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FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

THE POTENTIAL FOR ATRAZINE TO AFFECT
AMPHIBIAN GONADAL DEVELOPMENT

U.S. ENVIRONMENTAL PROTECTION AGENCY
CONFERENCE CENTER- LOBBY LEVEL

One Potomac Yard (South Building)

2777 S. Crystal Drive

Arlington, Virginia 22202

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1 U.S. ENVIRONMENTAL PROTECTION AGENCY
 2 FIFRA SCIENTIFIC ADVISORY PANEL
 3 OPEN MEETING
 4 OCTOBER 10, 2007
 5 DR. BAILEY: I think we're going to get
 6 going here. Welcome back to the second day of the
 7 FIFRA Scientific Advisory Panel Meeting. And this
 8 meeting is looking at the potential for Atrazine to
 9 effect amphibian gonads development. I want to thank
 10 Dr. Heeringa for being here as the chair. For anyone
 11 who wasn't here yesterday, I'll just run over a few
 12 things.
 13 The FIFRA SAP is an advisory committee. It
 14 only provides advice and recommendations. The agency
 15 is responsible for making all decisions and
 16 implementation of those decisions.
 17 We have asked the panel to complete standard
 18 government financial disclosure forms; and I, along
 19 with the deputy ethics officer, have reviewed these
 20 forms to ensure that all ethics requirements are met.
 21 We have established a public docket for this meaning,
 22 and all the materials that were handed out yesterday
 23 should be in the docket bind today. And they haven't
 24 gotten them here, yet, but they should be available
 25 very shortly.

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1 We will prepare meeting minutes at the, after
 2 the closing meeting. They should be available in,
 3 approximately, ninety days after the close of the
 4 meeting. And one final note, we are recording the
 5 meeting again today.
 6 So, if you have comments to make, please give
 7 your name before you make those comments so that we
 8 can
 9 clearly hear on the recording who's making the
 10 comments. I think that's it, and at this point, I'll
 11 turn it over to Dr. Heeringa, the chair for today's
 12 session.
 13 DR. HEERINGA: Good morning everyone, and
 14 welcome back to the second day of our FIFRA Science
 15 Advisory Panel meeting on the topic of a potential for
 16 Atrazine to affect amphibian gonadal development.
 17 As Joe mentioned, I'm Steven Heeringa. I am
 18 from the University of Michigan, where I am a
 19 statistician specializing, primarily, in population-
 20 based research studies. I'd like the other members of
 21 the panel to also introduce themselves, and maybe a
 22 little background, and I'll turn it over to Ken
 23 Portier, and we'll move around the table.
 24 DR. PORTIER: Good morning. I'm Ken
 25 Portier, Director of Statistics at the American Cancer
 Society National Home Office in Atlanta. And I'm a

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1 member of the permanent panel, and my expertise is in
 2 general applied statistics and probabilistic risk.
 3 DR. CHAMBERS: I'm Jan Chambers with the
 4 College of Veterinary Medicine at Mississippi State
 5 University. My area is pesticide toxicology with
 6 emphasis on neurotoxicology and metabolism.
 7 DR. SCHLENK: My name is Dan Schlenk.
 8 I'm in the Department of Environmental Sciences at
 9 University of California Riverside, and my, I'm a
 10 member of the permanent panel. And my expertise is in
 11 aquatic toxicology.
 12 DR. BUCHER: I'm John Bucher. I'm the
 13 Associate Director of the National Toxicology Program
 14 at NIHS. I'm a member of the permanent panel. And I
 15 have a background in carcinogenesis and general
 16 toxicology issues.
 17 DR. ISOM: Good morning. I'm Gary Isom
 18 from Purdue University and Professor of Toxicology. My
 19 area of interest is neuromechanisms of, or molecular
 20 mechanisms of neurodegeneration, and I'm a permanent
 21 member of the panel.
 22 DR. GREEN: My name is Sherril Green, and
 23 I'm from Stanford University. And my area of expertise
 24 is in *Xenopus laevis* amphibian biology and disease.
 25 And I'm an SAP member, and had some discussions with

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1 United yesterday about a sixty-eight person standby
 2 list, so I finally made it today. Thanks.
 3 DR. PAULY: My name's Bruce Pauly. I'm
 4 with Environment Canada in Ottawa, Ontario. I'm a
 5 wildlife biologist with a particular interest in the
 6 effects of pesticides on wildlife. And I've been doing
 7 studies on pesticide effects on amphibians for the last
 8 while.
 9 DR. SKELLY: My name is David Skelly.
 10 I'm a professor of ecology at Yale University. And my
 11 interests include the study of natural populations of
 12 amphibians, including developmental deformities.
 13 DR. DENVER: I'm Robert Denver from the
 14 University of Michigan. I'm Professor of Molecular
 15 Cellular Developmental Biology. And I'm a
 16 neuroendocrinologist. And I study hormone action in
 17 amphibian development.
 18 DR. FURLOW: My name is David Furlow.
 19 I'm a Professor of Neurobiology Physiology and Behavior
 20 at the University of California Davis. I am an
 21 endocrinologist, specializing in thyroid hormone and
 22 steroid hormone action, and including in amphibians.
 23 DR. YEATER: My name is Kathy Yeater, and
 24 I'm an area statistician with the U.S. Department of
 25 Agriculture, Agricultural Research Service, where I



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1 specialize in applied statistics of biological and
 2 agricultural statistics.
 3 DR. BAILEY: Tim Bailey, Professor at
 4 Iowa State University in Department of Statistics.
 5 DR. DELORME: Peter Delorme. I'm a
 6 Senior Science Advisor in the Environmental Assessment
 7 Division of the Pest Management Regulatory Agency of
 8 Health Canada.
 9 DR. LEBLANC: Gerry LeBlanc. I'm a
 10 Professor of Toxicology at North Carolina State
 11 University with research interest in endocrine
 12 toxicology.
 13 DR. MILLER: Debra Miller. I'm from the
 14 University of Georgia College of Veterinary Medicine,
 15 and I'm a veterinarian pathologist.
 16 DR. PETINO: I'm Reynoldo Petino. I'm
 17 with the U.S. Geological Survey Texas Cooperative Fish
 18 and Wildlife Research Unit in Lubbock, Texas. And I'm
 19 a comparative and reproductive endocrinologist and
 20 physiologist.
 21 DR. HEERINGA: Thank you, again, members
 22 of the panel for your willingness to participate in
 23 this multi-day meeting. We, again, we recognize very
 24 busy schedules at work, academia for many of you, and
 25 appreciate the fact that you're able to be here and

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1 participate. So, at this point, before we turn back to
 2 the proceedings, John Bucher mentioned that he had one
 3 clarification from yesterday that he wanted to get in
 4 the record.
 5 DR. BUCHER: Thanks. John Bucher.
 6 Yesterday, I was asking a question about the relative
 7 doses of estrodyle used in the Syngenta studies versus
 8 the Hayes studies. And I misread my notes and
 9 indicated that the Hayes positive control list,
 10 actually, concentration of half of what Syngenta was
 11 using, and in fact, that's incorrect. And the dose
 12 that he was using was much higher than that. So, I
 13 withdraw that question.
 14 DR. HEERINGA: Thank you very much for
 15 that clarification. At this point, just to review
 16 where we've been, I asked the panel. We have had our
 17 first of initial overview presentations from the EPA
 18 scientific staff.
 19 We have, then, had a period of extensive
 20 public comments, including a description of the data
 21 call-in studies, represented by Syngenta crop
 22 protection. We've had additional public comments from
 23 representative of the Agriculture and the Natural
 24 Resources Defense Council. And, so, we've had a fairly
 25 good introduction to the topic.

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1 I understand that, while I was in College
 2 Park teaching, that you've managed to finish up the
 3 presentations on the studies and the statistical
 4 analysis of those studies, and that, at this point, we
 5 are ready to approach the conclusions, I think, that
 6 would be presented by Dr. Steeger. And, also, we have
 7 one additional presentation on the power analysis that
 8 was done for the Syngenta DCI study.
 9 So, we'll turn to those in order, but we'll
 10 complete our discussion and presentation with EPA
 11 scientific staff. And then we'll go to the power
 12 analysis presentation. And then we'll have a general
 13 set of wrap-up questions for clarification from the
 14 panel on any remaining items that you'd like to have
 15 discussed before we move to the charge question.
 16 So, at this point in time, I'd like to turn
 17 to Dr. Thomas Steeger of the Office of Pesticide
 18 Programs at the EPA, and ask him to, maybe, introduce
 19 the staff that's with him this morning, scientific
 20 staff, and then, also, to proceed with the conclusions
 21 and the EPA's reviews.
 22 DR. STEEGER: Okay, good morning, and
 23 thank you for the opportunity, again, to address the
 24 FIFRA Scientific Advisory Panel. Sitting with me at
 25 the table today is Mary Frankenberry, a senior

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1 statistician with the Environmental Fate and Effects
 2 Division; Dr. Sig Degitz, who is a Research Biologist
 3 and order at the Office of Research and Development,
 4 The Mid-Continent Ecology Division.
 5 Next to him is Arthur, Arty Williams, who is
 6 the, I'm sorry, Acting Director of the Environmental
 7 Fate and Effects Division; Dr. Stephanie Irene, who is
 8 a Senior Advisor at the Environmental Fate and Effects
 9 Division; and Anita Pease, who is a Senior Biologist in
 10 the Environmental Fate and Effects Division. All of us
 11 are co-authors on the 2007 white paper, and many of us
 12 were co-authors on the 2003 white paper.
 13 Yesterday, we spent most of the day
 14 discussing the data that the agency considered relative
 15 to the effects of Atrazine on amphibian gonad
 16 development. While focus has been on the registrants
 17 submitted studies that are in response to the agency's
 18 data call-in for tiers, tier one studies, the agency
 19 has continued to look at open literature.
 20 Over the past four years, since the last
 21 FIFRA SAP on this same issue, laboratory and field
 22 studies have continued to examine potential effects of
 23 Atrazine on amphibians.
 24 The Agency has determined that none of these
 25 studies have taken into account the recommendations



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1 made by the Agency and the 2003 FIFRA SAP to reduce
 2 potential sources of variability that could effect the
 3 endpoints being measured in the studies. Taken at face
 4 value, though, both the laboratory and field studies
 5 have failed to demonstrate a consistent, dose-dependent
 6 effect due to Atrazine exposure.
 7 Some authors, that claim to show a consistent
 8 effect in both the laboratory and field studies in
 9 previous studies, now demonstrate no effect on
 10 Atrazine, of Atrazine on timed metamorphosis or gonadal
 11 development, and claim that the lack of response is due
 12 to biological variability.
 13 The Agency is obliged to follow specific
 14 processes in its risk assessments; and yesterday, we
 15 discussed the risk assessment proc-, the paradigm used
 16 by the Agency. Ecological risk assessment process is
 17 further described in the document entitled, "The
 18 Overview of the Ecological Risk Assessment Process" in
 19 the Office of Pesticides Program document.
 20 In considering open literature, the risk
 21 assessor adheres to guidance to assure this consistent
 22 approach is used to evaluate all studies.
 23 The criteria used to evaluate open literature
 24 were discussed yesterday and include, experimental
 25 design; study protocols and quality assurance

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1 measurements; the strength and shape and cause, of the
 2 cause and dose, cause and effect relationship; whether
 3 there was a dose response; whether observed effects
 4 have a plausible mechanism of action consistent with
 5 the known; what is known about the chemical; and
 6 finally, whether the measured effects are ecologically
 7 relevant. These same criteria are applied to guideline
 8 studies, as well as non-guideline studies.
 9 Contrary to what was suggested yesterday, the
 10 EPA cannot dictate to non-registrants how to conduct
 11 studies. Nor does the agency, typically, require
 12 researchers, other than the registrants, to provide raw
 13 data. The agency has to rely on the journal peer
 14 review process to serve as the primary reviewers to
 15 open literature. For the previous SAP, the agency did
 16 try to work with researchers outside those that are
 17 regulated by the agency.
 18 That process proved to be resource intensive
 19 and completely unproductive. As well as-, as will be
 20 discussed-, as was also discussed yesterday, the agency
 21 makes use of multiple lines of evidence to determine
 22 whether the use of a pesticide represents a threat to
 23 human health and the environment.
 24 The prim-, the Agency primarily relies on
 25 guideline studies that are intended to provide

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1 information on whether the chemical affects acute
 2 mortality or chronic survival, reproduction, growth of
 3 non-targeted plants and animals. Guideline studies
 4 include both laboratory and field studies.
 5 The Agency also relies on nine guideline
 6 studies, either submitted by the registrant, and or
 7 recorded in open literature, to determine whether
 8 additional effects, not measured by guideline studies,
 9 are associated with the use of a particular chemical.
 10 Field studies are typically used to determine whether
 11 effects observed in the laboratory are apparent in the
 12 field on their actual use conditions.
 13 It is possible that well designed field
 14 studies can identify chemical effects prior to
 15 laboratory studies; however, it is likely that the
 16 laboratory studies would then be required to verify
 17 that the effects, to verify the effects, and to better
 18 allow the determination of the dose of response; and if
 19 necessary, establish the mechanism to which the effect
 20 occurs.
 21 In 2003, the Agency appeared before the FIFRA
 22 SAP to discuss its assessment of data for non-guideline
 23 laboratory and field studies. Based on the open
 24 literature studies, the Agency recommended that, while
 25 there was sufficient information to conclude that the

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1 Atrazine causes gonadal effects in amphibians, there
 2 was insufficient information to formulate a hypothesis,
 3 or there was sufficient information to formulate a
 4 hypothesis. The agency proposed, and the FIFRA SAP
 5 concurred with, the tiered process for examining
 6 whether Atrazine exposure results in effects on
 7 amphibian gonadal development.
 8 We are meeting here this week to discuss the
 9 results of studies designed, based on recommendations
 10 from the EPA and the FIFRA SAP, to address whether
 11 exposure to Atrazine effects amphibian gonadal
 12 development. We have, also, discussed the available
 13 open literature. Yesterday, Mary Frankenberry provided
 14 an overview of the statistical analysis and of the DCI
 15 studies.
 16 Based on that statistical analysis, the
 17 Agency has developed the following conclusions. The
 18 agency, again, has reviewed the total of thirty-six
 19 documents, representing both interim and final reports
 20 from open literature and registrant simulated studies
 21 related to the potential effects of Atrazine on gonadal
 22 development.
 23 In doing so, the agency used experimental
 24 designs, study protocols, extent of quality assurance
 25 and control, strength of the shape, strength and shape

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1 of the cause-effect, and or dose-response relationship,
 2 both classical and U-shaped dose response curves,
 3 mechanistic plausibility, and ecological relevance as
 4 evaluation criteria.
 5 Based on the available data, the Agency
 6 concludes that Atrazine does not produce a consistent
 7 reproducible effect across the range of exposure
 8 concentrations and amphibian species tested. The lines
 9 of evidence do not support that hypothesis, that
 10 Atrazine exposure causes effects on amphibian gonadal
 11 development.
 12 In 2003, in the white paper, and as
 13 previously described by Dr. Degitz, the Agency proposed
 14 a tiered approach to determine the effects of Atrazine
 15 on gonadal differentiation in anuran amphibians. At
 16 that time, the FIFRA SAP concurred that the approach
 17 was reasonable.
 18 In response to the recommendations made by
 19 the SAP, consistent with what was proposed in the 2003
 20 white paper, the agency required the technical
 21 registrants of Atrazine to conduct tier one studies to
 22 test for apical gonadal effects. During this SAP, the
 23 agency has provided its analysis of the registrants
 24 submitted studies that were responsive to the data call
 25 in.

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1 The agency has also provided its analysis of
 2 the open literature studies and its effects, and the
 3 effects of Atrazine alone on amphibian gonadal
 4 development. Based on its analysis, the agency has
 5 concluded that Atrazine is not affecting amphibian
 6 gonadal development. The agency is also concluding,
 7 based on the tiered study approach proposed in 2003,
 8 that since the tier one studies reveal no effect on
 9 apical endpoints in *Xenopus laevis*, no additional
 10 testing of amphibians for amphibian gonadal effects is
 11 warranted.
 12 The 2003-, in 2003, the SAP was asked whether
 13 there was anything that would preclude the use of the
 14 African clawed frogs as surrogates for amphibians, and
 15 whether there were any important differences to
 16 conclude that any developmental processes of *Xenopus*
 17 *laevis* would not, also, occur in ranids. In response
 18 to these questions, the SAP could not identify any
 19 differences. Therefore, the agency concludes that
 20 additional tier one testing with other amphibians is
 21 not warranted.
 22 Consistent with the Agency's iterative
 23 process for evaluating ecological risk, the Agency will
 24 continue to evaluate data as it become available; and
 25 where necessary, the Agency will develop appropriate

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1 measures to mitigate risk to ensure human and
 2 environmental health.
 3 Similar to what was done in 2003, the Agency
 4 is seeking input from the FIFRA SAP on the Agency's
 5 evaluation of the available literature. In the next
 6 presentation, Dr. Stephanie Irene, Senior Advisor in
 7 Environmental Fate and Effects Division, and co-author
 8 of the 2003 white paper; and Ms. Anita Pease, Senior
 9 Scientist in the Environmental Fate and Effects
 10 Division, will read the charges to the panel.
 11 The SAP is being asked to comment on the
 12 Agency's evaluations and conclusions regarding the
 13 effects of Atrazine alone on amphibian gonadal
 14 developmental data. Additionally, the SAP is being
 15 asked to comment on the Agency's conclusion that higher
 16 tier testing is not warranted. The SAP is also being
 17 asked to comment on the Agency's conclusion that no
 18 additional testing on other amphibian species is
 19 warranted. Thank you.
 20 DR. HEERINGA: Thank you very much, Dr.
 21 Steeger. Members of the panel, any questions for Dr.
 22 Steeger at this point? Not seeing any additional
 23 questions, at this point, I'll have one more
 24 opportunity before we move to the charge questions. I
 25 would like to propose that we move to the-, you're

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1 finished with the presentations for the formal portion
 2 at this time?
 3 DR. STEEGER: That's correct.
 4 DR. HEERINGA: If that's correct, we move
 5 to the final item, which was left for me yesterday
 6 afternoon, which was requested by the panel, and that
 7 is a discussion or presentation on the power analysis
 8 that was conducted for the Syngenta study, and then
 9 permitting that is a, I think, an important
 10 contribution to the statistical understanding of the
 11 study data analysis. So, I think, at this point, Mr.
 12 Hosmer, Bob Sielken, will be doing that presentation.
 13 Panel members, you should have, I think, a handout with
 14 the slides in front of you.
 15 DR. SIELKEN: Thank you, Mr. Chairman.
 16 DR. HEERINGA: He wants you to mike,
 17 Robert.
 18 DR. SIELKEN: My name is Robert Sielken.
 19 I'm a statistician with Sielken and Associates, and a
 20 consultant to Syngenta Crop Protection. Thank you, Mr.
 21 Chairman, and thank you to the rest of the panel for
 22 this opportunity. Dr. Portier raised the question
 23 yesterday about power, and I do have a presentation on
 24 that, on the power studies that we did for the Atrazine
 25 study. I have in the slides, the post hoc power



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1 calculations that we did, and I'll discuss those.
 2 I'd like to, also, indicate that prior to the
 3 conduct of these DCI studies, and in fact, prior to the
 4 conduct of the preliminary estrodyle studies that were
 5 used to kind of iron the issues of conducting these
 6 Atrazine studies, we did do a power analysis for those
 7 estrodyle studies. We had been involved with the Karr,
 8 et al, and some of the earlier studies sponsored by
 9 Syngenta, and we had familiarity with the type of
 10 experiments that were being done.
 11 Drawing on that experience, for example, that
 12 we were seeing a background rate of what went on, a .04
 13 percent in some, or .4, point four percent, in some of
 14 the back-, or 4 percent in the background for some
 15 effect, like newt six, in some of the earlier studies,
 16 we used that as a nominal background rate, and asked
 17 some power questions, relative to that background rate
 18 as a planning step for the estrodyle studies. And we
 19 looked at a range of tanks from four tanks per
 20 treatment, up to about forty tanks; and then, also
 21 looked at a range of animals from two per tank to eight
 22 per tank.
 23 So, we did an analysis early on to see what
 24 would be reasonable. We concluded that using eight
 25 tanks in the controls and eight tanks in the treatment

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1 groups would be a reasonable, give us reasonable power
 2 relative to a four percent background.
 3 Then that would give us the opportunity to
 4 detect anywhere between ten and twenty percent of, say,
 5 something around the mid-six, if it were to occur
 6 against a four percent background. And we would be
 7 able to detect ten to twenty percent with an eighty
 8 percent, we'd have eighty percent chance of detecting
 9 such and end effect.
 10 In the estrodyle study, this was the first
 11 study that EPL and Dr. Wolf were going to be asked to
 12 look for abnormal findings in the gonadal development
 13 area. And in order to accommodate, give him a
 14 background in what was normal, we included, in that
 15 early estrodyle study, a reference group. I don't want
 16 to call it a control, because it wasn't used for
 17 treatment control comparisons.
 18 But it was a reference group that was
 19 untreated that would, allowed him to look at those
 20 animals to see, to give him an index of what was kind
 21 of normal. So, the, we did end up with sixteen tanks
 22 being untreated, that eight of those were really
 23 controls, and eight of them were a reference
 24 population.
 25 When we went to the design of the Atrazine

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1 study, it was logistically just as easy to go with
 2 sixteen controls, as it was to go with eight, given the
 3 dimensions of the room and what was going to have to be
 4 done to actually accomplish that study.
 5 So, they went, in the planning stages, with
 6 sixteen controls to give them, sort of, a cushion, a
 7 security, if you will, that if something should happen,
 8 that the whole study wouldn't just disappear. So,
 9 that's the reason why there was sixteen and not eight
 10 in the planning stages for the Atrazine study.
 11 Now, let me talk about, in terms of the
 12 slides that I have, what the power was, looking back,
 13 now, at the eventual design and eventual fate of the
 14 Atrazine study. And as most of you know, here, power
 15 is the probability of getting data that yields a
 16 significant result. It's a property of the design of
 17 the study.
 18 It does not equate to what was the least
 19 significant difference that was observed in the study.
 20 It's, the power is, rather, the probability of getting
 21 a significant result if something is true. And as the
 22 curve indicates up there with the horizontal axis being
 23 the size of the effect that might be there, you're
 24 likelihood of detecting, your power of probability of
 25 detecting it increases as the effects configure.

