think are imperative to standardizing the

17 conditions. And this should be done in Phase 1. So right from the

18 get-go we can do the best we can.

19 One of the things that struck me yesterday about the housing

20 and the husbandry conditions was the extreme variability and the fact

21 that one of the things we were measuring was growth rate and trying

1 to make a connection between the effect of atrazine on growth rate
2 and then on the growth rate in the control tank when between labs and
3 within the lab there were specific issues with water quality and
4 feeding and other things that could account for variability in growth
5 rate alone whether atrazine was there or not.
6 So I'm going to take the opportunity to make a plea. And part
7 of this bleeds into Question Number 4, which I was actually waiting
8 to answer, about two issues.
9 And the first one is with water quality. This is not pertaining to
10 measuring levels of atrazine. But I think we should make some
11 attempt to define the stocking density first off for both embryos and
12 adults and juveniles for xenopus laevis.
13 Unfortunately, that hasn't been standardized in Laboratory
14 Animal Medicine even yet, but there are some recommendations.
15 The proceedings for the National Academy of Sciences has
16 recommended one to two liters for adults. And there are various
17 textbooks and original papers for tadpole stocking and density. I
18 think we talked about that a little bit already. So that would be one
19 thing.
20 One thing that was lacking in the descriptions of many of the
21 papers was the kind of water that was used. I couldn't tell. Was this

1 deionized or R O treated or reconstituted salt water. Was it well water
2 from a source. Was it chlorinated or chloraminated, potable tap water
3 that had been filtered.
4 And I think all those water sources are perfectly suitable. But
5 it needs to be stated and, if possible, standardized so that everybody
6 who runs the test on atrazine uses the same kind of water.
7 The other thing would be water quality testing. For this
8 purpose, because there is evidence in the literature that atrazine and
9 nitrites interact and that they have some by-product of ammonium
10 metabolism, when the ammonia levels get high in tanks, it may falsely
11 elevate the detrimental effects, if any, that any other chemical has in
12 there. It could be chlorine. It could be atrazine.
13 So water quality should be measured on a regular basis.
14 Depending on the duration of the experiment and the stocking density
15 and the water turnover, I would say once a day. It could be done with
16 a quick dip stick that is a relatively inexpensive test. It doesn't have
17 to be the Hawk (ph) analytical test every day, but some notation of
18 water quality parameters on a regular basis.
19 That would include pH, conductivity, water temperature,
20 ammonia, nitrate, nitrite. And because we want to know what kind of
21 water is used, we should be measuring for chlorine and chloramine

1 and the heavy metals that we know interfere with tadpole development
2 like copper and some of the other things.
3 So I would like to see that in most of these reports at least have
4 been part of the quality control.
5 So water quality is one issue in Phase 1 that I think if we could
6 standardize. And you can't go to the literature and find this.
7 There is a paper that was published in the Laboratory Animal
8 Medicine Journal that reported a survey across the nation of what
9 most xenopus users are doing right now that you can use as a guide.
10 I have the reference here that I'll add to the list for Dr. Kelley
11 on all these parameters.
12 The second issue that I wanted to bring up was feeding. I'm
13 sure Dr. Kelley will bring this up as well.
14 But Dr. Hayes pointed out that he felt that maybe some of the
15 animals in the Syngenta studies had been underfed. And I looked at
16 that -- I went back and looked at that paper and looked at some of his
17 publications on what he was feeding.
18 It struck me that he is feeding rabbit chow. I find this a little
19 bit disconcerting because adult xenopus are strict carnivores. And
20 that is an herbivore diet.
21 DR. HAYES: The adult xenopus were fed trout chow, not rabbit

1 chow.

2 DR. GREEN: Dr. Hayes in the back said his adult xenopus were

3 fed trout chow. But if we continue these studies into grow-out

4 experiments where juveniles and adults are used, then we should

5 probably feed them a diet that's at least 14 percent protein, which is

6 more appropriate for a carnivore.

7 There are references. Kevin Wright has published in another

8 textbook, Amphibian Medicine and Husbandry, a chapter. In that, he

9 recommends that we not feed adult and juvenile xenopus laevis,

10 anyway, diets for omnivorous fish and turtles or for herbivores.

11 So you can go to a feed company, there are several reputable

12 ones, that will make xenopus chow for you or you can buy it from the

13 big distributors. Nasco and Xenopus 1 have their own that they sell.

14 I think in an attempt to standardize across labs, that's probably

15 the appropriate food for that age.

16 Now, tadpole feeding -- those companies also sell food, if I'm

17 correct, for younger metamorphs and juveniles as well. And they eat

18 phytoplankton.

19 I'm not so certain how important it is that they have a high

20 protein diet to maximize growth and health under these conditions.

21 But if you are going to make those comparisons in growth rates

1 and fecundity, then probably standardizing to a carnivorous
2 amphibian diet would be the best thing to do.
3 The companies also have based those diets and the amount of
4 feed on growth rate curves that they have developed. I believe they
5 will share them with you. I have asked for them and they have been
6 very helpful in advising me.
7 They also make recommendations on how much to feed per
8 animal. That's based on known anuran kilocaloric requirements at
9 different temperatures.
10 So if we hold tanks for some of the juvenile and adults at 19
11 degrees versus 25, we should feed them accordingly. There are ways
12 to do that.
13 If you look to the fish literature, as young fish grow, they are
14 regularly weighed once a month on mass. The tank feed is adjusted
15 accordingly.
16 I know that it is not standard practice right now to do that for
17 xenopus. Usually, you have about, whatever, 5, 50 or 100 in a tank
18 and you throw so much food and make sure they eat it all.
19 But in terms of water quality level, what is not eaten and the
20 amount they excrete will affect the amount of ammonia, which in turn
21 affects the amount of atrazine that is or isn't available or potentiated.

1 Those are two conditions, at least, the water quality and the
2 feeding that I think this Phase 1 should be spelled out as best we can.
3 And the temperature that we all agree would be the right place to start
4 with in conducting these experiments.
5 DR. ROBERTS: I think it will be important for us to convey
6 Dr. Green's recommendations in our report. I don't think it
7 necessarily belongs as part of 8 A, but we'll get it in there. I think it
8 is important to pass that information along to the agency.
9 With regard to the question posed by Dr. Bradbury, if you
10 couldn't do them all at once, what would you do?
11 Dr. Delorme.
12 DR. DELORME: I would concur with Dr. Kelley that probably
13 the first thing you want to do is -- if your goal is to move the atrazine
14 risk assessment forward, then I think you have to confirm the causal
15 relationship. That's the first thing you need to do. That would be the
16 first priority to do.
17 DR. ROBERTS: Dr. Coats.
18 DR. COATS: I think that the laboratory approach as described
19 in Phase 1 is probably the most obvious and least risky from a benefit
20 -- from the point of time invested and money invested.
21 And so that's where I think we should start.

1 DR. ROBERTS: Dr. Skelly.

2 DR. SKELLY: At the risk of boring everyone, I'm going to
3 reiterate that I think pushing ecological relevancy off to Phase 5
4 could be a mistake.

5 We're testing for apical gonadal effects in Phase 1. Then Phase
6 2 is sex steroid measurements. Phase 3 is aromatase activity
7 measurements. Phase 4 is aromatase inhibitor study. Phase 5 is
8 ecological relevance.

9 I think there could be a giant woops there if we get to the end
10 and find out that the population level effects aren't what we're all
11 being concerned about here.

12 DR. ROBERTS: Dr. Delorme.

13 DR. DELORME: Just as a point. If you look at figure one in
14 the white paper, it actually shows after the test for apical gonadal
15 effects and a yes being found, there is a line off to the side with
16 Number 5.

17 Can EPA comment on what was intended with that? It wasn't
18 clear whether or not you intended the ecological relevance test to
19 start at that point.

20 DR. SKELLY: Is ecology fifth level priority or not?

21 DR. TIETGE: It just so happens that we used numbers. We

1 could have used colors I guess to indicate what order to do them in.
2 Clearly, as Dr. Delorme pointed out, we had that split in the
3 sequence to suggest that if you get effects at the individual level, then
4 it may be more prudent from a risk assessment point of view to
5 conduct some field work.
6 I wouldn't read into the ordinal numbers here very much.
7 DR. ROBERTS: Does that help, Dr. Skelly?
8 DR. SKELLY: Yes. If I can follow up.
9 I'm fine with that with one important caveat. That is, we're
10 going to get to this a little bit farther down. But if the panel feels
11 that there are significant concerns about context dependence between
12 species, and at least in my case I think we have seen some evidence
13 that that could be the case, then I don't see that a no on apical gonadal
14 effects in xenopus necessarily means that we shouldn't be thinking
15 about an ecological relevance study for native species.
16 DR. ROBERTS: Dr. Bradbury.
17 DR. BRADBURY: Just to get a clarification, because the white
18 paper talks about using xenopus to get started as a biological model
19 bla, bla, bla, but also talks about other species to also be looked at in
20 the context of phase one style experiments.
21 I guess the clarification that if xenopus didn't reproduce the

1 previous results, would the panel feel it would be useful to try it out
2 in rana in the Phase 1 style experiment or to immediately go to a long
3 term reproductive study or go out to the field to look at rana or other
4 native species?
5 DR. ROBERTS: Expression of preference on that?
6 Dr. Kelley.
7 DR. KELLEY: Well, you know, I'm for parallel processing.
8 Let's face it. We don't care here in America about the survival of our
9 xenopus because they are well taken care of in the lab.
10 But we care about the survival of our native frog species. You
11 are just going to have to start the field studies right away. But I do
12 agree that it would be useful to see if we could run some rana
13 experiments in the lab.
14 Of course the problem is rana is a lot harder to run in the lab.
15 And rana is about a zillion times more difficult to work on than
16 xenopus and so forth.
17 But that isn't to say that it shouldn't be attempted, because it
18 will be very informative. There are folks that are good at rana. I'm
19 not one of them. But I know there are people that are.
20 It would be worthwhile to try in a limited way to see if we
21 could get that to go. But it is going to be a lot harder. The

1 background literature that is available for xenopus is just not
2 available for rana.
3 It will be somewhat more difficult to interpret the results. But
4 those are the results we care about. Because these are our frogs, our
5 North American frogs. We care a lot about them.
6 We should study them along with the indicator species, which
7 has many, many advantages, but is not native to our country.
8 DR. ROBERTS: Other thoughts?
9 Dr. Richards.
10 DR. RICHARDS: I concur with what Dr. Kelley has brought up
11 here. I think that ultimately the agency is going to have to go there
12 no matter what answer we get from xenopus. And it could lead us
13 down some very good trails.
14 Ultimately, the question is going to come to is what does that
15 mean to ranid species. And if we don't start now in trying to further
16 hone or develop methods that are appropriate for them, it is just going
17 to prolong the whole process.
18 DR. ROBERTS: Dr. Tietge.
19 DR. TIETGE: I think what we have to keep in mind is that
20 using the rana species or the intent of using the rana species is to do
21 the confirmatory work.

1 But you do the hard work I think in xenopus because it is a
2 more useful model at this point in time. So I don't think we meant one
3 or the other.
4 I think -- I agree with what I'm hearing here that you probably
5 will end up doing it in both species at least in that Phase 1 study.
6 But the intent of the xenopus is to go faster and farther to
7 understand, for example, mechanistic pathways that you would then
8 go back and in a more focused approach look at with the rana species.
9 DR. BRADBURY: Just to help clarify because you've been
10 getting some understandably different kinds of views.
11 At one point we heard we should try taking account of
12 variability associated with some of the current studies to try to see if
13 we could replicate what had happened in the past.
14 And all there is a ranid study. There are several xenopus
15 studies. That would imply get started with xenopus to see if you can
16 get it to happen in xenopus again. Start with ranid.
17 Again, I know you are probably thinking this through as we go.
18 But as the panel deliberates, it will be helpful to get, if not definitive
19 answers, at least some thoughts of the cost benefit, that's not the right
20 word, but the ups and downs, the trade-offs associated with some of
21 the different choices in the pathway.

