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)

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Page 2

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FIFRA SCIENTIFIC ADVISORY PANEL
OPEN MEETING
OCTOBER 9, 2007

MR. BAILEY: Good morning everyone. We're a few minutes so I'd like to go ahead and get started here.

My name is Joe Bailey and I'm serving as the designated federal official for this meeting.

As you know this is a four day meeting on the Potential for Atrazine to Affect Amphibian Gonadal Development. And as the DFO for this meeting I serve to ensure that the provisions of the Federal Advisory Committee Act are met.

The FIFRA SAP is a federal advisory committee that provides independent peer review to the Agency on pesticide related issues. No one provides recommendations and advice. It's up to the Agency to make the decisions and implement those decisions.

Part of my responsibility is to ensure that all provisions of the Federal Conflict of Interest Laws are met, and to that end each of the panel members have filled out a standard government form and we have reviewed those forms and also the panel members have been briefed on the ethics requirements.

Page 3

There is a public comment period established for the meeting and it will begin midmorning. And anyone who has not signed up for public comments, please see me. And if you have not made prior arrangements I would ask that you limit your comments to five minutes today.

There is a public docket that's established for the meeting as well and all of the background materials that have been presented to the panel, as well as presentations that will be made today will be placed in that docket and the number is referenced on the agenda if you're interested in seeing what's in the docket.

We will prepare final meeting minutes after this meeting is over. Within 90 days we will do that and the final meeting minutes will be posted on the website as well.

If there are any press individuals here who have any questions or anything, I think we do have a press person who is supposed to be in the office, Dale Kemery. I haven't seen him yet this morning but he is supposed to be here. So if anybody from the press has any questions we will try to track him down and have him address any of your questions.

One final note is, this meeting is being

Page 4

audio recorded, so when you have a comment to make I would ask that you please give your name and affiliation so that we can have a clear recorded transcript of the meeting.

That's all of my comments and at this time I am very pleased to introduce Doctor Heeringa to my left who will be serving as Chair for this meeting.

DR. HEERINGA: Good morning everyone and welcome to this meeting of the FIFRA SAP on the topic of the Potential for Atrazine to Affect Amphibian Gonadal Development.

I'm Steve Heeringa. As Joe said I am the current Chair of the FIFRA Science Advisory Panel.

I am from the University of Michigan. I am an applied statistician who specializes in population based research. I hold no specific expertise on this topic. My job is primarily to see that the proceedings of this meeting move smoothly, and that we have a full and complete discussion of the topic at hand.

But to support us here are certainly a panel of experts in this field and I'd like to have them introduce themselves at this point and I want to begin on my left with Doctor Ken Portier.

DR. PORTIER: Good morning, I'm Ken

Page 5

Portier, Director of Statistics at the American Cancer Society, National Office in Atlanta. My expertise is in environmental sampling and probabilistic risk among others.

DR. CHAMBERS: I'm Jan Chambers with the College of Veterinary Medicine at Mississippi State University. I'm one of the members of the permanent SAP. My area of expertise is pesticide toxicology with emphasis on metabolism and neurotoxicology.

DR. SCHLENK: My name is Dan Schlenk. I'm in the Department of Environmental Sciences at the University of California, Riverside. I'm also a member of the, a permanent member of the SAP. And my research interests are in aquatic ecotoxicology.

DR. BUCHER: I'm John Bucher, I'm the Associate Director of the National Toxicology Program at NIEHS. My research interests are in carcinogenesis and of toxicology and I'm a member of the permanent panel.

DR. HANDWERGER: I'm Stuart Handwerger from the Departments of Pediatrics and Cell Biology at the University of Cincinnati College of Medicine. My clinical expertise is in pediatric endocrinology. My research is in developmental and perinatal endocrinology.

Page 6

DR. ISOM: Good morning, I'm Gary Isom from Purdue University. I'm a neurotoxicologist. My area of interests include molecular mechanisms and neurodegeneration. And I am a permanent member of the panel.

MR. PAULI: Good morning, my name is Bruce Pauli. I'm with Environment Canada. I'm a wildlife biologist with a special interest in the effects of pesticides on wildlife. I've been studying the effects of pesticides on amphibians for the last few years.

DR. SKELLEY: My name is David Skelley. I'm a Professor of Ecology at Yale University and my research interests include the ecology of amphibians and notably developmental deformities in wild populations.

DR. DENVER: Good morning, my name is Robert Denver from the University of Michigan and I am a Professor in the Department of Molecular, Cellular Developmental Biology. I'm a neuroendocrinologist and my research interests are in hormone action of the developing brain and I study amphibians also.

DR. FURLOW: My name is David Furlow. I'm with the University of California at Davis, Section of Neurobiology, Physiology and Behavior. I'm an

Page 7

endocrinologist as well, also studying amphibians and my expertise is in thyrohormone control of gene expression.

DR. HEERINGA: And we'll move over to Doctor Yeater.

DR. YEATER: I'm Kathy Yeater, I'm from the Department of Agricultural and Agricultural Research Service. I'm an applied statistician specializing in biological and agricultural life.

DR. BAILEY: Ted Bailey from Iowa State University. My interests are in statistical methods and design of experiments.

DR. DELORME: Peter Delorme from Health Canada, Pest Management Regulatory Agency. I'm with the Environmental Assessment Divsion as a Senior Science Advisor. I'm interested in environmental toxicology.

DR. LEBLANC: I'm Gerry LeBlanc from North Carolina State University. I'm a Professor in Toxicology and Department Head in the Department of Environmental and Molecular Toxicology with a research interest in endocrine toxicology.

DR. MILLER: I'm Debra Miller from the University of Georgia and I'm a veterinary pathologist and I do work amphibians.

Page 8

DR. PATINO: I'm Reynaldo Patino with the U.S. Geological Survey, Texas Cooperative Fish & Wildlife Research Unit. I'm a comparative endocrinologist working mainly with fish and some with amphibians as well.

DR. HEERINGA: Thank you very much. And I'm sure you'll agree with me we have assembled I think a fairly complete set of expertise to address the questions at hand. And I want to express my appreciation in advance to all of the panel members for participating here this week on this very important topic.

Just a few notes to add to Joe's comments earlier with regard to the proceedings.

One thing that I'm going to try to do as we get into conversation it's sort of easy just to come to the mike. I'll try to acknowledge all speakers. When you do begin to speak if you would just state your name. Because of the transcription it'll make it a lot easier to identify the speakers on the transcription if you just state your name before making your comments. So just a minor thing but it's important in terms of the capturing of the proceedings.

So at this point I think we're prepared to begin and I'd like to introduce Mr. Bill Jordan who

Page 9

is the Senior Policy Advisor from the Office of the Pesticide Programs at the EPA. Good morning, Bill.

MR. JORDAN: Good morning, Doctor Handwerger excuse me, Heeringa

DR. HEERINGA: Good morning.

MR. JORDAN: and Doctor Handwerger and all the rest of the SAP permanent members and ad hoc members.

As Doctor Heeringa suggest, I'm bill Jordan and I work in the Office of Pesticide Programs. The Office Director, Deborah Edwards is on travel this week and asked me to extend her best wishes to you and to welcome you to EPA on her behalf.

And I want to add also my welcome and say how much we appreciate the time that you are taking to help us sort out some very important scientific questions.

We understand that you have many other things to do and that spending nearly a full week with us represents a significant commitment of time, not to mention the amount of time that you will spend also in getting prepared for this session and in contributing to the development of the report on the work that you do collectively.

So we greatly appreciate the

Page 10

contributions that you are making here.

I also want to say a thank you to Steve Knott and Joe Bailey and the other members of the secretariat for the Scientific Advisory Panel. I know hard they have worked to get ready for this meeting for finding such a distinguished group of panel members, and then in helping us in the Office of Pesticide Programs get our materials ready and distributed to you for this meeting, setting up all the logistics and handling so many of the details. So Joe and Steve, we greatly appreciate your efforts as well.

I'd like to extend a welcome also to the members of the public who have come to listen and some of them to make comments to this particular SAP meeting.

We find that the engagement with our stakeholders across the full range of interest groups that are affected by and people who are interested in the regulation of pesticides to be a very helpful and constructive process. And we are delighted that we have a very full audience today.

I have, I was sitting here talking with Artie Williams about old t.v. shows and I realized that I've been around here for a long, long time. In fact I was working at EPA back before there was an SAP. And I

Page 11

was reflecting over the weekend about how the SAP came into existence.

For those of you who have not been around this process as long as I have, I'd like to take just a couple of minutes and offer some observations.

In the early '70s there was a lot of controversy about the regulation of pesticide products. There were actions being taken by the Agency that folks thought were motivated by political considerations and were not consistent with sound scientific analysis of the available information. And so the Congress, in an effort to make sure that the Agency didn't run amuck and do silly things, directed, passed a law that directed EPA when we were making important scientific decisions, important regulatory decisions that were grounded on controversial scientific propositions, to seek out the advice of the experts. They said that we needed to take our analysis to an independent scientific body, the SAP, who would review it and comment on it and then we had to think seriously about and address those comments before we went ahead.

And I was thinking about that and I decided, you know, things don't change very much. We still find ourselves in a situation where there's a lot of controversy about pesticide regulation, a lot of

Page 12

controversy about the scientific underpinnings of EPA's regulatory decisions, and that there is still a lot of value to be had from going through a process of independent scientific peer review of the Agency's decisions.

And so today we are bringing to you for your expert review and commentary, our assessment of a large body of information concerning the Affect of Atrazine on Amphibian Gonadal Development.

We have found over the years, since the '70s when we began this process, that the SAP's that we've had on a wide variety of subjects have really made a valuable contribution to our understanding of the science and to the development of sound scientific positions underlying our regulatory process.