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1 And, so, to talk about power, you're really
 2 talking about a power curve. However, in order to
 3 facilitate conversations, it's pretty convention to
 4 talk about the effect size that gives you an eighty
 5 percent power, or a probability of .8 to detect
 6 something, and to use that as an index of this whole
 7 curve, but bear in mind that you do have a greater
 8 probability of detecting effects that are larger, and
 9 correspondingly smaller power to detect smaller
 10 effects.
 11 I'm going to talk about power in two
 12 contexts. One is for the measurement employs, which
 13 are the continuous variables like age in metamorphosis,
 14 body weights, snout vent length, gonadal image area,
 15 those types of endpoints. And, initially, I'm going to
 16 be talking about effect size or some people would call
 17 this a standardized effect size. And it's really what
 18 mean difference from control can you detect with the
 19 eighty percent power in looking at that mean de-, mean
 20 difference as a ratio, with the underlying between
 21 tanks standard deviation.
 22 And so, in the studies, we've got power
 23 numbers for both IGB, which ended up with sixteen
 24 control tanks, and the Wildlife International studies,
 25 which ended up with eight control tanks. And starting



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1 first with the IGB number of 1.5, that's saying that
 2 in, for a measurement endpoint, if the true mean
 3 difference was one and a half standard deviations, we
 4 would have eighty percent power of detecting that. And
 5 that's a, really a result, it's a simulation of the
 6 whole process.
 7 And by the whole process, I mean that for
 8 most of these endpoints, you go through a protective F
 9 test, and if that's significant, you follow with a peer
 10 wise comparison at five percent level.
 11 And, so, we've got that sequential testing,
 12 and if you go through that whole system, the
 13 probability of detecting the difference is eighty
 14 percent, if the, if you're talking about a difference
 15 that's one and a half standard deviations. On the, for
 16 the Wildlife International study, that number moves up
 17 to 1.6.
 18 So, you have a little bit less power when you
 19 have eight control tanks. It's not a dramatic shift in
 20 the power as you from sixteen to eight. In fact, I can
 21 hardly see if there's much shift at all.
 22 This is, although, this might seem a little
 23 bit surprising at first, there is two things going on
 24 here. There's sort of a point of diminishing return,
 25 standard deviations act like the square root of the

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1 sample size, but also, here, we're still talking about
 2 having, at both IGB and Wildlife, eight tanks per
 3 treatment.
 4 And the next slide is also in response to
 5 something that is, more than one of you mentioned, but
 6 I remember Dr. Portier mentioned specifically, was what
 7 was the observed variation in these two studies. And
 8 could I give something that kind of gave a feel for
 9 what was the variability in these studies.
 10 And this slide shows the coefficient of
 11 variation, which is the ratio of the standard deviation
 12 to the mean, so, giving an idea of the relative amount
 13 of variation. And that is different, of course, for
 14 the different endpoints, because there's underlying
 15 different standard deviations for age, body weight,
 16 snout vent length, and gonadal image area.
 17 But looking, for example, at Wildlife
 18 International, the standard, the coefficient of
 19 variation is about 2.1 for females, 3.4 for males, and
 20 then 3.4 and 3.9 at IGB. And so, you can look at these
 21 numbers and, yes, they're not identical, of course, but
 22 they're fairly close. They're fairly comparable.
 23 Which is saying something about the comparability of
 24 these two studies. And there are some numbers here on
 25 this slide for you to look at. I apologize that I

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1 added this after Dr. Portier's request. This one slide
 2 is not in what I distributed, and I will reproduce it
 3 and get it back to the panel this afternoon.
 4 SPEAKER: Thank you very much.
 5 DR. SIELKEN: All the other slides are in
 6 there and in order. The earlier slide where I showed
 7 that in will, if you had power, eight percent power at
 8 1.6 standard deviations for will, and 1.5 for IGB.
 9 Well, what does that translate into, in terms of
 10 percent differences from the control.
 11 And, for exam-, and these are the numbers
 12 that, kind of translate standard deviations, if you
 13 will, into percent differences from control, if that
 14 makes it kind of easier to understand. And that's
 15 really just a product of that effect size expressed in
 16 terms of a ratio difference in standard deviation,
 17 times, or factoring in the coefficients of variation,
 18 gives you these numbers.
 19 And these would be the numbers if, in fact,
 20 the observed coefficients of variation are then true
 21 underlying coefficients of variation, and the, render
 22 then powers of theoretical calculation.
 23 Okay, and you can see that these numbers,
 24 like age, that you're looking at in the will study,
 25 being able to decipher the percent of power of three to

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1 five percent change in age, depending on whether you're
 2 talking about females or males, and in IGB, it's five
 3 to six percent, right. Now, in case, I mean, and this
 4 gives you the idea that these are really fairly
 5 comparable, I mean, you've got 11 and 9.8 for body
 6 weight, and two elevens for IGB.
 7 So, these are fairly comparable. They're,
 8 this calculation shows that the age in sixteen didn't
 9 really make too much difference in terms of power. It,
 10 also, shows that, you know, with a little bit more
 11 variability at IGB, that little bit of variability,
 12 coupled with a few more control tanks, really made
 13 these numbers quite comparable.
 14 So, it's a coupling with both of those
 15 factors in there. In terms of a power curve, not just
 16 looking at the eighty percent point, you can, actually,
 17 draw a power curve, the power increasing on the
 18 vertical axis, and the true difference, in terms of
 19 difference divided by standard deviation, on the
 20 horizontal axis.
 21 The solid line is the power curve for sixteen
 22 control tanks. And the dotted line is eight control
 23 tanks. This particular picture is for a measurement
 24 endpoint. So, the point of this picture is, you know,
 25 to remind you that these are power curves. And also,

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1 that the difference between sixteen and eight is not a
 2 dramatic difference, and to quantify that.
 3 When it comes to looking at incidence, that
 4 is, the things that we evaluated at percent, presence
 5 or absence, and what percentage was present, what
 6 percentage did you have in the treatment, what
 7 percentage did you have in the control, and here,
 8 effect size is not relative to a standard deviation,
 9 but just difference in those two percents. So, that
 10 first and, your power would differ, of course,
 11 depending upon whether you're using something that
 12 involves all frogs, a one-sided test or a two-sided
 13 test, or whether you're using males and females
 14 separately. All of these cases were, you know, in one
 15 analysis or another. We've got lots of analyses, and
 16 they're one of these types.
 17 Here, the power actually depends upon the
 18 background rate. At a low background rate, which I've,
 19 I couldn't do exactly zero. I did a nominal, something
 20 close to zero, .1 percent, and asked, what difference
 21 from .1 percent, how big would the percentage have to
 22 be in order for me to have eighty percent chance of
 23 detecting it. And it will, it's 3.5 percent or 2.7
 24 percent there in that first line. And you can see that
 25 there IGB has slightly more power than will, and you

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1 reasonable to compare the results in the two studies
 2 from the perspective of power.
 3 The other types of comparisons that are being
 4 made at different times, at least in the white paper in
 5 Syngenta's reports, is to compare what happened for the
 6 positive controls, the estrodyle or E2, and what
 7 happened for the Atrazine. And for a lot of endpoints,
 8 there was an effect for E2, but you did not see that
 9 same effect for Atrazine.
 10 So, the positive controls were responding.
 11 The Atrazine, there was not that same adverse effect.
 12 And, in order to illustr-, in order to, for that
 13 comparison to be fair, it has to be true that the power
 14 for the E2 statements and the power underlying the
 15 Atrazine statements are fairly comparable. I mean, it
 16 wouldn't be fair to say, I found differences in one
 17 place if I had a lot of power, and I didn't find them
 18 over here when I didn't have power. That wouldn't be
 19 fair. So, it's important to show from the power
 20 perspective that the powers were comparable, and that
 21 it was a fair comparison to say, we sought the positive
 22 controls. We did not see it in Atrazine.
 23 And, there are a whole bunch of cases that I
 24 could have enumerated, and of course, in the finite
 25 time available, I chose to just give a few examples.

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1 can quantify the magnitude at those differences. And,
 2 kind of, if you go all the way down the page, they're
 3 comparable. They're not identical.
 4 This calculation, also, involves the, since
 5 we're dealing with tanks as the unit of analysis, it
 6 involves, this calculation requires to specify a
 7 correlation within the tanks. We've picked the number
 8 here of .02, which is about, is the upper confidence
 9 limit of the correlation that we estimated.
 10 So, this is a fairly conservative value for
 11 the correlation. And then, of course the great the
 12 correlation, the less the power, because the
 13 correlation effectively reduces the sample size. And
 14 these, the power for incidence endpoints would change
 15 the picture as you move to a higher percent
 16 backgrounds.
 17 From these first few slides, you can see
 18 that, even though the number of control tanks in the
 19 will study did end up being reduced from sixteen to
 20 eight, four are lost to a microbial blurb, and four
 21 lost to trace Atrazine contamination. The power of the
 22 will study was not significantly compromised.
 23 And that the power of the will study is
 24 still, approximately, equal to, or maybe marginally
 25 less than, the power of the IGB study. So, it's still

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1 And, really, I've got examples of where Atrazine
 2 compares then to its slightly greater power than the
 3 E2.
 4 Some where they were, approximately, equal,
 5 and some where Atrazine had slightly less. In almost
 6 all of these comparisons, you'll see very little
 7 practical difference in the powers, but let me just
 8 illustrate that for you. Here is a couple of the
 9 endpoints where Atrazine had comparisons at slightly
 10 more power than the E2 comparisons. And, what you're
 11 looking at is, really, in the bottom is those pairs. I
 12 can point to them one place, but of course, I can't
 13 point to them everywhere. And from here, apparently, I
 14 can't point to anything.
 15 So, that's all right. Since I can't point to
 16 three screens, and I am not real sure how to use the
 17 mouse here, I'll just say, look at that line for will.
 18 And in that line for will, that age for metamorphosis,
 19 it says that in order to have an eighty per-, I mean,
 20 80 percent. You have 80 percent power, thank you.
 21 It's working, but it just doesn't go to that far out.
 22 A little more powerful. Yeah, there we go. Thank you
 23 for all.
 24 The 6.4 says that if you had the, if you have
 25 80 percent power to detect, and age at metamorphosis



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1 that's increased by 6.4 percent over the controls, when
 2 you're talking about until you, the power for, you
 3 would only need to be 5.4 percent increase in detecting
 4 Atrazine. And I'm not, that's really not much
 5 difference. I wouldn't make much of an issue of it.
 6 And for IGB, you're looking at 6.3 versus 5.9, and you
 7 can see the numbers on the right-hand side as well.
 8 The point here is not the exact numbers, but
 9 the comparability of what you get for E2 and for
 10 Atrazine. And the same, I mean, there are some that
 11 are really almost exactly the same numbers for both E2
 12 and Atrazine. And there are also some where Atrazine
 13 is slightly more powerful. That first column is a
 14 little confusing, because if you're talking about
 15 percent male, you have 80 percent power.
 16 If the percentage had dropped from controls
 17 down to 29, you could detect it at 80 percent. And
 18 from 48 down to 27, it would have to be how much it
 19 dropped before you pick it up with Atrazine. So, those
 20 two numbers are fairly comparable.
 21 For a member of the course that, when you're
 22 talking about percentages, the points in the middle,
 23 around 50 percent, are the most difficult to detect
 24 changes. So, that's why those numbers would go from,
 25 like, 48 or around 50 down to around 30, because it's

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1 very difficult to detect changes in 50 percent. If you
 2 go from, say, 1 percent to 5 percent, that's a lot of,
 3 it's a fivefold increase. That's a lot easier to
 4 detect, than, say, to go from 50 percent to 55, which
 5 is a relatively small change, and given that things are
 6 quite variable at 50 percent.
 7 So, the whole point of these slides was to
 8 say that, if you're looking at the Atrazine
 9 comparisons, and you don't find it in Atrazine, and you
 10 do find it with estrodyle, it's not because they were
 11 different in the power. It was different in the way
 12 estrodyle works and Atrazine works.
 13 In the DCI studies and in EPA's follow up to
 14 those studies, we not only looked at a protective test
 15 followed by a pair wise comparison. We, also, did
 16 trend tests on everything. And we did those trend
 17 tests regardless of the protective F test or the
 18 protective Kruskal-Wallace test.
 19 Trend tests were done across the board. And
 20 they were done to increase the likelihood of finding
 21 something, if something was really there. And, I'll
 22 just illustrate that.
 23 Here, I really haven't assumed a linear
 24 effect with dose. I've just said, if you've got
 25 linearity, in terms of dose order, something like that,

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1 an increase with the doses. And what does that do, if
 2 you had an underlying trend like that, what would that
 3 do to your power, or how was the power to, how was
 4 power affected by having that sort of trend actually
 5 existing in the data.
 6 And what it does is, if there is a trend, the
 7 trend test has more power to find a difference. Here,
 8 you can see in a comparison, the first column there,
 9 where it says comparison to control, if that's the
 10 analysis, a variance of a Kruskal-Wallace comparison,
 11 those tests had an effect size of around 1.5 standard
 12 deviations. You had 80 percent power to pick that up.
 13 Trend tests will pick it up at 1.1 or 1.2. So, if
 14 there is a trend, if underlying trend, the trend test
 15 will give you a little bit of power, more power to pick
 16 that up.
 17 Now, this slide is to restore your faith in,
 18 kind of, what's going on with both types of tests. On
 19 the left hand side, there's a scenario where you only
 20 have a high dose effect. Okay, and I've chosen this
 21 maximum high here, and then the point begins 80 percent
 22 power. That's 1.4 standard deviation in this scenario.
 23 And then, I took the same maximum difference over here,
 24 but I had a different sort of trend.
 25 The protective Kruskal-Wallace or F test

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1 followed by T contrast for the analysis and variance of
 2 the Wilcoxon, and when they, for the Kruskal-Wallace,
 3 it has 80 percent power at this departure in this
 4 scenario.
 5 It has almost the same power, even in this
 6 dose order situation. It's slightly less. And that's
 7 because an F test looks for differences among
 8 treatments. It doesn't look strictly at treatments
 9 versus controls, and do an F test, and you make it a
 10 difference in treatments, because two of the dose,
 11 treatment doses are different, and not, necessarily,
 12 different but the controls.
 13 On the other hand, if there is a trend,
 14 although the trend test doesn't pick up much over here,
 15 where there isn't a trend, if there is a trend, the
 16 power of finding a difference jumps up to 94 percent in
 17 this case. So, the point here was that, by including
 18 both non-trend tests and trend tests, you've got an
 19 increased chance of finding a difference if there was
 20 one there.
 21 Since I mentioned protective tests, it's
 22 important to say that we used a reasonable protective
 23 test methodology. And as I've indicated, we did a, for
 24 example, the measurement endpoints when we do a
 25 analysis of variance.



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1 If that F test was significant, we would then
 2 do peer-wise comparison in the 5 percent level for each
 3 of those. If you do that, the, you really have a
 4 little bit higher false positive error rate using that
 5 procedure than something like a Dunnett's test, or a
 6 Bonferroni correction.
 7 So, we've kind of used a little bit of a
 8 protective test that was conservative in the sense of
 9 increasing the false positive error rate, and by
 10 consequence, also increasing the power. So the
 11 screening method we did choose, with some added on
 12 power, to choose something that would give us a little
 13 bit more power than say Dunnett's or a Bonferroni type
 14 correction to deal with multiple comparisons.
 15 Lastly, we were concerned about tank effects,
 16 because we'd seen tank effects in the earlier studies.
 17 And what this slide shows is that, as the within tank
 18 correlation increases, what does that do. That can
 19 have a dramatic impact in your false positive error
 20 rate. So, the tank effects, if they exist, they, it's
 21 important to incorporate them into the analysis, and
 22 the tank effects may have an effect, even though you,
 23 they're harder to detect.
 24 Our solution for the tank means, and since
 25 there are four statisticians on the panel, I'm sure

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1 that they, any one of them could come up with some more
 2 complicated approaches, nested models, mixed models, to
 3 address this issue of tank effects.
 4 And so, it's probably important for me to
 5 say, why didn't we do that. And the reason why we
 6 didn't do that is, by going to the, kind of, simpler
 7 approach of going just to the tank means, and using
 8 that as our unit of analysis, that would work for both
 9 the measurement data and the incidence data. Whereas,
 10 some of the other models, particularly the mixed and
 11 nested models, they have, they struggle when incidence
 12 rates get low.
 13 And so, they're very, they're a problem when
 14 the incidence rate gets low. But tank mean analysis
 15 will work in both situations. Also, the tank means,
 16 because their averages tend to be a little bit more
 17 normally distributed, and we weren't forced to do one
 18 transformation for one endpoint, and a different
 19 transformation for another endpoint. So, we did
 20 consider those other approaches, but we thought that
 21 the tank means themselves made an intuitive,
 22 understandable, workable approach that applied across
 23 the board.
 24 The other thing that's relative, perhaps, to
 25 power, a little bit more than biological veins and the

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1 statistical vein, is the choice of *Xenopus laevis*. And
 2 what this slide shows is, starting up at the top, and
 3 on the far right, these are looking at the dots, the
 4 black dots are looking at the static studies, looking
 5 at the literature, and asking, what was the smallest
 6 dose reported in the literature that gave 100 percent
 7 females. And who fell up there at the top is one where
 8 you need the largest dose to get 100 percent with
 9 females. And moving on down, that's kind of a
 10 cumulative plot, if you will, all the way over, and you
 11 can see that *Xenopus laevis* is down there at the end,
 12 where it requires less dose to get to 100 percent
 13 females. And those are in the static studies. So,
 14 picking *Xenopus laevis* was at the more responsive end
 15 of this curve, as far as 100 percent females, and that
 16 was an important consideration.
 17 The green dot to the left of the black dot
 18 represents the study by Knotts, et al, in 1999, where
 19 they were looking for 75 percent females. And that was
 20 in a static system.
 21 The similar number in the flow through system
 22 for the *Xenopus laevis* in the DCI studies was around
 23 .2. So, *Xenopus laevis* is at the more responsive end
 24 of that curve, as far as the species selection, and in
 25 a biological way, then, likely to have more power of

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1 turning up something in the doses that we've studied
 2 than some of these other species.
 3 The last thing that I have that is the last
 4 slide, if I can figure out on how to get there. Sorry,
 5 bear with me for just a second. I prepared something
 6 that I hope would be helpful for you. It was something
 7 that I found helpful. And it's a one page overview of
 8 all of the DCI results.
 9 It makes a lousy slide, but it makes a great
 10 reference sheet. And, I'm, I give it to you because it
 11 helps you, me to see what was going on with E2, what
 12 was going on with Atrazine, what was going on in will,
 13 the Wildlife International Study, and what was going on
 14 at IGB. And I won't belabor this. This is for your
 15 reference, but it's a cheat sheet that I found helpful,
 16 and I thought that you might as well.
 17 And what this is made up of is, obviously,
 18 there's two columns for will, Wildlife International,
 19 and two columns for IGB, results for E2 in the column
 20 marked E2, and the results for Atrazine there.
 21 Within a column, if there was no peer-wise or
 22 comparison that was significant, there's nothing. If,
 23 for example, under number three, the endpoints are
 24 indexed on the left, for age at completion of
 25 metamorphosis, in 42, the males were significantly

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1 different than the controls.
 2 So that's why there's an M there. Similarly
 3 at E2, looking down here, gonadal image area for males
 4 was significantly different for E2. Looking down for
 5 mixed tissue types, there was no sex specificity. I
 6 mean, it was a test using all the animals. It says
 7 none, but that just means that there was no sex.
 8 It was significant there, but there wasn't a
 9 sex to put there, so none should not be taken to mean
 10 not, as not significant, but just that it wasn't a sex
 11 specific test, so it's a no sex, but it was a
 12 significant result. Under Atrazine, for gonadal image
 13 area, the males were significant at dose of 100, and
 14 that's it. So, it's looking at all five, and it's
 15 saying only 100, is saying that there was not
 16 significant at 25 one, .1 was a .01. So, wherever it's
 17 significant, it shows on this table.
 18 The one page I gave you allows you to look at
 19 gross on the top half of the page. Histological at the
 20 bottom half, the left side is the non-trend test
 21 comparisons. The right side of the page is trend
 22 tests. So, I'm not going to make any comments.
 23 I just thought you might find that a handy
 24 sheet. These results are consistent with what's in the
 25 EPA report. They're consistent with what's in the DCI

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1 study report. There were a couple of cases where EPA
 2 used a one-sided test. I have marked those in
 3 parenthesis where those differences occur.
 4 And they, for example, down there under
 5 gonadal segmental translucence, it shows a two-sided
 6 test is what the DCI study was, when, and EPA F in
 7 parenthesis would mean that EPA found female to be
 8 significant at one side of the test. So, this is just
 9 a handy page that you're free to use or discard at your
 10 pleasure, but it helped me, and so I give it to you,
 11 okay.
 12 The other thing that relates to power is the
 13 sticky wicket that came up yesterday about the cluster
 14 effect. And Mr. Chairman, since that does relate to
 15 the power discussion, I have just a few words - -
 16 DR. HEERINGA: I'll allow it, yes.
 17 DR. SIELKEN: Thank you.
 18 DR. HEERINGA: Dr. Sielken, please
 19 proceed.
 20 DR. SIELKEN: Okay. As I've indicated,
 21 we did use the tank as the unit of analysis. And I'm
 22 just going to say it that, as a unit of analysis. We
 23 did, when we were considering the design of this study
 24 and the layout of this study, we were aware of the
 25 layout of the room that was going to be necessitated by

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1 the pumping system that led to what was identified as
 2 clusters and has been discussed as clusters.
 3 So, we were aware of that element of the
 4 design, and we discussed with the principle
 5 investigators, well, why don't you just simplify this
 6 whole thing and put one pump for every tank, and spread
 7 all the tanks around the room. Well, we got a lot of
 8 feedback on that, and, of course, as a statistician, I
 9 thought that that was the simplest solution. But,
 10 then, we listened to the discussion about why not do
 11 that.
 12 Well, first of all, they said, well, you
 13 know, if we put all those pumps in the room, we're
 14 going to have additional heat. We're going to have to
 15 figure out a way to deal with that. We're already
 16 rebuilding the rooms to accommodate this study, but
 17 those extra pumps are going to be a problem. And, they
 18 said, well, you've got to look at the overall error
 19 rate in the study.
 20 Since everything had to be blinded, instead
 21 of having sixteen things to control, you'd have sixty-
 22 four things to handle all the equipment, and handle all
 23 of the preparation of what goes in each. And they,
 24 basically, convinced us that running a lot higher error
 25 rate for all of these other things that could go wrong,

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1 they didn't want to do the study blinded, but for
 2 scientific reasons, it had to be blinded.
 3 But the blinding and all of those extra tanks
 4 was going to create a problem and, probably, increase
 5 our error rate. So that was one of the reasons that
 6 they argued to try and break up the number of tanks
 7 into, I mean to keep a certain amount of clustering,
 8 just from a practical point of view.
 9 We did try and make the number of tanks per
 10 cluster as small as workable. There was, at one point,
 11 a discussion of having them all being fed by the same
 12 thing, having eight tanks instead of four in cluster.
 13 The difficulty with that was a practical consideration
 14 that, that was difficult, that if we lost something
 15 there, we lost everything.
 16 I mean, if we, if, as it turns out in this
 17 study, the things that they were worried about, we had
 18 seen in the earlier studies of Karr, et al, back in
 19 those days, that the tanks themselves, there was a lot
 20 of problem with the tanks, and you could lose tanks.
 21 And we, you know, and here we had a microbial bloom,
 22 and that caused us to lose one cluster, and did not
 23 cause us to lose everything.
 24 Similarly, by having the clusters, we had a
 25 little bit better control over cross-contamination,



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1 where, as the sixty-four tanks was increased and the
 2 blinding would have been sixty-four clusters, if you
 3 will, the breaking it up into one tank per pump, would
 4 have given a greater chance for cross-contamination,
 5 and we were trying to avoid that.

6 There was also some discussion, and I'll let
 7 you turn to others if you want for whether this was
 8 consistent with the aquatic tox guidelines and using
 9 the tank as a replica, it certainly was. We did, of
 10 course, randomize the tadpoles to tanks. They did come
 11 in and were randomized.

12 The tadpoles were randomly assigned to the
 13 tanks. Since we were aware that we did have these
 14 clusters, of course, that creates the possibility of
 15 some dependence, so we did tests for cluster effects.
 16 We, the tests were not greatly powerful, but we did
 17 test for them. And, we tested all endpoints, so when
 18 you're looking for cluster effects, you know, we didn't
 19 just look at one. We looked at every endpoint tested.

20 As I mentioned yesterday, we had one out of
 21 176 of those tests that is significant at the 5 percent
 22 level. And I really looked, not just at that, but this
 23 5 percent is just a bright line in the sand, this would
 24 be sort of meaningless.

25 If you look at the bulk of the test, were

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1 there any others that were close to 5 percent. The
 2 next closest was 8 percent. There's one of those.
 3 There's one in ten. And then there was 18 and up, it's
 4 only three of them out of 176, were under 10 percent.
 5 And most of them were 18 percent and above, so, a P
 6 values of .18 or above. So, there really wasn't much
 7 close to being significant.

8 So, what we concluded was, was that, although
 9 the tanks are not technically independent, they were a
 10 reasonable approximation to independence. And we
 11 treated them as the iterative analysis, with that
 12 understanding that where they were as close as we could
 13 get to independence of the situation and have a viable
 14 study.

15 We did notice that we did get largely
 16 comparable and parallel results with the E2 positive
 17 controls, with this method of analysis, in treating the
 18 tanks as the unit of analysis. We also asked
 19 ourselves, well, what would be the effect of a cluster,
 20 the fact that it was there.

21 And, as I mentioned yesterday, the effect
 22 would be, with the cluster effect, you would be, kind
 23 of, overestimating the number of real tanks that you
 24 had. So, you would be pretending that you had a bigger
 25 sample size than you actually would have had. And that

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1 would increase the false positive error rate. And of
 2 course, when you increase the chance of rejecting a
 3 null hypothesis, that's true, that where there was no
 4 differences, you're actually increasing the power, the
 5 likelihood of detecting something as different. So, we
 6 felt that it was a conservative approach in that, if it
 7 did occur, we were only going to be increasing the
 8 false positive error rate. We weren't going to be
 9 hiding anything.

10 It was a robust design. We thought it was a
 11 robust design and gave us a certain amount of
 12 protection against the things that the experimenters
 13 themselves were worried about.

14 Turns out, it was a darn good thing we did,
 15 because they did have some of the problems that were
 16 expected. And because it was a well designed study,
 17 they had the opportunity to actually detect these
 18 problems and survive. I mean, they detected the plume.
 19 They detected the trace contamination. They were able
 20 to detect those things and still have a viable, strong
 21 experiment when they were done. So, that's it, thank
 22 you.

23 DR. HEERINGA: Thank you very much, Dr.
 24 Sielken. What I would like to do is to give
 25 opportunity for a few questions of clarification.

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1 Remember, we're going to discuss the statistical
 2 analysis for your charge question 9B, but if there's
 3 anything about the information that Dr. Sielken has
 4 just presented, if there are any questions on that,
 5 now, but again, let's try to keep our comments or, kind
 6 of, dialogue on this for the actual response. Yes, Dr.
 7 Yeater.

8 DR. YEATER: Kathy Yeater. Could you
 9 clarify on the paralyzed comparisons, 'cause that was
 10 kind of confusing yesterday. Did you use any
 11 adjustment correction or are these strictly all
 12 paralyzed comparisons. And then, you looked,
 13 individually, at the T tests then for your comparison
 14 and estrodyle to the control and the Atrazine to the
 15 control, or did you use a Dunnett's or a Bonferroni or
 16 any, or any type of that thing?

17 DR. SIELKEN: Okay, thank you for the
 18 question. This is Dr. Sielken. We did, for the
 19 measurement endpoints, do a protective F test. For the
 20 incidence endpoints, there was a protective Kruskal-
 21 Wallace test.