1 DR. ROBERTS: Dr. Denver and then Dr. Green.

2 DR. DENVER: Let me sort of echo the comments of Dr.

3 Richards and play devil's advocate.

4 What if you are unable able to replicate the xenopus results and

5 you are unable to show in fact any effects of atrazine? What decision

6 would you then make?

7 Would you then decide to go on to ask the question in North

8 American ranid species or would you conclude that, in fact, there is

9 no basis for concern?

10 DR. BRADBURY: I think some of that dialogue -- some of the

11 issues that one would face in making that decision are some of the

12 latter questions we have in the series of question eight which gets at

13 some of the issues about toxico dynamic and toxico kinetic

14 differences among frogs.

15 And to not dodge the question, I think I would benefit greatly

16 from the panel's dialogue on those issues that could drive interspecies

17 extrapolation.

18 DR. ROBERTS: Fair enough.

19 We've had a lot of dialogue. I hope Dr. Richards has been

20 keeping up.

21 Let me see if we can decide whether or not the -- is there some

1 sort of consensus in terms of priority or are there still differences of
2 opinion?
3 Dr. Richards, what is your sense?
4 DR. RICHARDS: I'm hearing that most of the panel seems to
5 feel comfortable that some of the -- the Phase 1 experiments need to
6 be replicated, techniques cleaned up. That work needs to go forward.
7 I'm hearing that from most people.
8 I have heard from a little bit smaller number that some aspects
9 of Number 5 need to go forward in terms of grow out or some of the
10 other basic ecological studies. Not necessarily full ecological
11 studies.
12 DR. ROBERTS: I sort of heard unanimity in the desirability for
13 the Phase 1 studies to proceed. And some support for, if possible,
14 beginning the Number 5 ecological studies, because recognizing that
15 that -- demonstrating that relevance would be very important for the
16 risk assessment.
17 Does somebody else have a different take?
18 Dr. Gibbs.
19 DR. GIBBS: I would concur. I thought Dr. Kelley had outlined
20 a sequence that is really quite doable in a fairly short time frame in
21 terms of looking at the fitness consequences with intersex in normal

1 males and then you could actually put those in a natural environment
2 and look at the consequences and mating with maybe even molecular
3 genetic markers getting the tadpoles or metamorphs.
4 But I don't think until there is -- rather than a full-blown
5 ecological study, 100 wetlands, et cetera, we're not talking about that
6 necessarily for Phase 5. I think just a shorter term study like Dr.
7 Kelley outlined I think is quite doable.
8 DR. ROBERTS: Does that sound reasonable to the panel? All
9 right. Good. Then let's try and have the minutes the reflect that
10 recommendation.
11 Any follow-up questions from the agency? Dr. Green and then
12 Dr. Isom.
13 DR. GREEN: I wasn't clear now. Was the decision that ranA
14 and xenopus experiment should be run in parallel, if possible?
15 DR. ROBERTS: I think we're going to take up the species --
16 DR. GREEN: Later on?
17 DR. ROBERTS: Yes. As we go a little bit further down.
18 Hopefully, after we have that discussion, then we'll be able to clarify
19 our recommendation for that aspect.
20 Dr. Isom.
21 DR. ISOM: I wasn't clear also with regards to the first study.

1 A lot of data, mechanistic data, can be obtained, related
2 mechanistic data can be obtained from that study. Are we just
3 advocating looking at, say, anatomical gross abnormalities in these
4 animals, the survivability, or are we also saying we should be looking
5 at blood levels of hormones and other associated effects in those
6 animals?
7 If you have those tissues of those animals, why not get the
8 mileage out of them?
9 DR. BRADBURY: 8 B starts to get at some of the very issues
10 you are bringing up.
11 DR. ROBERTS: I guess my initial response, Gary, would be to
12 encourage doing superimposed mechanistic studies while they are
13 doing these.
14 DR. ISOM: It wasn't clear.
15 DR. BRADBURY: In fact, Question Eight B charged the panel,
16 very explicitly asks the panel for advice and counsel on the attributes
17 of these studies.
18 DR. ROBERTS: Then let's go to Eight B.
19 DR. STEEGER: Please comment on whether the agency's first
20 set of proposed studies has accounted for the major sources of
21 uncertainty associated with the potential effects of atrazine on anuran

1 sexual differentiation.

2 In addition to the time to metamorphosis, gonadal

3 abnormalities and sex ratios in the proposed Phase 1 assays, please

4 comment on any other endpoints that should be considered in this

5 initial phase.

6 DR. ROBERTS: Dr. Richards.

7 DR. RICHARDS: No. The first part I think we pretty much

8 elaborated on, it seems to me, in several of the other questions. No,

9 we haven't accounted for the major sources of uncertainty associated

10 with potential effects.

11 And I'll let the discussion open up in terms of the other

12 measures as Dr. Isom and others have initially brought up.

13 DR. ROBERTS: Dr. Green would like to comment on that.

14 DR. GREEN: I think I already commented basically on the

15 husbandry issues. But in terms of in addition to time to

16 metamorphosis gonadal abnormalities and sex ratios, please comment

17 on any other endpoints, and we addressed that with the 10 different

18 estrogenic biomarkers.

19 I'm not clear if the goal here is to get these studies done quickly

20 and in what level of detail we want to look at these things. Because

21 all 10 of them would be a lot of work. The first four or five, which

1 are observational and easy to see, I wouldn't suggest would be the
2 place to start.
3 DR. BRADBURY: I think that kind of a discussion would be
4 helpful. What would be the experimental investments, time, effort
5 associated with adding the different endpoints in, what is the kind of
6 information that is gained as one gets that information.
7 Some of the logic in the different phases, probably isn't the
8 right word, but the components or the trying to sequence things is sort
9 of an approach to if we get the frank apical effects, then -- part of this
10 question is getting at are there ways to maybe blend some of the
11 concepts that are in the analysis plan and try to link some things up
12 more quickly. That's the question.
13 Getting some insights on what some of the efforts would be and
14 some thought about what would this experimental design start to look
15 like I think is important.
16 DR. ROBERTS: Go ahead and follow up, Dr. Green.
17 DR. GREEN: We're trying to decide here -- in Phase 1 let's say
18 we're going to start with xenopus laevis. And in Phase 1 we're going
19 to grow them out to three months. That was something Dr. Kelley
20 suggested. And I concur that would be the minimum because then we
21 could see some of the secondary sexual characteristics start to emerge

1 or not.

2 And we would look at different stages and ages along the way

3 out to three months. Is that correct?

4 DR. KELLEY: Let me just talk about a moment the sex ratio.

5 You are not going to be able to interpret the sex ratio unless

6 you have a uniform genotype. Here is what I would do. I would get

7 Nasco or Kelley Evans at Xenopus 1 to establish for me a stock

8 colony for researchers to draw their animals from.

9 They will be willing to do this. I would like to point out to the

10 audience that these places are agricultural facilities in their home

11 states. They support the greater agricultural goods. Xenopus is a

12 farm product.

13 Regulate is a farm product in many states. We're growing frogs

14 here. It's not corn, but frogs.

15 Anyway, so you have to start with ZZ animals, otherwise the

16 sex ratio becomes very difficult to interpret without long breeding

17 experiments. It takes two years to get a female to breed. You don't

18 want that. It will really slow you down.

19 How long will it take to get ZZ animals. If you get donated ZZ

20 animals that people have, you would have to confirm they really are

21 ZZ by mating them to normal males and having all male offspring.

1 That would take three months.

2 If you have to make them, that will take six months. Three

3 months minimum, six months maximum.

4 Then you would run your experiments in the presence and

5 absence of atrazine and various other things, and you should be able

6 to get data on that in, I would say, six months. That's a year.

7 Nine months to a year minimum. With things going wrong,

8 that's 12 months basically for sure and maybe 18 months.

9 So 18 months to replicate with a known population under

10 defined growing conditions. That's what that would take in a lab that

11 was up and functional.

12 In a lab that had never dealt with frogs before, though, I would

13 like to point out that it will take longer. If you contract this out or

14 something to people who have never raised xenopus, it will take

15 longer because there is a learning curve, learning how to keep the

16 animals and keep them happy and so forth. Just catching them.

17 Learning to put lids on so they don't hop. These are major things.

18 Is that the sort of thing you want? That's how long it is going

19 to take just with xenopus.

20 DR. BRADBURY: In the white paper we laid out attributes that

21 may or may not be consistent. Some may be, some probably not

1 consistent with what you just described.

2 On the screen, we have laid out some of the details of that.

3 DR. KELLEY: I disagree deeply with some of the details.

4 DR. BRADBURY: Again, it was a plan to get this kind of

5 dialogue going. I'm trying to figure a way see if I can get the

6 chairman to help us sort of systematically explore some of these.

7 DR. ROBERTS: I think there is lots of issues. In CS for range

8 and spacing and number of concentration, some of those things are up

9 here -- you are looking for feedback on what should be done in these

10 experiments. And I think we can go sort of go through those aspects.

11 I think your first question was in B is what endpoints should we

12 look for. And so let's answer that question. And then we can go on to

13 provide you with feedback on other aspects of the experimental

14 design.

15 You asked for a little bit of dialogue for us in terms of if you

16 did this, it adds this, but it costs this kind of thing. Not only in terms

17 of dollars, but just to give you some sort of feedback in terms of what

18 you get for what extra effort and resources are invested in terms of

19 adding different endpoints.

20 If I understood you correctly, Dr. Bradbury, that's the kind of

21 advice they are seeking from the panel right now as part of this

1 particular question.

2 So if we have some suggestions for them in addition to the

3 endpoints that are identified here, some information about what else,

4 pros and cons.

5 DR. KELLEY: Since you are concerned about fertility, you

6 have to add a rate of fertilization of eggs as an endpoint. And there

7 are a number of different ways to do that.

8 Some less variable than others. But that's an important

9 endpoint. And that will require growing animals out longer. Six

10 months.

11 So that will add to the cost of it. But it is an important

12 endpoint for your goal. I would argue the most important endpoint for

13 your goal.

14 Do you need more detail on how to do that? There are 42

15 different ways.

16 DR. ROBERTS: I don't think at this point. But I think that's

17 exactly what -- provide you with this information which is important,

18 but it means the experiment has to go longer.

19 DR. KELLEY: Right. But it is not very fancy. You don't have

20 to learn to do a vitellogenin induction assay.

21 You get the testes and mush it up and fertilize the eggs, you

1 know.

2 DR. ROBERTS: Other suggestions? Dr. Isom and then Dr.

3 Skelly.

4 DR. ISOM: We have talked around this and some of our

5 speakers have mentioned this. I think perhaps this accounts for some

6 of the variability we have seen among studies.

7 That is, clear definition of the endpoints. We really -- even to

8 define sex ratios, how are we doing that? There is all kinds of

9 variability in the terminology, and I would really encourage

10 somebody to sit down and come up with some standardized

11 terminology and endpoints and how you are going to evaluate those

12 before these studies are conducted, at least this phase one.

13 DR. ROBERTS: I think that's something that everyone on the

14 panel will concur with, is that the agency, as you begin this effort, is

15 going to have to standardize the terminology for the endpoints by

16 convening a workshop or whatever mechanism is the best way to do it.

17 But there has to be some terminology that everybody is using

18 and agrees upon. We clearly saw the issues as the agency has. This is

19 not something new. You are not surprised by this recommendation.