And as a consequence of that I think that we have by and large at EPA made much better decisions, regulatory decisions about what is acceptable and what is not acceptable. That there has been a greater breadth of acceptance of those decisions, in no small measure because of the continuing good advice that folks like you have given us over the last thirty years.

So we are looking forward eagerly with perhaps a little bit of nervousness about what you'll

Page 13

have to say about our quality of our scientific work. And we're also looking forward to having the input of public commenters as well so that you too will hear some of the kinds of concerns that are on their mind.

With that I'll say thank you again for coming and I look forward to being here for the rest of today and hearing the beginning of the process.

Thanks.

DR. HEERINGA: Thank you very much, Mr. Jordan.

At this point I'd like to introduce Director Jean Williams who is the Acting Division Director of the Environmental Fate and Effects Division of the Office of Pesticide Programs.

MS. WILLIAMS: Thank you Doctor Heeringa and members of the panel for taking the time out of your schedules to be here and assist us with this important issue.

On behalf of the Environmental Fate and Effects Division I would like to welcome you to the new facility that we have and hope you are finding it enjoyable and will continue to throughout the long week that we're all going to spend here.

The FIFRA Scientific Advisory Panel serves as our primary scientific peer review mechanism

Page 14

for the Office of Pesticide Programs. Its purpose is to provide scientific advice, information and recommendations to the Agency's administrator on pesticides and pesticide related issues and regulatory actions, and in particular, those that have impacts on health and the environment.

As the title of this SAP indicates, we are meeting this week to discuss the potential for the pesticide Atrazine to affect amphibian gonadal development.

As will be described in later presentations, this is the second time that the Agency has relied on the FIFRA Scientific Advisory Panel to review our analysis and interpretation of data related to this potential affect.

Also as will be reiterated throughout the Agency's presentations, the specific focus on the affects of Atrazine on amphibian gonadal development is based on recommendations made by the FIFRA Scientific Advisory Panel in 2003 when they initially addressed this issue.

Based on those recommendations the Agency required the technical registrant for Atrazine to conduct studies to determine whether Atrazine affects amphibian gonadal development.

Page 15

Additionally, research of this affect of Atrazine on amphibian gonadal development has also been reported in the open literature since the 2003 review that we conducted.

We have reviewed all of this information in our 2007 white paper and concluded that across multiple lines of evidence Atrazine does not affect amphibian gonadal development.

Additionally since no affects could be consistently demonstrated in laboratory studies using the African Clawed Frog, a common amphibian in laboratory tests, the Agency has concluded that testing with other amphibian species is not warranted at this time.

Consistent with the process identified in the Agency's 2003 white paper and with the recommendations made by the 2003 FIFRA Scientific Advisory Panel, since no affects were demonstrated in the laboratory studies, the Agency has also concluded that no additional testing is required with respect to the potential affects of Atrazine on amphibian gonadal development.

Finally, since the multiple lines of information do not provide evidence that Atrazine affects amphibian gonadal development, the Agency concludes that no changes in its interpretation of Atrazine's ecological risks are warranted at this time based on the potential affects, based on this potential affect of Atrazine.

Page 16

As with all regulated pesticides, we'll continue to review information as it becomes available and we'll reevaluate our scientific position where that is warranted.

The SAP members have been provided copies as Joe mentioned, of the Agency's 2003 white paper and our most recent 2007 white paper examining the affects of Atrazine on amphibian gonadal development. The SAP members have also been provided copies of the full study conducted by the registrant in response to recommendations made to the Agency by the SAP in 2003.

Copies of open literature articles reviewed in the 2007 white paper have also been provided to the panel members. Unfortunately we were unable to obtain permission from all of the relevant journals to broadly distribute copies of all of the open literature.

Over the remainder of this week the panel members will have an opportunity to listen to public comments as Joe mentioned regarding these

Page 17

affects, followed by the Agency's analysis and conclusions regarding the subject.

Afterwards we'll review specific charge questions that the SAP has been asked to consider and address regarding the Agency's analyses and conclusions.

As stated earlier, the Agency relies on the FIFRA Scientific Advisory Panel as a means of scientific peer review. These public external peer review meetings assist the Agency in making sound scientific decisions.

We're looking forward to a candid and open exchange as we proceed with this FIFRA SAP meeting and I thank you for the opportunity to address the panel and for your efforts on behalf of the Agency and the public that it serves.

Thank you.

DR. HEERINGA: Thank you, Director Williams. And I will promise you that we will certainly devote our full attention to the scientific issues that are presented to us this week and we look forward to it as well.

So at this point I think we're ready to actually move into a presentation on the historical perspective on the issue of the Potential for Atrazine

Page 18

to Affect Amphibian Gonadal Development, and to present that is Doctor Thomas Steeger of the Environmental Fate and Effects Division, Office of Pesticide Programs. Doctor Steeger.

DR. STEEGER: Thank you very much. I'd like to thank you for this opportunity to address the FIFRA Scientific Advisory Panel regarding the Agency's evaluation of recent data on the affects of Atrazine on amphibian gonadal development.

As Doctor Heeringa mentioned, my name is Tom Steeger, I'm a senior biologist in the Environmental Fate and Effects Division and I've also served as the coauthor of the 2003 and the 2007 white paper.

During this presentation I will provide a brief overview of the paradigm that the Agency uses to conduct ecological risk assessments. Afterwards I will discuss the factors leading up to the 2003 SAP, the studies reviewed at that time regarding the affects of Atrazine on amphibian gonadal development, and the 2003 white paper and FIFRA SAP recommendations.

And finally I will provide a brief overview of what has occurred subsequent to the 2003 SAP that has led to the development of the 2007 white paper and the affects of Atrazine on amphibian gonadal

Page 19

development.

This figure depicts the ecological risk assessment paradigm followed by EPA in assessing risks to non-target animals from stressors such as pesticides.

The process consists of three major phases, the problem formulation, analysis and risk characterization. The process is intended to be, as more information becomes available, it is integrated with the existing information, the problem formulation including its conceptual model may change. As a result, the additional data may be required for estimating either exposure or affects.

In turn the Agency's assessment of potential risks may change.

In the slides that follow, various components of the risk assessment paradigm are depicted. Although many of the arrows appear to be unidirectional, in practice the process is iterative as data analysis informs both problem formulation and risk characterization.

Whether additional information is required depends on the risk management decisions under consideration.

Atrazine was first registered in 1958.

Page 20

In 2003 the Agency issued an interim re-registration eligibility decision for Atrazine. In the interim re-registration eligibility decision, the Agency concluded that Atrazine may continue to be used provided that all precautions are implemented to reduce risk to drinking water.

The decision was based in part on an analysis of both human health and ecological risks in the currently registered uses of Atrazine as a herbicide.

In response to a consent decree the Agency considered the potential affects of Atrazine on amphibian development. In 2003 the Agency reviewed studies on the affects of Atrazine on amphibian development that had been conducted up to that point in time.

The Agency's review was summarized in the 2003 white paper and was presented to the SAP in June 2003. At that time the studies focused primarily on the affects of Atrazine on amphibian gonadal development.

In 2003 the Agency reviewed a total of seventeen studies that were submitted as of February 28th of that year. Twelve of the studies were sponsored by the registrant and five were drawn from

Page 21

the open literature.

Registrant submitted studies received more scrutiny during the Agency's review since more detailed information was available. Although none of the studies were fully compliant with good laboratory practices or their standards, many of the studies had standard operating procedures and some level of quality assurance in place.

Additionally, for studies where raw data were available the Agency conducted an independent statistical analysis of those data.

Since most of the published studies reviewed in 2003 did not have standard operating procedures, nor were raw data available for review for the majority of the open literature studies, the open literature studies were evaluated at face value with the understanding that these published studies would have been subject to some degree of scrutiny already through the normal journal peer review process.

In 2003 as well as today, formal Agency guidelines are not available for specifically examining the affects of Atrazine on gonadal development in amphibians. As a result the Agency relies on other aquatic and terrestrial animal tests for which there are guidelines to serve as surrogates for estimating

Page 22

risks to amphibians.
Additionally many of the measurement end points such as inter-sex, sex ratio, laryngeal muscle area examined in previous studies differ from those regularly utilized by the Agency to estimate acute and/or chronic risks.
However the Agency is not confined to using the study requirements to identify potential hazards.
As part of 158 of the Code of Federal Regulations which outlines data requirements for the registration of pesticides, if data are insufficient to permit the Agency to evaluate the potential risks of a pesticide to cause unreasonable adverse affects, additional data requirements above those required by the Code of Federal Regulations can be imposed.
In determining whether additional data are required the risk assessment team relies on the professional judgement and available lines of evidence to determine whether toxicological end points can be linked to assessing end points in a reasonable and transparent manner.
As I said, in 2003 a total of seventeen studies were submitted for review. Seven of the studies were conducted in the laboratory exclusively

Page 23

while ten of the studies were conducted in the field. Field studies included Florida, Indiana, Iowa, Illinois, Michigan, Nebraska, Utah, Wyoming and South Africa.
Consistent with the Agency's process for evaluating the studies, each of the seventeen studies were evaluated using the following criteria. Experimental design, study protocols and quality assurance mechanisms, the strength and shape of the cause and effect relationship, whether there was a dose response, whether the observed affects have a plausible mechanism of action that is consistent with what is known about the chemical, and finally, whether the measured affects are ecologically relevant.
A range of amphibian species were tested in the studies. While the sum of the laboratory studies relied on non-native species, each of the field studies examined species within their native or introduced ranges.
Thus, cane toads in Florida, bullfrogs were studied in Iowa, norther leopard frogs were studied in Wyoming, Utah, Nebraska, Indian, green frogs were studied in Michigan, cricket frogs were studied in Illinois and African clawed frogs were studied in South Africa.