22 If those tests indicated at the 5 percent
 23 level if it was two-sided, or 10 percent if it was one-
 24 sided, if they indicated a significant difference in
 25 looking at the group of treatments or the group of E2



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1 versus control, that second group.
 2 If, at a group-wise level, there was a
 3 significance somewhere, it rejected the null hypothesis
 4 of equality, then we followed with a peer-wise T
 5 contrast, or a peer-wise Wilcoxon in with the test, at
 6 the 5 percent level, there was not any protection
 7 beyond the initial test. So, everything beyond that
 8 initial test was a 5 percent significance for that one
 9 test.
 10 DR. HEERINGA: Dr. Bailey?
 11 DR. BAILEY: You said that you did
 12 examine for cluster effects, and could you tell me how
 13 you made that test, what, how you actually carried that
 14 out. Was it a T test or an F test or, and what did you
 15 use for an error turn?
 16 DR. SIELKEN: Okay, good question. This
 17 is Dr. Sielken in response. When we did that test for
 18 treatments, there would be two clusters. And we would
 19 look at the, do it, do a comparison of those two
 20 clusters at a particular treatment level, control or
 21 treatment. We'd look at those two and ask the
 22 question, whether those two were the same or different.
 23 That's only a sample of size two. We did
 24 that for every dose, got a significance level, combined
 25 those significance level producing Tippett's minimum P-

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1 value as a method of combining those, and then
 2 evaluated whether that Tippett's minimum P-value was
 3 less than 5 percent or not. So, it was a, looking at
 4 the pair of clusters, followed by, then, looking across
 5 all the treatments and controls, and then combining
 6 those P values, using Tippett's minimum P-value method.
 7 DR. HEERINGA: Dr. Sielken and Dr.
 8 Bailey, my experience in these panels on an item like
 9 this is, I would encourage you, maybe, just during the
 10 break, to sit down with a pad and paper, and so that
 11 you're very clear as to exactly how this was done.
 12 Don't you agree? Or are you comfortable with it. If
 13 you would be willing to do that, I, and we'll come
 14 back, and we'll report specifically on that, but I
 15 think, I don't want to, avoid any misunderstanding
 16 later on on that, so. It's a critical question. I
 17 know you've raised it earlier yesterday, too, so. Dr.
 18 Portier?
 19 DR. PORTIER: Bob, thank you for the
 20 presentation. It was really good and answered a lot of
 21 questions. Kind of, the only, kind of, remaining
 22 global question would be combining the data from the
 23 two studies into one overall analysis, and looking at
 24 the data that we could see, we don't see a lot of
 25 differences in variability.

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1 A lot of effort was put into making the two
 2 sides as comparable as possible with similar protocols,
 3 similar sources of the frog larvae. So, I wondered if
 4 you, actually, went off on the side and did that
 5 analysis, like a one way anova for all of those things,
 6 both sides, kind of, combined together?
 7 DR. SIELKEN: This is Dr. Sielken in
 8 response. That's a good question, Dr. Portier. Of
 9 course, it is possible to create an analysis that would
 10 combine the two laboratories. We did not do that. We
 11 discussed.
 12 We discussed some of the complexities
 13 involved in doing that. And we, also, discussed what
 14 could be gained by doing that. And the feeling was
 15 that what we wanted to strive for was two comparable
 16 studies. And then, as you've indicated, they are quite
 17 comparable, and they were designed to be that way.
 18 They were designed more to be reproducible results.
 19 And intended to be portrayed as reproducible results,
 20 rather than, rather than combining them.
 21 We tried to design the studies to be powerful
 22 enough, each on their own, to stand alone, so that the
 23 usual argument for combining, namely to increase power,
 24 would not be as relevant, given that they were strong
 25 enough on their own. And, so, that was the intent of

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1 leaving them as separate reproducible studies, and not
 2 combining them, but we did not do that analysis.
 3 DR. HEERINGA: Additional questions for
 4 Dr. Sielken? Well, I would like to thank you very much
 5 for this presentation and clarification. I think there
 6 are, have helped considerably. And I think, if Dr.
 7 Bailey and Dr. Sielken would like to meet briefly, just
 8 to discuss the test for the cluster effect, so that the
 9 panel has a clear understanding, and we'll rely on Dr.
 10 Bailey for that, and it's my best position to do that.
 11 DR. SIELKEN: Thank you. I will have
 12 that sidebar discussion.
 13 DR. HEERINGA: At this point, I'd like to
 14 call back to the table, the representatives of the
 15 EPA's Scientific Assessment team. And we'll move on to
 16 any final questions for them from the panel. And, then
 17 turn to the, and actually, we'll probably take a short
 18 break and go on.
 19 But, at this point in time, are there any
 20 remaining questions of clarification or points from the
 21 panel that you would like to address to Dr. Steeger
 22 before we move the charge questions? Obviously, if
 23 something comes up during the charge questions, we will
 24 certainly permit points of clarification there, too,
 25 but I'd prefer to get most of that out of the way at

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1 this point, if there are no objections. Yes, Dr.
 2 Delorme?
 3 DR. DELORME: Dr. Steeger, I was just
 4 wondering if you could clarify something for me. When
 5 you look at field studies, I think you're talking more
 6 about experimental field studies, where somebody's
 7 going out and trying to do the experiment in the field.
 8 Given that, would you use the result of these field
 9 studies, if they are done appropriately, to determine
 10 in effects endpoint for use in risk assessment?
 11 DR. STEEGER: We would use well- defined,
 12 well-described, properly conducted field studies in our
 13 assessments. But it is necessary when we're able to
 14 determine the relationship between the measurement
 15 endpoint and the, or the Agency's assessment endpoints.
 16 Our assessment endpoints are for acute studies, that
 17 would be mortality; and for chronic studies, whether
 18 reproduction, survival, or growth are impaired.
 19 One of the problems that's been present with
 20 the current battery of studies that we've had on the
 21 effects of, potential effects of Atrazine on gonadal
 22 development has been trying to link this phenomena of
 23 intersex or hermaphroditism to our assessment endpoints
 24 of reproduction and growth and survival.
 25 The, none of the studies that we're aware of,

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1 although, intuitively, you might think that
 2 hermaphroditism could be problematic for animals, it
 3 would impair their reproductive capacities, we don't
 4 have that many studies that, actually, demonstrate
 5 that.
 6 And, in fact, in many of the studies where
 7 field collections were made, apparently, the
 8 researchers did not have a problem collecting animals
 9 where hermaphroditic animals were relatively common.
 10 So, it's unclear how hermaphroditism would impact the
 11 reproductive capacity of the animal.
 12 DR. DELORME: And just another question,
 13 in some of the field studies that you reviewed, you
 14 indicate that the Atrazine concentrations weren't
 15 properly characterized to allow an analysis. Can you
 16 just share with us a little bit what would be a
 17 properly characterized in a field study, what do you
 18 consider properly characterized determinations of
 19 whatever the stressor is?
 20 DR. STEEGER: Well, ideally, we'd like to
 21 know what the concentration of the chemical in question
 22 is at each of the sites. And where you have reference
 23 sites, ideally, we'd like to see that the chemical is
 24 not present at the same concentration that it is at the
 25 treatment sites.

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1 It would also be important that you
 2 demonstrate that other pesticides or chemicals that are
 3 capable of impacting the measurement endpoint are
 4 characterized, and ideally not present at your
 5 reference sites, so that, if you're going to make a,
 6 draw a conclusion that the levels of a particular
 7 chemical are correlated with a particular measurement
 8 endpoint, there has to be data from all the sites that
 9 you've collected those levels from, to demonstrate that
 10 from where you've had a zero concentration, to where
 11 you have high concentrations, you're not having overlap
 12 in the measurement endpoints themselves.
 13 DR. DELORME: And what about temporal
 14 aspects?
 15 DR. STEEGER: Well, temporal aspects is
 16 studies that was problematic for a lot of the field
 17 studies that we reviewed from the open literature and
 18 registrants submitted datas, where field collections
 19 were made of animals in different, that would clearly
 20 have been in different stages of their reproductive
 21 cycles. And the animals were combined and an effort
 22 was made to draw conclusions regarding plasma steroid
 23 levels, the condition of the gonads, when in fact, it
 24 would have been very difficult to say that all the
 25 animals were at the same stage of development.

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1 Because, clearly, they were collected at different
 2 periods, and they were of different sizes, different
 3 ages, and so their reproductive capacity would have
 4 been different, just by age structure alone.
 5 DR. DELORME: Okay, thank you.
 6 DR. HEERINGA: Dr. Miller.
 7 DR. MILLER: Debra Miller, EGA. I'm not
 8 sure if this is an appropriate time to ask this
 9 question, because it's probably really to Dr. Wall. I
 10 know that some extra histopath was examined. And I was
 11 just curious if things like, specifically, the brain
 12 and the adrenal glands, were they looked at?
 13 DR. STEEGER: To our knowledge, the only,
 14 the, during the gross morphology stage of the study, a
 15 number of organs were looked at. The histopathology
 16 only focused, to my knowledge, on the gonad and the
 17 kidney was sectioned.
 18 DR. HEERINGA: Dr. Denver.
 19 DR. DENVER: Bob Denver. I'm curious if
 20 the EPA is aware of any studies that have been done,
 21 either in the public literature submitted by the
 22 registrant, on Atrazine metabolites or degradates on
 23 amphibian development?
 24 DR. STEEGER: I'm not aware of any
 25 studies that have been conducted on the three major



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1 degradates, relative to gonadal development.
 2 DR. FURLOW: Have any analysis, analyses
 3 been done of the accumulation of such degradates or
 4 metabolites on either static renewal or flow-thru
 5 systems of significant or significant to you?
 6 DR. STEEGER: There is one study that was
 7 discussed yesterday that Dr. Solomon was presenting on
 8 where they did autoradiography suggested that
 9 metabolites were not accumulating to any significant
 10 effect to any significant extent, at least based on
 11 radio labeling, in animals in a bioconcentration study.
 12 We have not reviewed that study to great
 13 extent, though. So, in answer to your question, no,
 14 I'm not aware of, to the extent to which those
 15 degradates are accumulating in static or flow-thru
 16 studies looking at amphibians.
 17 DR. HEERINGA: Dr. Skelly.
 18 DR. SKELLY: David Skelly. Are there,
 19 it's my, well, let me just you this question. Are
 20 there any new field studies that EPA has reviewed since
 21 the 2003 meeting, and if there are not, is the EPA's
 22 assessment of field study evidence different than it
 23 was in 2003?
 24 DR. STEEGER: All the field studies at
 25 EPA reviewed since the 2003 SAP represented the final

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1 versions of internal reports that have been reviewed in
 2 2003. So, there have been no new studies submitted for
 3 our review. Our conclusions have not changed regarding
 4 those studies.
 5 DR. HEERINGA: Dr. Petino.
 6 DR. PETINO: Reynoldo Petino. We had a
 7 brief discussion yesterday about this, the effect for
 8 solution, I guess, I was told that's the theme, the
 9 term to use of, when it comes to some of the
 10 categorical variables that were measured, especially
 11 things that was the presence or absence, and that given
 12 that the analysis was done bisex, that the sample size,
 13 there was no variable between ten and fifteen
 14 individuals for those analysis.
 15 And, therefore, the effect for solution can
 16 be as low as 10 percent. And, so, I'm just curious,
 17 not being a statistician, what is, is there an
 18 acceptable level or a guideline for, that the EPA has
 19 to, you know, how, what, you know, about the effect for
 20 solution, how low can it be before there's a problem
 21 with the analysis?
 22 DR. STEEGER: None of the studies that we
 23 were reviewing, or guideline studies, that's one of the
 24 difficulties with looking at these endpoints. However,
 25 our guideline of studies in effect is considered a

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1 significant at the .05 level.
 2 So, that's, regardless of the frequency, if
 3 it's statistically significant, that's where we would
 4 flag it as meaningful. But, the risk assessor is then
 5 expected to try and determine whether that
 6 statistically significant effect is biologically
 7 significant.
 8 And that's what we're hoping to have input
 9 from this panel on, as to whether many of the
 10 histological endpoints that are reported in the
 11 studies, many of which were statistically significant,
 12 they're occurring in very low frequencies, are they
 13 biologically significant. And even those that were not
 14 statistically significant, was it just because, as you
 15 say, the frequency at which the animals were, either
 16 male or female, in the tank, were such that it would
 17 have been difficult to detect a significant effect.
 18 I'm not a histologist.
 19 These are new endpoints to me. It's been
 20 very difficult to try and link them to what, do these
 21 represent some primordial stage that an animal, it
 22 might be in, as it's moving towards becoming an
 23 hermaphrodite or a mixed sex animal. I don't know, but
 24 that's why we're asking the panel. What's your opinion
 25 as to whether these low frequency events, whether

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1 they're significant or not, should be meaningful in our
 2 assessment of these data.
 3 DR. HEERINGA: Good questions, both
 4 statistical and biological. Unfortunately, it's hard
 5 to separate out. Any other questions at this point
 6 from the panel? Seeing none at this stage, what I'd
 7 like to do is, I'd like to thank everybody, the EPA
 8 scientific staff that's prepared this white paper, all
 9 the commenters and the representatives from Syngenta
 10 who presented their data yesterday for your
 11 contributions to the initial information session of
 12 this panel. I'd like to call a break at this point
 13 for, say, twenty minutes. We'll reconvene at 10:15,
 14 and at that point in time, we'll turn to the charge
 15 questions that have been posed to the panel.
 16 (WHEREUPON, there was a break.)
 17 DR. HEERINGA: I'd like to welcome
 18 everyone back to the late morning session of second day
 19 of our FIFRA Scientific Advisory Panel meeting on the
 20 topic of the potential for Atrazine to affect amphibian
 21 gonadal development. At this point, we have had the
 22 presentations from the EPA scientific staff, and the
 23 period of public comment. And, I believe, that we are
 24 ready to proceed with the first of the charge questions
 25 to the panel. And I think I'll leave it to Dr.

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1 Steeger. I believe that my near-sightedness, I'll have
 2 to - -
 3 DR. IRENE: This is Stephanie Irene.
 4 DR. HEERINGA: Yeah, Stephanie Irene is
 5 going to be reading - -
 6 DR. IRENE: Stephanie Irene with EPA.
 7 DR. HEERINGA: Dr. Irene is going to be
 8 reading the charge questions into the record. And
 9 also, Dr. Pease, I believe. And we will respond in
 10 turn. We will have a lead discussant who will begin
 11 the initial response of the panel.
 12 Followed by associate discussants that have
 13 been identified. And then we will open it up to the
 14 panel at large for their comments. After we've
 15 successfully completed each response, we'll move on to
 16 the next charge question, obviously. So, Dr. Irene.
 17 DR. IRENE: Thank you. The Agency now
 18 requests that the panel discuss the first charge
 19 question, which is, in reviewing the available
 20 laboratory and field studies, the Agency used a number
 21 of criteria to evaluate individual investigations.
 22 Criteria such as experimental design, test protocols,
 23 and quality assurance information were used to evaluate
 24 the reliability of the study's ability to adequately
 25 assess the hypothesis, that Atrazine elicits

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1 developmental effects in amphibians, and if so, the
 2 nature and strength of the associated dose-response
 3 relationships.
 4 A. Please provide comments and
 5 recommendations regarding the EPA's approach, and
 6 criteria used to evaluate the studies; and
 7 B. Given the evaluation criteria employed by
 8 the Agency, please comment on the EPA's overall
 9 application of these criteria for the currently
 10 available studies.
 11 DR. HEERINGA: Dr. Green is the lead
 12 discussant, but since she was delayed on her flight
 13 yesterday, I asked Bruce Pauly to step in, but I have
 14 not determined quite where we came down on that.
 15 DR. PAULY: And I've passed it on to Dr.
 16 Schlenk to it.
 17 DR. HEERINGA: That's appropriate
 18 committee work. Everybody will have their say, but
 19 let's begin with Dr. Schlenk.
 20 DR. SCHLENK: Yeah, Dave Schlenk, UCI.
 21 Just to fill everybody else in, Dr. Pauly and I met
 22 this morning, and we met Sherril this morning as well.
 23 So, hopefully, we'll give it a first crack here, and
 24 then, please feel free to add in as needed. So, I'm
 25 just going to, what I did is, I sort of combined A and

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1 B together as addressing both of them, sort of
 2 intermittently.
 3 So, with regard to experimental design, EPA
 4 noted several issues that prevented their utilization
 5 of previous reports in open literature regarding the
 6 risk assessment of Atrazine on gonadal development in
 7 amphibians.
 8 To reduce uncertainties, the 2003 SAP
 9 recommended that a tiered approach be utilized to
 10 determine causality between exposure of Atrazine and
 11 adverse affects on the model amphibian. Standard
 12 aquatic toxicology methods, with endpoints associated
 13 with apical effects were recommended.
 14 In most cases, the experimental design
 15 implementing these recommendations and the criteria
 16 were sound. A tiered approach is a logical step in
 17 determining causality. However, it should be noted
 18 that laboratory studies may not always exclude
 19 causality in field effects. And, I think the 2003
 20 panel addressed that in one of their papers.
 21 Ten being a great example of where you see
 22 100 percent of the animals showing endocrine
 23 destruction in the field, but very difficult to
 24 replicate that in a lab. A lot of metalloids types of
 25 chemistry, selenium, it's very difficult to replicate

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1 those studies in a lab. But, of course given the
 2 situation here, and due to the lack of a proved field
 3 of studies and cost considerations, the laboratory
 4 approach seemed like a sound alternative in this
 5 particular case, but it shouldn't rule out completely
 6 field studies, I guess.
 7 As previous studies focused exclusively on
 8 static exposure systems, often in containers of
 9 questionable and confounding material constructs, the
 10 use of a flow-through system in class is valid,
 11 especially since it appears that the life history
 12 organism does not seem to be impaired under these
 13 conditions.
 14 One of the things I thought was very good
 15 about this study was the replication of the experiment
 16 in two concurrent laboratories. I thought was an
 17 excellent approach. And, actually, in contrast to what
 18 Dr. Portier mentioned about combining the two, I think
 19 the strength is actually in the separation of the two,
 20 as far as the different effects. And I'll give an
 21 example of that here in a minute.
 22 The test organism with a well-characterized
 23 genetics and life history was utilized. This allowed
 24 efficient exposure of the toxicant during a previously
 25 determined sensitive window of exposure, which again



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1 maximized efficiency in the laboratory test.
 2 Since adverse effects were previously
 3 observed at the .1 and 25 microgram per liter
 4 concentrations, exposure concentrations bracketing
 5 those were utilized in this experiment, which was a
 6 sound approach, I think.
 7 While, however, while one laboratory was
 8 successful in conducting exposures at the critical
 9 window of sexual differentiation, bracketing the 0.1
 10 microgram per liter, which again was identified in
 11 these previous studies, the other laboratory was less
 12 successful in reaching this nominal concentration. And
 13 we'll deal more with that when we get to question four.
 14 So, as an example of the benefit of this
 15 inter laboratory comparison, it was, basically, you
 16 could see that the effects for particular, the gonadal
 17 hypoplasia that was observed in the males of the .1
 18 concentration, which was observed in the WLI, but not
 19 the IGB, were determined, were measured concentrations
 20 for consistently below the .1, because you only saw 50
 21 percent of the nominal, one wonders whether this
 22 prevented replicated response in the IGB study.
 23 However, this effect was not dose dependent at WLI,
 24 where the exposures at the .01 microgram per liter were
 25 adequate in the sensitive window of development, and

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1 the effects were not considered significant. In
 2 addition, this effect was not confirmed by other
 3 measures, such as gonad area or histological
 4 abnormalities.
 5 Thus, utilizing multiple endpoints to confirm
 6 adverse effects was a strength. And clearly, the
 7 quality assurance protocols that were implemented
 8 allowed the observation and measured concentrations
 9 during critical windows of development were successful,
 10 at least in one laboratory.
 11 Talking about the positive control, having a
 12 positive control is an essential component of the DCI
 13 study, and estrodyle is an effective positive control
 14 for feminization, and the responses of estrodyle on
 15 this organism have been well characterized, which is,
 16 again, another strength of using that particular
 17 organism.
 18 Given that the tested hypothesis was based
 19 upon up regulation of aromatase, even though, at least
 20 to my knowledge, increases in estrodyle have never been
 21 observed, which would lead to an increase in estrodyle
 22 during critical windows of development. That's the
 23 hypothesis. However, given the knowledge base for this
 24 compound and this species, it was probably the most
 25 cost effective choice for a positive control.

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1 So, overall, the evaluation criteria seemed
 2 to be extremely focused on gonadal development, which
 3 was a plus, but I think, may have also been a minus,
 4 and maybe some of the other panel members can chime in
 5 on that. And the studies did not focus upon other
 6 endpoints, which may also be important with regard to
 7 the toxicity of Atrazine. However, with the exceptions
 8 of the caveats mentioned above, EPA appropriately
 9 applied their criteria in carrying out these particular
 10 DCI studies. Bruce.
 11 MR. PAULY: Bruce Pauly, Environment
 12 Canada. Yeah, I'm just picking on one of the final
 13 points that Dr. Schlenk made. One of the things we
 14 discussed this morning was the fact that the charge
 15 question off the bat reads that the studies were
 16 evaluated to determine whether or not Atrazine elicits
 17 developmental effects in amphibians. And, I think we
 18 all realize that it's more narrow than that.
 19 The open literature, which was reviewed for
 20 the white paper, included studies which focused on
 21 Atrazine alone and on gonadal developmental effects
 22 alone. So, the fact that we're only asked to, or the
 23 review only included studies on Atrazine alone and
 24 gonadal developmental effects alone, narrows that
 25 charge question, in my opinion, to a certain extent.

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1 So, I think the charge question is the one that was
 2 used in 2003. I think this year's charge question is
 3 na-, it should be narrowed. Because we are only
 4 considering gonadal effects, not developmental effects.
 5 That said, and as Dr. Schlenk mentioned, the
 6 studies were evaluated to determine the effects on
 7 gonadal development. Every study was looked at for
 8 experimental design protocols to update equality and
 9 then, eventually, if there was, they had to go further
 10 for dose-response relationships and ecological
 11 relevancy. I think we would probably agree with the
 12 2003 panel response that the reviews were thorough.
 13 The approaches and criteria were appropriate.
 14 What we discussed to a certain extent, too,
 15 was that when these reviews were done on all of the
 16 open literature studies, at least, these criteria that
 17 were established were very strictly applied. And, for
 18 instance, where a recommendation was made for a
 19 particular test method or protocol, if any individual
 20 study did not employ this design element, those were
 21 grounds, that was sufficient enough for the study to be
 22 removed from further consideration. And one of the
 23 things that we specifically discussed there was flow-
 24 through methods.
 25 All of the studies, except for the DCI



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1 studies, were static renewal, so they were immediately
 2 removed from future consideration. So, the open
 3 literature studies were reduced quite substa-, well,
 4 completely, basically, by those considerations on their
 5 design elements. There were other ones that we, I've
 6 already heard about, other considerations, in terms of
 7 the open literature studies, which effectively removed
 8 them from consideration. High loading was one.
 9 Few Atrazine concentrations, so a dose
 10 response wasn't possible to determine. And poor
 11 responses to positive control was another criteria for
 12 evaluating the studies. High mortality, and I think
 13 the final one that I had written was inadequate study
 14 design to overcome high variability in the endpoints,
 15 was another reason for taking a lab study out of
 16 consideration.
 17 And we already heard, again, from Dr.
 18 Steeger's presentation, yesterday, presentation three,
 19 there are studies, or criticisms of the field studies
 20 as well, which removed all of them from future
 21 consideration.
 22 So, what happened there was that, applying,
 23 strictly applying the criteria, and I think we're going
 24 to talk about this in the next question as well,
 25 strictly applying those criteria resulted in an

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1 effective study of one, a study that we're, basically,
 2 considering in this process. And, while we say that
 3 the evaluation criteria are appropriate and were
 4 appropriately applied, I think we'd like to recognize
 5 that, as a result of that, we're, basically,
 6 considering only one study for this process. Thank
 7 you.
 8 DR. HEERINGA: Thank you Mr. Pauly. Dr.
 9 Green, who we recognized was delayed due to airline
 10 problems, but what's your response at this point in
 11 time?
 12 DR. GREEN: I concur with the comments
 13 made by the other discussants so far, and I'd like to
 14 bring to the attention of the panel and the people in
 15 the room today that one of the criteria listed on the
 16 to-do list from the previous session in 2003 was that
 17 studies be performed such that bio-residues of Atrazine
 18 in the specimen studied are looked at.
 19 Tissue levels, plasma levels, if possible, to
 20 document that these animals did, indeed, absorb and had
 21 the substance in their body at the time that the
 22 gonadal abnormalities were observed histologically.
 23 There was a dearth of studies submitted where that kind
 24 of dose response testing and toxicological analysis was
 25 performed. I believe there was just one that reported

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1 a half-life that was very short for Atrazine in the
 2 animals that were under study.
 3 So, in addition, grow out studies were
 4 recommended in the past, I believe, in 2003 by the
 5 panel. And there were very few studies that reported
 6 that.
 7 So, the interpretation of that criteria would
 8 be difficult, and despite the fact that some of these
 9 studies have shown no effect, or an effect, of Atrazine
 10 on gonadal development, we cannot, at this time, use a
 11 criteria that allows us to report on functionality as a
 12 result of Atrazine exposure. In other words, would
 13 these animals go on and lay eggs that can be
 14 fertilized, and then produce more healthy animals. So,
 15 other than that, I have no further comments.
 16 DR. HEERINGA: Comments from other
 17 members of the panel on this first charge question.
 18 Yes, Dr. Delorme.
 19 DR. DELORME: Just following along on
 20 what doctor, or Bruce Pauly said, and what Dr. Schlenk
 21 said, with respect to the field studies, I think it
 22 might be helpful for EPA to develop some general
 23 guidance on what's expected in field studies to help
 24 the people who are doing them. And, essentially, to
 25 help the overall success rate of field studies.