20 But I think that's something we'll make in our minutes.

21 Other endpoints? Dr. Skelly.

1 DR. SKELLY: Just to build on what Dr. Kelley suggested, if
2 you are going to grow animals out and add that extra investment, I
3 think it is going to be important to beyond castrating the males and
4 mashing up their testes to figure out, and I don't know enough about
5 xenopus to know how this could work, but in the species I work with,
6 it would be possible to measure reproductive behavior and
7 reproductive endpoints of whole living animals.
8 That would measure their reproductive function. Because what
9 Dr. Kelley suggested is taking a look at some index of male fertility.
10 But we don't know what is going on in this animal's brain and whether
11 it is going to act like a male or female or what it is going to do. I
12 think that's critical to getting beyond the kind of physiology to the
13 behavior in the ecology.
14 DR. ROBERTS: So you are suggesting another possibility is to
15 add reproductive tests that involve behavioral component, but the
16 downside is that there is -- is the methodology relatively standard or
17 this something they would have to work through?
18 Dr. Kelley is -- I'll let you respond to this.
19 DR. SKELLY: Before Dr. Kelley builds on something that I
20 say, just let me say that I don't know that the methods are
21 standardized. They probably vary quite a bit from species to species,

1 because you are turning on frogs. They all have their own little
2 things they like.
3 But it's certainly possible to do. And I think it adds
4 significantly too, and then you can mash their testes up.
5 DR. ROBERTS: Dr. Kelley.
6 DR. KELLEY: So that's what we study, the reproductive
7 behavior of male frogs and female frogs. You can do it. And we have
8 standardized assays for doing it.
9 There are uncertainties associated with this. I find in my lab
10 that the more things you try to get out of a single animal, the greater
11 the possibility that you will compromise one of the measurements.
12 I don't like killing animals. I always try to get everything
13 possible from the animal if it is going to give its life in the name of
14 science. But there are problems with running that kind of experiment
15 where you end up confounding it.
16 If you have a protocol for stimulating the testes that is very
17 reliable and then you take that same animal and test it behaviorally, I
18 think you could do that. And you could test clasping for which there
19 is normative data. The papers are on the list. You could test the
20 vocal behavior quite easily.
21 So you could do that. It would add to the study. It will be, to

1 be honest with you, behavior is hard and more variable than numbers
2 of fertilized eggs. It will add a level of uncertainty and a level of
3 interlab variability that will not be trivial.
4 But it is doable. I agree with you that it is important.
5 On the other hand, you have to say to yourself, hey, suppose
6 you could never get these male xenopus to clasp. Is that going to be
7 something that you are going to regard as a valid endpoint or not.
8 I'm a little bit worried about that, particularly since it won't be
9 very easy to transpose that same paradigm to rana where the hormonal
10 requirements for male reproductive behavior are rather different.
11 Very different.
12 DR. ROBERTS: Dr. Green, did I see your hand up? And then
13 Dr. Denver.
14 DR. GREEN: I was just thinking along the lines you brought
15 up, Dr. Skelly, that an endpoint might be to just see if these animals
16 can naturally mate in a laboratory. It is a simple easy way to do. It
17 doesn't require castration or anything.
18 And the evaluation of that would be the number of fertilized
19 eggs at the end of the time they have been in the bucket together or
20 something simple like that. There is a lot of variability in that.
21 I know. I have seen that. And the females will eat the eggs

1 sometimes and that kind of thing. But one way to assess their
2 behavior, and I'm sure you can elaborate on this, Darcy, would be if
3 you put them together after hormonal priming, can they -- do they
4 mate like controlled animals do.
5 DR. KELLEY: So again, I stress that you need a very
6 well-defined stock to do that experiment with. You need to make sure
7 that your controls and your experimentally treated animals are
8 equivalent in establishing that group that everybody agrees are going
9 to be the -- whatever those mice are, C J B 57 or whatever, you know,
10 of the xenopus world is going to take a little bit of work.
11 DR. ROBERTS: I think as we make some comments, it is
12 important to give the agency some advice about how straightforward
13 these assays are. Let's be honest, some things may be possible. But if
14 there is one laboratory in the world that can do it because they have
15 enough experience, that's an important practical consideration for the
16 agency as opposed to other techniques that are more straightforward
17 that depending upon who happens to do it, they are likely to get
18 decent results as opposed to something that is trickier.
19 I think it's maybe important to make it clear to the agency as we
20 discuss these things, give them some sort of sense about that.
21 DR. KELLEY: I can give you a protocol for watching mating. I

1 have this long boring paper if you want to go read it from my
2 dissertation on hormone levels, amount of time spent in amplexus,
3 number of clasp attempts and controlling for females and so forth.
4 It is spelled out very clearly. I believe it could be replicated,
5 although I noticed nobody has ever wanted to do it. I think it is
6 possible to do.
7 In my experience, killing the animal and mushing up the testes
8 is far and away the easiest way to get a first, easy measurement.
9 I think you would have to take eggs from a variety of females
10 and you will have to have a good control and you will have to make
11 sure it is done double blind bla, bla, bla.
12 But you will end up with if you fertilize X number of eggs, you
13 get X number of offspring. You will get a quantifiable measure that
14 can be subjected to robust statistical procedures as opposed to
15 nonparametrics requirements, which are required for these
16 noncontinously distributed variables like percentage of males
17 clasping, which is what I used in my original studies.
18 I'm happy to teach anybody how to watch frogs clasp each other
19 and score it. I think it's pretty easy to do. I'm just telling you that it
20 would be about a zillion times easier, even for me, to do a fertility
21 assay first.

1 I'm not against studying reproductive behavior. It is what I do.
2 I'm just telling you it is a more variable endpoint in a field in which
3 the endpoints are already very variable.
4 DR. ROBERTS: My point in raising this is I'm not trying to
5 discourage any particular kind of assay. I just want to disclose as best
6 we can the advice -- take advantage of the experience of you
7 individuals and experts who have done these kinds of things to let
8 them know how easy or how hard they are to do.
9 Dr. Richards, Dr. Green and then Dr. Delorme.
10 DR. DENVER: I want to say that getting an estimate of
11 reproductive output when the putative species is the male is going to
12 be really difficult.
13 On the face of it, it sounds fairly straightforward to do invitro
14 fertilization with oocytes, mash up the testes. But you don't need a
15 lot of sperm to get a reasonably efficient rate of fertilization.
16 Also, it depends on the quality of the oocytes, as you know. So
17 standardizing something like that I think would be a nightmare. I
18 think -- if the target species were a female, it would be a lot easier to
19 quantify, say, yolk deposition or other aspects of female fecundity.
20 But I think that given it is a male, we may need to think about
21 other ways to do that. Maybe the behavioral tests might be more

1 informative or more capable of standardizing the --

2 DR. KELLEY: I have the behavior paper with me. You guys

3 can read it and see what you think.

4 DR. DENVER: But what do you think about that, Darcy? You

5 mentioned the invitro fertilization as a way to measure --

6 DR. KELLEY: You are absolutely right. It is a problem. The

7 other way to do it is simply to do histology on the gonads and look at

8 the spermatozoa. You can stage spermatozoa. It is not as well worked

9 at as mice, but you could stage it.

10 If you get a big effect, it is never a problem. If you have a

11 marginal problem, it is always a problem. Right? What I would like

12 to do is, if you wouldn't mind, to go back and reread my dissertation

13 paper from 1975 and see what the error bars are like, basically. See

14 how variable the measure is for looking at clasping.

15 Calling is not going to do you any good. Either they call or

16 they don't. That's it. You could look at amount of time calling at

17 various levels, but that reflects internal androgen. And calling is

18 really difficult. To get robust callers at certain times of year is really

19 hard. But clasping is much less difficult, the amplectant (ph)

20 position.

21 Let me go back and look at the clasping data and get back to

1 you.

2 DR. ROBERTS: Dr. Richards, Dr. Green, Dr. Delorme.

3 DR. RICHARDS: I just want to point out I have never worked

4 with behavior in amphibians, but I have done a little bit with fish and

5 invertebrates.

6 Doing behavioral experiments in the lab are problematic. They

7 are very complex. They are difficult to anticipate outcomes often

8 times.

9 And I think behavior is best viewed in the field. I think that

10 some of the mashing and counting things might be better

11 measurements at this point in the game.

12 Plus, I'm not sure that federal employees are allowed to watch

13 clasping behaviors.

14 DR. ROBERTS: Good point.

15 Dr. Green.

16 DR. GREEN: You had asked to indicate how feasible, how easy

17 some of these proposed experiments might be to do.

18 Just to communicate to you, in our animal facility, natural

19 matings are quite common just for the purpose of collecting the

20 fertilized eggs and studying the eggs.

21 And usually in the fall, we have the arrival of 150 or so new

1 graduate students and post docs who have never dealt with frogs
2 before. I'll go through the frog rooms and there may be as many 20 to
3 50 buckets lined up on the floor where animals are paired. They have
4 been primed hormonally. They are put together and essentially left
5 overnight undisturbed. Some labs have video cameras to watch. I
6 don't know why.
7 So there is a way to record the behavioral aspects of the mating
8 without disturbing the frogs. And then the students come back the
9 next day hopefully early enough that the females haven't eaten the
10 frogs, but there are ways to prevent that from happening with a mesh
11 grid at the bottom.
12 And then they take the eggs on upstairs. And as I mentioned, I
13 think that seems relatively straightforward. I know all about the
14 variability in that. You go to the dissecting scope and you look for
15 the ones that are fertilized, and you could carry that one step further
16 to see how many actually become viable tadpoles.
17 The endpoint could go on and on and on. But that basic part is
18 fairly simple to do.
19 DR. ROBERTS: Dr. Bradbury.
20 DR. BRADBURY: I just wanted to do a check-in to see if I'm
21 synthesizing the dialogue properly.

1 At one point there was a statement that seemed to be accepted
2 by several, at least, that there should be an attempt to try to replicate
3 the studies that have been discussed over the last few days, the lab
4 studies. And that that was an important task at hand.
5 And that xenopus and/or rana simultaneously or -- we're going
6 to think about that a little bit later, the Phase 1, and again, don't get it
7 too sequenced, was a proposal to get out to the panel that wasn't
8 intended to be a detail by detail replication of the previously
9 published studies in part because we had concerns about growth and
10 water quality and those kind of things.
11 But in the spirit of many of the discussions over the last few
12 days, that if a response seems to be consistent and is occurring in a
13 reproducible, logical pattern, that study designs don't have to be
14 identical, but there are certain principles they are holding to to see if,
15 in fact, they are getting concordance of the information development.
16 Phase 1 is designed in that spirit. And seemed -- was sort of
17 our proposal for your consideration in terms of "repeating what had
18 been done in the previous studies."
19 Am I to interpret that that is one aspect of an experiment or a
20 study that would go on, and some of the other discussion we have
21 been hearing would be in the context of aspects of studies we have

1 been using as phase five? Or am I to interpret that not only do you
2 want us to come up with an experimental design that can capture
3 "what has been previously studied," but also include all these
4 additional endpoints?
5 I could imagine an experimental design that would do that. But
6 I would be curious if that's what you mean. Or are we talking about a
7 series of separate studies?
8 DR. ROBERTS: Responses? Dr. Delorme, actually you were
9 next up anyway. DR. DELORME: Actually, I was going to
10 bring up that point, because I was getting confused as to what is going
11 on.
12 And I flipped back to the slide on phase one which is, test for
13 apical gonadal effects, and the objective was to determine if atrazine
14 exposure results in gonadal effects in males and females in brackets.
15 When I had put in my consensus that repeating the experiments,
16 basically, that's what this one is about, and then the side bar with the
17 ecological relevance is sort of the next thing, I mean, bringing it back
18 and wearing my risk assessor hat, because that's what I'm doing, I
19 think we have to recognize that EPA wants to move this risk
20 assessment forward.
21 And the first step in doing that is determining whether or not

1 that cause effect between atrazine and gonadal effects is there.

2 So perhaps maybe we want to consider an experiment which

3 would try and replicate, not replicate, but at least firm up that cause

4 effect.

5 DR. ROBERTS: Dr. Green.

6 DR. GREEN: I think the issue was whether or not those

7 gonadal effects that you see morphologically have any physiological

8 consequence. It is hard to answer that question if all you are looking

9 for is gonadal abnormalities.

10 That would be the endpoint, I guess, and a place to start in

11 phase one. If they do, then maybe go on with other experiments to

12 then test the hypothesis as to whether or not that has any effect on

13 fertility and fecundity.

14 DR. ROBERTS: Dr. Bradbury.

15 DR. BRADBURY: The way that discussion was going is good. I

16 think it would be helpful for us to hear you explore is it sort of a

17 sequential -- get this information, check in what to do next or do you

18 try to do it all at once.

19 And I think you opened up dialogue that would be good to hear

20 some more about.

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

1 Coats.

2 DR. DELORME: You may be able to design a study that you

3 could actually phase the results where you get the results of the initial

4 study but you have enough animals left over that, if you need to take

5 it to looking at the physiological consequences of having disrupted or

6 abnormal gonads whether or not that has effect.