Page 24

Although most of the laboratory studies relied on tadpoles, field studies examined both larval and adult animals.
End points measure in the laboratory and field studies included time to metamorphosis, growth in terms of length and weight, presence of gonadal abnormalities, laryngeal muscle area, sex ratios, plasma steroid concentrations and brain/gonad aromatase activity.
As stated previously the majority of studies reviewed in 2003 focused on activity and gonadal development.
Each of the studies evaluated in 2003 contained uncertainties or inconsistencies in the way data were collected. Evaluation focused primarily on the methodological issues rather than on statistical analysis of the data.
In other words, there were sufficient uncertainties in how the data were collected and it made it difficult to put the data into any perspective.
As mentioned previously there were seven laboratory studies and ten field studies. Most of the field studies included some laboratory analysis.
Collectively the following issues were identified in the laboratory studies. Atrazine

Page 25

contamination of the controls, poor water quality, poor growth development and survival of the test species, high variability in end point measurements, a lack of reproducibility and unresponsive positive controls.
With respect to the field studies, the Agency recognizes that field studies can be difficult to conduct since researchers are not able to control environmental conditions. Also the Agency recognizes the difficulty in identifying sampling sites that can be considered true replicates of one another and/or devoid of factors that can potentially confound analysis.
Of the field studies submitted there was considerable variability between the sampling sites. Similar to some of the laboratory studies, Atrazine parent compound and/or its derivatives was present in reference groups, the reference sites.
Additionally other trizine herbicides and chemicals, other pesticides were present but not always well characterized.
Where pesticides were characterized the concentrations were in some cases relatively high and it's unclear what impact they may have had on the study.
In some studies there was unusual

Page 26

environmental conditions that may have impacted the study, such as unusually high rainfalls and increased depredation due to introduced species were problematic in some of the studies.

In spite of all the issues identified in the available studies, the Agency believed that the laboratory and field studies provided some useful information in terms of how to improve study designs. The studies provided sufficient information with which to formulate a hypothesis on the potential affects of Atrazine on amphibian development.

They provided insight on the potential sources of variability and they provided insight on future test species and study conditions.

Although many of the studies did not demonstrate any affect of Atrazine on amphibian development, there were sufficient data to suggest that Atrazine alone may be affecting developmental and more specifically, amphibian gonadal development.

Thus the hypothesis was that Atrazine exposure may result in affects on amphibian gonad that may ultimately impact secondary sexual characteristics and reproductive fitness.

However there were not sufficient data to refute or confirm the hypothesis that Atrazine alone

Page 27

may cause gonadal affects in amphibians because of the collective uncertainties associated with the studies.

Uncertainties included whether the cause/affect is real and can be readily repeated in different laboratories, a lack of a clear and consistent dose reponse relationship, the mechanistic plausibility of Atrazine exposure causing a given affect, the inability to readily extrapolate laboratory affects to the field and the uncertain ecological relevance of the measurement end points.

Without addressing these uncertainties it was not possible for the Agency to determine whether a particular affect could be consistently expected to occur at a particular exposure level, whether the affect, if real, could be expected to occur in other animals, and whether the affect were likely to reverse the affect in animals' reproductive fitness.

In 2003 the Agency concluded that none of the studies fully accounted for the environmental and husbandry factors capable of influencing measurement end points.

Based on all seventeen studies the Agency concluded in its 2003 white paper and the SAP concurred that Atrazine exposure did not produce consistent reproducible affects across all species

Page 28

tested. Therefore the lines of evidence suggested that Atrazine exposure did not impact gonadal development.

However there were lines of evidence from the laboratory and field studies that supported the formulation of a plausible hypothesis that Atrazine exposure may result in developmental affects in amphibians.

The studies also provided useful information of the potential sources of variability. This information will be critical to the design of future studies.

Because there were insufficient data to refute or confirm the affects of Atrazine on amphibians, the Agency recommended and the SAP concurred that additional studies be initiated and that these studies build on the body of information available in 2003.

The Agency proposed, and the SAP concurred that a tiered approach be used to examine the cause/affect, dose response, mechanistic plausibility and ecological relevance of any affects observed following the exposure of Atrazine to amphibians.

As will be discussed in later presentations, the white paper identified an analysis plan where the initial tier of testing focused on first

Page 29

establishing whether Atrazine exposure results in affects on amphibian gonadal development.

As of the 2003 SAP, or after the 2003 SAP the Agency required the technical registrant for Atrazine to conduct studies to examine the potential affects of Atrazine on amphibian gonadal development.

In November of 2004 the Agency issued a data call in requiring the Agency requiring the registrant to conduct the tier one amphibian studies. In response to the data call in, the registrant provided the Agency with a study protocol that incorporated all the design elements identified in the 2003 white paper.

The Agency provided comments on the protocol and the registrant adjusted the protocol to reflect the Agency's input.

Additionally, during the course of the studies, EPA inspected the laboratories to verify that the protocols were being followed and that quality assurance and quality control procedures were operational. As part of the inspection EPA verified the data were accurately recorded.

In June 2007 the registrant provided the Agency with a complete final report of the tier one amphibian studies.

Page 30

In addition to the registrant's submitted studies that were responsive to the DCI, the Agency reviewed open literature studies completed after the 2003 SAP.

A total of nineteen studies have been reviewed since 2003 and white paper has been developed, summarizing the Agency's interpretation of the available data.

Including the studies reviewed for the 2003 SAP, a total of thirty-six documents representing both interim reports, final reports and published open literature have been reviewed examining the affects of Atrazine on amphibian development. The vast majority of these studies examined amphibian gonadal development, primarily in the African clawed frog.

While other potential affects of Atrazine have been reported in the open literature, the Agency's focus with regard to the current white paper and this SAP meeting is on the affects of Atrazine alone on amphibian development alone.

This week the Agency will present its analysis of the open literature and the registrant submitted studies in response to the data call in and the Agency will ask the SAP to comment on its analysis and conclusions.

Page 31

DR. HEERINGA: Thank you very much, Doctor Steeger. Recognizing that Doctor Steeger and the scientific staff of the EPA will have extensive presentations tomorrow morning in support of their white paper and its findings, are there any comments or questions for Doctor Steeger on this historical perspective on the problem at hand?

Okay, we are actually ahead of schedule but I think this will be a floating agenda. We're schedule to follow the printed agenda through Friday, but we will progress at a pace which covers all of the issues but also means that we will float a little bit with regard to time.

At this point in a little bit of a difference from past SAP meetings we have placed the public comment period up front and we've done that more recently in another SAP meeting and it worked quite well I think in terms of stimulating the conversation and sort of setting the tone for the meeting.

And so I'd like to enter at this point the public comment period. And before we do that though I want to take a quick look. I think our first public commenter will be Syngenta Crop Protection and they're scheduled for approximately an hour and a half, and that was negotiated with the DFO, Joe Bailey in

Page 32

advance, and there are two speakers.

What I would like to recommend to the public commenters is that we will begin your public comments but I would plan to take a break about halfway through. So if there's a logical breaking point and that's acceptable, I think with an hour and a half we have to probably permit that. So that would be my plan at this point.

So before we invite the presenters for the first public speaker to the podium here, or to the table, I'd like to turn to the Designated Federal Official, Joe Bailey for some initial comment on the

MR. BAILEY: Thank you, Doctor Heeringa. Joe Bailey here. During today's public comment period we anticipate hearing about Syngenta's sponsored study that was conducted by Doctor Vern Klaus who is a former SAP member. And some of the written public comments for this meeting have addressed Doctor Klaus' role in the conduct of the study. And I just wanted to make a couple of comments regarding post-employment restrictions for former SAP members.

First, once an SAP panel has completed its work, former panel members are free to engage in any outside employment they desire, with one exception. And that is, under certain circumstances former SAP

Page 33

members may not represent a third party back to the U.S. government on the same issue that they addressed as a member of the SAP.

And I understand from discussions that to avoid raising questions regarding this restriction, Doctor Klaus will not be present this morning at the meeting to participate in any of the public comments.

Thanks very much.

DR. HEERINGA: Okay, at this point then I'd like to begin the public comment period and welcome the representatives from Syngenta Crop Protection, Doctor Keith Solomon and Doctor Glen Van Der Kraak.

MR. OSMER: Mr. Chairman and panel members, good morning. I'm Alan Osmer with Syngenta Crop Protection and I served as the GOP Study Director for the two studies that are the subject of the scientific evaluation and we appreciate this time this morning.

In the function as Study Director it was my responsibility to assemble and coordinate a team of scientific experts capable of delivering the data required to address the question of Atrazine's potential to affect gonadal development amphibians.

Also present is Doctor Keith Solomon, University of Guelph and Doctor Glenn Van Der Kraak,

Page 34

University of Guelph. Both of these gentlemen have served on the Atrazine ecological endocrine panel and in addition Doctor Van Der Kraak was the scientific advisor on the current study.

We'd like to provide two fairly brief presentations, the first by Doctor Solomon to provide some additional results of work that Syngenta has funded since 2003, but work that is not being considered by this panel at this time.

Then Doctor Van Der Kraak will present findings of the current studies.

These two studies have evolved over eighty scientists and technicians in multiple locations and we have a number of people here today to address the questions which you may have concerning the studies.

And these also include Doctor Jeff Wolfe, a certified veterinary pathologist from Experimental Pathology Laboratories. This is the gentleman that did one hundred percent of the histopath work for both of the studies.

We have Doctor Tim Springer, aquatic toxicologist from Wildlife International.

Doctor Ilga Lutz, comparative endocrinologist from IGB in Berlin.

Page 35

Doctor Bob Silken who performed of Silken & Associates, who did the statistical analyses.

And also present and involved in the studies, present in the room is Doctor Larry Holden of Silken & Associates.

And Doctor Hank Kruger, terrestrial toxicologist with Wildlife International.