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1 I think we've seen from this that there
 2 hasn't been much success. A lot of resources expended,
 3 but I'm not sure that they resulted in anything. And
 4 to improve the utility of the studies. So, I think
 5 some general guidance from EPA to, whether it's
 6 registrants or universities researchers, on what's
 7 expected and what's needed, what the criteria are for
 8 evaluation might help. And that would be a
 9 recommendation.
 10 DR. HEERINGA: Dr. Chambers.
 11 DR. CHAMBERS: Just to respond to an
 12 earlier comment, I'm really not too bothered by the
 13 fact that there weren't any residues in the animals.
 14 If they were exposed in the water column, such as an
 15 animal in the environment would be, as they were in
 16 that experiment, then, you know, they were subject to
 17 the exposure. And I think, I think it's reasonable not
 18 to have that data set as a real concern.
 19 DR. HEERINGA: Dr. Green.
 20 DR. GREEN: If I can make a comment to
 21 that.
 22 DR. HEERINGA: Sure, absolutely.
 23 DR. GREEN: I think, in certain studies
 24 where the exposure was chronic, and we do or don't see
 25 effects, those are well-designed studies. But if there

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1 was a brief exposure for a short duration at a short
 2 time point in an animal's life, that may or may not
 3 have an effect.
 4 If the half-life truly is as short as it is,
 5 that wouldn't allow for accumulation or chronic
 6 exposure over the time, and for us to see the results,
 7 which I think would probably be more likely to parallel
 8 what you would see in a field study, where animals are
 9 growing up in a pond, and spend their whole life
 10 exposed through periods of rain and runoff, and
 11 repeated applications of the chemical.
 12 DR. HEERINGA: Dr. Chambers.
 13 DR. CHAMBERS: Jan Chambers. The, this
 14 was continuously renewed in the tanks, though, so they
 15 were continuously exposed and, really, probably, more
 16 continuously exposed than they would have in rain,
 17 runoff events, I would think. And this is the critical
 18 time for the gonad development, which is the subject of
 19 this particular question, this particular study.
 20 So, again, I'm really not concerned that the
 21 analytical chemistry on the animals was not performed.
 22 The analytical chemistry on the water column was
 23 performed, and the compound was there at, pretty close
 24 to the nominal amount, so I'm not bothered by the lack
 25 of analytical chemistry data in the animal.

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1 DR. HEERINGA: Other comments from the
 2 panel. Yes, Dr. Furlow.
 3 DR. FURLOW: David Furlow. So, one thing
 4 that came out of discussions with Dr. Steeger was, I
 5 think I said I was disheartened by the fact that he
 6 found the investigators in the academic lab or his
 7 laboratory said that those discussions either that they
 8 were non-responsive, or it was unproductive. And, I
 9 guess, I'd like to go on record as saying that I wish
 10 that could be improved in some way.
 11 If we could come out, come up with some means
 12 for the EPA or other regulatory agencies to improve
 13 their interactions with academic scientists. I know
 14 that in my world, not in terms of regulatory agencies,
 15 but, say, if there's some sort of database or
 16 repository, I know that in microwave data, there's a,
 17 you have to submit microwave data to, the raw data,
 18 say, okay, here it is.
 19 Here's our evaluation of the microwave data,
 20 but the raw data is available for everybody to look at
 21 and make their own conclusions. And here are the
 22 criteria that we use to do the experiment. And, maybe
 23 some of my toxicology colleagues can comment on that,
 24 so if there is some sort of, maybe I'm not aware of it.
 25 Some sort of open toxicology database for quality

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1 assurance that studies can be submitted to, that is in
 2 addition to the peer review, where it's only one or two
 3 peer reviewers, right, the times with the journal.
 4 So, I think it can be a strong, that it
 5 should be a strong level of control at the peer review
 6 level, but sometimes it is not. And I wonder if there
 7 is some sort of database or if that's something we can
 8 discuss to help the interaction between the EPA and
 9 academic institutions.
 10 I guess, just the one last comment I had
 11 about that is that, if we are only restricted to
 12 considering the studies that are in DCI, really, when
 13 it comes down to it. If that's what we're left to
 14 really consider as what is acceptable to the EPA, in my
 15 heart of hearts, I don't see any conflict of interest.
 16 I haven't seen any evidence of that at all.
 17 But the close interaction between the EPA and
 18 those studies might have the appearance of conflict of
 19 interest. And I don't that's something that we want to
 20 have, even the appearance of it. And, so, if there was
 21 some closer contact with, and trust and interaction
 22 between academic scientists and the EPA outside of the
 23 industry to study, from the studies, would be, I think,
 24 beneficial to all of us, just as a general comment.
 25 DR. HEERINGA: Yes, Dr. Steeger, please,

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1 I think it's important to respond.
 2 DR. STEEGER: Yeah, I'd like to make a
 3 couple comments. In the 2003 SAP, prior to it, we did
 4 make an extraordinary effort to contact the researchers
 5 that were generating some of the data that were showing
 6 adverse effects. We requested, we don't have the
 7 authority to require researchers to provide data, nor
 8 do we have the authority to tell them how to conduct
 9 their studies.
 10 But, we were, we, actually, went to the lab,
 11 and to the study site, and we requested, on three
 12 occasions, to have access to those data. There were
 13 four staff scientists that attempted to review the
 14 data, and the researchers are provided the data in the
 15 way that they, typically, would record data. It's not
 16 the way that we would require data to be presented to
 17 us.
 18 So, we're at something of a disadvantage from
 19 the get-go, but, at no time, were the data supporting
 20 the researchers conclusions. Each time we received a
 21 dat-, a different data set, each time the data could
 22 not be related to the study or its conclusions. By the
 23 third time, the Agency had determined that it had spent
 24 enough resources attempting to make sense of this, and
 25 we ended up evaluating the study at face value.



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1 The other researcher with whom we
 2 communicated with had noted very dramatic changes in
 3 histology after very short exper-, exposure periods,
 4 and eventually, some of the more provocative results
 5 that were recorded, in follow up discussions with that
 6 researcher, it turned out that they had been artifacts
 7 of histology.
 8 So, again, rather than get into
 9 contradictions between EPA's analysis of an open
 10 literature study versus the author's interpretation of
 11 it, we just let it go and said that we would just
 12 evaluate the studies at face value.
 13 The, so we do make an effort, we did make an
 14 effort to evaluate, or to work with the study
 15 researchers, but it did not prove to be productive.
 16 Again, we have no authority to tell people how to do
 17 their studies.
 18 And with the Syngenta studies, as we
 19 indicated in our presentations, we had extensive Q-A
 20 going on throughout the study. EPA wanted to assure
 21 that the protocols were properly developed, that they
 22 were consistent with what EPA had required, and that
 23 the SAP had recommended in 2003.
 24 I have never participated in a st-, in a Q-A
 25 process that has been more extensive than the one

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1 that's been applied to these particular, these two
 2 particular studies. It is unusual for the agency to
 3 have access to a study as it's actually being conducted
 4 to know exactly what is going on at any moment of the
 5 study.
 6 When the Atrazine contamination took place,
 7 we knew about it within a day. And, so, it's our very
 8 clear understanding of what was expected, what actually
 9 occurred, and the results of those studies, that we've
 10 put such an incredible amount of weight on what appears
 11 at face value to be a very small number of studies,
 12 relative to what is available in the open literature.
 13 And to follow up on a previous commenters
 14 statements, the criteria that were applied, a single
 15 criteria was not implied to discount the utility of the
 16 open literature. We attempted to, did they follow the
 17 recommendations that were made by the SAP, that they
 18 did or they did not.
 19 That would not have discounted them from
 20 further considerations. It was the concordance of
 21 information from each of the studies that would have
 22 allowed us to either use it or not use it in
 23 determining whether Atrazine was having an effect on
 24 gonadal development. And most of it had to do with,
 25 you were, the, you were either not seeing dose res-,

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1 not, in addition to not just seeing, following the
 2 recommendations that were made, it was a matter of not
 3 getting a dose response, that the response has changed,
 4 and were inconsistent. So, it was a concordance of
 5 information, not a single factor that deemed the
 6 acceptability of the studies.
 7 DR. HEERINGA: Dr. Furlow, please.
 8 DR. FURLOW: So, thank you, Dr. Steeger.
 9 I just wanted to clarify that, 'cause earlier you said
 10 other, I think I had it down as non-responsive versus
 11 unproductive, and I just wanted to clarify what the
 12 interaction was, for the record, with the academic
 13 researchers, so we do, more about the nature of that
 14 interaction, so I think what you said was helpful to me
 15 in that regard. So, I just want to improve the
 16 process, too. I mean, that's something we can discuss
 17 later.
 18 DR. HEERINGA: Thank you, Dr. Furlow. I
 19 think that's a very important point. Steve Heeringa,
 20 just to make a general comment that I think the study
 21 that, the studies that are in the general literature
 22 review and the studies that are being reviewed here are
 23 a little bit of an exception in the normal process that
 24 I have observed, in terms of the involvement of
 25 academic research, and the collection of peer review

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1 literature, it's input to processing.
 2 I think a lot of that was, as a member of the
 3 2003 SAP, and I'll let others contribute here, it was
 4 very much a result of the deliberations in that meeting
 5 where we systematically went through a review of the
 6 scientific quality and the weight of evidence from all
 7 of these studies, and concluded that what was really
 8 needed was a consistent, certainly to begin with, a
 9 consistent and well-conducted with the criteria laid
 10 out, laboratory-based study.
 11 Not to the exclusion of field studies or
 12 other studies to supplement that, but we really needed
 13 to get back to a fundamentally direct and accurate
 14 laboratory-based study before the decision making on
 15 this could proceed on it.
 16 That's my personal view, and other people
 17 from 2003 SAP could, but generally, the process, I
 18 think, that, that has gone through with the SAP, and
 19 with the EPA sciences, is broadly inclusive at
 20 academic based research. And so the criteria here,
 21 that sort of sweep away a lot of the previous
 22 researches. It's a little bit atypical, but it was in
 23 large part reviewed and endorsed by the 2003 SAP. Yes,
 24 Bruce Pauly.
 25 MR. PAULY: Bruce Pauly. Just, real



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1 quick, clarification, if you had a study that had good
 2 water quality, low mortality, low loading, and if it
 3 was static renewal, a static renewal studies are, would
 4 be acceptable?
 5 DR. STEEGER: Static renewal studies
 6 would be acceptable, provided they measured the
 7 concentration of the test material, and that the water
 8 quality parameters were well reported.
 9 DR. HEERINGA: Dr. Delorme.
 10 DR. DELORME: Just following up on what
 11 Dr. Heeringa said. I think it's important to note that
 12 the criteria that the EPA used are based in science.
 13 I think another thing that's important to
 14 note is that studies on amphibians are not the norm in
 15 pesticide regulations. And, you know, the protocol
 16 work that was done in developing this, I think, is
 17 going to help us in the long run, not only for
 18 Atrazine, but for other compounds in the future.
 19 So, when they're looking at, you want porta-
 20 pseudo-protocols during the study for the first time.
 21 Maybe they're going to be a little bit harder on it,
 22 but you know, I think that the criteria that they've
 23 used is appropriate.
 24 DR. HEERINGA: Comments from other panel
 25 members. Turn then to Dr. Steeger to see whether you

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1 have any, require any additional clarification. Do you
 2 feel the panel has addressed these two points of
 3 question number one?
 4 DR. STEEGER: I agree that the panel
 5 addressed the two questions, and I, also, agree that
 6 the scope of the question is intended to focus on
 7 amphibian gonadal development.
 8 DR. HEERINGA: Thank you for putting that
 9 on the record, too. Implied, certainly, and that
 10 correction. Okay, at this point in time, I'd like to
 11 turn to either Dr. Irene and Ms. Pease to read the
 12 second question, the tag team this year, so Doctor or
 13 Ms. Pease.
 14 MS. PEASE: Yes, Anita Pease here. The
 15 second question to the panel deals with questions
 16 concerning open literature studies.
 17 The Agency has concluded that the information
 18 contained in the open literature published in 2003 SAP
 19 does not provide any additional information that could
 20 be used to refute or confirm the hypothesis that
 21 exposure to Atrazine alone causes adverse developmental
 22 effects in the amphibian gonads. This is a three part
 23 question.
 24 A. Please comment on the comprehensiveness of
 25 the Agency's literature reviews relative to the

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1 potential effects of Atrazine alone on amphibian
 2 gonadal development.
 3 B. Please comment on the Agency's evaluation
 4 of the open literature studies, and the Agency's
 5 conclusion that the data derived from laboratory
 6 studies, both individually and collectively, are not
 7 sufficient to refute or confirm the hypothesis that
 8 Atrazine exposure causes developmental effects in
 9 amphibian gonads.
 10 And C. The Agency concluded that that the
 11 field studies are not adequate for assessing the
 12 hypothesis at hand. Please comment on the Agency's
 13 conclusion. If the SAP concludes one or more of the
 14 field studies do provide the means to assess the
 15 hypothesis, the Atrazine exposure results in effects on
 16 amphibian gonadal developmental. Please suggest
 17 interpretive and statistical methods that should be
 18 employed to evaluate the data.
 19 DR. HEERINGA: An important, Dr. Skelly
 20 will be our lead discussant. I'll leave it up to you,
 21 David, to, whether to do three parts together or
 22 individually.
 23 DR. SKELLY: Okay, this is David Skelly.
 24 I think what I'll do is I'll run through A, B, and C;
 25 and then ask my co-discussants to add in their

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1 comments.
 2 On the first question on the
 3 comprehensiveness of the Agency's literature reviews, I
 4 am not aware and the other people that I've spoken to
 5 are not aware of additional studies that have examined
 6 the potential effects of Atrazine alone on amphibian
 7 gonadal development.
 8 And, in fact, if you examine the review that
 9 EPA has done, they consider studies that went beyond
 10 this specific charge as well. So, in that sense, the
 11 review was thorough. There are certainly other studies
 12 that have been conducted on Atrazine and amphibians
 13 since then, but not, specifically, on Atrazine alone or
 14 amphibian gonadal development.
 15 For part B. to comment on the Agency's
 16 evaluation of open literature studies derived from a
 17 laboratory, in general, I agree with the review. There
 18 are certainly many concerns with the open literature
 19 laboratory studies that have already been gone over.
 20 We don't need to go over them again in
 21 detail. I will just reiterate one point, so, many of
 22 these studies involve a lack of information. So,
 23 tadpole food was not tested for the presence of
 24 Atrazine in many of, if not most of the open literature
 25 lab studies. And this is viewed as a deficiency.

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1 Many of them used static renewal techniques
 2 that Bruce Pauly talked about. And that is also viewed
 3 as a deficiency. So, it seems like there's a, there is
 4 an ongoing gap between these expectations and what is
 5 being done in the open literature. I was reassured by
 6 Dr. Steeger's response that these alone would not have
 7 refuted, or did, completely discounted the studies;
 8 nevertheless, all of the studies in their estimation
 9 were discounted.
 10 And there was a comment yesterday referring
 11 to GLP standard studies. I guess that means good
 12 laboratory practice studies. Dr. Steeger said that the
 13 open literature cannot hope to compete with that
 14 standard. And I, actually, hope that that's not true.
 15 I think that it's actually very important for these
 16 standards to be developed in a way so that scientists
 17 that are not doing things in close collaboration with
 18 EPA can participate in this process.
 19 I think it's, that's actually very important.
 20 If that's statement is taken at face value, it's going
 21 to be very difficult for open literature studies to be
 22 used as part of the basis for decision making in
 23 context like these, and by default, that means the GLP
 24 studies are going to predominate in a situation where
 25 weight of evidence is important. And so, I hope that

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1 we can do some more thinking about that, and it's a
 2 part of our, the SAP's recommendations, maybe, come up
 3 with some advice that we can offer to EPA to try to
 4 increase the participation of scientists working in the
 5 open literature.
 6 One comment I wanted to make about the
 7 laboratory studies that goes back to something that Dr.
 8 Leblanc said yesterday, was this idea of reviewing the
 9 open literature studies in the context of what was seen
 10 in the data call-in study. So, without getting in, too
 11 much, into the, we're going to have plenty of time to
 12 discuss the data call-in study. One of the patterns
 13 that was observed in a number of open literature
 14 laboratory studies was the influence of Atrazine on
 15 growth and development of larval Xenopus and other
 16 species.
 17 So, this result was confirmed in the portion
 18 of the DCI study, and it was confirmed in the
 19 laboratory that would have had the best ability to
 20 detect such an effect. And I appreciated the
 21 presentation on power today, but what I would really
 22 like to know is, what the power of detection of that
 23 effect was. Because it might have been less than 1.6
 24 or 1.7 standard deviations of an effect size. So, but
 25 we know that at least the trend was that if we have the

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1 best ability to see that in the Berlin lab, and it was
 2 there, and it's been seen in other places.
 3 And the only other comment I would add to
 4 that is, is that, that effect was dismissed as an
 5 anomaly in the DCI study, and yet, it's something that
 6 we've seen in these other studies. And so, I don't
 7 think it's fair to characterize it as an anomaly, and
 8 the other argument that was made is that, this isn't
 9 biologically significant. And, in fact, if you go to
 10 the ecological literature, and try to estimate the
 11 influence of a 6 percent decrease in body weight and
 12 metamorphosis, I think you'll find that studies have
 13 shown that post-metamorphic survival and the size at
 14 maturity, and so on, can be influenced by an effect of
 15 that size.
 16 So, while the growth and development result
 17 is not consistent among the open literature studies,
 18 and it's not even consistent within the DCI study, it
 19 has emerged frequently enough that I think we can't
 20 treat that as an anomaly.
 21 As for part C. field studies, earlier this
 22 morning I asked Dr. Steeger the question, have there
 23 been any new studies done since 2003. And,
 24 essentially, the answer is no. So, the answer to the
 25 overarching question that we're supposed to be

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1 entertaining here is, the Agency has concluded that the
 2 information contained in the open literature published
 3 since 2003 SAP does not provide any additional
 4 information.
 5 So, in fact, there is no additional
 6 information on the field side of things. And, I view
 7 that with some concern, because while I certainly agree
 8 that there are significant issues with each of the
 9 field studies as they have been published, the white
 10 paper fails to acknowledge something that I think is an
 11 important observation that was also raised in the 2003
 12 SAP report.
 13 And that is, that several field observational
 14 studies, or surveillance studies, conducted by
 15 independent research groups, have detected gonadal
 16 abnormalities in wild populations of North American
 17 amphibians.
 18 And in at least some of those studies, those
 19 patterns were heterogeneous across landscapes where
 20 either the expected or confirmed application rate of
 21 pesticides, including Atrazine, also differ. So, it
 22 does, these kinds of studies, I think, are probably
 23 incapable of giving us that gold standard type evidence
 24 that you can get in a study design like the DCI study.
 25 But, what they do show is that these kinds of



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1 gonadal abnormalities are not simply an artifact of a
 2 lab practice of some kind. They, they seem to be
 3 happening out in the field. We don't have a good
 4 understanding of background rates, but what we do know
 5 is that it varies in space. And it varies in space in
 6 a way that, at least, raises the possibility that was
 7 expressed in the 2003 deliberations, that this
 8 hypothesis is worth entertaining. And rather than
 9 viewing the field part of all of this as subsidiary to
 10 the development of a laboratory study, at least some
 11 members of the 2003 SAP viewed those as complimentary
 12 efforts.

13 In other words, these observations of gonadal
 14 abnormalities in the field stand on their own. And so,
 15 in my estimation, they constitute a lot of evidence
 16 that's worth further evaluation. That may or may not
 17 lead us to a conclusion that Atrazine's even involved.

18 As Dr. Steeger and others have pointed out,
 19 it's very difficult to tell, in most of these studies,
 20 what the driver may be, because of various problems in
 21 the way those studies have been carried out.
 22 Nevertheless, the abnormalities are there. They're in
 23 the field. And, there, we're not here to talk about
 24 limb deformities, but there are also studies of limb
 25 deformities that show similar kinds of patterns.

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1 So, none of what we're talking about here
 2 today, with the DCI study, takes away from the fact
 3 that there's something going on with frogs out in
 4 nature. And we need to pursue that. And with that, I
 5 will turn it over to my co-discussants.

6 DR. HEERINGA: Dr. Denver is the first
 7 co-discussant.

8 DR. DENVER: Well, first I have to say
 9 that I concur with the points that Dave Skelly made. I
 10 don't know of any other literature that is available,
 11 open literature that deals with the effects of Atrazine
 12 alone on amphibian gonadal development. However, I
 13 want to point out that there is a body of literature
 14 that deals with Atrazine effects on other aspects of
 15 amphibian life history development and survival that's
 16 not being considered. And as an environmental risk
 17 assessment, gonadal development is only one poss-, but
 18 not the most important potential endpoint to be
 19 analyzed.

20 Regarding part B., the data that are recorded
 21 in the open literature are derived from studies that
 22 vary in their experimental design, and the questions
 23 being asked, the endpoints analyzed, et cetera. And
 24 there clearly are flaws in these published studies.
 25 But, I, my opinion is that this does, in and of itself,

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1 disqualify all of the open literature studies for
 2 applications in the testing of the central hypothesis.
 3 Several lab studies, in fact, studies that we
 4 considered in the 2003 SAP, suggested that there may be
 5 effects of Atrazine on amphibian gonadal development,
 6 of course that prompted the subsequent DCI. A major
 7 difference between the published studies and the DCI
 8 studies are static renewal versus a flow-through
 9 system. And I think that, I think that, and I agree
 10 that the flow-through system provides much greater
 11 quality control, but I would like to have seen a
 12 comparison of the static renewal versus the flow-
 13 through. Because those data that are in the published
 14 literature are still out there, and still need to be
 15 explained. I don't think they can be completely
 16 discounted.

17 Now, studies in which the conclusions were
 18 negative are not informative in that there are
 19 significant flaws in the experimental design. There
 20 are many problems with the field studies. Unpublished
 21 studies, I think, are sufficient to refute the
 22 hypothesis. However, some of the published studies do
 23 provide support for the hypothesis, despite flaws in
 24 their design. That is, these flaws may or may not be
 25 fatal flaws.

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1 Whether such flaws are sufficient to remove a
 2 study from consideration is arguably a subjective
 3 determination. The possibility that components of
 4 these studies may provide some relevant data appear to
 5 not be considered. Limitations and flaws may render
 6 some data suspect, but I find it remarkable that,
 7 virtually, all of the published data are being
 8 discounted. I think that it is misleading to suggest
 9 that all conclusions of all of the studies reviewed for
 10 the 2003 SAP are suspect.

11 And finally, part C., I agree that the field
 12 studies are not adequate for testing the central
 13 hypothesis; but I also agree with David's assessment
 14 that it requires further analysis before concluding
 15 that there is no risk of not showing it in populations.

16 DR. HEERINGA: Okay, Dr. Denver. And the
 17 final discussant is Bruce Pauly.

18 MR. PAULY: Bruce Pauly, Environment
 19 Canada. I agree on question Dave, with Dr. Skelly and
 20 Dr. Denver. I'm not aware of any studies that have
 21 been published that would help to address this question
 22 published in the open literature.