7 I guess that would be possible. But again, I come back to what

8 Dr. Green said earlier. The more things you try and get out of a

9 study, then you risk having uncertainty creep in or losing animals or

10 whatever.

11 So you are going to have to evaluate the relative merits of what

12 you are going to get out based on the design.

13 If you want to design a study where you could initially try and

14 replicate or look at the causal effect with the idea that you are going

15 to have enough animals built into it that you can take it beyond that

16 should you find out that there is effect, certainly you could do that.

17 DR. ROBERTS: Dr. Skelly.

18 DR. SKELLY: Dr. Delorme said much of what I wanted to say.

19 I guess one way to phrase the recommendation back to EPA would be

20 in the context of doing a power analysis and thinking about how many

21 animals need to be available at each stage. How many of them are

1 going to have to be sacrificed at each stage.

2 Some things that we're not doing when we're sitting here

3 talking about it may become obvious one way or another that it makes

4 sense to economize and break those up or it makes sense to economize

5 and put them together.

6 DR. ROBERTS: Good point. Dr. Coats.

7 DR. COATS: My thinking is that phase one is presented up

8 there and as described in the book is pretty much where we need to

9 start. But that there has been discussion of maybe adding one more

10 endpoint on to the end of that or two if they are not too complicated.

11 DR. ROBERTS: Others? Dr. Gibbs.

12 DR. GIBBS: If I can just add one point. Molecular genetics

13 gets right to the heart of the matter, which would be paternity. There

14 are simple ways without getting into behavior. For male frogs,

15 paternity is the bottom line. However, they get there. And I think

16 you could devise some fairly simple experiments, toe clips from all of

17 your adults and all of the metamorphs and you've -- genotyping them

18 and assigning paternity. And you have got some good data. It gets

19 right at the issue.

20 DR. ROBERTS: Any other comments? What do you think, Dr.

21 Bradbury, is that -- we have sort of honed in on it a little more?

1 DR. BRADBURY: Yes. I know you are going to be writing. I now
2 have a feeling that I can imagine some of the dialogue that will be on
3 paper that will be helpful based on this last round of discussions.
4 DR. ROBERTS: I hope Dr. Richards can also imagine that
5 dialogue. Dr. Coats.
6 DR. COATS: One point had not been discussed very much was
7 the dose response possibility as described in the tier one, phase one
8 there.
9 And I think it is an extremely important part of it, and would be
10 very informative to elaborate on the dosing scheme. That may be
11 discussed at a later point.
12 DR. ROBERTS: I think it comes up in C as I would interpret C.
13
14 Have we finished then with B, Dr. Richards? Do you want to
15 poll the panel? Do you have a pretty good feel for where we are on
16 this?
17 DR. RICHARDS: I don't know if the point about conditions and
18 this thing about the ASTM standards and flow through --
19 DR. ROBERTS: I think we're going to get to that.
20 DR. RICHARDS: -- is that going to come later?
21 DR. ROBERTS: I think so. We're still sort of what components

1 need to be in that phase one and that sort of thing.

2 Dr. Delorme.

3 DR. DELORME: I just want to ask for a clarification. You

4 have replication up there as two. Does that mean two tanks or two

5 species or two xenopus?

6 DR. TIETGE: It was meant to represent two tanks. I think we

7 have since -- this is an older version of the slide for some reason.

8 DR. DELORME: Do you want to comment on whether or not

9 you think that's appropriate given the context of the discussion?

10 DR. TIETGE: No. In the context of the power analysis and the

11 discussion that's been going on, it is probably not sufficient.

12 It is a commonly used approach in aquatic toxicology, at least

13 two.

14 DR. ROBERTS: Should we go on to 8 C then?

15 DR. STEEGER: Please also comment on the range, spacing and

16 number of atrazine concentrations that should be employed in the

17 proposed testing sequence to resolve uncertainties in the shape and

18 nature of the dose response relationships for any observed

19 developmental effects.

20 DR. ROBERTS: I think for this particular question -- I'm sort

21 of looking ahead. We have some questions -- most of the rest of them

1 deal rather specifically with the species involved and differences and
2 those kinds of things.
3 I think this is probably the best question to raise not only issues
4 regarding to spacing and dosing of atrazine concentrations, but
5 perhaps some of these other things about experimental design that we
6 have been sort of eager to bring up.
7 With that said, let's go to Dr. Richards.
8 DR. RICHARDS: I'm going to open it up to those first
9 statements on range and spacing. I know some of you had some
10 stronger feelings about that if you want to just jump in here.
11 DR. ROBERTS: Dr. Heeringa had to leave for the airport. But
12 he did give me typed up comments. With your forbearance, let me
13 read them, because I think that's probably the best way to get them
14 into the record. And I would not dare try and paraphrase his points
15 for fear of not getting them right.
16 In regard to 8 C, he says, this question must be answered in the
17 context of a
18 presumed constraint on the cost and effort that can be devoted to a
19 single replication of the study to determine if aqueous concentrations
20 of atrazine bears a relationship to gonadal irregularities and any
21 associated mechanicism for endocrine disruption in frogs.

1 The scope of the study is determined by the number of
2 concentrations and controls tested, the number of intralab
3 replications, for example, tanks for each control concentration level,
4 and the number of test animals per experimental replication.
5 Range in spacing of the experimental concentration levels is
6 obviously related to the number of feasible experimental points.
7 Range in spacing should also be governed by the specific test
8 hypothesis concerning the potential shape of any underlying
9 concentration response relationship.
10 Consider the components of this design in the following order.
11 One, selection of controls. Two, range of observations for
12 experimental concentrations. Three, number of independent
13 replicates per treatment. Four, number of test animals per
14 experimental replicate. And five, number in spacing of experimental
15 concentrations.
16 Number one, selection of controls. The experiment should
17 include untreated control replicates and a positive control under
18 which test animals are exposed to a concentration of estrogen. I
19 support the EPA statement that a positive androgen control group is
20 not needed, although would be beneficial if laryngeal muscle
21 measurement is included as an endpoint.

1 As a protective factor in the test of hypothesis concerning the
2 dichotomous or polyotomous, (ph) Dr. Hayes' classification, gonadal
3 deformity endpoint, which is based on existing data, suggests a one-
4 sided test. The sample size for the untreated control should be
5 increased beyond the levels that would be assigned to concentration
6 points in the nonzero domain of the test range.
7 Number two. Range of observations. Setting aside the positive
8 controls, the range of experimental concentration should span zero or
9 untreated atrazine concentration across ecologically relevant
10 concentrations and extend to concentrations that meet and at least one
11 point exceeds the upper percentile bounds that have been measured in
12 natural aquatic environments.
13 Number three, number of replications for each experimental
14 treatment. For the xenopus, and that's Hayes and
15 Carr studies, and the rana studies, Hayes, estimates of the empirical
16 intrareplication or intratank correlation should be obtainable.
17 Based on concordance of the values or maximum of estimates if
18 highly variable, the estimated intraclass correlation should be used to
19 determine the number of replicates per treatment arm and the
20 allocation of total sample size to replications and test animals per
21 replication.

1 This should be based on the best empirical data from the
2 existing studies. Underestimating the intrareplicate correlation and
3 planning the sample size allocation may seriously attenuate the true
4 power of the test of the hypothesis concerning the chosen endpoints
5 and in particular test of hypotheses concerning a dichotomous that is
6 deformity outcome or the differentiated polytomous classification of
7 deformities proposed by Dr. Hayes in his presentation to the panel.
8 Number 4, number of animals. Subjects per replication having
9 established a working value for the intrareplicate correlation for the
10 class of outcomes of interest and a desired level of statistical power
11 for a specific hypothesis test.
12 The determination of the optimal number of animal subjects can
13 be determined jointly with the determination of number of treatment
14 replicates.
15 This allocation is obviously constrained by bio-loading and
16 water quality considerations that are discussed in the EPA white
17 paper.
18 Finally, Number 5, optimal determination of the number in
19 spacing of treatments as governed by the hypothesized shape of any
20 underlying concentration response relationship.
21 The panel has determined that data from existing studies lend

1 support to further testing the hypothesis of a relationship between
2 atrazine concentration and gonadal abnormalities in frogs.
3 Dr. Hayes provided data and arguments that the relationship is
4 not monotonic potentially in inverted response. But this has not been
5 replicated in other studies.
6 At this stage, there is insufficient basis to set the spacing of
7 treatments to optimize the experimental design for a functional form
8 for a potential concentration response curve.
9 A robust design would use multiple concentration points to
10 accommodate the possibility that any effect is monotonic or
11 alternately that there is a simple nonmonotonic or convex
12 relationship.
13 There was also an advantage to retaining concentration points
14 that have been used in the prior research by Hayes, Carr, Hecker and
15 others, 0, 0.01, 1, 10 and 25 micrograms per liter, and adding an upper
16 concentration level that exceeds the 25 microgram per liter value, at
17 which Dr. Hayes and Carr studies have detected increases in the
18 number of abnormalities, such as basing of concentration treatments
19 should be sufficient to test the hypothesis of an effect and to
20 secondarily test whether any real effect is monotonic or
21 nonmonotonic.

1 Obviously, these concentration levels would not permit testing
2 threshold responses below the .01 microgram per liter level.
3 I will give these to Dr. Richards to work into our comments.
4 Dr. Green, did you want to --
5 DR. GREEN: I was just going to say I support his
6 recommendations, and I particularly like the dose range that he has
7 proposed in that.
8 DR. ROBERTS: Other comments. Dr. Coats.
9 DR. COATS: Yes. I liked some of the comments. I don't think
10 that dose range is going to depict the curve any more clearly than --
11 concentration response any better than what we have already seen
12 other than one laboratory doing all those concentrations, which Dr.
13 Hayes has mostly done.
14 I think if there is an unusual response at which point .1 part per
15 billion is a significant concentration, that points near that, above and
16 below ought to help delineate the response curve if it is a curve.
17 And that if you are going by orders of 10, orders of magnitude,
18 you would miss that.
19 DR. ROBERTS: Dr. LeBlanc.
20 DR. LEBLANC: I agree completely with Dr. Coats. I think we
21 need to recognize here or at least acknowledge what the intent of the

1 experiment is and design it appropriately.

2 We can design the experiment to replicate earlier experiments,

3 that is, determine whether or not atrazine is eliciting the effect, or we

4 can try and characterize the shape of the concentration response

5 curve.

6 But I don't know that we can do both unless we get lucky. And

7 I would suggest that probably the first time around we follow the

8 recommendations of Dr. Heeringa recognizing that we're probably not

9 going to characterize the concentration response curve, but will have

10 a good design to replicate previous observations and, if we get lucky,

11 perhaps we'll gain information on the concentration response curve as

12 well.

13 DR. ROBERTS: Dr. Green. Then Dr. Delorme.

14 DR. GREEN: The aspect I liked about the dose responses or the

15 doses that he has proposed is that they do include the ones that have

16 been looked at previously.

17 And I agree. They could be added to. And I like the fact that

18 he exceeded the maximum dose that was looked by both labs. I can't

19 recall how many fold, how many times he said higher than the 25.

20 What was the next value? Did he give one?

21 DR. ROBERTS: Based on some percentile, I believe, of

1 observations from the field.

2 DR. GREEN: That seems important to do.

3 DR. ROBERTS: Dr. Delorme.

4 DR. DELORME: I just wanted to ask a clarification of EPA.

5 Were you intending this as an inova (ph) design study which is a

6 hypothesis testing or one which -- a linear design -- or a regression

7 type of design where you actually want to get a dose response?

8 Because that actually makes a difference on how you space your

9 doses and how many animals you have in your replicates.

10 This actually goes to some of the other comments that have

11 already been made.

12 DR. ROBERTS: Dr. Tietge.

13 DR. TIETGE: To the extent possible, I think dose response, but

14 I think recognizing what Dr. LeBlanc said is you may not be able to

15 hit the proper range of concentrations to achieve that.

16 DR. ROBERTS: As Dr. LeBlanc said, often times when you do

17 these kinds of studies, especially if there is a sharp inflexion in the

18 curve, then you have to go back and start loading in in that critical

19 range. So it wouldn't be surprising.