And Doctor Robert Yopeley, an analytical chemist with Syngenta and responsible for all of the Atrazine analyses, the water samples.

So Mr. Chairman, depending upon the nature of the questions from the panel I would request that the appropriate people be permitted to come as needed to address those questions.

DR. HEERINGA: We'll certainly permit that and I'll allow you to sort of moderate that if you would.

MR. OSMER: Thank you.

DR. HEERINGA: Again, what we would like to do is throughout the public comment period for all of the public commenters I will give the panel time for exchange to pose questions for clarification or additional insight into the presentation, so we'll handle it that was and between you and I we'll keep track of time and progress.

Page 36

MR. OSMER: Okay. I appreciate that.

MR. BAILEY: Excuse me, Joe Bailey here, just a quick note, for the panel we are going to get copies of these slides for you. We thought we'd have them but we're a little ahead of time so as soon as they get there we'll get the presentations to you, hard copy.

MR. OSMER: Okay, very good. With that then I will turn it over to Doctor Solomon.

DR. SOLOMON: Thank you very much, Alan. Mr. Chairman, members of the panel and members of the audience, I am Keith Solomon, I'm a Professor and the University of Guelph where I do research on environmental toxicology and risk assessment.

And what I'm going to present is an overview which will be, or has been made available to you in written form that summarizes a large number of studies, including many of them that have been conducted under the purview of a group of us that formed a panel to address this issue.

To introduce the panel and acknowledge the members, it's sort of kind of like an orchestra when you're on one of these panels, everybody plays a different instrument, you all have your own expertise, I've served on many of these in my life and it's been a

Page 37

wonderful experience.

But I would certainly acknowledge our Chair and then also the key members of the panel on the left side of the screen. Some of these individuals are here today, including Doctor Louis Dupree from South Africa who would be able to answer and all of these individuals would be able to answer additional questions should they be needed.

I'd also point out that a number of students have been participating in this project. Several of them now are actually professors in their own right and there are a large number of reports that have been written and an equally large number I think of publications have been published in the literature. And some ancillary studies have been done that really have nothing to do with Atrazine, but have helped us illuminate some of the issues that we're dealing with here.

To put this in a larger context, and I'll come back to this later, we took what we call the guidelines for causality that were developed from some of the old principles as espoused by Koch, Hill, Dahl and more recently in the IPCS document on endocrine disruptors.

But looking here at temporality,

Page 38

strength of association, consistency biological plausibility of recovery, these are all essentially similar issues that were pointed out by Doctor Steeger in his introductory comments.

So this is the way we've looked, or we've tried to look at this data.

We've also looked at a large range of end points and I don't have the time to go through all of these but they are in written form that we made available to you, ranging from the basic principles of acute toxicity, developmental tests such as the FETAX test on Xenopus, things through limb deformity, sex ratio, sexual development of the testes, aromatase, which I'll deal with in a bit more detail and some other issues, all the way up to the level of the population.

This is probably the most scientifically appropriate to look at an issue such as we are dealing with here, because obviously affects on reproduction can have affects all the way up to the population level.

I'd like to spend a little bit of time on what I call the aromatase theory. Aromatase is the enzyme that converts testosterone to estradiol and the ratio of these hormones and related hormones depends,

Page 39

at least in amphibians, results in the expression of female or male characteristics, depending on the ratios and concentrations of these hormones.

And one of the theories that's being put forward, that to explain some of the results that we observed with Atrazine in terms of gonadal development, have been based on the early work of Sanderson and others which showed that you could induce aromatase in cancer cell cultures, both Atrazine and a number of related trizines.

A couple of important points to notice here in this study. This was seen only in cell cultures. They looked at tissue slices from fish and didn't find any affects. The EC50's are, occurred in relatively concentrations and as is typical with all induction responses, it's a monotonic concentration response, not an inverted view. So any downstream affects of this would most likely follow the same monotonic dose response.

In other studies, not in this one that I'm referencing here, these affects have not been seen in all animals, but certainly it's been reported in tissue culture systems.

To go into that in some detail, this is work from Katie Cody of Michigan where she looked a

Page 40

aromatase activity in Xenopus laevis and obviously large differences, pink for females and blue for males, the females have much higher innate aromatase activity. And this is responsive to estradiol exposures because it's a study with a regulated process. And that's the reason for the significant difference on the left side of the screen.

But as you see on the right side of the screen, both in males and females where it's not easy to measure, there's no concentration response to Atrazine and no significant differences as well in this particular study.

The result of aromatase activity would be expressed in estradiol and you can see from the same study the results with plasma estradiol, there were significant differences but again no concentration response from a range .1 to 25 micrograms per liter of Atrazine.

One of the downstream affects of androgens and estrogens and interactions between these is the development of sexual characteristics such as the laryngeal muscle in humans as well as in amphibians. And what you see here are results from a laboratory study where the laryngeal muscle, at least an area was measured, and again you can see affects of

Page 41

DHT which stimulates the development of the larynx and the or then what's consistently significant but the affects of estrogen that decrease it as well in other studies.

But the important message here is no significant responses in terms of Atrazine exposure through that range of concentrations.

We were fortunate enough to be able to study Xenopus in the field. Xenopus is a native of Africa and it is found widely through southern Africa where it occurs in ponds and cornfields where it has been exposed to Atrazine over many decades now of mace production in southern Africa. So these were frogs, adult frogs collected from field sites across the reference areas where no corn growing was taking place in the watershed where no Atrazine was being historically used and the corn growing areas where Atrazine had been used for many years and the concentrations were measured in these systems.

And again you see the difference between males and females in the sense of larynx size, but no significant differences between the reference sites, either between the males or the females.

The bottom line of this I guess is that based on the response of aromatase and the lack of any

Page 42

significant downstream affects, there seems to be little support for the aromatase hypothesis.

Another issue that's come up certainly in some publications has been that of uptake and bioconcentration. And given its water solubility and particularly its optimal water partition coefficient, one would not expect Atrazine to bioconcentrate to any great extent in aquatic or terrestrial organisms.

But this has implications for static renewal and flow through type studies.

So in order to further eliminate this issue a study was conducted where the uptake and elimination of labeled Atrazine was studied in Xenopus laevis, this had not been done before in this particular species.

Now this is work of Etington and Neuro and what you see here is the results for Atrazine. The uptake is indicated by the red bar and the depuration phase when the animals were moved to clean water, this was in Stage 66, is shown by the green bar. And you will see rapid equilibration between the solution and then a very rapid depuration once the animals were removed to fresh water.

Atrazine residues were not detectable in the frogs after 22 hours and when you used the uptake

Page 43

and depuration kinetics to estimate the BCF it was 1.5. In other words the concentration in the animals would be one and a half times that of the surrounding water. The half life of Atrazine was 48 minutes and it was also shown in this study to be rapidly metabolized to several known metabolites as well as some unknown metabolites.

So it is not a chemical that would accumulate over time. The animals are in very rapid equilibrium with their environment and the exposure to the environment are probably the most important ones in terms of assessing any affects.

In this same study we were able to also look at the distribution radio label within the tissues of the frogs. The top two pictures here show sections of whole animals done using freezing sections so that the location of the radio label was not disturbed by solid extraction in the normal procedures. And this allowed us to identify the various organs and tissues that could then be studied using radioautography techniques where the intensity or the amount of radio label is indicated by increasing color. And you can see a scale on the bottom here to give you some representation of that.

Now this process also actually allows

Page 44

you to count and quantify the disintegrations in a particular area and so you can actually do numerical evaluations here.

If you enlarge that you can see here that the radio label, which of course is a mixture of Atrazine and any of its metabolites, is present in the gallbladder and the GI tract. So this is consistent with metabolism and also excretion via the bile.

There was no concentration observed, or no untoward concentration observed in tissues or other tissues of the organism. We can see the eye and the brain up in the top there.

So this was I think very useful information in terms of understanding exposures in these organisms and how to interpret them.

Another issue that's come up is a testicular ovarian follicle. This is perhaps a new term to some of you. We ourselves have recently realized that this is the more correct terminology. We used to call these testicular oocytes which is a little bit easier to say, but an abbreviation of TOF or TOF's might be appropriate.

What are these? It's basically a female tissue in the testes, you can see the testes tissue surrounding this testicular ovarian follicle with a

Page 45

nucleus and you can also see the epithelial cells associated with it, which is the reason for calling it a follicle.

These have been commonly observed in the literature in all sorts of situations. They've been seen in fish. There was a recent study published that looked at control fish, Japanese Padica and it was seen in unexposed control animals with some indication of specificity to the strains for the labs that were doing these studies. It's been seen in reptiles, in snapping turtles, either exposed or unexposed to Atrazine, there was no concentration response.

It's also been reported in laboratory studies with frogs. They're either absent, depending on where the study is done or there is no concentration response observed at concentrations as you'll hear later on up to 100 micrograms per liter.

We've not even seen a concentration response in the generational study that I will focus on shortly.

One study reported an occurrence of these in the field sorry, in the lab and the field at concentrations less than .1 microgram per liter Atrazine.

There have also been a number of field

Page 46

studies and again no concentration response was observed in the field studies.

This sort of led us to wonder if the testicular ovarian follicles are really a natural phenomenon. We've seen them in exposed/unexposed organism without a concentration response. They've also bee observed historically in a number of frog species listed here.

And just to focus in on one of those studies by Amy Reader from the University of Illinois, this was inter-sex incidents in cricket frogs from museum specimens. It was kind of an interesting study to go back almost on an archeological hunt to look for this. And what you can see, this represents deviation from expected which is the mean of the entire data set, that these occurred well before the introduction of Atrazine and which occurred roughly there. And in fact one might argue that there's been a decrease. I don't know if that's significant or not.

With this background and with differences in studies reported on the Xenopus laevis, we were interested in seeing if we could find Xenopus laevis in South Africa that were truly removed far enough form the use of Atrazine in mace production that they would be true reference sites.