23 I also agree with Dr. Skully and Dr. Denver
 24 on question B. I guess, I also have some concerns, and
 25 I guess maybe that was why I was trying to determine

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1 how, which criteria would trigger no further
 2 consideration of a study. I think there is some, there
 3 is something going on, and it's been seen, effects have
 4 been seen in so many studies, that I think that it, it
 5 would behoove us to try to see, maybe, doing a
 6 consideration of published studies versus the DCI.
 7 In one study in particular, I'm not sure if
 8 we'd want to go into it in any detail, I'm, the one
 9 study that seems to have disappeared, to a certain
 10 extent, from 2003 to now was the Karr study, where it
 11 was static renewal.
 12 There was some water quality issues, but
 13 there seemed to be, to a certain extent, acceptable
 14 mortality. There was effects seen, not a dose
 15 response, but I think, I'd, just maybe, like to
 16 reiterate that, when these studies are done, things are
 17 seen. And, we have to try and reconcile that with a,
 18 you know, an evaluation of all of the existing
 19 information that we have at hand.
 20 Finally, on C., again, I agree with Dr.
 21 Skully and Dr. Denver. Field studies are problematic,
 22 and I think we need to do them. I think we need to do
 23 them for ecological relevance, and I think we need to
 24 do them to figure, try to figure out what's going on in
 25 the real world. And I think I'd agree with Dr. Delorme

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1 from the previous questions that we need a certain
 2 amount of guidance and instruction on how we might
 3 conduct a field study that would be acceptable for a
 4 risk assessment paradigm that requires rigid
 5 considerations of the protocols, and the data quality.
 6 DR. HEERINGA: Thank you much, Bruce.
 7 Comments from other members of the panel on this
 8 particular question, charge question. Yes, Dr.
 9 Leblanc.
 10 DR. LEBLANC: Being on both the 2003 and
 11 the current SAP, as several members here, I've
 12 participated in both. I struggle with the fact that
 13 with the 2003 SAP, we had data, we evaluated data, and
 14 we tried to reconcile the data when we struggled. And
 15 we felt that there was something going on.
 16 We just didn't know what it was. And the
 17 best we could do was to agree that the data was
 18 sufficient first to hypothesize that perhaps Atrazine
 19 elicited effects on gonadal development in the anurans.
 20 And the recommendation was that we now proceed and
 21 conduct well-designed GLP studies to reconcile these
 22 ambiguities, these uncertainties that we were
 23 struggling with, and that's been done. And we have
 24 that data in front of us now.
 25 What bothers me is that we're still

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1 struggling with people from literature data that we
 2 struggled with back in 2003, with all the uncertainties
 3 that are associated with it. I agree that we can't
 4 ignore it.
 5 We didn't ignore it then. We made
 6 recommendations. We've moved forward, and we have
 7 data now to evaluate. And, as I said yesterday, I
 8 think there would be value added if we revisited the
 9 open literature data now, and compared it to the GLP
 10 studies, but I truly feel that our mission, at this
 11 SAP, is to look at these GLP studies and look at the
 12 spread and what they have to say.
 13 DR. HEERINGA: Yes, Dr. Petino.
 14 DR. PETINO: Reynoldo Petino. I just
 15 want to say that I agree that the field studies,
 16 despite the problems that they may have, that they
 17 should not be just discounted.
 18 That, you know, they either conform or don't
 19 conform to a hypothesis may not serve to prove it or
 20 disprove it, but they may provide some information to,
 21 in support of the laboratory studies. So, they should
 22 not be just discounted, I mean. Then, on the other
 23 thing, and I don't know if this, you know, if I read
 24 correctly the report, the white paper, that there may
 25 be, may have been an error in the white paper in the

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1 review of the Hayes pesticides mixture or study.
 2 But, the analysis of the white paper states
 3 that this paper found no significant effects of
 4 Atrazine on the size of metamorphosis. And, by, if I
 5 read the paper correctly, they had a figure three that
 6 had that met, Atrazine actually did affect the size of
 7 metamorphosis, to a degree that was very comparable to
 8 one of the laboratory results of the DCI studies.
 9 So, I don't know if we may want to look at
 10 that, and maybe correct the white paper if necessary,
 11 but if I read it correctly, you know, that on page, at
 12 the top of page 65 of the report, that statement is
 13 made that the Hayes, et al, paper did not find an
 14 effect on size, when, in fact, they did do a report of
 15 an effect of Atrazine alone on size. So, I just wanted
 16 to point that out.
 17 DR. HEERINGA: Additional comments, yes,
 18 Dr. Williams.
 19 MS. WILLIAMS: Thank you. I'm not a
 20 doctor, but thank you.
 21 DR. HEERINGA: Just on tv.
 22 MS. WILLIAMS: Not even on tv. I
 23 appreciate all of the comments and the struggle with
 24 literature studies. And I think one of the things that
 25 was pointed out a minute ago is, kind of, where we're



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1 coming from on these, this point.
 2 For this particular question, the open
 3 literature studies that were available were not
 4 discounted. They, in fact, did form the basis for the
 5 hypothesis that had led us to where we are today. So,
 6 I just don't want to leave without saying, that we're
 7 not ignoring the information. It was not adequate to
 8 form a conclusion, we didn't believe, and the 2003 SAP
 9 agreed with that.
 10 I also wanted, though, to point out, because
 11 we're focused so discreetly on this one issue, in
 12 talking about open literature studies, the non-GLP
 13 studies, we're talking about it in the context of this
 14 issue. There are other issues in which we have used
 15 well-conducted field studies, and in fact, some of
 16 those issues related to this particular chemical, where
 17 there was a body of mesocosm studies that were very
 18 well conducted that we were able to use in a regulatory
 19 context.
 20 So, I appreciate the time to be able to just
 21 articulate that, that when we're talking about these
 22 things, we're talking about them specifically in the
 23 context of the issues before us today. And I hope you
 24 don't take it more broadly to imply that there are no
 25 field studies that are of value, and that nothing that

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1 isn't done in a laboratory under GLP standards is of
 2 use to us, 'cause that's just not the case. Thank you.
 3 DR. HEERINGA: Thank you very much. Yes,
 4 Dr. Bucher.
 5 DR. BUCHER: It's John Bucher. I think,
 6 though, that the issue that's been brought up about
 7 evaluating the new studies in light of the older
 8 studies is, still has value to me. Because, for
 9 example, I think that some of the static renewal
 10 studies that might be reevaluated in light of the fact
 11 that nothing was seen in a flow-through study.
 12 So, you might want to begin to look at
 13 metabolites and things of that nature. You know, the
 14 issues of buildup of potential metabolites as being
 15 responsible for some of those other effects seen. So,
 16 I think there is, certainly, certainly value in going
 17 back and looking at the old literature and trying to
 18 understand it, in light of this new literature. And I
 19 would agree with those statements.
 20 DR. HEERINGA: Yes, Dr. Bailey.
 21 DR. BAILEY: Open, the field studies, I
 22 think, have a very important role. They serve a
 23 different role than lab studies, but for people who are
 24 examining this kind of work, having that kind of data,
 25 the field studies is very important. Otherwise, you'd

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1 just have lab work, and you try to talk to other people
 2 about policy and so forth, they say, well, that was
 3 just lab work.
 4 DR. HEERINGA: Thank you. Dr. Delorme.
 5 DR. DELORME: I just wanted to ask Dr.
 6 Denver for a bit of a clarification perhaps, or a bit
 7 more information. You indicated in your comments that
 8 you thought some of the lab studies would be, or could
 9 be useful. And I was just, I'm sitting here looking at
 10 the study deficiencies that EPA has identified. There
 11 is numerous ones for some of those, and looking at this
 12 from a risk assessment process, as a risk assessor, you
 13 know. There's enough concern expressed in those that I
 14 would have a hard time using them to set an effect
 15 endpoint to do a risk assessment.
 16 DR. DENVER: Right, so I'm looking at it
 17 from the perspective of a scientist trying to test an
 18 hypothesis. And what I'm considering is whether there
 19 are any data in the public studies that might be
 20 considered in providing support for or against the
 21 hypothesis.
 22 DR. DELORME: Okay.
 23 DR. DENVER: Not in a risk assessment.
 24 And so, that's what I'm referring to. So, for example,
 25 Professor Petino just mentioned data in the paper that

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1 were, actually, supportive or perhaps parallel to the
 2 DCI study. So, the question becomes, then, what do you
 3 do with those data.
 4 Do you cherry pick, or do you consider data,
 5 you know, in one study and not another. Or, you know,
 6 are there some data in some studies that you might
 7 consider reliable, or at least might consider as a
 8 basis for, either formulating a hypothesis, or a test
 9 for a hypothesis, or design the experiments to further
 10 test the hypothesis, so that's what I meant.
 11 DR. DELORME: Okay. Thank you.
 12 DR. HEERINGA: Ms. Pease.
 13 MS. PEASE: Yeah, I just want to clarify
 14 the Hayes study that you're referring to that did find
 15 treatment related effects at Atrazine at the .1 PTD
 16 level for growth in the snout vent lengths. And the
 17 problem with that study is that they compared that
 18 treatment to an ethanol control, and there was no
 19 negative control tested along with it. So, it kind of
 20 confounds our ability to discriminate between potential
 21 treatment related effects and solvent impacts. I just
 22 wanted to clarify that. Thanks.
 23 DR. HEERINGA: Thank you for that
 24 clarification. Dr. Steeger.
 25 DR. STEEGER: I'd like to make a couple

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1 of clarifying statements. On the rejection of studies
 2 because of static renewal conditions, many of the
 3 studies that employed this methodology were using the
 4 static renewal after three days, and only 50 percent of
 5 solution changes at that time.
 6 The studies where we had data on water
 7 quality for following similar methodologies, and Karr
 8 was one of those studies, indicated that the quality of
 9 the water, given the loading rates that, the high
 10 loading rates that were used, and the infrequent
 11 solution changes, resulted in a compromise of the
 12 animal's ability to develop. And that was apparent
 13 through the lack of metamorphosis; the animals weren't
 14 undergoing metamorphosis, in many cases; decreased
 15 weight with increased time to metamorphosis; high
 16 mortality rates; and poor response to positive
 17 controls.
 18 And I think that the final effect that I just
 19 that I mentioned was poor response to positive
 20 controls, where you weren't seeing any developmental
 21 effects in the animals suggested that the animals were
 22 simply trying to survive, and that their development
 23 was seriously compromised. And that was associated
 24 with this static renewal phenomena, where you have
 25 three days of flow, or three days of static, and only a

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1 50 percent change. And, many of the open literature
 2 studies were following that protocol.
 3 The, I want to clarify something. The field
 4 studies, when I was asked are there any new field
 5 studies that we were aware of. Several of the studies
 6 that were reviewed in 2003, were still in the process
 7 of being completed, and were two to three year studies,
 8 and only one year of the study had been completed. But
 9 the problems that had been identified with the study
 10 were methodological problems, and those methods had
 11 not
 12 changed in the way the studies had been conducted, even
 13 into their second and third year.
 14 And so the issues still remain. I will say
 15 that, in the growth study, he did, eventually, look at
 16 Atrazine in the control sites. That was one of the
 17 issues that had been identified in the interim report.
 18 That, as I indicated yesterday, there are a number of
 19 other confounding factors that, really, limited our
 20 ability to use those studies, in addition to what had
 21 been identified in 2003 SAP.
 22 On the comment regarding GLP and inability of
 23 universities or academia to compete with that standard,
 24 in '75, when I was working as a bench biologist at
 25 Stanford, I had the misfortune of doing experiments for
 26 EPA.

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1 And, at that time, the GLP was just in its
 2 infancy. And I told them that it really wasn't worth
 3 the money that you could get from EPA to impose the
 4 GLP
 5 standard on any study. It, at that time, in '75, it
 6 added \$10,000 dollars to the cost of the study. And it
 7 was just a nightmare to try to satisfy the EPA. And,
 8 ironically, now I'm imposing it on other people.
 9 DR. STEEGER: It's, to expect that
 10 standard. I'm not saying that universities, academia
 11 cannot meet that standard, but the monies that are
 12 involved in accomplishing it. Here you have right in
 13 front of you contract labs that regularly function
 14 under GLP, and even trying to meet that standard is
 15 difficult for a contract lab.
 16 It's very difficult to imagine university
 17 labs from the get-go being able to meet that standard,
 18 and at a cost that would be competitive with contract
 19 labs. I'm not saying it can't, but it's a difficult
 20 standard to meet. A study does not have to GLP for us
 21 to consider it, though. A well-conducted study would
 22 be given heavy weight in any type of analysis that EPA
 23 has done.
 24 And, okay, that's, I do have one question to
 25 the panel, though, in follow up to your comments
 26 regarding the laboratory studies. Can the panel

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1 suggest methods of statis-, or statistical methods to
 2 consider these studies, given the limitations that have
 3 been identified in the methods that were used to
 4 collect the data?
 5 DR. HEERINGA: A question posed to the
 6 panel, this is snap quiz, possibly. We can do one of
 7 two things. We can try to address it now, or we can
 8 certainly return to it after a little thought has been
 9 given to it. I think Dr. Denver has raised this issue,
 10 and I think about the possibility of looking back at
 11 some of these now in the light of the DCI studies, but
 12 I'm not sure whether any of the statisticians or other
 13 panelists present here would have a suggestion.
 14 DR. BAILEY: Ted Bailey. I'd like to
 15 answer that, in our current discussion about university
 16 research. Is it possible to consult with those
 17 researchers, and maybe you did do that for the DCI
 18 studies, is it possible to consult with experts about
 19 the design and the type of analysis, as well as the
 20 treatments in the conduct of the experiment? Thank
 21 you.
 22 DR. STEEGER: I'll give you my personal
 23 perspective. I am more than happy to talk to
 24 researchers that could potentially undertaking studies
 25 to address this effect. I attended an amphibian



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1 conference earlier this year. And a message that I
 2 brought to those researchers was that, you know what
 3 the Agency's standards are that are being applied to
 4 these studies.
 5 If you hope to generate information that is
 6 going to be used in a regulatory context, understand
 7 the process that we use. Don't be surprised that your
 8 study doesn't meet that, those standards.
 9 When we're applying the same criteria to
 10 evaluate your studies as we apply to registrant
 11 studies. It's a level playing field. I, personally,
 12 have 27 chemicals on my plate that are active.
 13 Atrazine is only one of them. I have a 2300 page
 14 report that I have to analyze in a narrow period of
 15 time, and that's only one of my chemicals. To, we're
 16 willing to work with researchers to develop, help them
 17 understand how we evaluate studies, but as I said
 18 before, we cannot tell them how to conduct their
 19 studies.
 20 DR. HEERINGA: Dr. Portier.
 21 DR. PORTIER: I would like to attempt to
 22 address your question about statistical method design.
 23 And I'm addressing it from a background of twenty plus
 24 years of consulting with researchers, both in
 25 agriculture and health and ecology on study design.

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1 And the problem is, when you have studies that have
 2 confounded factors that are important, and they don't
 3 do anything in the study design to take that into
 4 account, by either measuring co-factors, so we can
 5 adjust for them in the analysis, or blocking, or
 6 controlling for them at the design phase, you can't do
 7 anything statistically. You have the data, the
 8 confounding is a fact of life, and it's what leads to
 9 all of this discussion that we're having.
 10 Every one of these criteria you look at is a
 11 confounding issue that can only be fixed by another
 12 study, the next study. You know, in an academic
 13 setting, the studies are small, so we tell the graduate
 14 student who usually makes this mistake, right, to go do
 15 it again and get it right, or take additional
 16 measurements. When you're doing \$20 million dollar
 17 field study, and you have confounding, which often
 18 happens because you don't have the control in field
 19 studies that you have in lab studies.
 20 You really got to think these things through
 21 very carefully before you step in the field. And I
 22 don't, and once it's started, and you haven't accounted
 23 for it, the data's going to be only marginally useful.
 24 It'll be useful for hypothesis generation and raising a
 25 lot of questions, but it's not going to answer any

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1 questions. And that's, that's my take on it. It's a
 2 fact of life.
 3 DR. HEERINGA: Dr. Delorme.
 4 DR. DELORME: Just to echo a few things
 5 that have been said already here, as I sit here and
 6 look at the study deficiencies that have been
 7 identified, in, it's one of the tables in the white
 8 paper, there's some things that can be recovered from.
 9 Unfortunately, at the time many of these studies were
 10 done, there was no protocol for looking at
 11 developmental effects on Xenopus. And we know have,
 12 at
 13 least the beginnings, of a protocol.
 14 And our conclusion in 2003, and as a member
 15 of that panel, we decided that there was enough
 16 evidence that there could be something, and that's how
 17 we came up with the hypothesis, to go forward and try
 18 to clarify it.
 19 To retroactively go back and look at these,
 20 you might be able to find some support for the
 21 conclusions that we've made, but beyond that, I'm not
 22 sure what you're going to get. I mean, many of the
 23 issues that EPA have identified were issues that were
 24 identified by the panel as well as being confounding
 25 factors, as Dr. Portier has pointed out. I don't know
 what you could do to actually make the data more

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1 useful.
 2 Things like high loading rates, high
 3 mortality controls, contamination, I mean, I would
 4 contamination controls a fatal error, and that would
 5 take any study out. If you have nothing to compare it
 6 to, how can you use that data.
 7 So, and I think you have to give a little bit
 8 of consideration that, you know, people at EPA have
 9 looked at these and as Thomas has pointed out, Dr.
 10 Steeger's pointed out, it's not usually one thing, but
 11 it's a series of things that are going to knock a study
 12 out. It's unfortunate. We look at this from our
 13 perspective now in 2007. You know, having seen a study
 14 that was very well conducted with the protocol.
 15 But try and put that in the framework of the
 16 studies that were done back in five, four or five years
 17 ago, they didn't have that. We've used the knowledge
 18 that we gained. We've looked at it. We've recognized
 19 what those mistakes were and moved forward.
 20 DR. HEERINGA: Dr. Denver.
 21 DR. DENVER: I just want to respond to
 22 that. I want to make it clear that I don't disagree by
 23 and large with the evaluation of the studies that are
 24 in the white paper by the EPA.
 25 Because, in fact, many of those studies, you

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1 know, and many of the same issues we raised and
 2 discussed at the SAP in 2003. I think it's a matter of
 3 respect of risk assessment, as you point out, and the
 4 process of doing science, which is often dirty, you
 5 know.
 6 And, you know, we take a result and we use
 7 that to formulate hypotheses. So, that's where I'm
 8 coming at it from, that, in fact, there are results in
 9 the literature that suggest that there could be an
 10 effect.
 11 And so, as a scientist, I'm curious why those
 12 results were obtained. They weren't obtained simply
 13 because of the flaws in the experimental design, right.
 14 So, there are flaws in the experimental design that
 15 make us wonder whether the results are reliable or
 16 repeatable or dose response, that sort of thing. Okay,
 17 so that's it.
 18 DR. HEERINGA: Very good discussion, and
 19 I appreciate that, encourage that, I guess, as we move
 20 through these other questions. Yes, Dr. Williams.
 21 DR. WILLIAMS: Can I please ask a
 22 clarifying question of Dr. Denver?
 23 DR. HEERINGA: You sure can.
 24 DR. WILLIAMS: What I heard is the
 25 following, and you can tell me if I heard it correctly

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1 or not. For effects other than the one that we're
 2 looking at, there's literature that shows those
 3 effects, and the study showed the potential for those
 4 effects. Are you suggesting that it's those that we go
 5 back and look at as different effects? That's what I
 6 heard, rather than going back and comparing the DCI
 7 study to the literature for the effects that we're
 8 focused on right now.
 9 DR. DENVER: Now, so, I think you're
 10 referring to my reference to the point that was made by
 11 Professor Petino, and the, is that what you mean?
 12 DR. WILLIAMS: No, I'm referring to the
 13 comment that you most recently made. I guess I just am
 14 not, I guess I'm not understanding - -
 15 DR. DENVER: My point of view. So, my,
 16 as I said, my point of view is of a skeptic, as a
 17 skeptical scientist wondering if there is literature,
 18 or if there's data, that points to some type of
 19 response that needs to be entertained.
 20 So, I'm not making any comments about how the
 21 EPA would use the literature for regulatory decisions.
 22 I'm just simply approaching it from the perspective of
 23 a bench scientist, who would look at results and decide
 24 whether there's something there or not to - -
 25 DR. WILLIAMS: I appreciate that. I

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1 guess the question that I'm having in my mind is that I
 2 think that this effect that the EPA is focused on right
 3 now, I think we did do what you're talking about,
 4 because that very literature helped to form the basis
 5 of the hypothesis that we brought to this group. So, I
 6 guess, what I'm wondering is, are there other effects,
 7 that you've seen in the literature, that you're
 8 suggesting are more points of curiosity that we ought
 9 to be exploring?
 10 DR. DENVER: Well, yeah, I mean there are
 11 lots of, there's a lot of literature, and, that goes
 12 beyond amphibian and points them out, that suggests
 13 that there may be effects. So, but, as far as gonadal
 14 development, which we're focused on?
 15 DR. WILLIAMS: Yes.
 16 DR. DENVER: I don't know of any other
 17 literature that deals with that, maybe other than the
 18 literature that's been reviewed.
 19 DR. WILLIAMS: Okay, thank you.
 20 DR. HEERINGA: Any additional comments
 21 for the panel? Dr. Steeger.
 22 DR. STEEGER: I'd like to follow up on
 23 that, Dr. Denver. I would, as a scientist, wonder
 24 about the fact that you still have people who are
 25 generating effects data showing Atrazine and gonadal

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1 development.
 2 Right now, it's my understanding, based on my
 3 review of the literature that Dr. Hayes has
 4 demonstrated in the past, that exposure to Atrazine,
 5 at various concentrations, results in various levels of
 6 an effect. In his most recent effort, though, to study
 7 even the same species, ranid, rana pipiens, there is no
 8 effect. And when I try to understand how does, how
 9 does he get a response like that, since it seems to be
 10 so clear in his original work; and yet, when he
 11 reproduces it, he doesn't get it. And when we have a
 12 study that attempts to control for all the sources of
 13 variability, we don't seem to see the effect.
 14 Yet, there his study is, the original one,
 15 where there is an effect. And I really am at a loss as
 16 to how to explain that. And I believe that, that might
 17 be what you're getting at, that you do have some
 18 investigator showing that there's an effect there, and
 19 it's, it stands. I don't know. I can't explain it.
 20 DR. DENVER: I can't explain it either.
 21 And, I, you know, and I'm actually going to mention
 22 this in a moment, but there, there are effects that
 23 appear, and in fact, in the Karr setting, there's also
 24 an apparent effect that is similar to what Hayes found.
 25 I don't know why he, he has not repeated it in other

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1 species.
 2 But, and I'm not trying to defend his work,
 3 or anyone's work. I'm simply coming at that this as a
 4 skeptic trying to look at the data. I think that, you
 5 know, I'm still puzzled by results that are reported.
 6 And I wonder why, you know, why did they, we find these
 7 results.
 8 Is it something that is related to the
 9 experimental design. Now, the experimental design in
 10 the DCI studies are arguably impeccable. I mean,
 11 there's, you know, they're highly controlled, following
 12 the STM standards, but nature is not like that.
 13 In fact, the static, you know, the static
 14 renewal studies, which had been done, I wonder whether
 15 there's some aspect of those studies that led to the
 16 generation of an effect in some labs and not others.
 17 And that's a curiosity that I have. And I don't know
 18 the answer to that.
 19 DR. STEEGER: I share your curiosity, and
 20 I struggle with what kind of conditions could have
 21 accounted for that. And I, I also struggle for, if a
 22 researcher has, or had, if we've identified what could
 23 be potential sources of variability in the study, how
 24 much you eliminate them, why someone would proceed to
 25 conduct a study knowing that those are potential

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1 sources of variability, and not make any effort to
 2 correct for it.
 3 The use of plastic mouse cages, and for those
 4 of us that have worked in labs with mammals, with
 5 rodents, know that as you work with plastic mouse
 6 cages, they have a limited life span in the lab,
 7 because they become brittle with washing, and
 8 eventually, they become useless. They'll just crack.
 9 And the reason for that is they're leaching out,
 10 they're plasticizers.
 11 EPA very strongly recommends the use, or
 12 discourages the use of plastics in aquatic studies,
 13 because of the potential for plasticizers to leach out
 14 of the container. There are published literature that
 15 indicate that phthalate ester, it's a common
 16 plasticizer, can produce gonadal deformities, and to
 17 continue, knowing that, and to continue to do a study
 18 that could potentially be confounded in that way, is
 19 amazing to me.
 20 Yet, even in his, like I said, if, that
 21 aside, if you look at, as the researcher continued to
 22 produce the effect that he claimed occurs under
 23 thousands of times of experimentation, thousands of
 24 times, tens of thousands, the answer is no. And it's
 25 attributed to biological variability. And I say var-,

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1 biological variability might account for a decrease in
 2 effect, but not account for an elimination of an
 3 effect.
 4 DR. HEERINGA: I'd like to thank
 5 everybody, yes, this has been a very healthy
 6 discussion, and certainly one that we'll, I think will
 7 be reflected in the report of the panel. And I
 8 appreciate the contributions from everybody on this
 9 topic, because I think, clearly, it's critical to the
 10 consideration, not only of the current experimental
 11 results, but the general issue of scientific evidence
 12 here. Okay.
 13 What I'd like to do at this point, is to move
 14 on to charge question. Let me just look at the panel.
 15 Let's tackle charge question number three, and we may,
 16 we'll take the appropriate time, but, obviously, if we
 17 get to 12:30 and we haven't finished, I may call for a
 18 lunch break and resume. But, Dr. Irene, if you could
 19 read charge question three into the record, please.
 20 DR. IRENE: Stephanie Irene, question
 21 three. The following questions will concerning the DCI
 22 study. Please comment on the Agency's evaluation of
 23 the final study design.
 24 For example, the Agency concluded that the
 25 minor changes in the experimental design, such as,

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1 omitting Atrazine degraded analysis for DACT, DEA,
 2 and DIA, and not conducting differential cell counts
 3 for ovarian and testicular histology, did not
 4 compromise the means to assess the hypothesis that
 5 Atrazine exposure can affect amphibian gonadal
 6 development.
 7 If the SAP concludes that the alterations in
 8 this study design preclude, or significantly
 9 compromise, the ability to assess the hypothesis,
 10 please discuss the extent possible, to the extent
 11 possible, how the specific design modifications could
 12 impact the means to assess the hypothesis. Please
 13 provide comments on other aspects of the Agency's
 14 evaluation as well.
 15 DR. HEERINGA: We'll return to Dr.
 16 Denver, who is the lead discussant of this question.
 17 DR. DENVER: Okay. So, omitting the
 18 Atrazine degraded analysis, that is for Deethyl
 19 Atrazine DEA, Deisopropyl Atrazine DIA, and
 20 Diaminochlorotriazine DAC, of the water, in my opinion,
 21 in itself, did not compromise the means to assess the
 22 hypothesis that exposure to Atrazine alone, and I
 23 emphasize alone, can affect amphibian gonadal
 24 development. The limited analysis that was done
 25 suggested that the metabolites are at or below the



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1 limit of detection, and subsequent water analysis were
 2 not conducted.
 3 We know that Atrazine is rapidly metabolized
 4 by frogs. It's unclear whether the tests have been
 5 conducted with tadpoles, but I know that frogs have
 6 been analyzed. And there's little bio-accumulation in
 7 fish or frogs. So, it's like that if metabolites or
 8 degradates were generated to any significant degree
 9 that they would be rapidly cleared in the flow-through
 10 system.
 11 Thus, these studies were able to test whether
 12 Atrazine, in its native form, affects amphibian growth
 13 and development, without potential confounding effects
 14 of Atrazine metabolites or degradates. And I just want
 15 to read for a moment from a document that was submitted
 16 from the eco risk panel that is the effect of Atrazine
 17 on aquatic wildlife, a critical review.
 18 DR. HEERINGA: This document, by the way,
 19 is in the docket for this particular panel meeting, and
 20 panel members have been provided a copy as of yesterday
 21 morning.
 22 DR. DENVER: Well, just a couple of
 23 sentences. Measurements of a persistence of Atrazine
 24 transformation products are limited, however, aqueous
 25 half-lives of Atrazine degradates and metabolites are

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1 not available.
 2 Bioconcentration data for Atrazine
 3 transformation products are also limited, as studies
 4 reporting biological concentration focus on metabolite
 5 production in biota upon exposure to Atrazine, rather
 6 than uptake a few degradates from the environmental
 7 media.
 8 Based on the results of the studies in
 9 mammals and other organisms, these metabolites should
 10 be considered in assessing potential risks. So,
 11 therein lies the rub. One wonders whether the flow
 12 through paradigm that was used in the DCI studies,
 13 versus the static real paradigm that they used in all
 14 the open, in the open literature studies, which we've
 15 discussed already, and have significant flaws.
 16 And prior registrant sponsor studies could
 17 account for the differences in the results among the
 18 studies. And that's what, that's what I wonder about.
 19 For example, one cannot rule out the hypothesis that a
 20 metabolite or degradate of Atrazine is responsible for
 21 the xenobiotic activity of the compound that's been
 22 observed in some other studies, some, but not all
 23 studies.
 24 Now, these effects could be minimized in the
 25 flow-through system, since such compounds would be

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1 rapidly cleared. And to my knowledge, the effects of
 2 Atrazine metabolites have not been tested for effects
 3 on amphibians. Correct me if I'm wrong. Nor have
 4 these metabolites been routinely analyzed in exposure
 5 studies. These metabolites have been measured in sites
 6 where amphibians live in South Africa and other areas.
 7 So, they do occur, and they are present in
 8 the environment. They have been shown to have effects
 9 in other species. For example, they have been shown
 10 to, perhaps, delay puberty in rats. This is work of
 11 some of the EPA lab in North Carolina. So, there are
 12 other precedents for considering the activities of
 13 metabolites, degradates. One of the better known being
 14 the breakdown product of DDT. It is DDU, which has
 15 demonstrated activity, endocrine destructive activity.
 16 The second part of the other question was the
 17 omission of cell counting for ovarian and testicular
 18 histology could have provided a means to backup the
 19 hypoplastic scores of the gross morphological level.
 20 However, as was pointed by the participating
 21 histopathologist, on stage 66, it's very difficult to
 22 differentiate primordial germ cells and primaries for
 23 that value. He suggested that it was impractical and
 24 of minimal value to do differential sub-counting of
 25 those omissions.