20 But I think at least the recommendations by Dr. Heeringa span

21 the right range of doses.

1 Any other recommendations or comments about spacing, et
2 cetera? Dr. Delorme.
3 DR. DELORME: Just out of curiosity, are you worried about
4 the low end of the dose response curve or the upper end? From a
5 regulatory perspective, often times what we're looking for is near to
6 no effects or what we would putatively call acceptable effects.
7 DR. BRADBURY: I think we're sort of getting into a phase
8 within a phase. I think the first question is can you reproduce the
9 effect and can we use that in dose basings that were recommended I
10 think are reasonable to get some sense of consistency across studies.
11 Give me a concentration response curve to start work even if
12 it's crude, then we can start talking about those kinds of things. I
13 think it's premature until we get a response relationship.
14 DR. ROBERTS: Using the chairman's prerogative, I'm going to
15 change my mind about talking about other experimental design
16 aspects at this time. Let's go through the rest of the questions, and
17 then at the end if there are recommendations regarding other aspects
18 of the experimental design or other points, let's go ahead and bring
19 them in at that time.
20 Are there any other comments, then, on the specific issues
21 raised in this particular question on the range, spacing and number of

1 atrazine concentrations?

2 Let's move on, then, to D.

3 DR. STEEGER: Please comment on the agency's

4 recommendation that xenopus laevis be used as the primary biological

5 model in the proposed studies and whether or not the mechanisms

6 involved in sexual differentiation of the ranid and pipid species are

7 sufficiently similar to predict effects and associated dose response

8 curves for rana and/or to efficiently design rana studies.

9 DR. ROBERTS: Dr. Richards, do you want to lead off or throw

10 it open for discussion?

11 DR. RICHARDS: Let me make a comment. I think that the

12 agency and others have indicated there's lots of reasons you use

13 xenopus, for a variety of laboratory tests and quick techniques and so

14 forth.

15 I think we have also previously identified some need to start

16 initiating a tighter rana procedure in the laboratory.

17 But I would like to throw it over. I hope Dr. Kelley will

18 respond on the differences in the differentiation.

19 DR. KELLEY: I don't think we know enough to be able to

20 answer this question. We know a huge amount about xenopus laevis,

21 and we know so much less about rana that I couldn't tell you. I

1 couldn't answer this question.

2 DR. ROBERTS: Dr. Skelly.

3 DR. SKELLY: I guess Dr. Kelley's point suggests to me that --

4 just to support what EPA has proposed, that rana is used as a

5 corroborating species for these early experiments.

6 DR. ROBERTS: Any other comments or any comparison of the

7 species or the suitability of one to serve as a model for the other? Dr.

8 Denver, and then Dr. Green.

9 DR. DENVER: I just want to concur with Dr. Skelly that the

10 ranid species do need to be considered early in the game.

11 DR. ROBERTS: Dr. Green.

12 DR. GREEN: I think it is probably obvious, but we could

13 probably all predict that there will be differences between the two

14 species.

15 DR. ROBERTS: Any other comments on this one? Dr. Gibbs.

16 DR. GIBBS: Only that the rana maybe should be clarified. It

17 should be a North American rana. There's rana on different

18 continents.

19 DR. ROBERTS: Then let's let our minutes reflect that

20 clarification.

21 Let's go ahead and go to E which is sort of the flipside of the

1 question.

2 DR. STEEGER: In this regard, are there important differences

3 between the species to conclude that any affected developmental

4 processes observed in xenopus laevis would not occur in rana?

5 DR. ROBERTS: Dr. Richards.

6 DR. RICHARDS: I will throw that one open.

7 DR. ROBERTS: Dr. Kelley.

8 DR. KELLEY: Every species develops in its own way, but they

9 all develop their gonads, and they differentiated, you know, not too

10 roughly inappropriate times.

11 There actually are, although I'm not sure how valid, there are

12 tables that relate, the tables of normal development in xenopus to

13 roughly equivalent stages in rana. So it is possible to normalize a

14 little bit in that way.

15 The developmental biologists believe, although they now

16 mostly study xenopus, in the old days they studied rana, and the

17 developmental biologists believe that the fundamental processes are

18 extremely similar, shpay mons (ph) organizer, induction and so forth.

19 I don't know of any strong species difference that would lead

20 me to believe that there would be some fundamental reason -- some

21 fundamental difference at the developmental level that would lead

1 anything affected in xenopus to not necessarily be affected in rana.
2 So that's a very couched statement, but I believe that the
3 developmental biology community would agree with me.
4 DR. ROBERTS: Dr. Skelly.
5 DR. SKELLY: I will support everything Dr. Kelley just said
6 with just an added comment. It seems like timing is important here.
7 And one aspect that xenopus differs from almost all other frogs is in
8 how, say, the onset of reproduction and the development of
9 reproductive morphology differs with respect to the onset of other
10 sort of metamorphic characters in almost all other frogs.
11 So there are things that just never happen in xenopus.
12 Transitions that either don't take place or take place differently in
13 xenopus than they would in rana and almost all other frogs that
14 undergo metamorphosis.
15 That suggests that either the genes that control development
16 and some of the developmental pathways, and I'm getting way out on a
17 limb, I'm going back to grad school to remember this stuff, but
18 suggest to me that developmental pathways and timing of things are
19 somewhat different.
20 And since timing of the exposure to atrazine or whatever can
21 happen at different times relative to developmental sequence, even

1 though there are these tables that relate xenopus development to rana
2 development, what is going on inside and when genes are turning on
3 and those sorts of things, I don't know that we know that much about
4 that right now.
5 I guess that's just a note of caution in assuming that these
6 things are going to happen similarly. I will just finish by saying that
7 I think we have seen pictures anyway that suggest that some of the
8 outcomes in gonadal abnormalities seem to manifest themselves
9 somewhat differently in the two species.
10 DR. ROBERTS: Other points?
11 Should we go to F which is again related to the same --
12 different way of sort of tackling the same kind of issue?
13 DR. STEEGER: Alternatively, are there developmental
14 pathways in rana but not in xenopus laevis that raise concerns about
15 using xenopus laevis as the primary biological model in any future
16 atrazine studies?
17 DR. ROBERTS: Dr. Richards. You were going to defer to the
18 person who just walked out the door.
19 DR. RICHARDS: Like a flipside of what we just spoke of here.
20 DR. SKELLY: I guess I'm getting tired, but I was hoping to
21 make the comment I made for E for F.

1 You can move that.

2 (Thereupon, the time was 5 o'clock p.m.)

3 DR. ROBERTS: I think we could sort of take these up kind of

4 collectively. Because if I understand it correctly, I think the agency

5 is trying to ask in different ways to what extent can we extrapolate

6 data from xenopus to rana, and does it serve as an effective surrogate

7 or is there any reason to be concerned if we used it as our primary

8 model that it would mislead us about what was going on in rana.

9 Dr. Denver.

10 DR. DENVER: I don't have any evidence that would suggest

11 that the basic developmental pathways would differ, although, there

12 are distinct differences, obviously, in life history and physiology and

13 we have documented differences in the development of the stress axis

14 when the stress axis becomes responsive, the production of stress

15 hormones in the two species throughout development, things like that.

16 So the timing of things are different in the two species as Dr.

17 Skelly pointed out. But I don't have any evidence to suggest that the

18 basic mechanisms are that different. So I think the main point there

19 is to realize that the critical periods or the sensitive periods may

20 differ between the two species. So that needs to be considered in any

21 experiments that are designed.

1 DR. ROBERTS: Other points? Dr. Delorme.

2 DR. DELORME: Just stepping back and taking the broad
3 picture, I guess what I'm hearing is that you can make the assumption
4 that there may be not too many differences, but you are making an
5 assumption.

6 And at some point, you are going to have to test that
7 assumption. It may not have to be right away. But certainly,
8 sometime in the future you are going to have to do some work to work
9 on it.

10 DR. BRADBURY: I think I'm picking up the message that
11 xenopus is a reasonable biological model to further confirm a
12 toxicological signal related to gonadal development and test that with
13 atrazine. That that's a reasonable biological model to get started.

14 Does that mean that all amphibians will respond exactly the
15 same way as xenopus does? I realize we're not saying that. But just
16 as we do our aquatic toxicology testing, we don't have the luxury to
17 test all the thousands of species of fish in North America. We have to
18 settle on a few surrogate species to give us a sense of the
19 toxicological potential of the chemical and then through other
20 analyses one deals with species extrapolation and other aspects of life
21 history to refine our risk assessments as needed.

1 So for that matter, we have a handful of mice and rats that we
2 use to go to humans. But at least we have several species to go to one
3 species. Ecological toxicology, we're going from a handful of species
4 out to potentially several or many.
5 So what I think I'm hearing is that xenopus is a reasonable
6 biological model to get a handle on the consistency of previous
7 studies in terms of the potential for atrazine to initiate these
8 developmental effects.
9 The proposal, then, is to use a North American species to get
10 some sense of species variability and to be using a species of North
11 America. Does that represent all the North American species? No.
12 But at this point, I would say that we're sort of in the venue of
13 all of the challenges we have in ecological risk assessment across the
14 board in how to extrapolate across many species when you only have
15 data for a few, unless the panel wants to probe that a bit.
16 DR. ROBERTS: I think I heard not only just now but
17 discussions earlier during the last couple of days that there are some
18 -- since a primary objective as we just discussed at least initially is to
19 get a handle and do some well controlled studies, that there are a
20 number of advantages in using xenopus to do that because it's
21 well-characterized. There is a lot of experiments and it lends itself to

1 doing those kinds of experiments that really need to be done initially.
2 But as you say, we'll also have to do at least some experiments
3 on rana because of uncertainty about the extent to which xenopus
4 would be representative.
5 Does anybody else want to add anything?
6 DR. DENVER: I just wanted to add a related point. That came
7 up earlier with regard to measurements of uptake of atrazine and
8 body burdens and that sort of thing.
9 These two species have very different feeding ecology as
10 tadpoles. And that could translate into differential rates of uptake
11 and different exposure to the compound.
12 And so I think that at the outset it is important to address those
13 issues. I think as already been -- that point has been made, I don't
14 know that has been made today, but it has been made previously that
15 that be measured and perhaps compared between species and the
16 studies.
17 DR. ROBERTS: Dr. Denver, can you provide a reference that
18 we can include in our minutes so we can be sure and pass that
19 message?
20 DR. DENVER: A reference to the previous discussion --
21 DR. ROBERTS: No, to the different feeding behaviors.

1 DR. DENVER: Oh, yes, sorry.

2 Xenopus tadpoles are filter feeders whereas ranids tend to feed
3 on -- try to sit at the bottom of the pond. So they have quite different
4 modes of feeding. And so their rates of uptake of compounds from the
5 environment may be different.

6 DR. ROBERTS: If you can draft a couple of lines or two and
7 throw in a few citations for a report, that would be very helpful.

8 Any other comments on this?

9 DR. ROBERTS: Dr. Kloas.

10 DR. KLOAS: Maybe if you want to generalize the effects on
11 sexual differentiation on amphibians, I think I would like to mention
12 that we have also another order of amphibians, oradales (ph), which
13 have different androgens.

14 If you would like to have some comprehensive studies, of
15 course I agree fully that you should go ahead to start out with
16 xenopus. But if you would expect something different, then you
17 should more go to oradales to look in this -- all of amphibians.

18 Because they have probably also a little bit differences or more
19 pronounced differences concerning sexual differentiations in
20 comparison to xenopus and ranids.