Page 47

So with this in mind Louis Dupree, a colleague from South Africa conducted a study where he collected frogs from the major mace producing area where much of the previous work had been done, this is very close to Doctor Dupree's university, through down towards Cape Town and then also across the Cape Fold Mountains to several sites on the other side in what we call the Cape sites to differentiate it from the northeast sites by the Cape Fold Mountains.

So this is the area of mace production and where Atrazine might be used and in these areas Atrazine and mace, this is a semi-desert area where Atrazine is not used because the mace is not grown. And this is also upwind of these sites. The prevailing winds are from the southwest.

We also measured concentrations in these sites. At the time of collection of these specimens there was Atrazine present in this site but not in any of the other sites.

We found testicular ovarian follicles in all of these sites, although Atrazine was not present in three of them and there was no indication of a spatial trend in terms of the numbers, although it's only really four sites.

When we went to the Cape sites we found

Page 48

no testicular ovarian follicles in any of the animals collected from there.

So this begged the question is, were there physiological and perhaps genetic differences between these species. But we were very fortunate to have at our disposal at the University of Guelph, a program called the Bar Code of Life which uses mitochondrial DNA to type species in a very rapid way.

And working with these people we were able to develop a mitochondrial DNA fenogram which was also confirmed with nuclear DNA as well to look at the distribution of the various Xenopus species. You'll see at the bottom, tropicalis, muelleri and gilli, which separate out quite distinctly from a large group that is traditionally know Xenopus laevis.

These as you know are distributed all the way through south of the Sahara down to the Cape.

But what was most interesting was that there was a very clear genetic difference between the animals from the Cape region southwest of the Cape Fold Mountains and the animals from the region northeast of the Cape Fold Mountains.

Just to focus in a little bit on there, these organisms here from the Cape are the source of importations of test organisms in Xenopus One and

Page 49

Xenopus Express and we also tested those strains from American, samples from the American distributors and they also have fitted into this group. Whereas the other group were a distinct type that is quite different.

These were used in the studies you'll hear about later which I've called the Osmer, et al studies, and these were used in the Dupree and other studies conducted in the northwest province. Atrazine is not used in the Cape so we can't study the other species under field conditions. But they obviously have been studied in the laboratory.

This really bets the question about, you know, what about Xenopus used in other studies? What is the provenance of cultures? And I know there have been some recent issues around leeches and the provenance of leeches that are used in various physiological and neurophysiological studies as well, for the same reason that they may be the same type or species.

We don't see any relationship to Atrazine exposure. The background incidence of these seems to be genetically determined. We feel this has serious implications for the use of certainly testicular ovarian follicles as a marker of endocrine

Page 50

modulated responses and OECD is proposing that this be the protocol and this species be used in the protocol for this type of response.

And we also feel that at best, studies may be confounded unless we know the genetic background of the frogs being used.

The last study I'd like to focus on is the Growout study because this addresses an issue related to population and other issues. A paper published in 2005 in Environmental Science & Technology looked at a microcosm study where frogs were taken from four days old through Stage 66 and through to ten month old juveniles.

These animals were used to look at the end point of testicular ovarian follicles as well as other developmental affects and they were exposed to a range of concentrations of Atrazine in these field, semi-field microcosms. We extended through the lab in South Africa and Doctor Dupree's department took these animals out to 24 months with continued exposure. So the F1 generation in this study was exposed to Atrazine all the way through 24 months.

And then we used these animals to assess reproduction and development. We did this by crossing

Page 51

of the responses we had seen earlier had been seen in males, we focused mainly on those and we crossed, exposed males to referenced females, but we also did one cross between the high concentration males and the high concentration females.

And we took the progeny of the F2 generation, looked at numbers of eggs hatched, development, various size parameters, and also testicular ovarian follicles.

This was done, these animals moved indoors during the wintertime and exposures were continued as they were in the field, the same water source and the same concentrations of Atrazine. You can see the tanks with the adults on the left and the tanks for the larvae on the right.

Looking at a number of end points, hatch, time to metamorphosis, first metamorphosis, last metamorphosis, survival, there were some statistically significant differences here in some of these, but no concentration responses in relation to exposures of the parental generation, the F2 generation who were not exposed to Atrazine in these studies.

If you look at some more parameters related to size there were no significant differences.

In terms of the F2 generation, frogs

Page 52

with testicular ovarian follicles, there were, this is the total number of frogs out of the 40 that were randomly selected from the pairings for histological analysis, you see certainly no significant, at least no clear concentration response and no significant difference here but quite a bit of variability in the number of testicular ovarian frogs sorry, ovarian follicles per frog.

So we see general conclusions from this. No evidence to suggest any trans-stimulation of relay affects in terms of and also development of the young Xenopus. This is consistent with robust populations in the areas where Atrazine was used in southern Africa. It's also consistent with most other studies where no affects have been found associated with exposure to Atrazine and it doesn't support hypothesis that Atrazine affects reproductive fitness development in frogs.

To go back to the temporality, strength of associations and other guidelines that we started with, we see in terms of temporality, no correlation between occurrence of gonadal affects and introduction of the use of Atrazine.

In terms of strength of association there's no clear concentration response. If you

Page 53

convert the postulate to address chemicals we see no evidence of causality there. The incidence in wild populations is very inconsistent and in many cases other confounders have not be specifically addressed. We don't know what all of those might be but there may be some other ones out there.

In terms of consistency, the outcomes were inconsistent between one laboratory and another and from laboratory to the field.

In terms of biological plausibility there is no evidence of affects to the estrogenic and androgenic mechanisms.

And in terms of recovery which is one of the postulates, we've not been able to address that because we have not been able to produce consistent and robust responses from which we can see the recovery.

So our final conclusion if you want to think of this as a symphony is environmentally irrelevant concentrations of Atrazine are not demonstrated to affect growth, sexual development, reproduction and survival in amphibians.

And with that I'd like to thank you. I believe it might be better if we held the questions until Doctor Van Der Kraak has made his presentation if that's your wish, Mr. Chairman.

Page 54

DR. HEERINGA: I was going to ask you the same question and you seem to have given the response already. I will accept that.

We are at ten minutes of ten and I would like to if we could, use this opportunity to take a break. And we'll return to hear Doctor Van Der Kraak's presentation and then we'll entertain questions and comments from the panel.

Is that acceptable. I guess the full team will be there.

MR. OSMER: I believe that works fine.

DR. HEERINGA: Okay. Let's take a fifteen minute break. I have 9:48, we'll reconvene here at let's say 10:05.

(WHEREUPON, there was a recess.)

DR. HEERINGA: Prepare to start again in a minute or so. Photocopies of the presentation materials are being circulated to the panel members and for the audience and the public those will be available on the docket for this particular panel meeting.

Okay, welcome back everyone to the second half of our first morning session on the Potential for Atrazine to Affect Amphibian Gonadal Development, FIFRA Science Advisory Panel Meeting.

At this point we are in the process of

Page 55

beginning our period of public comment. We've heard from Doctor Solomon on the conclusions drawn from a series of additional studies and I think, Mr. Osmer, at this point we're going to hear from Doctor Van Der Kraak and then I'd like to stop to give the panel a chance to pose some questions on these presentations.

So if you'd like to go ahead at this point.

MR. OSMER: That would be fine and for your time management we anticipate that Doctor Van Der Kraack's presentation would be about 25 to 30 minutes and then use the remainder of our time to address any questions from the panel.

DR. HEERINGA: That will be fine.

MR. OSMER: So I'll turn it over to Doctor Van Der Kraack.

DR. VAN DER KRAAK: Thank you, Alan and Mr. Chair. I'm very pleased to have the opportunity to present the results of two studies that have assessed the potential affects of Atrazine on growth, metamorphosis and sexual differentiation of Xenopus laevis.

To put this in context and to summarize if you will what Doctor Steeger spoke of this morning, in 2003 seventeen studies were evaluated and the result

Page 56

of those were such that the results were inconclusive as a result of issues associated with design deficiencies and uncertainties, questions about water quality and husbandry and inconsistent procedures across the various studies.

However these studies established that Xenopus laevis was an appropriate model to move forward with additional tests to evaluate the affects of Atrazine on gonadal development.

Part of the EPA requirements and through the data call in instructions to the sponsor, Syngenta, there was the need for the development of the standard operating procedures to do these tests in flow through, to meet ASTM water quality and testing standards, to verify exposure, to deal with the terminology and make it standardized with respect to gonadal structures and to conduct the study under good laboratory practice standards with quality assurance.

Now, in order to achieve this, and part of this has been introduced, there was a large study team that was assembled. The in-life studies which were conducted at Wildlife International and IGB Labs in Germany and Doctors Springer, Klaus and Lutz as the principal investigators.

Jeff Wolfe who is at the table was with,

Page 57

or is with EPL Labs and he was the principal investigator responsible for the zoological evaluation of the gonads.

Larry Holden who is here along with Bob Silken from Silken Associates were responsible for the statistical analysis.

Alan Osmer on my left was the GLP study director.

And I had a role, a very minor role throughout the project as a scientific advisor to this group.

In terms of restating the objectives, the objectives were to evaluate the potential affects on gonadal development in Xenopus laevis and this was conducted over two parts.

And the first part was conducting estradiol pre-exposure studies to address design and method deficiencies, to confirm the appropriateness of the test systems and to identify the concentration of estradiol that would be used as a positive control level for the studies evaluating the affects of Atrazine.

And then specifically the main study was to determine whether a wide range of exposures to Atrazine during early development would affect aspects

Page 58

of survival, growth, metamorphosis or sexual differentiation of Xenopus laevis.