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1 DR. HEERINGA: Our second discussant
 2 would be Dr. Furlow.
 3 DR. FURLOW: So, first off, I have to say
 4 that overall, the study design was, in fact, impressive
 5 to me, in terms of the controls that were included, and
 6 the rigor to which the water quality control and health
 7 of the animals was maintained. And that is certainly
 8 something that has confounded amphibian research in the
 9 lab for years and years and years.
 10 And, I think that the setup that was used in
 11 the DCI experiments was justified and was, well, you
 12 can see by the consistency between the two labs, one in
 13 the States and one in Germany, that the amazing, to me,
 14 having worked with amphibians for a while, in laevis
 15 Xenopus, in particular, the amazing degree of overlap
 16 between the two labs is really a tribute to the careful
 17 conduction of the study.
 18 And I think, Peter Delorme's earlier
 19 statement that we really need some sort of standardized
 20 testing setup for an amphibian, I think this is an
 21 excellent starting point and framework, with which to
 22 continue to test potential endocrine disrupting or
 23 other kinds of chemicals that compromise amphibian, all
 24 kinds of aspects of amphibian physiology.
 25 It's my understanding that, at least part of



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1 the original design of the flow-through system, maybe,
 2 actually, Dr. Davis can mention something about this,
 3 was to develop a system that would give you extremely
 4 reproducible time to metamorphosis. And as someone
 who
 5 studies that particular aspect, I appreciate that, and
 6 I'm jealous at the tight error bars that have, and the
 7 times metamorphosis, and the, actually revealing
 8 potential effects on body weight in the snout vent
 9 length of Atrazine. Small effects, but nonetheless, I
 10 think it's physically significant ones.
 11 So, all of that being said, I think, I
 12 definitely agree with what Dr. Denver said earlier, in
 13 the terms of does the study design allow us to test the
 14 hypothesis of whether or not Atrazine alone effects
 15 amphibian, or at least, let me say, Xenopus laevis
 16 gonadal development. I think so. And I think the data
 17 produced list was remarkably good. However, I do agree
 18 with Dr. Denver that, in trying to reconcile some of
 19 the earlier studies, that metabolites should have been
 20 considered.
 21 In addition to the studies that Dr. Denver
 22 mentioned, it's also, I think, reasonably well
 23 established, that DDT metabolites to a safe DDE can
 24 actually be a potent anti-androgen. Whereas, DDT is a
 25 weak estrogen. And so, I think these things certainly

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1 need to be accounted for.
 2 In addition, I was actually impressed by the
 3 mitochondrial DNA testing that was mentioned in the
 4 earlier presentation, and actually getting at actual
 5 potential subspecies differences in Xenopus laevis, and
 6 differences in where animals were collected from South
 7 Africa, and where people actually get their animals to
 8 do these studies. That, actually, was very impressive
 9 to me, and very important work. It was, in fact,
 10 curious that the animals that were, sort of in the
 11 maize growing regions, and north, east South Africa, in
 12 fact, were sensitive.
 13 Or, I should say, showed more of these
 14 testicular oracites than the animals were actually,
 15 most of them were collected down near the Cape for, to
 16 supply the animals for, for virtually all of us. But
 17 it does raise the question of where the animals come
 18 from, all of those earlier studies.
 19 Now, the large majority of them are known to
 20 come from Nasco, is that Xenopus One, or Xenopus
 21 Express, but to my understanding, the Hayes studies
 22 were using his own colony, and it's unclear where those
 23 animals came from. Were those animals, in some way,
 24 more sensitive for whatever reason. So, this is
 25 something that, at least, ought to be considered.

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1 The other thing is just a general comment,
 2 and that is, in the design and, this is something that
 3 goes back before Hayes presentations, and back to the
 4 original suggestions made by the 2003 SAP, I think.
 5 And something that was brought back to my mind by
 6 something Dr. Steeger said. And that is, mixtures are
 7 not being considered, and I think that for, I can
 8 understand and appreciate the EPA's stance on this, or
 9 at least, approach to the Atrazine question, that it,
 10 you start to open, I would say, a Pandora's box, when
 11 you start thinking about, well, maybe it's not just
 12 Atrazine.
 13 It's Atrazine in combination. We touched on
 14 this, in terms of the metabolites, but it is also
 15 formerly possible that Atrazine is, essentially,
 16 sensitizing the system to the effects of other
 17 compounds, including possibly estrogens.
 18 If there are phthalates potentially coming
 19 from the containers, perhaps Atrazine is sensitizing
 20 the animals to the effects of phthalates. This is
 21 possible, but I don't think it can be ruled out. I
 22 think that it's becoming more of an important problem
 23 that we're going to have to address sooner rather than
 24 later about the effects of mixtures.
 25 All of that being said, I think that the

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1 study design was, in fact, in my mind, remarkably
 2 robust. It touched the specific hypothesis about
 3 Atrazine, and pretty much, Atrazine alone on Xenopus
 4 gonadal development. And I'll stop there.
 5 DR. HEERINGA: Dr. Miller.
 6 DR. MILLER: Debra Miller, UGA. I agree
 7 with the other discussants, and I do, also, want to say
 8 that your design was very good. But I, also, would
 9 like to say that I, it would be a great advantage, I
 10 think, to look at parallel studies of flow versus
 11 stagnant or, a stagnant type of system. Simply
 12 because, in other situations, we are starting to design
 13 studies that way and are noticing a difference. And
 14 most of those are with dizzy studies, but it may be
 15 similar here just because of the degradate component.
 16 And, as far as the differential cell counts,
 17 I understand, and I totally agree with omitting them,
 18 and I don't really have a problem with that. The
 19 problem I do have is how you dealt with the scoring,
 20 because for, as a pathologist looking at the results,
 21 it's really hard for me to look at, okay, so you have
 22 significant differences here, but not here, but what
 23 does that really mean. Because we can see such a wide
 24 range of variation in changes.
 25 So, if you have hypoplasia of the tubule, for

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1 example, looking at it histologically, did you just
 2 have, like, less than 1 percent, and you might see that
 3 in all of them, and that is going to be a standard.
 4 And so, then, that becomes a presence versus absence.
 5 But how many did you see were most of the tubules were
 6 affected, or 50 percent of them.
 7 So, that becomes a real issue when you're
 8 looking at where there was no variation with increasing
 9 dosages. Because you don't really, you've got to be
 10 able to break that apart. And I understand the
 11 variation, and I am so amazed with the numerous slides
 12 that you went through to look at these and grade them,
 13 that it's really quite a feat in and of itself.
 14 But, I totally, also, understand that if you
 15 review a slide more than one time, yes, you can easily
 16 see differences. And then, you have to go back and
 17 say, well, what do we need to do to compensate for
 18 that, rather than just omit it. Does that mean that
 19 your pathologist has to then go through the slides two
 20 more times and take the average. Which would be, I'm
 21 sure Dr. Wolfe would have absolutely no hair left by
 22 that time.
 23 Or, do you need to have a panel of
 24 pathologists, then, to, you know, to say, well, we'll
 25 have two others. And Dr. Wolfe is the lead, and so

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1 then, we can compare those. So, for me, omission of
 2 that was more of a factor than omitting the
 3 differential cell count.
 4 DR. HEERINGA: Dr. Petino.
 5 DR. PETINO: Reynoldo Petino. I,
 6 generally, agree with what has been said. I think that
 7 the lack of the information of the degradates of
 8 Atrazine, even if one takes a, that the effect of
 9 Atrazine was the main interest, does not effect the
 10 ability of the study to determine whether the Atrazine
 11 is having an effect or not.
 12 I am, I also, share, though, the concern that
 13 the change from static to a flow-through, and what the
 14 level of metabolites, and you know, that accumulated in
 15 those two systems, and whether that might be a factor.
 16 And, you know, and perhaps, you know, the effects being
 17 different now than, you know, some of the studies in
 18 the past that reported effects. But, I think we're
 19 providing information.
 20 The answer does show that the level of
 21 metabolism was quite low. So, I don't know that I'm
 22 terribly concerned about that, but I agree, it's a
 23 question, it's a question mark, and you know, when you
 24 change from a static to a flow-through, or that would
 25 definitely affect in relation to metabolites.

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1 The lack of the differential cell counts, I'm
 2 not bothered too much about that either, especially
 3 because there was some other measurements made, you
 4 know, gonadal image area, for example, where there
 5 seems to have been some effect where they were small,
 6 and if I remember correctly, they were different of
 7 the, there was a trend towards negative values in one
 8 live, and an effect of the high concentrations won't
 9 effect the other live, so if the effects were
 10 inconsistent, and were small.
 11 So, I mean, if it had been otherwise, I would
 12 have said that maybe the differential cell counts would
 13 have been important to consider. But, it's not, so I'm
 14 not too concerned. I would like to make a point,
 15 though, that perhaps, if the concerned one is the, you
 16 know, the, the effects of Atrazine or any compound on
 17 gonadal genesis, and the ability, you know, the
 18 productive potential of the individuals as they grow as
 19 adults. That probably, other endpoints would have been
 20 other than, I guess, I wasn't here in 2003, or I wasn't
 21 a member of this panel.
 22 But, if there would have been other endpoints
 23 probably, it would have been more appropriate to
 24 measure. If the concern was the effects gonadal
 25 genesis future potential, and if they're commenting on

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1 that, for example, size and age to maturity are, you
 2 know, important parameters, they have also an
 3 ecological relevance. But, actually, those are common
 4 measures that people in the fisheries field make. So,
 5 you know, is there an impact on those, too.
 6 I think, the, that we were presented with a
 7 study yesterday, a grow up study that can, attempted to
 8 address that concern, and I think it's, that study has
 9 a lot of value in determining, for example, the effects
 10 of the, even, lifetime of exposures on reproductive
 11 potential.
 12 But, the, if the impact is, you know, if the
 13 question is fecundity, you know, germ cell, numbers of
 14 germ cells, I think that the way that study was
 15 conducted, probably, was not appropriate to address
 16 that question, because the frogs, after the exposure
 17 was conducted, and they became mature, the frogs were
 18 selected because they weren't mature, even, they were
 19 mature.
 20 They were, in other words, it was a biased
 21 sampling for selection of the rootstock in order to
 22 determine the F2 generation effects. So, I think
 23 points such as age, size of reproduction are important,
 24 would, could provide some information in view of the
 25 this differential sub-colonies. But, that would be

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1 useful, that information would be useful there, too.
 2 DR. HEERINGA: Dr. Green.
 3 DR. GREEN: Sherril Green, and I just
 4 wanted to make a comment. I concur that I'm not too
 5 bothered, particularly, if the histological data was
 6 not recorded as, or collected as recommended, but I do
 7 think omission of the metabolites is probably something
 8 that should be considered as important.
 9 One of the reasons, as I recall the 2003
 10 recommended the flow-through tank testing system to
 11 start was because, at the time, the papers and the data
 12 that we had had largely been collected from animals
 13 that were in static tanks, and as Dr. Steeger pointed
 14 out, the water quality analysis was, either, not done
 15 or what was reported, reported some parameter changes
 16 in waste materials that would have prohibited normal
 17 growth in that tank anyway, or compromised the animal's
 18 health.
 19 And we did have one paper, at that time, that
 20 showed, in the presence of nitrate, Atrazine did have
 21 some effect on those animals. So, before we could
 22 begin to sort things out, that recommendation was made
 23 that we try to begin basic studies in flow-through
 24 tanks. And I think that data has been done, and it's
 25 pretty good data.

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1 And, again, in a static pond in an
 2 environment, though, in the field, animals might not
 3 get the benefit of a flow-through, wash away effect of
 4 Atrazine or metabolites if they're toxic, or the
 5 metabolic waste that you would find normally, or any
 6 other chemical in conjunction with Atrazine. So, I
 7 would make the recommendation that those studies be
 8 reconsidered as important. And the studies I'm
 9 referring to are the ones where the metabolites are
 10 evaluated as well.
 11 DR. HEERINGA: Dr. Chambers.
 12 DR. CHAMBERS: I'm a little confused
 13 about the metabolites, and I guess we don't know for
 14 sure that the tadpoles can create metabolites at their
 15 level of development, but any in vivo study, of course,
 16 studies not only the parent compound, but any
 17 metabolites that the organism forms while the parent
 18 compound is being metabolized in them.
 19 So, if metabolism was possible by these
 20 tadpoles, then obviously, that the, the studies studied
 21 not only the parent compound, but any metabolites that
 22 the tadpoles formed. So, that was part of the study
 23 design. Now, the degradates that, environmental
 24 degradates, that's another question altogether.
 25 DR. GREEN: I should correct and say

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1 degradates, instead of necessarily, metabolites.
 2 DR. HEERINGA: Dr. Leblanc or Dr.
 3 Delorme.
 4 DR. LEBLANC: Jerry Leblanc. In regards
 5 to metabolites, I think there certainly would be value
 6 in, if we had information on metabolites from these
 7 studies, with respect to, perhaps, explaining
 8 ambiguities between studies, between the flow-through
 9 and the static renewal studies. Unless, I'm
 10 significantly less concerned about the relevance of
 11 metabolite data with respect to trying to assess
 12 ecological hazard of the material.
 13 If, and I was a little bit confused as well.
 14 If we're talking about biotransformation products,
 15 metabolites, I think it's addressed in these studies.
 16 The animals were taking up Atrazine in a flow-through
 17 situation, and they're constantly being exposed to
 18 Atrazine. And they're little machines. They're
 19 producing these biotransformation products, and so
 20 they're getting a good dose of them.
 21 If we're talking about environmental
 22 degradates, then certainly in a static renewal
 23 situation, these things could be accumulating. But,
 24 we're talking about a little aquarium, as opposed to a
 25 big pond, and I think that the pollution, a,

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1 considerations there would be again, would certainly
 2 reduce my concern.
 3 The other thing is that, and I could be wrong
 4 here, but I, but it's my understanding that the
 5 degradation products of Atrazine are no mo-, have no
 6 more of propensity to bio-accumulate than does
 7 Atrazine. And Atrazine doesn't bio-accumulate. So,
 8 it's not like these animals were being cumulated. They
 9 may be exposed, but they won't be accumulating
 10 significant amounts of these materials.
 11 And I, I just want to add a voice of support
 12 to something that Dr. Furlow had stated with respect to
 13 variables. The ability of Atrazine or the toxicity of
 14 Atrazine to, perhaps, be confounded by other
 15 components in the exposure environment, perhaps,
 16 metabolites, perhaps, estrogens, perhaps, nitrates,
 17 whatever.
 18 Certainly, this is an issue, and again, it
 19 may be, in time, it may turn out to be the issue that
 20 explains a lot of the uncertainty that we're struggling
 21 with. But this isn't unique to Atrazine. It's
 22 something that EPA has to deal with with respect to
 23 every chemical that they're charged with regulating in
 24 the environment, and it's something that they need to
 25 get a hold of and address, but I think it's warranted.



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1 DR. HEERINGA: Dr. Delorme.
 2 DR. DELORME: And with respect to
 3 metabolites, or environmental transformation products,
 4 whatever you want to, metabolites I usually associate
 5 with the organism, itself. And transformation product
 6 is what's in the open environment. EPA might want to
 7 consider looking at the existing monitoring data to see
 8 to what extent larval amphibians or eggs might even be
 9 exposed to determine whether or not the concentrations
 10 in the real environment are high enough to warrant
 11 that. If you consistently find high concentration, it
 12 might warrant some further investigation.
 13 Obviously, when we, when the panel in '03
 14 looked at and recommended a flow-through design, it was
 15 the water quality parameters that were uppermost in our
 16 mind, trying to reduce the number of confounding
 17 factors so we could get some clear answers to some of
 18 the questions that we had posed.
 19 But I think that this is a case where maybe
 20 there is some field data available that could help you
 21 make a decision as to whether or not you need to move
 22 forward on that.
 23 And it doesn't, necessarily, mean you have to
 24 repeat this kind of, or the details spending with those
 25 as well. There may be other methods that can tell you

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1 whether or not there's potential for finding to
 2 estrogen receptors or whatnot and help you out in that
 3 to begin with.
 4 So, you might want to consider looking at the
 5 existing monitoring data for the metabolites. I know
 6 it exists out there, to see, you know, what the state
 7 of the environment is, and what role or what exposure
 8 there is.
 9 DR. HEERINGA: At this point, I guess
 10 I'll turn to Dr. Steeger for any questions of
 11 clarification or comments.
 12 DR. STEEGER: I just have one comment on
 13 the scoring issue. Out of fairness to Dr. Wolfe, the
 14 veterinary pathologist, when he was asked to repeat
 15 those scorings for the benefit of the new panel member,
 16 during an inspection, EPA inquired that the pathologist
 17 re-read at least six to ten of his slides. I had his
 18 original results in front of me, and I just tracked on
 19 how well he was able to duplicate his results. And for
 20 the actual lesions, he was able to duplicate them very
 21 well.
 22 But the scoring, in terms of the severity of
 23 the lesion, was where he deviated. Those deviations
 24 were not striking. If it got a score of a one versus a
 25 two, that type of difference might have occurred. If

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1 it was a high number like a four, he did not score it
 2 down to a one.
 3 So, it was not a striking difference in his
 4 scoring abilities. But, at that time, I viewed as
 5 somewhat as a subjective interpretation, and because of
 6 that, I recommended that the scoring would be
 7 collapsed. In doing so, it made it more likely to be
 8 able to detect effects, but the registrant themselves
 9 actually conducted their statistical analyses, based on
 10 the score data, as opposed to the collapsed data.
 11 DR. HEERINGA: Dr. Frankenberry.
 12 DR. FRANKENBERRY: This is Mary
 13 Frankenberry. I think we collapsed the upper levels
 14 into a more severe score and then a less severe score,
 15 or, and I think it was, actually, all effects and then
 16 the most severe. And with regard to Dr. Miller's point
 17 about it's contributing to the variability, I think we
 18 did, we probably eyeballed that only before making the
 19 decision to combine them. And, my recollection is that
 20 the numbers were so infrequent that it, we didn't think
 21 it would affect them. On the other hand, if they were
 22 extreme enough, I suppose that could have happened, and
 23 we should go back and we'll look at that.
 24 DR. HEERINGA: Dr. Miller.
 25 DR. MILLER: Just to clarify, then, so

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1 you did or did not include them in the statistical
 2 analysis?
 3 DR. FRANKENBERRY: We combined
 4 everything
 5 into the effect or no effect. The company looked at
 6 severe effect or any effect.
 7 DR. HEERINGA: At this point, it's 12:05,
 8 and we all deserve lunch. So, I'm going to recommend
 9 that we break now until, let's make it until 1:30, and
 10 we'll reconvene at 1:30 and pick up with charge
 11 question number four. But we're making good progress
 12 with the agenda, and I think we're having a fairly
 13 thorough discussion on each of these items, and so, I'm
 14 very pleased. Thank you. See everyone at 1:30.
 15 (WHEREUPON, the morning session was concluded.)
 16 DR. HEERINGA: This is the meeting of the
 17 FIFRA Science Advisory Panel on the topic of the
 18 potential for Atrazine to affect amphibian gonadal
 19 development. At this point in the process, we have
 20 begun to consider the charge questions that have been
 21 presented to the panel, and we have completed
 22 discussion of charge questions one through three, and
 23 are about to move on to question four.
 24 But before we do, Dr. Steeger and Joe Bailey
 25 and I had a discussion. Dr. Steeger indicated that one
 of the studies that was mentioned in the discussion of

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1 the charge question this morning, and relevant to the
 2 literature on this topic, the study published by Dr.
 3 Carr, that there's been some re-analysis. And Dr.
 4 Steeger has asked permission for Dr. Carr to present
 5 that re-analysis at this point in time, and we've
 6 granted that, I've granted that at this point.
 7 DR. STEEGER: The original work done by
 8 Dr. Carr on the analysis of whether Atrazine affected
 9 amphibian gonadal development was based on gross
 10 gonadal morphology. Subsequent to the publication of
 11 his article, Dr. Carr worked with experimental
 12 pathology labs with Dr. Wolfe and, actually, conducted
 13 histology on those same animals. And I've asked Dr.
 14 Carr if he would be willing, along with Dr. Wolfe, to
 15 provide a very brief overview of what the results of
 16 the histological analysis of those study samples were.
 17 DR. CARR: Jim Carr, Texas Tech
 18 University.
 19 DR. WOLF: Jeff Wolf, EPL, Incorporated.
 20 DR. CARR: There was some discussion this
 21 morning about some of the previous studies.
 22 And one of the things that came up was the
 23 question of intersex gonads in the Carr, et al, report
 24 from 2003. And what we have done, subsequent to that
 25 study, is taken slides that were read for that study of

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1 animals that had ambiguous gonads, that we could not
 2 tell were male or female, based on gross morphology,
 3 and sent those to EPL for them, and this was done
 4 blinded, for them to do their analysis, in an attempt
 5 to harmonize terminology, make sure that we're calling
 6 things the same when we see them at the microscopic
 7 level, and EPL prepared a report, based on the re-
 8 analysis of those slides. And they were also sent
 9 slides from the Cardell study from Michigan State,
 10 which was also reviewed as an interim report for the
 11 2003 SAP. And, I think we can make a copy of that
 12 report available to the SAP.
 13 In the 2003 Cardell paper, we used the term
 14 intrasex at the gross morphology level to identify
 15 animals that we couldn't sex as male or female at the
 16 gross morphology level. That was the initial protocol.
 17 And upon subsequent histological analysis, as we
 18 reported in our paper, we could tell at the histology
 19 level that they were males and females. This was about
 20 twelve out of three hundred animals. It was about 4
 21 percent of the 25 PPV study group.
 22 But we could tell that they were males and
 23 females. And, so, subsequent to that study, with EPL
 24 developing these more rigorous criteria for evaluation
 25 of intersex, as well as a lot of other things, we sent

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1 those slides to Dr. Wolf. And, do you want to mention
 2 what you found?
 3 DR. WOLF: So, we received the slides,
 4 and we also received, although I was blinded to it at
 5 the time, the histologic assessments that had been done
 6 previously. And, essentially, I think except in one
 7 case, I agreed with their histologic assessments,
 8 which, actually, instead of saying ambiguity, they used
 9 terms like male-like, or female-like. And I confirmed,
 10 well, that is a male or that is a female. So, there,
 11 really, isn't that ambiguity there.
 12 DR. CARR: All right and we discussed
 13 that in the overview document that's on the docket that
 14 was submitted by the eco risk Atrazine panel, and
 15 that's on page 38, and we cite the EPL report. And I
 16 think the EPL draft report can also be made available
 17 to the panel, so that they can see this subsequent
 18 histological analysis.
 19 DR. HEERINGA: Thank you very much, Dr.
 20 Carr and Dr. Wolf. Panel members, any follow up
 21 questions on this. It seems as though the data is
 22 available to us in the eco risk report that was
 23 provided to us yesterday morning. And if there's
 24 interest we can apparently see the draft report of Dr.
 25 Wolf's analysis, also.