21 DR. ROBERTS: Dr. Delorme.

1 DR. DELORME: Just a comment on Dr. Denver's little
2 discourse there on uptake from feeding.
3 I don't know if there is any data, but it might be worthwhile
4 looking at the relative contribution from food and bioconcentration
5 from the water.
6 I don't know if one would outweigh the other, but certainly it is
7 a thing to consider, something to consider in the exposure, because
8 we're really treating the water here, not the food.
9 DR. ROBERTS: Any other points or comments on this, on the
10 appropriateness of the models?
11 Let's go ahead and take G, then.
12 DR. STEEGER: Assuming xenopus laevis and rana are
13 sufficiently concordant from a toxico dynamic perspective with
14 regard to potential developmental effects of atrazine, what critical
15 toxico kinetic processes should be considered for extrapolating
16 xenopus laevis dose response relationships to rana and/or for
17 designing subsequent studies with rana.
18 DR. ROBERTS: Who wants to tackle this one? Dr. Delorme,
19 then Dr. Green, then Dr. Coats.
20 DR. DELORME: I think we already touched on one of them.
21 That's uptake. The second one would be looking at whether or not the

1 degradation or the depuration from the animal is similar. I can't tell
2 you.
3 DR. ROBERTS: Dr. Green.
4 DR. GREEN: I can't answer this directly, but I know from
5 looking at pharmacokinetic studies again for the purpose of trying to
6 apply veterinary drugs to treat sick animals that there are differences
7 between rana pipiens and mammals. There have been some
8 comparisons in small rodents.
9 Differences between rana pipiens and X. laevis in
10 pharmacokinetic studies, the comparisons aren't usually directly
11 made. But the margin of safety for many drugs has been proposed to
12 be much lower for X. laevis because it's a fully aquatic species, which
13 means that when they get sick or weak, they can't get up to the surface
14 to gulp air.
15 So they die from drowning, which I might envision that if at the
16 higher ends of this dose range that we propose for atrazine, the
17 mortality perhaps, maybe not, could be higher in the juvenile and
18 older animals that are fully aquatic at that point, because they will get
19 sick and weak, and not being semiterrestrial like rana pipiens, you
20 might see more of them die earlier on in the study than you would for
21 rana pipiens.

1 That's speculative on my part. But there is some suggestion in
2 the literature that when it comes to some veterinary drugs, that's what
3 happens to xenopus laevis.
4 So you can't apply a drug dose for a rana to a xenopus to treat a
5 particular condition without being aware you could kill the xenopus.
6 DR. ROBERTS: Dr. Coats.
7 DR. COATS: I agree there is basis to probably expect different
8 pathways of degradation. Most of them probably are detoxifications.
9 There is possibility of some of them not being at detoxification and
10 still resulting in a molecule that would be bioactive. From the uptake
11 perspective, I agree that the water and the food are both important
12 probably and need to be studied.
13 And certainly absorption through the skin is something
14 relatively unique or at least feasible that would have to be looked at I
15 think in the aquatic forms.
16 DR. ROBERTS: Just to comment on my part, I think you could
17 do a lot of toxico kinetic studies in terms of absorption and
18 depuration. But an easy thing to do to sort of see what the summation
19 of those processes are is to look at the tissue levels in animals at
20 different concentrations, which really gives you sort of the
21 integration of the intake and outflow.

1 And we have already talked about the desirability, if possible,
2 of putting that, getting that information as part of the study so you
3 can see whether or not at a particular concentration of atrazine and
4 water you get the same concentrations in xenopus as you do in rana.
5 On a very simple level, that might be very useful for
6 extrapolations.
7 Dr. Delorme and then Dr. Kloas.
8 DR. DELORME: I guess my only concern with this would be
9 that the development of the different organs, especially the liver,
10 which is probably going to be the major detoxification organ, you
11 have to be aware of any differences between the two species and how
12 that can affect what is going on.
13 I don't know how you are going to do that. I'm not a
14 developmental biologist. It theoretically I guess could have an effect.
15 DR. ROBERTS: Dr. Kloas.
16 DR. KLOAS: For toxico dynamics I think during the larval
17 developments there shouldn't be a big difference between ranid and
18 xenopus.
19 But after metamorphosis, the skin of xenopus is relatively
20 impermeable. So there should be a big difference in toxico dynamics,
21 especially if some experiments for the modes of actions might become

1 designed for juvenile or adults. This should be taken in account that
2 there is severe differences between ranid species versus skin still that
3 keeps on being quite permeable in xenopus.
4 DR. ROBERTS: Good. Any other comments or suggestions on
5 this point?
6 Dr. Bradbury, is the input reasonably clear?
7 DR. BRADBURY: Yes.
8 DR. ROBERTS: This was the last part of the last question. But
9 during the course of some discussions, I think there have been some
10 aspects of potential experiments that the panel has been anxious to
11 recommend or offer their advice in terms of how some of these studies
12 should be performed. We have already heard some of them. And I
13 think Dr. Green has provided some excellent suggestions on issues in
14 terms of practicalities of doing studies and things that need to be
15 considered.
16 Maybe if we could go back to the slide which sort of laid out
17 the things in some of the initial phase one studies to sort of, to serve
18 as prompts.
19 I will ask the panel now as we sort of move beyond the
20 questions posed to use if there are any specific suggestions that they
21 might have that haven't been mentioned so far.

1 Dr. Green and LeBlanc.

2 DR. GREEN: I think we talked about water quality issue and

3 nutrition and feeding a little bit. But the issue of what kind of tanks

4 these studies would be performed in, whether they were flow through

5 or static renewal, I don't know if that was resolved.

6 I was just wondering if the EPA were to conduct these studies

7 in their lab, and I'm not saying that they would, what kind of tanks are

8 routinely used for such studies?

9 DR. TIETGE: We typically use glass tanks using flow through

10 conditions.

11 DR. GREEN: What is the water turnover rate in the flow

12 through?

13 DR. TIETGE: In terms of flow rate, it is 25 mils per minute,

14 which is about 36 liters per day.

15 DR. GREEN: And the total volume of the tank is how much?

16 DR. TIETGE: In that particular system, the standing tank

17 volume I believe is four liters and our stocking density is from 20 to

18 25 organisms.

19 And if you calculate the maximal, the approximate maximal

20 weight, the total loading approaches the ASTM standard of one gram

21 per liter per day.

1 DR. GREEN: I don't have a feel for how difficult this would be
2 for labs that might be asked to perform these studies to reproduce.
3 DR. TIETGE: Well, I kind of got prepared for this. I was
4 anticipating these questions last night while everybody else was
5 awake.
6 I have a brief little presentation here.
7 Perceived problems. We hear a lot of different problems
8 brought up. And one is system costs.
9 Actually, system costs are not necessarily high because there
10 are proportional diluter devices which work on hydrolic principles
11 that really cost probably a few hundred dollars to build.
12 These have been published. There is published designs for
13 these that date back to the 70s, I believe. One of them is called the
14 Mount Brungs (ph) Deluter. There is a Banoit (ph) Deluter. These
15 kinds of technologies are very old. We have many of them in our
16 laboratory.
17 I think the notion of cost is often considered an impediment
18 because there are fancier systems that can be computer controlled and
19 can do very complicated exposure or can be used to achieve very
20 complicated exposure designs.
21 But the basic systems can be very inexpensive and very

1 reproducible because you are relying on gravity to make them work.
2 They are very low cost systems.
3 There are concerns about operational costs. For example, waste,
4 chemical costs in terms of the test chemical, which I don't think in
5 this case would be a great impediment, and then costs for water. And
6 I'll get into a few of those a little bit more in a moment.
7 We often hear that it is suboptimal for anurans. And I guess I'm
8 wondering what the biological basis is for that. I have read
9 anecdotally that it is a suboptimal. All I know is empirically it seems
10 to work pretty well. So if someone has a biological basis for that --
11 Dr. Kelley mentioned yesterday that stimulation of lateral line could
12 relate to -- could relate to some stress response, and I have seen that
13 in the literature, but I have never seen it actually documented. It
14 seems to be kind of an informal opinion.
15 Just a quick history about these methods. As I mentioned, some
16 of the technology for doing the flow through methods were developed
17 back in the 1970s at the end of the period of aquatic toxicology where
18 it was called the kill them and count them period where acute lethality
19 in short term static tests were kind of the norm.
20 As water quality criteria, for example, became more
21 complicated or more, what do I want to say -- well, we started to move

1 toward more sophisticated approach to water quality criteria. And we
2 began using studies that were more capable of dealing with chronic
3 and subchronic sublethal endpoints.
4 That's where flow through systems really became developed,
5 was to achieve those ends. And when you get into more subtle
6 effects, such as endocrine disruption, these types of systems provide
7 highly reproducible exposures, which minimizes the variance in the
8 system.
9 Currently, EPA requires flow through studies in different
10 offices of at least 12 species. We just kind of threw these numbers
11 together last night. And typically, there is 300 studies submitted
12 annually. These are primarily fish studies.
13 But the point is that there is adequate facilities in the research
14 community to do this. There is the toxicological expertise and, in
15 fact, guidance such as in the ASTM guidelines on how to conduct
16 these study was amphibians as well as fishes.
17 In general, at our laboratory, we also maintain a very large
18 database for aquatic toxicity. And I can tell you that there is
19 thousands of studies that have been conducted over the last 20 years
20 using flow through methods with at least 30 aquatic species. This is
21 not anything new. That's my point here.

1 So there is two main lines of reasoning to use flow through
2 methods. The first is the biological rationale. Maintenance of water
3 quality. These issues have been brought up. Temperature, pH, et
4 cetera.
5 Reduced stress caused by repeated exchanges or handling of
6 organisms during static renewal studies. In a flow through study, you
7 don't need to do that.
8 And with most species, at least the species that we have worked
9 with, these methods have been demonstrated to promote survival,
10 growth and development.
11 Now, the toxicological rationale for the flow through methods,
12 first of all, is the maintenance of a stable chemical concentration.
13 Remembering that in this particular case we're not trying to achieve
14 an ecological approach, it is a toxicological approach, to reduce the
15 variables involved so we can, you know, assess the chemical in a
16 tightly controlled system.
17 It is particularly good for highly hydrophobic chemicals
18 because it eliminates the mass limitations that occur when you are
19 dealing with chemicals, let's say, that are in the 6 to 7 KOW range
20 with labile chemicals which may be -- will degrade due to
21 metabolism, hydrolysis, photolysis, or they might be volatile in a

1 system.

2 It also has the added benefit that it reduces or eliminates the

3 accumulation of chemical metabolites. That's of the parent, which

4 was brought up as an issue yesterday. And we get improved dose

5 response or concentration response data from that.

6 So at our laboratory in Duluth, we have flow through methods

7 that we have used for xenopus laevis. And we have gone through some

8 of these baseline studies that have been mentioned previously.

9 We have done loading studies to evaluate whether or not we

10 needed to approach the ASTM or utilize the ASTM recommendations.

11 And indeed, when we pushed the loading organism performance based

12 on mostly growth and development, it became problematic. So we

13 have stuck with the ASTM guidelines.

14 We have done some feeding comparisons. I could get into the

15 detail if anybody is interested. And we have done some baseline

16 developmental studies looking at developmental rate.

17 One of the added improvements that we see in the flow through

18 is we have better developmental synchrony, which makes the tests a

19 little bit easier to conduct and reduces some variation.

20 If you use the ASTM standards, you can conduct static tests or

21 you can conduct flow-through tests. And I have listed the biological

1 loading rates for the two there.

2 And the reason I was able to pull that number off the top of my

3 head a little bit earlier is because I went through the calculations last

4 night. In our studies, we typically use 25 organisms. Their maximal

5 weights are about one and a half to 1.8 grams. Occasionally, we'll get

6 a 2 gram organism. That usually occurs at about stage 61, 62, I think.

7 So we base our loading on our maximum weights.

8 And as you can see, if you go through the math, we're at about

9 one gram per liter per day.

10 One of the things to note here is if you choose the static route

11 and you are going to adhere to this guideline, because we're working

12 in the absence of other validated protocols, then you will see that if

13 you want to run 25 organisms, I did the calculation just for

14 comparison, you would need 75 liters per day for those 25 organisms

15 as opposed to in a flow through condition where you would only need

16 38 liters per day.

17 So I think if you make the decision that you are going to stick

18 to the guidance that was developed and first published in 1980 and it

19 had -- it has taken a lot of -- taken advantage of a lot of information

20 that was developed up to that point, then I think it is more efficient to

21 go with the flow through.