Now, this slide comes with the title that Xenopus laevis is the standard model for sexual differentiation in amphibians, and that certainly is the case. There is much that's known about primary sex differentiation in this species, including the affects of steroids. There's much that's know about many of the genes involved in steroid hormone biosynthesis and in other genes as well as information on secondary sex differentiation, including the affects of the major steroid hormones.

To put this in a little bit of a different context, sexually undifferentiated tadpoles will mature to males or females and they do so under the appropriate hormonal environment.

Much is know about how hormones affect sexual differentiation in Xenopus, such that there is information on a sensitive window or a sensitive stage over which hormones can direct sexual differentiation. And then beginning about Stage 55 through to Stage 60, one starts to identify and be able to very clearly morphologically distinguish the ovary and the testes.

In terms of this particular experiment, the exposure periods spanned from days 46 or pardon

Page 59

me, from Stage 46 through to the end of Stage 66 which is the time in which there is the final resorption of the tail.

In terms of putting this in chronology, if we start back here in 2003 there was the EPA Amphibian SAP which Doctor Steeger talked about and following that the registrant worked extensively on method development and lab selection for these definitive studies.

Wildlife International and the IGB Labs were selected and they went through a estradiol study in both locations where there was much work on protocol refinement and review of the procedures by the U.S. EPA.

This led to the definitive studies with estradiol as a positive control and Atrazine conducted at the two laboratories again and these were also subject to review, both in terms of the EPA Atrazine Protocol Review and quality assurance inspections by the EPA at the three major laboratories involved in the studies, IGB, Wildlife International and at EPL where the histology evaluations were conducted.

In terms of the good laboratory practice inspections and study audits, these were very extensive. There were inspections that were conducted

Page 60

at a governmental level and these occurred as I mentioned previously at all of the major laboratories through the EPA Office of Enforcement and Compliance Assurance, the EPA Office of Research and Development, and by the German GLP Federal Bureau which was associated with their Institute for Risk Assessment.

There were also a series of audits that were conducted by the internal quality assurance units in each of the major laboratories, including people coming from the registrant, Syngenta Crop Protection.

In all, there were 90 quality assurance audits that evaluated various phases of the study conduct and reporting.

So in terms of the experimental designs for the Atrazine studies at the two laboratories you're going to hear more about the treatments. This included a positive control which was 17 beta estradiol at .2 micrograms per liter, a negative control which was untreated and Atrazine in 5 different dose concentrations spanning a ten thousandfold difference in concentration.

The number of tanks were 8 per treatment for Atrazine. There were 8 tanks for the positive controls and 16 negative controls at each of the laboratories.

Page 61

The number of larvae that were put into these tanks were 25 and it's written here that the sex was unknown because they were put in before they were sexually differentiated. They were put in at a loading rate that met ASTM standards. And the exposure began 8 days post-fertilization and it ended at Stage 66 at tail resorption or up to 83 days post-fertilization.

Now, the Atrazine concentrations as I've mentioned, spanned four orders of magnitude from .01 through to 100 micrograms per liter. These bracketed the Atrazine concentrations for which affects were previously reported. And they included and exceeded environmentally relevant concentrations for chronic exposure.

They also covered potential low dose response or concentrations that would be appropriate for looking at potential low dose responses to Atrazine.

Now in terms of 17 beta estradiol, it was determined experimentally that .2 micrograms per liter would be the concentration of estradiol that would cause approximately an EC50 response for feminization of males. And this would mean that one would predict that this would be a concentration that resulted in about 75 percent females in the treatments.

Page 62

This was taken in part again as a positive control and the intent was to increase the likelihood of intermediate responses such as mixed sex individuals and inter-sex individuals. In fact, with going quickly to some data, the .2 microgram per liter was very close to the predicted response in terms of achieving 71 percent and 65 percent females in the two trials that we're going to report on, again very close to this EC50 concentration.

Now in developing and validating the test procedures the study design I think went well beyond some of the requirements laid out by the EPA. Much effort was made in the design of the experiment and in terms of making sure that the appropriate statistical power was achievement. The pre-studies which I've mentioned were important in establishing the methods that would be used. It also enabled inter-laboratory harmonization and verification of the experimental design.

A unique feature was the repeated independent experiments that were conducted at the two site. And there was considerable work that was expended in refining the methods for assessing both gross morphology and gonadal histology.

And in the process of doing this

Page 63

experiment there were recommendations that were put forward by the OECD and in fact and this was specifically for an amphibian metamorphosis assay and this study met the relevant water quality parameters.

To describe a little bit about the experimental set up, the experimental set up was such that it was a standard flow through system which is commonly used in aquatic testing. The tanks were glass, the tubing was such that it was selected to minimize the exposure to potentially, or to compounds that had previously been identified as ones that interfere with endocrine function. Any test solutions were made weekly and they were delivered to a mixing chamber and then these were delivered to the tanks such that there were 7 tank volume exchanges per day. The treatments, as you'll see in the next couple of slides were conducted blinded and they were, the tanks were distributed in a random fashion.

Now this is a picture of what the experimental layout looked liked at the labs at Wildlife International. If you look across the picture you'll see various different colors and they represent the concentrations of various test compounds. If you'll note very quickly, there were two sets of control tanks shown here and it's pair was up here,

Page 64

control two and control one which was pardon me, control two and control one, control two and control one, these were also completely randomized so that the experimenters would not know which tanks were associated with those particular treatments.

In terms of the design, so there was clusters of four tanks each for each treatment except for the negative controls which had four clusters. These clusters as I've mentioned were color coded and randomly positioned.

Now, in the course of the experiment and the monitoring of the levels of Atrazine, it turned out that one of the control tanks had recurring low levels of Atrazine contamination and the maximum concentration that was observed was below .013 micrograms per liter. But given that Atrazine was detected in these tanks, which it turned out were sandwiched between two of the highest concentrations of Atrazine in the treatment because of the randomization procedures, those tanks were removed from the analysis.

In the process of doing the study there were also microbial blooms which were identified in the two sets of tanks associated, one tank here specifically, tank six in the low group of Atrazine, that was removed as was this entire cluster of control

Page 65

tanks shown here. So those were removed.

This still left at the end additionally, additional control tanks so that at the end of the experiment, two negative control cluster were omitted from the analysis. However the robust study design permitted continuation of the study and there were eight tanks that were included for all treatments.

Now, the experimental layout at the IGB Labs was similar. There was the same pardon me, the layout was different in terms of the structure of the room, but the tanks that were included or the treatments that were included stayed the same.

There was an issue that was identified in this that Atrazine rather than estradiol was inadvertently used to prepare the estradiol stop solution on day 49 post-fertilization during the course of this study. Now this occurred after the sensitive developmental window closed for the species, that is to say this was occurring at Stage 56, and there were consistent results with what was seen in these tanks with what was evident at the Wildlife International studies. And this indicated there was no impact on the study and these tanks were included in the study.

Now this is a real important slide and what this shows is the measured concentrations of

Page 66

Atrazine at both the laboratories of Wildlife International and IGB. You'll not here that dosing started at day negative 5, so 5 days before, and then continued until the end of the experiment.

Now you'll notice that in some of these cases monitoring continued for longer time intervals and that relates to the times, or whether there was frogs in those tanks that hadn't completed metamorphosis. So if there were no frogs there was no point in further sampling the fish not the fish, the frogs.

In terms of the concentrations of Atrazine, there was a clear delineation and very close agreement with nominal concentrations, such that there was no overlap in concentrations of Atrazine over the four orders of magnitude of the concentration response. In terms of the control tanks the levels of Atrazine that were detected are listed as non-detectable and they were lower than the level of detection which was .005 micrograms per liter.

In terms of placing these data in a different context, one could comment on them in relation to the nominal concentrations and one could look at these in relation to what was the study mean over the course of the entire study and what was the

Page 67

concentration during the critical window of survivor the critical window for sexual differentiation.

Again there was a high degree of congruence between the levels that were nominal and the levels that were actually measured. As well there was measurement of estradiol concentrations and these again were, levels were achieved with high reproducibility across the study.

An issue that came up was whether there should be an analysis of Atrazine degradants. This was not part of the protocol per se and in part it wasn't there because under static conditions negligible amounts of the degradates would be expected based on EPA standard fate studies for aqueous photolysis, aerobic aquatic degradation or hydrolysis.

Now the study was in fact, you know, conducted under flow through conditions which would produce even less opportunity for degradate formation. As well, Atrazine stocks were prepared weekly and 7 exposure, or 7 tank volume exchanges occurred per day.

Nevertheless there was an analysis made of degradates in 6 of the tanks from the 100 microgram per liter samples taken from the IGB and Wildlife International studies. The analytes that were listed here were measured, but the concentration of these

Page 68

analytes at the highest treatment group turned out to be less than 1 percent, well less than 1 percent of the measured Atrazine concentrations.

Now, to get to some actual results of what happened in the experiment in terms of the biological end points, there were a suite of primary end points, and these related to survival, body weight, snout to vent length as a measure of gross development, time to metamorphosis as a key developmental measure. And then a series of the responses within the gonad, including sex ratio, the incidence of mixed sex individuals, inter-sex individuals and the testicular ovarian follicles which Doctor Solomon mentioned. As well there were gross gonad, liver and kidney features that were monitored.

Now, this slide, I'm going to just take a second because many of the slides will follow this same pattern. This is reported as the various treatment groups going from the positive control, the negative control to the various concentrations of Atrazine for Wildlife International and for IGB. This is the survival and it turns out that there were no significant differences in mortality between treatments.

And if you focus on the y axis, the

Page 69

survival was very high across the treatment, ranging from about 93 percent to above 98 percent for all treatments.

One of the other parameters that were evaluated was mean body weight at metamorphosis and in terms of the studies at Wildlife International for males in blue and females in this burgundy type color, there were no significant differences between body weight across the various treatments.

By comparison, in the studies at IGB there were some significant differences that were reported in some of the Atrazine treated groups, but only for females.