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1 But, not seeing any additional questions at
 2 this point, we appreciate that clarification and
 3 additional information on this point, and we'll
 4 certainly take that into consideration in deliberations
 5 here. Thank you Dr. Carr and Dr. Wolf. And I think,
 6 in our order of question entry, it's Ms. Pease is up to
 7 bat, here.
 8 MS. PEASE: Okay, question number four.
 9 The Agency has described exposure profiles for studies
 10 conducted in response to the DCI and has stated that
 11 mean measure concentrations in the studies were lower
 12 than the target nominal concentrations. However, the
 13 Agency concluded that the frequent analytical
 14 measurements provide a sufficiently comprehensive
 15 understanding of the exposure profile over the course
 16 of the studies.
 17 Please comment on the Agency's conclusion
 18 that the Atrazine exposure concentration profile is
 19 reasonably characterized and sufficient for documenting
 20 the potential effects of Atrazine over a broad range of
 21 exposure concentrations. In addition, provide comments
 22 on whether the actual concentrations were consistent
 23 and sufficiently stable to establish the means to
 24 analyze exposure concentration response relationships.
 25 DR. HEERINGA: Our lead discussant on



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1 this is Dr. Schlenk.
 2 DR. SCHLENK: Dan Schlenk, UC Riverside.
 3 Again, in conversations with Peter and Bruce, we, this
 4 is sort of a synopsis, but I'm sure they'll have their
 5 comments as well after this, but. As mentioned
 6 earlier, EPA attempted to bracket concentrations of
 7 previously demonstrated adverse effects in test
 8 species. The lowest of these was .01, or .1 micrograms
 9 per liter.
 10 In order to demonstrate a dose response
 11 effect, a 0.01 microgram per liter concentration was
 12 utilized. This was also the LOQ for the toxic end. It
 13 is very difficult to carry out an exposure at the LOQ,
 14 and WLI should be commended in this effort, since they
 15 were able to, actually, maintain appropriate
 16 concentrations during the window of exposure.
 17 Since IGB was unable to maintain the exposure
 18 concentrations consistently, multiple evaluations of
 19 water chemistry were essential in both laboratories in
 20 determining accurate exposure concentrations. Given
 21 the LOQ, it probably would have been more appropriate
 22 to use something like .05 micrograms per liters of
 23 bracketing concentration, as the IGB exposure failed to
 24 approach these values.
 25 In particular, the, and this is in the IGB

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1 study, the 0.01 microgram per liter was under the limit
 2 of detection, which is 0.005 micrograms per liter for
 3 tank seven on day thirteen, tank four, six, and eight
 4 on day twenty, and all of these were within the
 5 critical window of exposure.
 6 All tanks at the .1 microgram per liter
 7 concentration during the critical window from day
 8 thirteen and day twenty were below .1 microgram and
 9 were approximately .05, which is, would have been an
 10 appropriate value perhaps.
 11 But, of course, this is all sort of moot,
 12 since the adverse apical effects, again, in
 13 morphological and histological effects, were not
 14 observed consistently at the .1 microgram per liter in
 15 either lab. And the WLI laboratory was able to meet
 16 the exposure requirements during the critical window of
 17 differentiation for both concentrations.
 18 The Atrazine exposure concentration was
 19 adequately characterized when considering the studies
 20 together. And, again, this goes back to my earlier
 21 point. It was nice to, actually, have both studies
 22 done in both laboratories because, if the WLI hadn't
 23 been able to attain that, I think we would have had
 24 some issues with regard to the, that low dose, the
 25 lowest dose, or lowest concentration.

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1 So, and again, if the WLI had not been
 2 successful, a concentration of .05 might have been a
 3 better choice than, rather than the concentration
 4 equivalent to the LOQ. Again, one may wonder if this
 5 may be the reason why secondary effects were not
 6 observed in the IGB lab for the .1 microgram per liter
 7 nominal concentration, but just a thought. Overall,
 8 using a multiple chemical concentration analyses
 9 adequate to a allowed appropriate exposure over the
 10 range recommended by the 2003 SAP.
 11 DR. HEERINGA: Thank you very much, Dr.
 12 Schlenk. Dr. Delorme.
 13 DR. DELORME: And I'm just going to echo
 14 several things that Dr. Schlenk said. I'll just read
 15 what I've written.
 16 The frequency of analysis is sufficient to
 17 allow a good understanding of the exposure profile, and
 18 we have weekly samples over an extended period of time,
 19 gives us a good idea of what the exposures were in the
 20 different nominal concentrations each week. And it's
 21 well characterized over a broad range of
 22 concentrations, which encompass concentrations, which,
 23 in the past, have been associated with effects on
 24 gonadal development in *Xenopus laevis*.
 25 So, they did bracket properly the

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1 concentrations that before were of concern.
 2 I had some minor concerns with respect to the stability
 3 of the two lower exposure concentrations at IGB. These
 4 were considerably below nominal concentrations as noted
 5 by the EPA. And I had some concern that the measure of
 6 concentrations at 1 microgram per liter exposure
 7 concentration at IGB was a little low. It was reported
 8 at 72, a mean of 72 throughout, with a 74 percent in
 9 critical periods. I believe, normally, we would look
 10 for around an 80 percent of nominal to have it
 11 acceptable.
 12 So, that being said, for those three
 13 concentrations, you may want to consider in your
 14 report, reporting against a mean average concentration
 15 or time weighted averages of some respect, rather than
 16 the nominals, because they are so different. I think
 17 that would, you know, provide a little bit extra
 18 information.
 19 And as Dan noted, the establishment of an
 20 exposure level at the LOQ, I don't think is a really
 21 good thing to do, and would encourage or recommend that
 22 in the future, EPA consider of establishing a guideline
 23 for a minimum distance between lower doses and LOQ,
 24 just to avoid problems in the future.
 25 We both noted, I went through the raw data

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1 that was in the Syngenta report that we received from
 2 Syngenta. There are a number of instances that Dan
 3 noted where there were no detects at the lowest dose
 4 level. And had there been effects at that dose level,
 5 it could have seriously compromised your ability to
 6 interpret the results.
 7 So, there's a number of tanks where, during
 8 the critical period, there is apparently no Atrazine,
 9 based on the data that's in that document. So, I'd be
 10 careful about that. Other than that, everything else
 11 is.
 12 DR. HEERINGA: Bruce Pauly.
 13 MR. PAULY: I would concur with both Dr.
 14 Schlenk and Dr. Delorme. I think that the frequent
 15 analytical measurements provide a sufficiently
 16 comprehensive understanding of the exposure profile,
 17 but again, I might come back to the general question,
 18 as I did this morning.
 19 We are, we have got data on the parent
 20 compound, and that maybe the question should be that
 21 the exposure to parent Atrazine was adequately assessed
 22 over the course of the experiments, and I won't belabor
 23 the discussion we had this morning about the
 24 possibility that there are degradates, metabolites in
 25 there. That said, I think that I would concur that

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1 there are sufficient data to assess whether or not
 2 these, the exposure concentrations were well
 3 characterized.
 4 DR. HEERINGA: I just want comments from
 5 members of the panel. Dr. Steeger, I believe what I
 6 heard that we had a fairly clear and consistent
 7 response from all of the primary discussants of this.
 8 Are there any questions that you have, or do you feel
 9 that we've addressed this question satisfactorily?
 10 DR. STEEGER: I think the question has
 11 been satisfactorily addressed. I would comment,
 12 though, that the registrant notified us before the
 13 start of the study that the lowest test concentration
 14 would be at the level of quantification.
 15 They had originally proposed testing to .1
 16 micrograms per liter, which has, in previous studies,
 17 been identified with effects on gonadal development.
 18 The Agency insisted that they test at a lower
 19 concentration that suitably bracketed the effects
 20 concentrations in previous studies, and would account
 21 for a potential U-shaped curve, if one did, indeed,
 22 exist.
 23 My recommendation to the principal
 24 investigator was that he attempt to sample larger
 25 volumes of water, possibly using salt phase extraction,

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1 so that they could concentrate the sample and better
 2 count for their level of quantification. My
 3 understanding was that they were limited in the volume
 4 of material that could be transported back to the
 5 analysis lab, which was at Syngenta, with, using tandem
 6 aspect.
 7 But, we were aware going into the study that
 8 it was, that the lowest test concentration was close or
 9 at their level of quantification, but we had hoped that
 10 there would still be reasonable recovery. And at
 11 Wildlife International, they were, indeed, able to be
 12 fairly close to the nominal concentrations at 0.1
 13 micrograms per liter.
 14 DR. HEERINGA: Thank you very much. Dr.
 15 Schlenk has a follow up comment.
 16 DR. SCHLENK: Yeah, just, again, let me
 17 reiterate. I think you didn't have to be stuck on the
 18 .01 concentration. You could've gone up, you know,
 19 fivefold on that, and still been, and got the cursive
 20 view, if you were trying to demonstrate that dose
 21 response curve.
 22 DR. HEERINGA: I think that I'd like to
 23 move on, then, to question five at this point in time.
 24 And ask - -
 25 DR. IRENE: Stephanie Irene, question

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1 five. The Agency described Atrazine contamination of
 2 negative controls in one out of two studies, and
 3 concluded that, since the experimental design had twice
 4 the number of controls, relative to other treatments,
 5 the data from these Atrazine contaminating controls
 6 could be removed from the analyses without invalidating
 7 the statistical interpretation of the results.
 8 Please comment on the Agency's decision to
 9 omit half of the controls from the Wildlife
 10 International study in the statistical analyses, and
 11 on the conclusion that the study is still
 12 scientifically valid. If the SAP has an alternative
 13 approach to treating these control data in the
 14 statistical analyses, please provide specific
 15 recommendations.
 16 DR. HEERINGA: Dr. Portier.
 17 DR. PORTIER: I like easy questions. I
 18 concur with the decision to omit the contaminated
 19 controls from the WLI study, and have no alternative
 20 methods for treating or using these control data.
 21 Also, the control clusters does not invalidate the
 22 overall WLI study, nor does it have a large effect on
 23 the ultimate power of the study comparisons.
 24 DR. HEERINGA: I think that's the
 25 briefest I've heard you. Dr. Bailey.

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1 DR. BAILEY: The study is valid. Not
 2 using half of the controls may have been unfortunate,
 3 but it's not a problem. Thank you.
 4 DR. YEATER: Kathy Yeater. I also agree
 5 that there is no effect on the validity of the study by
 6 removing these tanks.
 7 I just wanted to comment, also, on how the
 8 nature of the study led to a discussion earlier on the
 9 appropriate laboratory layout of the tank, mixing
 10 container, pump setup. And I really appreciated the
 11 discussion on the possibility of, either, the one pump
 12 per tank, which could have resulted in increased heat,
 13 and also, by affecting increased confounding in the
 14 study; or one pump per eight tank cluster, which would
 15 have caused greater restriction and more problems, due
 16 to the fact that we had to toss some tanks.
 17 So, also regarding the comments earlier on
 18 the assessment of a possible cluster effect, I don't
 19 disagree with the use of the tank to be the unit of
 20 analysis, with the understanding that it is not a true
 21 experimental unit.
 22 DR. HEERINGA: Thank you very much, Dr.
 23 Yeater. Other comments on this particular question
 24 from the panel? Dr. Steeger, I think the answer was
 25 fairly concrete, and do you have any comments or

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1 questions?
 2 DR. STEEGER: No comments.
 3 DR. HEERINGA: Before everybody runs to
 4 re-book their flights, we won't -- So, we're on to
 5 charge question number six, Ms. Pease.
 6 MS. PEASE: Anita Pease. The original
 7 white paper from 2003 identified measurement endpoints
 8 that included the possible enumeration of specific
 9 histological structures, such as the number of oogonia
 10 in ovaries and the number of spermatids in testes.
 11 Such a detail analysis was not conducted in the studies
 12 that are in response to the DCI. Rather a qualitative
 13 assessment of the instance of ovarian and testicular
 14 gonad oocytes was conducted.
 15 The Agency has concluded that the lack of
 16 these data does not limit the means to assess
 17 hypothesis that Atrazine exposure affects amphibian
 18 gonadal development. Please comment on whether the
 19 lack of these histological data limits the utility of
 20 the available information to support the hypothesis
 21 that Atrazine exposure affects amphibian gonadal
 22 development.
 23 And B., if the SAP concludes that these data
 24 are necessary to adequately assess the hypothesis,
 25 please provide options to processing, processing and

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1 analyzing the data in an efficient and robust manner.
 2 DR. HEERINGA: Dr. Miller.
 3 DR. MILLER: Debra Miller, UGA. First of
 4 all, it's excellent to have multiple endpoints and
 5 generally good selection, because they're doable. As
 6 discussed in, back in number three, on the mission of
 7 the number of oogonia in ovaries and the number of
 8 spermatids in testes is acceptable, because, basically,
 9 they were not doable. And, qualitative assessment is
 10 acceptable, given that scoring was performed.
 11 With the clarification of the scoring
 12 variation, I don't have an issue with that. But I
 13 would recommend that you revisit the statistical
 14 analysis and do so with Dr. Wolf's input. Dr. Steeger
 15 is correct in part that pathology is very subjective.
 16 Pathology is often described to be more of an art than
 17 a science, but in research, we have to combine the two,
 18 and mold art into science, and scoring is one way that
 19 we do that.
 20 Combining the scores may be acceptable, but I
 21 would do so with Dr. Wolf's guidance. Example,
 22 yesterday, renal mineralization was brought up, and
 23 yes, no one wants mineralization in their kidneys or
 24 gonads.
 25 But, Dr. Wolf point out, renal mineralization

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1 is not uncommon in fish, and in my experience, it's not
 2 uncommon in amphibians or reptiles either. Thus, a
 3 renal mineralization score of 1 could easily be
 4 expected in almost all of the test subjects, because it
 5 can be a common response to various stresses on that
 6 organ.
 7 But a score of 2 may be significant, and
 8 thus, it may not be appropriate to group scores 1 and
 9 2, and similarly, for the various gonadal scores. So,
 10 you might wonder why to revisit the analysis with Dr.
 11 Wolf's guidance.
 12 It's because he can tell you whether or not
 13 it's appropriate to group those scores. With regard to
 14 whether the data collected support that hypothesis that
 15 Atrazine exposure alone affects amphibian gonadal
 16 development, and here, I need to state that I'm not
 17 really certain if using the term amphibian is really
 18 correct, because, are we including caught eggs in that,
 19 or just anurans and, perhaps, more specifically, should
 20 we keep saying Xenopus laevis.
 21 I think you are addressing morphologically
 22 and, or anatomically, if you will, development. But
 23 this does not, necessarily, equate to function. And to
 24 evaluate whether or not development was complete or
 25 successful, it's necessary to follow through to the



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1 adult stage and assess reproductive ability, i.e., was
 2 that individual able to reproduce, and was reproductive
 3 output changed, increased or decreased.
 4 As Dr. Petino stated, you presented a grow
 5 out study that was impressive, but there were aspects
 6 of that study that may fall a bit short, such as, what
 7 was the age inside sexual maturity, because I believe
 8 you waited until they were two years of age, and then
 9 you chose that were sexually mature for your study,
 10 which may enter in some bias.
 11 So, for B., as far as study design, as I
 12 indicated, I would recommend revisiting your analysis
 13 with, on the histological parameters, and, perhaps,
 14 consider incorporating size and age of sexual maturity
 15 into your broad study.
 16 DR. HEERINGA: Thank you, Dr. Miller.
 17 Dr. Green.
 18 DR. GREEN: Sherril Green, and I concur
 19 with Dr. Miller in all her points. The only other
 20 additional point I have to make is a very fundamental
 21 one, that I still think we should consider that we
 22 really don't know what is normal in *Xenopus laevis* in
 23 terms of some of these gonadal that we are, gonadal
 24 differences that we're calling abnormalities.
 25 And would this be something that we'd see in

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1 a very large population of healthy *Xenopus* in the wild
 2 that would go on to develop without incidence and
 3 continue to reproduce normally. And that's my only
 4 other comment.
 5 DR. HEERINGA: Dr. Petino.
 6 DR. PETINO: Yes, I agree with, Reynoldo
 7 Petino. I agree with what has been said so far, and
 8 that the lack of the histological data does not impair
 9 the ability of the study to determine, to assess the
 10 hypothesis.
 11 And the reason being, you know, we talked
 12 earlier about the same, the same situation, but you
 13 know, the numbers of cells and the types of cells
 14 present at the time that this anomalies were analyzed
 15 are, you know, that's a dynamic state of changes at
 16 that point in time, that also depends not only on the
 17 state of development, but also on the age of the
 18 animal. So, there's a, you know, counting cells, at
 19 this point in time, may not have a lot of value.
 20 So, and as we discussed, and it's been
 21 repeated that there's other ways to assess the effects
 22 on gonadal genesis and, or, I guess, more to the point,
 23 fecundity, or reproductive fitness, better ways to do
 24 that than, than, you know, this, this endpoint that was
 25 included in the study. So, not having that endpoint

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1 does not effect the study's conclusion, overall study's
 2 conclusions.
 3 DR. HEERINGA: Thank you, Dr. Petino.
 4 Other members of the panel have comments to contribute
 5 on this particular charge question? Dr. Portier.
 6 DR. PORTIER: I was just going to say, we
 7 are going to address some of the analysis issues
 8 related to this in question 9. B.
 9 DR. HEERINGA: Thanks. Dr. Steeger, I'll
 10 turn to you again, before we move on, to make sure that
 11 a clear interpretation of what was said, and if you
 12 have any questions for the panel, clarification on
 13 their comments.
 14 DR. STEEGER: My interpretation of what
 15 the panel said is that the endpoints that were measured
 16 were suitable. And that if additional endpoints, or
 17 additional information was needed, a further
 18 examination of the grow out study that the registrant
 19 conducted may be appropriate. Is that correct? Thank
 20 you.
 21 DR. HEERINGA: Dr. Green, do you concur
 22 with that? Your questions, of course, will, the final
 23 report will reflect the full discussion. Any other
 24 comments on this particular item from the panel? Let's
 25 move right along then to charge question. I'll ask Dr.

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1 Irene to read it.
 2 DR. IRENE: And as you said, moving right
 3 along. Question number seven. The Agency has
 4 described a number of measurement endpoints, for
 5 example, translucent gonads, unpigmented ovaries, and
 6 pigmented testes, based on the histology results that
 7 were reported in the studies.
 8 The Agency, however, based on its
 9 understanding of relevant scientific literature, could
 10 not conclude that these measurement endpoints are
 11 biologically relevant indicators of the effects on
 12 growth or reproductive success.
 13 That is, the Agency did not interpret these
 14 responses as adverse effects, per se; nor was the
 15 Agency aware of any information that established these
 16 responses as precursors to the apical endpoints of
 17 primary interest; time to size at metamorphosis, sex
 18 ratio, and the presence of mixed and or intersex
 19 animals. Please comment on the biological relevancy of
 20 these endpoints, and the extent to which they may
 21 reflect reliable measures in developmental
 22 abnormalities.
 23 DR. DENVER: Well, I'm not aware of any
 24 literature that links translucent gonads, unpigmented
 25 ovaries, or pigmented testes to effects on growth or



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1 reproductive success. That's not to say that these
 2 phenotypes are not associated with fitness
 3 consequences, but to my knowledge, as I said, such
 4 relationships have not been described.
 5 The lack of pigmentation could reflect
 6 abnormal migration of cells from the neuro crest, but
 7 presumably, that's not the case in these studies,
 8 because the exposure was initiated well after that
 9 developmental stage.
 10 Alternatively, it could be the failure to
 11 express melanin in the melanocytes. But, I don't know
 12 what the significance of internal melanocytes are on
 13 internal organs. And I'd like to ask anyone in the
 14 room if they do know. I think it's a fascinating
 15 question, but I have no idea what the significance is.
 16 DR. HEERINGA: Dr. Leblanc.
 17 DR. LEBLANC: The value to get these,
 18 what I view as secondary endpoints, are certainly to
 19 provide support when effects are observed on more
 20 apical endpoints. That is, endpoints that we, as
 21 biologists, can ascribe with a little bit more
 22 confidence, some ecological relevance, some effect on
 23 fecundity.
 24 So, for example, if we observe that male
 25 frogs have feminized testes, we're concerned about

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1 that, and that may go into the formula in describing
 2 ecological relevance; whereas, hypoplastic testes may
 3 not. However, in conjunction, it may provide
 4 additional support. It gives us confidence in our
 5 decision making. However, in the absence of effects on
 6 these more apical endpoints, I don't see much value to
 7 these secondary endpoints. So, I guess I concur.
 8 The, I can't we can't lose sight of the fact
 9 that there is always the possibility that there's some
 10 biological significance associated with these secondary
 11 endpoints directly, or that they are a precursor to
 12 some biologically significant effect. And I think EPA
 13 has acknowledged that. But at this point in time,
 14 that's only speculation. I don't, I'm not aware of any
 15 information in the literature to support that. That's
 16 all. Thank you.
 17 DR. HEERINGA: Dr. Petino.
 18 DR. PETINO: I agree, Reynoldo Petino. I
 19 agree, I don't think I have much new to contradict, so
 20 I'll just agree with what's been said. Thank you.
 21 DR. HEERINGA: Dr. Miller.
 22 DR. MILLER: Debra Miller. I agree with
 23 what's been said, too, and I will just comment.
 24 Pigmentation is one of those things that we don't
 25 really have a good grasp on why it occurs or doesn't

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1 occur. We don't know the biological significance of it
 2 in many cases. There is some thought that it might
 3 help with UV exposure and things like that. But we do
 4 see, however, and it's a good point, that you always
 5 have to combine these things and put together the whole
 6 picture.
 7 Because, if you see some sort of irritant to
 8 a specific organ, pigmentation in amphibians and fish
 9 and reptiles, it's one of the things that tends to
 10 increase in many cases, or it can decrease in the skin,
 11 depends on the organ. And so, it can be an indicator
 12 that that organ is distressed in some way. But again,
 13 you always have to look in a big picture. In this
 14 case, you know, I agree with what's been said, in that,
 15 we don't really understand the biological significance
 16 of it.
 17 DR. HEERINGA: Dr. Denver.
 18 DR. DENVER: I guess we don't know, but
 19 correct me if I'm wrong, whether there were any other
 20 pigmentary changes in these animals, other than the
 21 gonads. I don't know if that was recorded.
 22 DR. HEERINGA: You mean internally, Dr.
 23 Denver?
 24 DR. DENVER: Internally or externally.
 25 DR. HEERINGA: Dr. Steeger.

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1 DR. STEEGER: If there were changes in
 2 pigmentation, it would have been noted on a gross
 3 morphological basis for any of the organs that were
 4 evaluated, and for the external appearance of the
 5 animal. None of which is, was specific to either one
 6 of those.
 7 DR. HEERINGA: Comments from other
 8 members of the panel on this particular question. Dr.
 9 Delorme.
 10 DR. DELORME: I forget who it was
 11 yesterday, but somebody made the comment that
 12 without a
 13 grow out, there's always going to be some uncertainty
 14 as to the importance of these kinds of effects.
 15 So, just for the record, I mean, I just went
 16 back and looked at the original panel report from '03,
 17 and the panel did suggest that some grow out studies be
 18 started immediately as well. I understand the
 19 logistics behind it are horrific, but again, it's going
 20 to remain an uncertainty, I think. It may be nothing,
 21 but we won't know.
 22 DR. HEERINGA: Dr. Steeger, I think, get
 23 your reaction and any questions of clarification or
 24 follow up points on this charge question.
 25 DR. STEEGER: My interpretation is that
 the panel concurs that it's an uncertainty.



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1 DR. HEERINGA: Moving so fast here, I
 2 don't even keep up with the numbering. I only have to
 3 go even odd, 'cause I know that Dr. Irene did the last
 4 one, so Doctor, Ms. Pease, number eight, please.
 5 MS. PEASE: Question eight. The Agency's
 6 analysis of potential developmental effects and study's
 7 responses to the DCI has focused on histological data,
 8 as opposed to gross morphological data. Histological
 9 data from these studies were based on the analyses of a
 10 single certified pathologist. While this approach
 11 eliminates the potential variability associated with
 12 having multiple pathologists analyze histological
 13 slides, it may introduce an avidity bias.
 14 Please comment on whether a single
 15 pathologist is sufficient for interpreting the
 16 histology data. If the SAP believes that a single
 17 pathologist is not sufficient, please comment on the
 18 potential value of convening a pathology review board
 19 to evaluate findings of the DCI study.
 20 Please also comment on the potential value of
 21 having a pathology review board evaluate materials, for
 22 example, the digital images of gross morphology and
 23 microscope slides containing histological sections from
 24 studies published in the open literature. These data
 25 can be submitted voluntarily by the authors and could

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1 include slides to evaluate similarities or differences
 2 in identifying or describing histological features, and
 3 or describing and quantifying histological responses.
 4 DR. HEERINGA: Dr. Miller.
 5 DR. MILLER: Debra Miller. First of all,
 6 I do not expect to get through this question today.
 7 First of all, as far as, I believe it was a good choice
 8 to choose Dr. Wolf. There are very few pathologists
 9 that have a lot of experience with amphibians, and
 10 experience with fish, reptiles is always a good
 11 addition. Internal correction, and I mentioned this
 12 this morning, of a bias is possible if you have that
 13 one pathologist read the slides like three times.
 14 But if that, the best way to function, I'm
 15 not really sure that that is, and in many experiences,
 16 especially when you're talking about a study that's
 17 designed for regulatory purposes, a panel can be very
 18 advantageous, and we tend to use panels a lot when
 19 there are large scale studies.
 20 So, although it may be adequate or
 21 appropriate for a single pathologist, there's that old
 22 adage that if you ask five pathologists, you get five
 23 opinions. And that the panel of three or so
 24 pathologists can be best to, for studies designed for
 25 regulatory purposes.