1 At our laboratory, we have conducted approximately 35
2 toxicological studies. If you are interested, 12 to 15 of those studies
3 are published, and there are several in press. But the thing I want to
4 point out here is that we have worked with xenopus laevis, rana
5 pipiens, clamitans, sylvatica, septentionalis. And all those species
6 are amenable to these methods.
7 Some are better than others. I know someone was complaining
8 about a doing green frog work. I kind of had to smile because it just
9 takes them forever to develop. So I wouldn't recommend doing green
10 frog or mink frog work with these methods. But they can be done if
11 you are studying larval period.
12 But if you want to go through metamorphosis, I think the
13 xenopus laevis, rana pipiens and rana sylvatica would be the
14 organisms of choice.
15 We also hold in culture -- all of our cultures flow through. And
16 we have currently xenopus laevis and xenopus tropicalis in culture.
17 That's all in flow through conditions.
18 I would be happy to take any questions on that.
19 DR. ROBERTS: Are there any questions or comments on the
20 flow through versus static issue?
21 Dr. Green. I believe Dr. LeBlanc had indicated previously you

1 wanted to -- no? Then Dr. Kloas.

2 Dr. Green.

3 DR. GREEN: I'm in full support of using a flow-through

4 system, I think -- for the reasons you just pointed out there.

5 Although, I had many people complain about having to do that seems

6 like once they switched they are overall more satisfied.

7 And I can't find anything in the literature either except

8 anecdotal reports that it is detrimental to the development of either

9 xenopus laevis or rana pipiens.

10 One thing I did note was several people felt like, and these are

11 big commercial suppliers of these stock sources for these frogs, said

12 that they feel that laboratory reared and conditioned frogs actually

13 adapt quite well to being in a flow-through system.

14 You wouldn't want to take wild caught frogs or tads and put

15 them in a system. They probably wouldn't deal with it as well.

16 The question I had for you was what kind of water is it. Is it

17 reconstituted R O treated water?

18 DR. TIETGE: No. The water we use is for the most part

19 unmodified Lake Superior water, which is a relatively low

20 conductivity water.

21 It goes through several treatment -- well, filtration steps and

1 UV sterilization. But beyond that, it is Lake Superior water.

2 DR. GREEN: It is potable, chlorinated, filtered water that goes

3 through UV sterilization?

4 DR. TIETGE: It is not chlorinated.

5 DR. GREEN: Chloraminated?

6 DR. TIETGE: It is not chloraminated.

7 This is taken from the bottom of Lake Superior. We're located

8 right on the lake. And we have an intake that's about a quarter of a

9 mile, I think, from the laboratory out into the lake where there is a

10 specialized filter system that is built into the lake.

11 And then the water comes in, and then there is further

12 filtration, and then the final step is UV sterilization. There is no

13 chemical additives in the water whatsoever.

14 DR. GREEN: I know this is being detailed, but if we were to

15 try and reproduce this system or recommend that something like this

16 be used, when you talk about filtered, do you know -- you are filtering

17 particulates. Do you know what size -- the reason being is all the

18 runoff that goes into the lake you want to make sure you get

19 pathogens like --

20 DR. TIETGE: We have several types of filters involved in our

21 system. First of all, in the lake itself, there is a graded, gravel

1 sandbag that has fairly crude, I think maybe, 25 micron, I think, is the
2 cutoff range for that. But when it comes into the building, it goes
3 through some five micron filters, at which point it is UV sterilized.
4 And then prior to introduction to the system, it goes through a
5 more typical laboratory filter that I think is probably two and a half
6 or five microns or in that range.
7 One thing I meant to mention earlier in terms of efficiency is
8 from experience, I can tell you that these systems require much less
9 labor. And we actually elected to conduct a static renewal study
10 about two years ago for very specific purposes.
11 And after we got into it, we really regretted it because it was so
12 much work. These systems require very low maintenance.
13 DR. GREEN: That's what makes me a bigger fan too, low
14 maintenance.
15 DR. TIETGE: There are numerous universities and contract
16 laboratories that use these. It isn't necessary to use our lake water
17 supply. There is numerous examples of waters.
18 Of course, I think that, if you wanted to, you could modify a
19 water with additives, salts, whatever using the appropriate
20 technology.
21 DR. ROBERTS: Dr. Kloas.

1 DR. KLOAS: Did you perform -- not just for the performance
2 of the animals, this is quite convincing. I agree that it would grow
3 and so on.
4 But do you have any comparative study using positive controls
5 for instance in endocrine disruption, estradiol treatment, something
6 like this, where you can compare static renewal system with flow
7 through and the sensitivity of both exposure regimes?
8 DR. TIETGE: No, because we don't do static renewal. Our
9 work primarily is focused on thyroid axis disruption. So we've used
10 numerous chemicals to inhibit or to stimulate metamorphosis.
11 But we have also run a couple of chemicals, nonaromatizable
12 androgen, and we have also -- we did see androgenic effects. We have
13 also run estradiol where we do see -- off the top of my head, I don't
14 remember the concentrations of the study, but I know that we do have
15 feminization in that case.
16 DR. KLOAS: Because there is always some concern about if
17 it's the same or if you lose sometimes -- maybe you can also lose by
18 permanent loading of humero (ph) compliments, you may lose a little
19 bit sensitivities.
20 DR. TIETGE: Loss of sensitivity based on what? I'm sorry.
21 DR. KLOAS: In comparison to static renewal system. I can't

1 compare. We don't have flow through. But if you compare, some
2 people found -- I'm aware of one or two studies in a lab with a flow
3 through. They use same concentrations of estradiol but are getting
4 much lower sensitivity concerning feminization of xenopus and also
5 ranid species.
6 DR. TIETGE: I think I'm familiar with at least some of the
7 work you are talking about. I'm not sure if they used -- that was a
8 positive control. I'm not sure that they had analytical verification of
9 that.
10 We can talk about that. But I'm not sure.
11 One of the things that also I should mention has to do with the
12 rate of development in some of the studies that we reviewed for the
13 white paper. The developmental rates for xenopus were very long, in
14 our opinion.
15 And in our laboratory, using the flow through systems, we
16 typically have metamorphosis well underway at seven weeks. About
17 50 days post fertilization. And it is usually completed within three
18 or four days after that.
19 Certainly, within 56 days, we are generally done with
20 metamorphosis at that point, unless there is a chemical effect, and
21 then, of course, that is another story. But I'm referring to controls in

1 that case.

2 DR. ROBERTS: Dr. LeBlanc.

3 DR. LEBLANC: Joe, I agree with your recommendations for a

4 flow-through system as well as recommending the proportional

5 diluters.

6 But it seems that proportional diluters scare a lot of people. It

7 must be all those tubes and boxes or something. I was just wondering

8 if there are alternatives that you are aware of in terms of peristaltic

9 pump systems --

10 DR. TIETGE: Yeah. During the period that those were

11 designed, pump systems weren't quite as reliable as they are today.

12 There are many, many reliable pump systems that can be used.

13 And some of our newer designs do not use the proportional flow

14 through devices. They have simple dilution or, I should say, solution

15 cells that serve as a stock for a peristaltic pump that might have six

16 or eight lines coming out of it or other pumps.

17 We have very many systems in our laboratory that represent

18 different design ideas.

19 I think when you get into a more complex exposure paradigm,

20 then the system has to be more complicated to accomplish it. But

21 pumps work fine, actually.

1 DR. ROBERTS: Any other comments? Dr. Skelly.

2 DR. SKELLY: Just real quickly, Dr. Tietge.

3 We're talking about this I assume because this is going to be

4 what EPA recommends as people go forward or requires.

5 I think Dr. Bradbury or somebody said before that there doesn't

6 necessarily need to be a requirement that people that want to do

7 experiments that are going to be evaluated and used by EPA to make

8 judgments will use one method or another.

9 I thought it would be helpful if you could comment on that.

10 DR. TIETGE: I think in the absence of a validated protocol,

11 one needs to adhere to a standard. And the standard is right now this

12 most appropriate ASTM. There is the flow through recommendation.

13 And there is the static renewal recommendation.

14 I think if you take a look at the two, it is more efficient to do

15 the flow through. Because for ends that you need to utilize for your

16 statistical design, I think you would be in a lot of trouble with a static

17 test.

18 DR. ROBERTS: Dr. Skelly I think wanted to respond.

19 DR. SKELLY: Just to follow up. Maybe the economics of

20 working in a government agency versus a university can be a little bit

21 different where labor can be quite cheap in a university setting,

1 people getting credits or whatever, whereas equipment costs whatever
2 it costs.
3 I guess what I'm wondering is will the EPA have an all flowers
4 can bloom sort of attitude towards this? Or are they going to say flow
5 through or nothing.
6 DR. STEEGER: I think one of the things we're looking to see
7 happen is that the studies that are conducted for regulatory purposes
8 are consistent or as consistent as they can be with our guidelines.
9 And although we don't have any guidelines right now for
10 amphibian studies, we do have the A 50 guidelines for water quality
11 standards. And we would hope that whatever studies are conducted
12 adhere to those guidelines.
13 DR. BRADBURY: I think some of the sort of minimal issues in
14 terms of data quality that we're talking about are the ASTM
15 guidelines. They are measured concentrations.
16 As Dr. LeBlanc mentioned earlier today, if it is a static
17 renewal, you probably need to do more analytical chemistry in terms
18 of numbers of samples over time than one would have to do in a flow
19 through test to ensure that you really know what your concentrations
20 are. Because that's one of the other challenges of a static renewal,
21 can be, depending on the chemical and toxicokinetics, that aspect.

1 So I think it is more of the, if you will, performance issues,
2 loading, DO, ammonia, measuring the concentrations that are the real
3 important issues to the extent that some studies would be done
4 because people read these documents and think they would be like --
5 they would like to pursue some research, they will have some ideas on
6 the kinds of quality indicators that would be important for the agency
7 to use -- looking at -- when they look at the open literature to the
8 extent the registrant may be performing some of these studies.
9 That's sort of a different venue in which some of these studies
10 potentially could be done, if they are done, which gets at what Tom
11 was saying in terms of the kind of guidelines the agency requires for
12 data that's submitted as part of registration or reregistration.
13 DR. ROBERTS: Dr. Kloas.
14 DR. KLOAS: Concerning atrazine effect, obtained up to now
15 they have been just obtained in static renewal systems. Maybe
16 metabolism of atrazine and so on could also play a role for getting the
17 effect.
18 If there is really concern about atrazine and -- I agree with
19 several advantages. And from a logical point of view, I agree
20 completely. I would also like to have flow through in my lab to try
21 doing that.

1 But there is concern about what was done and results obtained
2 up to now. I think to repeat this should contain both at least static
3 renewal and maybe an advance study using the flow through. Because
4 otherwise, we cannot rule out if there is no effect in a flow through
5 anymore.
6 DR. BRADBURY: One point. I guess we would be curious to
7 hear the panel's response in terms of ASTM loading guidelines,
8 ammonia, D O, those kind of attributes that have to be met in terms of
9 evaluating the quality of the study regardless if it was done flow
10 through or static renewal.
11 I think the agency in putting out the document to get a response
12 was first understanding your impressions about basic data quality
13 parameters. How you get there. It could be static renewal. It could
14 be flow through.
15 DR. ROBERTS: Dr. Delorme, then Dr. Green.
16 DR. DELORME: Dr. Bradbury just answered my question. He
17 said that you had to meet the water quality standards that have been
18 set out.
19 But just as another note for the panel, as somebody who does
20 risk assessments and gets studies in, typically, when you get a study
21 in on fish or invertebrates, they have to provide information on the

1 water, which includes the presence of other contaminants, be they
2 heavy metals, pesticides or whatnot.
3 So the water is characterized typically at the labs that do the
4 studies, whether it be an industry lab or a contracting company.
5 DR. ROBERTS: Dr. Green.
6 DR. GREEN: I think Dr. Kloas's point is well taken too. There
7 may be something inherent with the static renewal system that mimics
8 wildlife pond situations closer than the flow through. Particularly,
9 with regards to ammonia and other things in the water at higher
10 levels.
11 And some of the studies we looked at yesterday clearly had
12 higher levels of ammonia. And that might play a role in interaction
13 with atrazine that if we put them in flow through systems and we
14 adhere to the guidelines, we might not see that.
15 If it's feasible, it seems a reasonable thing to, if you don't get
16 results in the flow through system that are repeatable, that perhaps a
17 static renewable system would be on a smaller scale something to look
18 at as well.
19 DR. ROBERTS: Dr. Bradbury.
20 DR. BRADBURY: I want to get clarification.
21 If our question at hand is to evaluate the hypothesis that