But again if you focus on the y axis the range of biological variability across those treatment groups was in fact very tight.

In a similar manner, snout to vent length was measured at the time of metamorphosis and again there were no significant differences from the negative control across the various treatment groups at Wildlife International.

Again, no affects in males but the same groups that appeared that were significant on the previous graph were also significant for snout-vent length for those groups treated with Atrazine at these

Page 70

varied concentrations.
Now, just to remind you a little bit about what this study was looking at, and one of the key factors was metamorphosis, and so as I had mentioned that tadpoles were initiated to the tanks, in fact even earlier than the stage depicted here which was Stage 48, and the experiment terminated at complete tail resorption at Stage 66. And in this example this would have been the progression of over 55 days of treatment.
This slide is a bit complicated and it reports cumulative numbers of individuals that had completed metamorphosis. And so this axis shows the composite number of individuals having completed metamorphosis and you'll note that this essentially goes to 100 percent, in that throughout the entire study, only 3 individual frogs did not complete metamorphosis.
Now, you'll notice this and of course if I was looking at my students having presented this I would be critical, and I would say I can't make out those lines. I don't think that's important in this situation, in that this represents the control individuals and the Atrazine treated individuals. And in males there was one group that was an outlier and

Page 71

that was the group that was treated with estradiol.
If you look at this, the slope of this line parallels the other lines. What this is suggesting is that estradiol is delaying the onset of metamorphosis.
In a similar fashion you see this at IGB for males. And if we look in females it turns out that the group that would be the far right hand group was also the estradiol treated group.
To put these data in a different context if you will to look at the mean age at metamorphosis, if one looks at the negative controls and the Atrazine treated groups, whether it's at Wildlife International or at IGB, there are no significant differences across these treatments.
There is however at both locations a significant increase in the age at metamorphosis in both males and females that were treated with estradiol.
Again though you'll not that there is particularly within the negative controls and the Atrazine treated frogs, a very tight range in terms of this parameter of age at metamorphosis.
Now, a major focus in this study was to look at gonadal differentiation and so you all should

Page 72

be experts by now and realize that this would be female with a normal ovary. This would be a normal testis and this would be a mixed sex individual and you'd very quickly recognize that there was an ovary there and a testis there on one of the gonads and a testis there and an ovary there on the other gonad.
These individuals though with these mixed sex conditions were ones that were treated with estradiol.
Now throughout the study the reliance on scoring gonadal development was not made on the basis of gross morphology, but rather was made on the basis of histological evaluation of the gonad. So all of the individual frogs were trimmed and embedded and then they were sectioned. And they were sectioned from the ventrum to the dorsum. And if you look at the bottom slide there you can see the sectioning occurring.
And then there were four micron sections and slides or sections at 12 micron intervals were evaluated. It turns out that all of the histological sections, that is to say greater than 100,000 of these were evaluated in a blinded manner by one board certified veterinary pathologist. And to add a little of humor to the day, Jeff who did this work in fact had hair when he started.

Page 73

Now, these are standard protocols for this type of analysis, but one might want to ask the question, having done this gonad sectioning and evaluation procedure, would significant findings have been missed using the methodology? And the answer to that is, I'm going to say no, things would not be missed. And I say that because the estrogenic responses that one would see in terms of a sex reversal and mixed sex gonads are obvious as I showed you a couple of slides back in terms of the gross morphology.
And the testicular leukocytes or testicular ovarian follicles as described earlier by Doctor Solomon, the smallest of these are 29 microns in diameter. And by taking 12 micron step sections, these are less than half of the diameter of the follicle. And so there's a high degree of confidence that one would not miss these should they be present.
So, having said that let's go to what was actually found.
And this is just what one of the histological slides would have looked like in terms of showing kidney, normal testis. In a similar fashion if one looks at the ovary one would have seen normal ovarian structure. But if one looks at what happens in those frogs that are treated with estradiol, one sees a

Page 74

series of different types of phenotype and this ranges from a normal ovary to very obviously altered ovarian structure that's associated with these large vacuolated areas to those individual frogs with normal testis structure to those with dilated tubules showing an obvious altered structure, and including those individuals that have a mixed sex gonad phenotypes.

In treating with estradiol we see that there is a increase in the proportion of individuals that have the ovarian phenotype and as you'll see in a few moments, an increase in the proportion of individuals that have the mixed sex phenotype.

So, this slide is a real key one because this slide reports the percentage of male, female and mixed sex frogs at the two locations. And if you focus in first of all on the Atrazine treated individuals there was no affect on the proportion of males or females at any of the Atrazine concentrations that were evaluated.

So looking here from the negative control through the high concentration of Atrazine, and again female in burgundy, male in the blue coloration.

If one continues on and looks at what happens with the estradiol exposure at this concentration that was selected to cause 50 percent

Page 75

feminization, there was a significant increase in the proportion of females here, and there was an increased number of individuals that were of the mixed sex phenotype.

Now I'm sure you're asking were any mixed sex individuals seen in the Atrazine treated groups? Yes, there was one individual frog that was of mixed sex, and that was in the 25 microgram per liter Atrazine treatment seen only at the IGB labs. And so this represents one individual out of approximately 2,400 Atrazine and negative treated control frogs.

In terms of other major or primary end points that were looked at, there were no testicular ovarian follicles and this was expected based on the data that Doctor Solomon had talked about in that frogs from Xenopus One which were the supplier of these frogs, come from the western Cape.

There was also no evidence of inter-sex and that is the left and right gonads being of the opposite sex, so no inter-sex were observed in this study in any treatment.

Now, in addition to the primary end points that were evaluated, and we've already talked about, there were a number of other histological that were reported. And this was the work of Doctor Wolfe.

Page 76

And so here in this case there were a suite of end points that were characterized and evaluated in this study.

Now, a question that came up in previous discussions associated with the EPA was the question of whether there should be differential gonad cell counting that evaluated cell types in these frogs. And that was not performed because the immaturity of Stage 66 gonads was such that there a very limited number of cell types that are available to enumerate.

These histological features though were ones that were selected and they represent the best effort to evaluate, is there something going on within the gonad that is remarkable and should be categorized?

Now, let's look at this. So, this figure shows statistical differences that were seen between estradiol treated and negative control frogs, and the corresponding results that were taken for Atrazine treated frogs.

Now, in terms of estradiol treated at both IGB and Wildlife International, I've already reported to you that there was a decreased percentage of males, an increased percentage of mixed sex individuals with estradiol treatment, and using this histological evaluation, there were clear affects of

Page 77

estradiol on the testes in terms of dilated tubules, dividing leukocytes, internal melanophores and in the ovary in terms of increased ovarian cavity size.

By comparison, if you look at the responses that were seen with Atrazine, all of these responses were non-significant.

So, the conclusion from this is that the findings associated with estradiol exposure were not observed in Atrazine exposed frogs and as such there is no evidence of feminization in the Atrazine exposed frogs.

Now, as I mentioned there were a suite of these other histological descriptors that were evaluated and so one should ask, what happened with these? So in terms of Atrazine treatments the incidence of these histological descriptors was low and it was low irregardless of the treatment.

There were sometimes inconsistent, or inconsistent and sometimes contradictory findings between laboratories for Atrazine, but none of these responses were significant in paralyzed comparisons.

Further analysis showed that only one of the end points, that being the fused kidneys, was significant at both IGB and Wildlife International in monotonic trend tests, and only when all of the doses

Page 78

were included.
The real take home message from this is that there was a lack of concentration response to Atrazine over four orders of magnitude in that Atrazine concentration.
So, let me just make a point here. In terms of the histological evaluation of the gonad of Stage 66 Xenopus, the evaluation that was conducted in this experiment was in my estimation far more extensive than anything that has been done in the past.
And the take home message from that was that the histological descriptors were not consistently significant across Atrazine treatments in the two studies. And the biological significance of those histological changes in gonad structure is truly not known.
So, to sum this up in terms of key findings, this study established a standardized procedure and protocol for evaluating sexual differentiation in Xenopus laevis and it was done in a manner that enabled a flow through exposure system.
The studies evaluated key end points, growth, metamorphosis, sexual differentiation and it was shown in these studies that all of these were highly responsive to the positive control, estradiol,

Page 79

affects of estradiol on all of these end points.
Whereas, treatment with Atrazine over four orders of magnitude, .01 to 100 micrograms per liter had no affect on these primary end points.
One of the charge questions and one of the discussion points was, what was the mechanism by which Atrazine was disrupting gonadal development?
Well, I'd like to leave you with the comment that in the absence of affects, we can report on a mechanism by which Atrazine disrupts gonadal development in Xenopus laevis.
Thank you, Mr. Chairman.
DR. HEERINGA: Thank you very much, Doctor Van Der Kraak. At this point Mr. Osmer, I think that I'm going to entertain comments but I would anticipate quite a few comments, and to keep some order to this we're going to hear about this data again tomorrow from the EPA and we'll have chances at that point to ask questions and I presume your team will be here if the EPA would like to call on you.
So what I'd like to do is I'd to return to the first presentation by Doctor Solomon and ask, are there any questions on the panel about the aromatase hypothesis and the results that were presented there?