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1 And in this case, one pathologist is usually
 2 designated as the lead, for example, Dr. Wolf, and then
 3 all the pathologists look at the slides separately, and
 4 write their findings, and the lead does this as well.
 5 And then distributes the findings to the others. And
 6 they all convene in a multi-headed scope to discuss
 7 discrepancies. And then, what generally happens after
 8 that is, the lead pathologist submits the group's
 9 consensus.
 10 And this is how histopath is generally done,
 11 like, with marine mammal species, which is one of the
 12 areas that I've worked, and this is how we've done it.
 13 And it's worked quite nicely. Or another way that you
 14 can do it is have the pathologists separately review
 15 the slides and submit them separately. And then you
 16 add in that into your statistical analysis. This may,
 17 you know, I'm not a statistician, but this may,
 18 actually, be more, add more soundness to the
 19 statistics.
 20 The second part, I'm not really sure exactly
 21 what you're suggesting here. As written, I'm not sure
 22 it would be necessarily appropriate, but, yeah, it
 23 would be great if you could researchers to send in
 24 their slides for a panel pathologist to review.
 25 However, the reason that I'm not sure it would be

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1 appropriate or all that useful is because collection is
 2 a huge factor.
 3 You have to make sure that everything was
 4 collected in the same way. That the serial sections
 5 were done in the same way. The stainings the same, it
 6 all makes a difference. Just, you know, one slide from
 7 a testes that's done at one facility can be totally
 8 different than what's done at another facility. And
 9 so, yeah, you could do it, but you would have to give a
 10 lot of weight to how you interpret those results.
 11 DR. HEERINGA: Dr. Green.
 12 DR. GREEN: On point A, I think ideally
 13 you would like to have more than one pathologist review
 14 those slides. There would be a, if it were me doing
 15 100,000 or 83,000 slides over a period of time, there
 16 would be some, I believe, the statisticians call it,
 17 inter-observer error after a while.
 18 So, to repeat the test, as Dr. Miller
 19 suggested, three times and taking an average or do the
 20 actual statistics to see how good that pathologist is
 21 in calling and recalling the same variations over and
 22 over, would be very useful.
 23 In terms of having a pathology review board
 24 look at the slides from a particular study, I think Dr.
 25 Wolf has done a fine job. It's, his interpretations I



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1 agree with. I think the slides are good quality. It
 2 seems relatively straightforward to detect gonadocytes
 3 and gross morphology differences as well.
 4 So, if you did have a panel of people
 5 reviewing that particular set of slides, then, again,
 6 as, although I'm not a statistician, I should think
 7 that it would add strength to the study statistically
 8 if inter-observer agreements were reported, so that we
 9 can get a standardized, this set of pathologists all
 10 looked at it and they're pretty close in agreement that
 11 this is what it is.
 12 But again, I think, Dr. Wolf has done a great
 13 job with that, and I feel fairly comfortable looking at
 14 most of those slides, and can see what he is pointing
 15 out. So, I think it is a straightforward evaluation.
 16 And I concur with Dr. Miller about the
 17 utility of having a panel of pathologists look at sets
 18 of slides from the open literature, because of the
 19 variations you're going to get in processing and serial
 20 sectioning and thickness and all that. It might not be
 21 so helpful. So, other than that, I have nothing else
 22 to comment on.
 23 DR. HEERINGA: Dr. Petino.
 24 DR. PETINO: Reynoldo Petino. I agree
 25 with what's been said. I think, I agree with the fact

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1 that normally you would want to have, when you,
 2 especially when you're ranking, providing grading ranks
 3 on categorical variables, it would be ideal to have
 4 more than one individual do the gradings. And then
 5 having, if there's any disagreements, have the
 6 individuals, I mean, this is standard practice, for
 7 example, reading otoliths for aging fish.
 8 If you have two individuals reading the
 9 otoliths and then, if there's a disagreement, there's a
 10 discussion, and if they cannot agree, well, there's
 11 procedures on how handle that data. But, so, ideally,
 12 then, it would have been nice to have more than one
 13 pathologist.
 14 But I also agree with the conclusions of the
 15 other two discussants, that, you know, the parameters
 16 of it were being looked at and all the catalog of
 17 slides provided for us to understand what the, what was
 18 being looked at was very clear here. So, I don't know
 19 what the value at this point in time would be of having
 20 those slides re-reviewed by a panel. But, that's not
 21 really my area of expertise, so I'm just saying that I
 22 rely on the bonafide pathologists here to give you that
 23 advice, but, that's what I think.
 24 DR. HEERINGA: Thank you, Dr. Petino.
 25 Dr. Portier.

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1 DR. PORTIER: I can't even contemplate
 2 looking at 100,000 slides, but one thing I can think
 3 about is that if you were going to do this, I would
 4 definitely recommend some kind of structure, stratified
 5 sampling approach, where they don't, you know, your
 6 panel doesn't have to recreate every slide that Dr.
 7 Wolf has looked at.
 8 It's clear that he's looking at certain
 9 things on certain slides, and so you get
 10 representatives of each of those things. And you just
 11 see how consistent your panels are. And that would
 12 give you your inter and intra observer variability, and
 13 that should give you a feeling for how reliable the
 14 overall study is.
 15 So, my recommendation would be to not
 16 recreate the whole thing, but actually, be a little
 17 smart and design a study that gets at resources of
 18 variation to figure out where you would have, expect
 19 variation among your experts, and where you would
 20 expect them to be pretty consistent.
 21 DR. HEERINGA: Dr. Yeater.
 22 DR. YEATER: Kathy Yeater. Just a
 23 further comment about getting a panel to read and a
 24 stratification setup, also, that would allow for more
 25 randomization to occur in the reading of the slides.

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1 As was noted in the Syngenta report, Dr. Wolf was
 2 unable to truly randomize the reading of the slides by
 3 treatment group, because he was getting them as they
 4 were coming at him out of the lab, and he just had to
 5 go through and start working through reading all those
 6 slides. So, I would think also that having a panel and
 7 some sort of stratification design would help in that
 8 randomization, so that you wouldn't have any sort of
 9 trend problems.
 10 DR. HEERINGA: Additional comments from
 11 the panel. Maybe for Dr. Yeater and Dr. Portier, a
 12 question comes to my mind. In recommending a
 13 replication or, essentially, an inter rater reliability
 14 test, even on a sample basis, the objective here would
 15 be, sort of, quality assurance, quality measurement,
 16 quality of assessment.
 17 Not something that would, ultimately, then,
 18 proceed to some sort of measurement error or adjustment
 19 in the data. It would just be an additional step in
 20 quality assurance.
 21 I heard a lot of confidence in Dr. Wolf's
 22 reading and quality of the preparation, but we had one
 23 assessment, so, no statistician could sit here and say
 24 we have est-, I mean, could be highly accurate and zero
 25 variance, or it could be zero variance and not so

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1 accurate.
 2 We don't know, but I think the general
 3 consensus is that it was probably done well, but in
 4 terms of external validity, at this point, there would
 5 be benefit in, from a Q-A perspective, and possibly, at
 6 least a sample base. I don't want to overstate things,
 7 but is that what you're suggesting? Additional
 8 comments, Ken.
 9 DR. PORTIER: I was going to say, this is
 10 an, this is different than what Dr. Miller was saying
 11 about averaging the results from multiple experts. I
 12 mean, if the goal is really to try to get the, get to
 13 the truth for each slide, then certainly, you're going
 14 to have to get more than one expert, look at each
 15 slide, and average the results to get the right, closer
 16 to the true value of the scale, right.
 17 But I'm, I think the feeling is, what you
 18 probably need is some kind of assessment of the overall
 19 quality of the pathology work that's done. Something
 20 that would support your vague feeling that he did a
 21 really good job. You might have something that would
 22 back that up that would be short of redoing his study
 23 all over again.
 24 I mean, it's got to be months of work to re-
 25 read all those slides, and a lot more work for somebody

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1 to go in and put them all together, and average them
 2 all, and I don't get the feeling that the cost of
 3 that's going to match the benefit of doing that study.
 4 But a smaller study that I'm talking about could be
 5 done pretty quick by even a larger panel.
 6 That way, you could have, although it doesn't
 7 sound like there's a lot of experts out there you could
 8 draw on your panels. You can get all the experts in
 9 the world to look at a small set of slides.
 10 DR. HEERINGA: Dr. Miller.
 11 DR. MILLER: Yeah, because I, you know, I
 12 agree that Dr. Wolf's assessment is, you know,
 13 fantastic. And, what you're suggesting is to take a
 14 subset, just to, just kind of do a generalization, a
 15 quality assurance type of thing, and that would
 16 definitely be doable.
 17 DR. HEERINGA: Additional comments on
 18 this particular question from the panel. Yes.
 19 MR. PAULY: Bruce Pauly, Environment
 20 Canada. I just wonder, just to clarify, would we
 21 follow the approach of Dr. Yeater, maybe, or would it
 22 be recommended that an approach is followed where, by
 23 sent, slides were selected by some mechanism that
 24 would, then, be the subset that would be given to all
 25 the world experts in amphibian gonadal histology for

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1 assessment of the quality?
 2 DR. PORTIER: I think we were kind of
 3 saying the same thing. I mean, the hard part would be
 4 stratifying the slides into typical classes that you
 5 could, then, randomly select.
 6 And the thing is, you could randomize the
 7 presentation of these slides to each of the panel
 8 members so that they're not seeing a sequence that
 9 might lead you into getting into a pattern of looking
 10 for certain things and not being challenged the right
 11 way to get, and that was, I think, Dr. Yeater's idea is
 12 that, we're going, we can do more randomization within
 13 a broader stratification. The stratification makes
 14 sure you get coverage of all the conditions that he
 15 saw, right.
 16 So, the panel is to challenge, with all of
 17 the different kinds of things, but they don't have to
 18 be challenged a hundred times on each of those things.
 19 And we may only need three or four to ascertain that
 20 they can rely, they pick up that item.
 21 DR. HEERINGA: Ask a question of Dr.
 22 Miller and Dr. Green. SAP's had a habit of, sort of,
 23 recommending additional work. I think this clearly has
 24 a benefit to the ultimate integrity and evaluation of
 25 the study, in terms of quality assessment. My sense,

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1 naively, is that we don't want to just sample slides.
 2 We want to sample organisms, and then have the review
 3 on all slides for that organism. Because the ultimate
 4 determination is whether the organism, a frog in this
 5 case, has a particular, in other words, we're not
 6 analyzing at the slide level. So, I assume, all of
 7 these different sections that are taking, that there
 8 would be multiple for a frog, or am I wrong here?
 9 DR. MILLER: If I'm understanding you
 10 correctly, no, basically for this part, because it's
 11 based on the histology. And so, we are looking at the
 12 slides. Now, to be able to review, you know, the
 13 sticky part is, slow as it would comes in, is that, in
 14 order to get an assessment, a true assessment, you've
 15 got to do it on that same, you've got to pick certain
 16 slides, and everybody has to look at that same slide.
 17 Does that answer your question?
 18 DR. HEERINGA: I believe so, and you're
 19 probably right. I'm confusing everybody in good shape.
 20 DR. GREEN: I think, though, you would
 21 look at the same set of slides from a group of randomly
 22 selected animals on the study.
 23 DR. HEERINGA: Exactly, so you look at,
 24 for a randomly selected set of animals, you'd look at
 25 all the sections. That's what I'm getting at. Not

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1 just a random selection of slides across animals,
 2 because - -
 3 DR. GREEN: A statistician could choose,
 4 but you know, in the studies I've participated in, when
 5 we've done subsets, so that we didn't have to repeat
 6 work on hundreds and hundreds of animals, it may be as
 7 few as ten animals. But they might have had twenty
 8 slides, but we would each review them independently.
 9 DR. HEERINGA: And you would review all
 10 slides for each - -
 11 DR. GREEN: Three times.
 12 DR. HEERINGA: Thank you. Additional
 13 comments on this particular question. Let's, I see Dr.
 14 Bailey smiling. And I know that we're moving ahead a
 15 little faster on these charge questions, and I think
 16 even I anticipated, and certainly some members of the
 17 panel, but what I'd like to offer here is that, I'd
 18 like to continue, because I think we're making
 19 progress.
 20 But I'll also mention to the panel members,
 21 and to the EPA staff, that we will revisit these later
 22 on, potentially, tomorrow morning, so that there's an
 23 opportunity to go back to any question in which the
 24 presenters either haven't had a full time to prepare,
 25 or would like to, sort of, extend their comments on a

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1 particular question. So, we'll proceed, but also,
 2 allow the possibility of revisiting those questions
 3 just in case. We don't want to race through them. We
 4 want to cover them thoroughly, so, but let's move ahead
 5 at this point, Dr. Irene, with question number nine.
 6 DR. IRENE: After an evaluation of the
 7 laboratory based studies submitted in response to the
 8 DCI, the Agency has concluded that these studies do not
 9 provide sufficient evidence to support the hypothesis
 10 that Atrazine causes adverse gonadal developmental
 11 effects in amphibians. In light of the responses to
 12 questions three through eight, please comment on
 13 whether the results from the study in response to the
 14 DCI are sufficiently robust to address the hypothesis
 15 that Atrazine exposure causes gonadal abnormalities in
 16 Xenopus laevis. If the SAP concludes that these
 17 results are not sufficiently robust, what
 18 recommendations can the SAP provide to efficiently and
 19 reasonably address remaining uncertainties. For
 20 example, if the SAP does not believe that the DCI study
 21 is sufficiently robust to assess the hypothesis, does
 22 the SAP believe either of the two experiments, or a
 23 specific component of the two experiments should be re-
 24 analyzed or repeated. Please provide the rationale for
 25 recommending any additional analyses and or

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1 experiments. Question B.
 2 DR. HEERINGA: Dr. Irene, I wonder, let's
 3 stop at point.
 4 DR. IRENE: Stop with that, okay.
 5 DR. HEERINGA: I really think question
 6 nine is two questions.
 7 DR. IRENE: Okay.
 8 DR. HEERINGA: So, let's take up 9.A.
 9 first, and Dr. Delorme is the lead discussant on 9.A.
 10 DR. DELORME: Okay, first off, I found
 11 the question a little bit confusing, because the first
 12 part talks about supporting a hypothesis that Atrazine
 13 causes adverse gonadal developmental effects in
 14 amphibians, which is general; but question A. and B.
 15 specify the DCI study. I believe that I do have some
 16 concerns about the general question, but I think I'll
 17 wait and address those in question number twelve later
 18 on. And just concentrate on the specific hypothesis
 19 that Atrazine exposure causes gonadal abnormalities in
 20 Xenopus laevis, which is identified in sub-question A.
 21 And with respect to that particular
 22 hypothesis, I think what we've heard this afternoon is
 23 that we have a well-characterized exposure. There was
 24 some minor concern about whether or not transformation
 25 products should be included or not, but I think there

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1 are other avenues to pursue on that.
 2 We seem to have reasonably well-characterized
 3 effects, on the apical endpoints are particularly well-
 4 characterized. There is some question, I believe, with
 5 respect to the histology, and maybe in my answer or in
 6 our answer, we can refer back to the questions, the
 7 results of question eight and what comes of that. And
 8 the statistical analysis seems to have been very good.
 9 The study was well designed, and you know, I think
 10 that, in general, we could say that, yes, you know, we
 11 can address the hypothesis in a robust manner with the
 12 data that's been presented from the DCI.
 13 Again, there was a number of minor concerns
 14 that I had whether or not the specific strain used is
 15 appropriate, in light of the data presented by Syngenta
 16 related to relative sensitivity for Xenopus laevis. I
 17 think that's, that's the question. But it's going to
 18 have uncertainty. But there's nothing you can do about
 19 that with respect to re-analyzing.
 20 And, I noticed that this question targets the
 21 gonadal development. There was, there did seem to be
 22 some effects on growth, but whether or not that was
 23 biological or be relevant or not, I'm not sure. But I
 24 think that needs to be looked at as well.
 25 And, since, again, I wasn't really expecting

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1 to get to question nine today, I'm going to pass this
 2 on to associates.
 3 DR. HEERINGA: Dr. Denver.
 4 DR. DENVER: So, I first want to say that
 5 I concur, and that these studies were very well
 6 designed and controlled and robust. And they, they
 7 certainly addressed the hypothesis. If the question is
 8 whether these studies fully tested the hypothesis
 9 sufficient to allow one to reject it, that is, that one
 10 can now accept the null hypothesis, I think that the
 11 answer is no from the perspective of the scientific
 12 method.
 13 These studies provide mostly negative
 14 results. And, as I mentioned earlier in the day, and
 15 was discussed earlier in the day, the flow-through
 16 design allows one much greater control over dosing,
 17 water quality, and maintains the health of animals much
 18 better than the static renewal. But, it does not
 19 follow that the flow-through design better mimics the
 20 characteristics of exposure that's encountered in
 21 nature. And I'm not saying that the static renewal,
 22 necessarily, better reflects that, but strictly
 23 speaking, it addresses the hypothesis, but I,
 24 personally, I do not think it fully tests the
 25 hypothesis such that it can be rejected.

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1 DR. HEERINGA: Dr. Leblanc.
 2 DR. LEBLANC: Jerry Leblanc. I recognize
 3 that it's not the place of an SAP member to modify the
 4 charge questions, but I really have to modify the
 5 question before I answer it, so that, so my question,
 6 my answers are relevant. The question posed to us
 7 were, are results from the DCI sufficiently robust to
 8 address the hypothesis that Atrazine exposure causes
 9 gonadal abnormalities in Xenopus. And I simply have to
 10 qualify my answers in stating that, at exposure
 11 concentrations as high as 100 micrograms per liter,
 12 that I, or I don't think anyone else here, can make any
 13 comments about what might be happening at higher
 14 concentrations.
 15 That said, if we evaluate, revisit some of
 16 the major considerations in testing the hypothesis, or
 17 the conclusions, or addressing how the conclusions were
 18 reached, I feel that the studies were sufficiently
 19 robust. And what I'm referring to, specifically, is if
 20 we look at the strength of the concentration response
 21 relationships as being one criteria, there was no
 22 evidence for concentration response relationships, with
 23 regards to the major apical endpoints, sex ratios,
 24 intersex gonads, et cetera.
 25 There were, there was evidence for

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1 concentration response relationships, however, for some
 2 of the secondary endpoints, as we discussed previously.
 3 Another criteria, the strength of the cause effect
 4 relationship. To me, there are two things that go into
 5 answering that assessment. One is the strength of the
 6 observations themselves, and in this case, the
 7 significance, the statistical significance that was
 8 observed, but the effects tended to be quite modest,
 9 quite low. The other consideration here is, when
 10 effects were observed, were they reproducible between
 11 the two labs that conducted the studies, and typically,
 12 they were not. So, my conclusion of that is that
 13 strength of cause effect relationship, based upon these
 14 studies, was weak.
 15 Mechanistic plausibility, I think this
 16 question's addressed in more detail in one of the
 17 subsequent charge questions, but based on the
 18 discussions that I've heard thus far, and based upon my
 19 own readings, I see no mechanistic plausibility. We've
 20 heard hypotheses that would certainly be aromatase
 21 hypothesis, I think, has been evaluated rarely
 22 carefully, and I don't think there's any plausibility
 23 for the reported actions of Atrazine. And while it's,
 24 perhaps, dangerous to speculate, I've got to assume
 25 that proponents of the aromatase theory have tried

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1 wholeheartedly over the past several years to use
 2 aromatase in frogs, and I've yet to see any
 3 demonstration of that if it occurs.
 4 And lastly, ecological relevance of the
 5 effects, and as I mentioned, effects were observed,
 6 subtle effects. But again, as we've discussed in some
 7 of the previous charge questions, the ecological
 8 relevancy of these effects are unknown.
 9 DR. HEERINGA: Dr. Furlow.
 10 DR. FURLOW: So, I'm left with the idea
 11 that, strictly speaking, under these defined conditions
 12 with the strain of Xenopus that there does not appear
 13 to be the effect of Atrazine on gonadal development.
 14 That said, proving a negative is hard. And we have two
 15 studies here that were conducted extremely well. The
 16 animals were, obviously, quite healthy, quite robust,
 17 and when I see the data on the pigmentation changes, or
 18 some partial changes on growth that may or may not be
 19 consistent between the laboratories, it leaves one
 20 wondering if the health of the animals, when they're
 21 exposed to Atrazine, may change their sensitivity to
 22 the compound. But that's purely speculative on my
 23 point, on my part. So, other than the concerns I've
 24 voiced before, I, generally, agree with what's been
 25 said. But I do, I guess, also, side with Dr. Denver



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1 about the fact that these are two studies. So, I'll
 2 just leave it there, okay.
 3 DR. HEERINGA: Very good. Thank you, Dr.
 4 Furlow. Additional comments. I want to, as Chair, I'm
 5 going to make the decision, I believe, to call
 6 proceedings for today. Because we have made very good
 7 progress and had, I think, excellent coverage of each
 8 of these topics. And discussion here has been fine,
 9 and people have done a wonderful job of responding on,
 10 sort of, short notice to the questions they anticipated
 11 later tomorrow afternoon or evening. So, what I would
 12 like to do is to call the proceedings for today, and
 13 ask everybody to return tomorrow morning at 8:30. We
 14 will have, I'll give you, Dr. Steeger, here a chance to
 15 respond, too, but I'd like to, Dr. Petino.
 16 DR. PETINO: Reynoldo Petino. Are we
 17 going to come back to this question tomorrow, or are we
 18 --
 19 DR. HEERINGA: Yes, we are. I think we
 20 can revisit question nine tomorrow morning. We'll
 21 start again, and I'll return to Dr. Delorme and the
 22 others who have just responded to see if there are any
 23 additional comments, but I don't want to get out ahead
 24 of our preparation. And this is a cumulative process
 25 of learning and preparing during the course of the

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1 multi-day meeting. And I think, in all fairness to the
 2 responses to the question and to the panelists, I think
 3 that we're well ahead of schedule, and I think we're
 4 certainly positioned to finish our deliberations
 5 tomorrow with a good day of work. And, so, Dr.
 6 Steeger, unless you object to that, that will be my
 7 proposal.
 8 DR. STEEGER: No, I do not object. I
 9 just have one question regarding the response to
 10 question nine, because it keeps coming. And that is,
 11 regarding the sensitivity of the strain of Xenopus that
 12 was used, the animal was collected at the Cape region,
 13 which, apparently based on genetic analysis, is less
 14 likely to exhibit testicular oocytes or mixed sex as a
 15 natural background in the population, as opposed to
 16 animals that were collected in the northeast of South
 17 Africa.
 18 The study that was done in response to the
 19 DCI, both studies, ran a positive estrodyle control,
 20 and the animals were responsive to that, indicating
 21 that, in an exposure to a chemical that is known to
 22 induce gonadal developmental effects, it actually does
 23 respond as expected. Am I to understand of that, that
 24 test, the positive control, is not indicative that the
 25 animal, the strain being used, is a suitable surrogate

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1 for amphibians. You don't need to respond to that.
 2 Tomorrow would be fine.
 3 DR. HEERINGA: If you would be willing to
 4 remind us of that question --
 5 DR. STEEGER: No problem.
 6 DR. HEERINGA: -- tomorrow morning.
 7 Sounds good, just to add that to it. At this point,
 8 then, I think that I want to thank everybody for a very
 9 productive day, so a shorter day, but I think we
 10 deserve a bit of a break here, but we'll return
 11 tomorrow. And, again, my anticipation is that we would
 12 complete our deliberations on the charge questions and
 13 wrap up tomorrow. And that the panel would probably
 14 have a writing session Friday morning. But just to,
 15 again, that's based on experience. We won't shortcut
 16 the discussion of any of the questions, but I think
 17 with the remaining five questions here, that we should
 18 be able to handle those quite nicely in a full day
 19 tomorrow. So, turn to Joe Bailey, the designated
 20 Federal official, and see if he has any additional
 21 comments if he's willing to close.
 22 DR. BAILEY: No additional comments. I
 23 just want to thank the panel and the Agency for their
 24 discussions today. Thanks very much.
 25 DR. HEERINGA: Okay, and thank you

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1 everyone in the audience for your participation today,
 2 too, and all of the other commenters or presenters, the
 3 EPA scientific staff. We'll plan to reconvene tomorrow
 4 morning at 8:30 here, and we'll start again with some
 5 initial comments from the EPA scientific team on
 6 anything that came up overnight. And then, also,
 7 return again to question number 9.A., just to make sure
 8 that the discussants want to stay with their initial
 9 comments or make additional comments. And then we'll
 10 proceed on to 9.B., 10, and so on. Have a good
 11 afternoon everyone. Could the panel meet briefly next
 12 door just to discuss the progress here and writing work
 13 on the questions that we have covered.
 14 (WHEREUPON, the session was concluded at 2:42 p.m.)
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