1 atrazine can cause developmental effects, sort of putting on my
2 toxicology hat, I would like to try to get a system that gives me the
3 cleanest possible way to interpret whether or not I'm getting a
4 toxicological signal. And then I'll get more complex as I need to.
5 I guess I would like to get a response to that.
6 DR. ROBERTS: I think Dr. Green's point is if the answer is if
7 you get a negative response, it is possible that atrazine under
8 conditions, under something less than clean conditions that may be
9 more relevant, in fact, to the environment might give a positive
10 response, you might want to check that before you stop, I guess.
11 I'm not speaking for Dr. Green. But that's what I think the
12 point is.
13 DR. GREEN: I think that's right. Otherwise, we'll be open
14 scientifically to the criticism that it wasn't a static and you get
15 negative results on a flow through, and we're back to phase one again.
16 DR. BRADBURY: Hopefully if some of our discussions about
17 the hill criteria hold up, we'll --
18 DR. ROBERTS: Dr. Richards.
19 DR. RICHARDS: This is partly a question, partly response.
20 I think maybe to step back a bit, some of the people I have
21 heard over the last few days express that the static was more

1 appropriate because maybe it more mimicked a pond-type situation.
2 I was trying to think back. I don't know a great deal about the
3 development of the ASTM standards, but when I think of all the
4 species of fish that have been used in the development of those
5 standards, how many of them might you place into the category of
6 being species that might inhabit these mesotrophic or eutrophic sorts
7 of ponds be exposed to relatively high ammonia levels, and that might
8 be considered a normal environment, as would be xenopus and
9 possibly rana pipiens too.
10 DR. STEEGER: Our test species are chosen again because of
11 their ability to be raised under laboratory conditions.
12 There are large mouth bass and blue gill sunfish. There are
13 other choices we get. Fathead minnows.
14 They can live under eutrophic conditions. But those are
15 challenges that can impact the signal that Steve is talking about in
16 terms of just trying to create a situation where we have removed as
17 many of those variables as possible to have as clean a signal as
18 possible, is what the agency uses for regulatory purposes.
19 Otherwise, it gets to be very difficult to sort out what the real
20 cause of effect was.
21 DR. RICHARDS: In partial response, am I hearing, then, that,

1 yes, other organisms like fatheads and so forth that do live often
2 times in mesotrophic or eutrophic situations seem to respond very
3 well and repeatably in flow through conditions.
4 DR. ROBERTS: I guess in response I would just say that if you
5 find yourself in the situation where you use flow through and you
6 don't get a response, the question will be, and I think I would be sort
7 of compelled to go back and see whether or not the reason you don't
8 get a response has something to do with flow through versus static
9 conditions, and then the question will come up, which is more
10 relevant.
11 I don't know -- of course, until you understand the basis for the
12 difference between static and flow through, I don't suppose you could
13 answer that question, but we can envision the situation where you
14 would be sort of drawn down that path.
15 And if you can't duplicate it in static conditions, you can just
16 say, well, we can't duplicate it under static or flow through.
17 If you get it under static, you don't get it under flow through,
18 then you are going to have to figure out why and figure out which one
19 is more relevant.
20 I guess we're just saying that.
21 DR. BRADBURY: Can I ask one more question?

1 Let's say we go down a path, you do a flow through and you
2 don't get the effect. Maybe that has something to do with the physics
3 of a static renewal is what is important to triggering whatever event
4 occurs.
5 This is just because I couldn't remember the dialogue. Would it
6 still be the recommendation of the panel that one would try to meet
7 ASTM standards in running that static renewal?
8 DR. ROBERTS: Dr. Kloas.
9 DR. KLOAS: I think it should be the same conditions whereas
10 people obtained the effect.
11 I'm completely surprised if I can -- 25 animals and 75 fetus or
12 something. It is hard to handle. But the effect already observed have
13 been used in volumes of four liters or something like that.
14 I think it wouldn't make sense to use another static renewal
15 system. Really in agreement with ASTM standards. I'm a dirty
16 endocrinologist. I'm sorry. I would like to have a study repeated
17 under the same conditions. Whatever it might make any difference.
18 I would like to have flow through and to see that there are
19 results. But if you change in another way and doing static renewal
20 under different conditions and you can't repeat again, it is the same
21 question. If you can't repeat it in flow through, then you are again

1 next step and one step backwards.

2 DR. BRADBURY: I just have this -- I get a feeling that I'm

3 going down a path that gets us back to some of the white paper's

4 analysis that it was very difficult to interpret the responses from the

5 previous studies because of concern over the ammonia levels, the

6 feeding issues, the DO issues.

7 Then we're being asked to try to replicate scenarios that -- I'm

8 trying to track the -- I feel like we've started getting into a do-loop.

9 DR. ROBERTS: I guess Dr. Green can respond.

10 We got I think some responses by at least a couple different

11 labs. I don't know they all had real high ammonia levels. I don't

12 know that that -- I don't know that you're going to have to really

13 make awful conditions to get --

14 DR. BRADBURY: I think some guidance on, if not ASTM

15 guidelines, where do you feel a reasonable static renewal in terms of

16 those issues, at least bracket that.

17 DR. ROBERTS: Sure. Dr. Green.

18 DR. GREEN: I don't think our intent was to recreate the dirty

19 water with a static renewal system. That wasn't it at all.

20 We have static renewal systems that house 400 and 500 adult

21 frogs. No matter how hard we work on it, we cannot produce ASTM

1 clean water to that degree.

2 I think what is important is the dynamics of the animal sitting

3 and reabsorbing the drug that they are absorbing and excreting for the

4 few hours that they do it in between water changes and for the gradual

5 increase in ammonia.

6 And certainly they don't have to go up to the toxic lethal levels

7 that one of those papers, I believe, had in it, but that system alone

8 would be something I think -- would be a justifiable attempt to

9 reproduce the results they got in the static system.

10 Try to keep the water as clean as possible. But you are never

11 going to get it as clean as you would with the flow through system. I

12 would say change the water as they did in the static renewal system,

13 as frequently as you need to to keep the ammonia down and all that

14 stuff.

15 And it is still going to go up enough that it is only speculative

16 that the interactions in the water chemistry dynamics will be there in

17 a way that they are not there in the flow through system.

18 DR. ROBERTS: Dr. LeBlanc.

19 DR. LEBLANC: It just seems like we're getting into the realm

20 of what if, what if we don't -- what if we get a negative result, what

21 do we do.

1 I just don't know that we should be crossing that bridge. I
2 recognize you need recommendations and you are looking for advice
3 and help. But I think we should proceed with the assumption that we
4 will replicate the results.
5 And if we don't, at that point we have to look at the new data as
6 compared to the old data and make some judgments as to why perhaps
7 we can't replicate the results. And then design some basic
8 experiments to address those variables. Leave it at that.
9 DR. ROBERTS: I want to ask if there are there other comments
10 by panel members regarding any of the topics -- this topic and the
11 discussions we have had over the last few days, any points that are
12 important you think that have not been made as yet but that would be
13 important to make and introduce into the minutes.
14 Dr. LeBlanc.
15 DR. LEBLANC: Quick technical point. The experimental
16 design that was up there, you had a positive control of estradiol. We
17 have seen some experiments with DHT. I don't see any reason why we
18 would be looking at DHT as a positive control.
19 But we are interested, I think, in some anti-androgenic effects,
20 not necessarily due to the ability of atrazine to compete at the
21 receptor level, but perhaps to interfere with conversion of

1 testosterone to DHT or just to overall cripple the synthesis of
2 hormones.
3 So you might give consideration to a control for
4 anti-androgenic properties. I guess you would have to look at an
5 anti-androgen. And one that I have seen in the literature with frogs
6 and I think Darcy or Sherril mentioned, it was cyproterone acetate.
7 That would be something to consider.
8 DR. ROBERTS: Dr. Kloas.
9 DR. KLOAS: I can add three suggestions. You can use
10 cyproterone acetate, which works as an anti-androgen, or you can use
11 para para DDE. Also, vinclozolin would work also.
12 DR. ROBERTS: Dr. Gibbs.
13 DR. GIBBS: I still have lingering concerns that far too
14 individuals use the found, the experimental larval populations in some
15 of these laboratory studies.
16 It is not my area of expertise, but as a population geneticist, it
17 really concerns me when three pairs are used. One onymous
18 individual with three pairs will potentially skew an entire experiment.
19 I think boosting numbers up into the 10s of pairs, I think it seems
20 like a good idea to me.
21 DR. SKELLY: Just a point that has been touched on a little bit,

1 but I wanted to make sure it made it clearly into the record.

2 That is that it may be standard practice in toxicology, but I was

3 struck by the kind of fluctuating use of different levels of statistical

4 units.

5 In some cases, people were using individuals. In some places,

6 people are using tanks as their units of analysis, even though all the

7 individuals are raised in group tanks.

8 And in ecology, that's just totally tabu. You don't do that. If

9 things are raised in a tank, they can influence each other. And you

10 don't use individuals as replicates in analysis if they came out of the

11 same tanks.

12 I don't pretend an SAP can change practice in an entire field.

13 But if that is standard operating practice and if we are going to get

14 into ecological relevance at some point and ecologists are going to be

15 evaluating this stuff, they are going to care about that a whole lot.

16 That alone will eliminate a paper from peer reviewed literature

17 in ecology.

18 DR. ROBERTS: Any other suggestions from panel members?

19 Dr. Bradbury, I think the panel has given you our best advice on

20 this subject. Are there any final clarifications or follow-up

21 questions?

1 DR. BRADBURY: No. I think it has been a very helpful
2 discussion this afternoon and mid morning. I appreciate all the hard
3 work and input.
4 DR. ROBERTS: I think as came through in our comments, I
5 think the panel was very impressed with the job that the agency had
6 done on the white paper and your analysis and your thoughts on how
7 to move forward.
8 I think we thought in general you did a very good job with that.
9 We had some suggestions here and there for things maybe to add and
10 consider. But overall, we thought the agency did an excellent job on
11 a very difficult subject area.
12 Before we close the meeting, I would also like to thank our
13 public commenters. We had many of them that travelled sometimes a
14 great distance to come and speak to us and share with us their data
15 and their viewpoints on this subject. We always welcome different
16 perspectives and viewpoints on the issues that we face. And we thank
17 the public commenters.
18 I would also like to thank the hearty soles in the audience who
19 have stayed with us now for a number of days through our
20 presentations and our discussions. I like to thank you.
21 And of course, always, I would like to thank the SAP staff for

1 putting this meeting together. They assembled an outstanding panel
2 of experts, got us all here, got us all the documents we needed and
3 have been very supportive.
4 Finally, I would like to thank the panel members, a very
5 impressive panel. Your expertise was obvious. The fact you came
6 prepared was obvious. We have had excellent discussion. Excellent
7 questions right from the beginning. I think you guys did a terrific
8 job.
9 Do we have any other announcements before we close the
10 session? Paul, anything you need to say?
11 MR. LEWIS: Just a few remarks. I want to first thank Dr.
12 Roberts for serving as our chair for the past three days. Did an
13 excellent job in managing the process for this meeting.
14 Members of the panel, it was a pleasure working with all of
15 you. And looking forward as we work in writing our report that for
16 the public's interest will be available in about four weeks, available
17 on our SAP web site and also in the docket.
18 And I thank the members of the public for listening to our
19 discussion and for the contributions they made as part of the
20 deliberations that we had in the past three days.
21 Finally, my colleagues at EPA, both in EFED (ph) and my

1 colleagues with the SAP staff in working with me, pleasure working
2 with you in getting this meeting off the ground. Thank you.
3 Dr. Roberts.
4 DR. ROBERTS: Immediately following the close of this
5 session, I would like the panel to meet in the meeting room for a short
6 closed session just to discuss the logistics of writing up our minutes.
7 As soon as we're finished here, if we could convene there for a
8 short meeting, that would be great.
9 If there no other announcements or no other topics to discuss, I
10 would like to now close this session of the FIFRA Scientific Advisory
11 Panel.
12 (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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