Page 80

Yes, Doctor Skelley?
DR. SKELLEY: Doctor Solomon, one of the, this is David Skelley, one of the study results you reported had to do with how rapidly Atrazine is cleared from amphibians and you mentioned that within 22 hours it could be undetectable, is that correct?
DR. SOLOMON: Yes, that's when you move them from an exposure situation to an unexposed situation.
DR. SKELLEY: Okay. So
DR. SOLOMON: Sorry, it's Keith Solomon for the record.
DR. SKELLEY: One of the studies that was submitted by the registrant is titled, Characterization of Atrazine Exposure and Potential Affects for Amphibians Inhabiting Sugarcane dominated Ecosystems in Florida", and the primary author is Timothy Gross.
And I'd like to just read one sentence out of the summary. The basic finding in the study was that 28 percent of the male frogs in sugarcane field associated locations had abnormal development of the Bidder's organ and this was about a fourfold increase over nonagricultural context.
And the sentence in the summary that I'd like to read is, "although the incidence of developed

Page 81

Bidder's organs was greatest in cane sites in the current study, results should be interpreted as an association between Atrazine exposure and the increased incidence of males with developed Bidder's organs since plasma Atrazine concentrations were not correlated with this anomaly at any site".
And I'd just like to ask you to comment on that conclusion relative to the statement you made.
DR. SOLOMON: Well first of all we have not studied the clearance of Atrazine from Bufo Marinus so I don't have actual data on the half life in those organisms.
It would depend on the most recent exposure, given that these are terrestrial and also aquatic, they share that habitat. Whereas the Xenopus is totally aquatic.
And our original study was designed to look at Xenopus as a model organism to see what clearance rates were in that. The, to do that kind of study, and I can't speak for Tim Gross personally because he's, I don't know enough of the detail of the study, but I would suspect that it would depend on the time of collection in relation to when the animals were last in the water and the sensitivity of the technique of analysis which is based on immunoassay and the

Page 82

possible issues with immunoassays, and given the other kinds of pesticides used in sugarcane production and in those agricultural areas.

I don't know that it's possible to draw any real conclusions about an Atrazine affect in that kind of mixed exposure situation.

DR. HEERINGA: Any other questions related either to the aromatase theory or to the uptake retention?

Yes, Doctor LeBlanc?

DR. LEBLANC: Gerry LeBlanc. Again Keith, regarding the accumulation of Atrazine, the information you presented which I assume was for Atrazine, or perhaps it was for radio labeled, and I just wondered if you could clarify that? Can we look at the information and conclude that Atrazine and its metabolites have a half life of about 22 hours?

DR. SOLOMON: In fact the paper which is, you can obtain from Environmental Science & Technology, the Atrazine was cleared quickly because it was metabolized as well as excreted. And the metabolites formed were also excreted from the animals and the half lives ranged for the metabolites, ranged from about the same time as Atrazine, roughly an hour, to around eight hours or something like that.

Page 83

But total radio activity was cleared relatively quickly as well, although there was some residual activity and one never knows, you know, it's bowel residue whatever that is, it could be EC14 incorporated into protein or unextractable conjugants, although they were hydrolyzed to see if they could be identified.

But we did identify several of the common metabolites but there were some unknown ones as well. That was done using chromatography radiotography.

DR. HEERINGA: Doctor Schlenk and then Doctor Isom.

DR. SCHLENK: Dan Schlenk, UCR. A question about the aromatase assays. We've done a lot of work with P450 assays and the turnovers that y0ou normally see with those assays are usually in the peak omoles, or hundreds of and this is in fish mostly, in mammals it's much higher, but the turnover rates are normally in the hundreds of peak omoles per minute per milligram. But yet your assays are fentomoles per hour per milligram and I'm kind of curious how that relates to say activity in the sinus which you also have activity there as well and how that compares to what you would see in say a rat or other organisms.

Page 84

Is that sort of consistent that you'd see such a low turnover rate and how does that compare to other species?

DR. SOLOMON: Keith Solomon. I would actually defer to people with more expertise in that area. Perhaps Doctor Van Der Kraak would be prepared to try that one.

DR. VAN DER KRAAK: It's Glen Van Der Kraak. The specific comparison, let me answer the question in two ways. The values that were reported for amphibians were consistent with other literature values for aromatase activity.

In terms of the cross species comparison, it becomes a little bit complicated as you move across species and particularly if you go to tissues like the brain and you do that in fish for example. The concentrations of aromatase in the brain there are very, very high.

The other issue is, is that you have a range of developmental stages across obviously groups of organisms.

And again I would come back to the fact that the values that were reported for the amphibians made biological sense as one looked across stage, so there was some consistency with what one would expect

Page 85

to see.

In terms of the fentomoles per milligram of protein and making comparisons with what happens in other species is that you also need to recognize, and you do, for the gonadal tissue that you're looking at an oviparous species, so the relative amount of tissue that is actually going to be the stereogenic tissue in an ovary of an oviparous species is much lower than it is in, you know, a mammal for example.

So I suspect that those are some of the reasons why there may be species differences but I think that I'm confident that the values that were reported for amphibians were, you know, appropriate with what's seen in the literature.

MR. OSMER: Mr. Chairman, Alan Osmer.

DR. HEERINGA: Yes, Mr. Osmer.

MR. OSMER: If there is additional interest in Atrazine and aromatase I would like to ask Doctor Jim Simkins to come to the table.

DR. HEERINGA: Let me turn to the panel. I guess I want to make sure that we proceed through the question period here within the allocated time.

Panel members, are there any other questions? Doctor Isom, you had a question regarding the aromatase?

Page 86

DR. ISOM: Well, Doctor Solomon, I'd just like to revisit your slide 29 and 30 and perhaps you could explain part of it in a little more detail than the conclusions on the slides. So that would 29.
DR. SOLOMON: Just give me a moment to get to that.
DR. ISOM: The upper right on survival?
DR. SOLOMON: Correct.
DR. ISOM: It appears to me that at least on the 1 microgram per liter exposure you did see a reduction?
DR. SOLOMON: Yes, there was a statistically significant decrease in survival there. And I guess under pressure of time, during the presentation this particular permit me to use other pointer so everybody can see it in this particular data set we had a significant difference at this concentration of exposure, that it did not show a concentration response which was what I was referring to down here.
In some of the other studies there was no significance in terms of the treatments and there was also no significant concentration response.
DR. ISOM: Okay. On the next slide, 30, again on the right side it seems that we do see some

Page 87

significance. And I think you said there were no significant differences?
DR. SOLOMON: There was no significant difference on this side and there was no significant difference here either.
You can see this rather high variance in the number of testicular ovarian follicles per frog. And I guess the other important message here was no concentration response.
This paper by the way is accepted in Chemosphere and I believe a copy has been made available to the panel. It's just been accepted so it was not circulated prior to this meeting.
DR. ISOM: In the upper right there's no indication of
DR. SOLOMON: No.
DR. ISOM: of variation in the
DR. SOLOMON: No, we took in sequence out of the tanks a total of 40 frogs because the histological work up is quite intensive.
So what I've just presented there is the number of frogs out of 40 that had testicular ovarian follicles in one or more of the testes. So I don't have any variance there.
Obviously if you count individual

Page 88

follicles in testis you can get some idea of variance which is in the lower panel.
DR. HEERINGA: Doctor Bailey?
DR. BAILEY: Yeah, Ted Bailey. I'm interested in are those error bars coming up from the heights of those?
DR. SOLOMON: Keith Solomon again, yes, these are standard errors of the mean.
DR. BAILEY: And do standard errors of the mean depend on the treatment?
DR. SOLOMON: Well, possibly. We did not actually look at that response.
DR. BAILEY: I would have expected a pool there, I would have expected those bars to be the same across all treatments unless you had evidence of heterogeneity in your error turn.
DR. SOLOMON: I will differ to statistical advice later on that if you don't mind.
DR. HEERINGA: We, may we revisit that unless we're prepared at this point. We can revisit when we have a little more time.
Doctor Denver, please.
DR. DENVER: Bob Denver. I noticed in reading the papers on the aromatase activity that the assays were conducted at a temperature of 37 degrees

Page 89

and the frogs, Xenopus laevis has a thermal optimum that's around 25 degrees.
So I wonder if you can comment on that?
And also there were a couple of papers that were published this year by Fan and colleagues that looked at the affects of Atrazine on aromatase activity in these cancer cells and propose a mechanism whereby it might be interacting with a transcription factor SF-1 and you didn't mention this, so I'd just like to hear what you have to think about it.
DR. SOLOMON: If I can, Keith Solomon again, those studies are actually addressed in the overview document, the Fan study and some of the other studies.
In terms of temperature, as an appointed senior citizen to make the presentation I'll hand over to one of my younger compatriots if you don't mind.
DR. HEERINGA: Absolutely. Mr. Osmer, if you'd like to comment.
MR. OSMER: I don't know if I can answer that question directly in the sense that in part with the relatively low activity of aromatase in amphibians, some of the tests were done, or the tests were done at 37 degrees.
And these were done consistently across

Page 90

all treatment groups so that if there was a bias, the bias would have been such that it was comparable across treatment groups.

As those particular studies that you're referring to were conducted in Doctor Gesey's laboratory at Michigan State University, I'd like to defer to trying to get some information about it from Doctor Gesey as to whether he did a thorough evaluation of responses at that range of temperature.

DR. GESEY: I'll just comment that given the Q10 affects that, you know, temperature has on enzyme activity, I would predict that the amphibian enzymes would be very unstable at that temperature.

MR. OSMER: Mr. Chairman.

DR. HEERINGA: Mr. Osmer.

MR. OSMER: Alan Osmer, it does seem that there is continued interest in aromatase.

DR. HEERINGA: Absolutely.

MR. OSMER: And if Doctor Simkins could join us

DR. HEERINGA: He may.

MR. OSMER: I would appreciate it.

DR. HEERINGA: He may, yes, I agree. Just to remind everybody too, we have probably approximately 30 minutes additional for this. But,

Page 91

Doctor Simkins.

DR. SIMKINS: Jim Simkins. I'm a Professor and Chair of the Department of Pharmacology and Neuroscience, University of North Texas Science Center. I am here on behalf of Syngenta.

With regard to two issues that came up, one was cross species comparison, I certainly am not qualified to talk about the aromatase assays in frogs, but am in mammals.

We have done those kinds of assays and I will report, or would like to point out to you that Ralph Cooper's spent a great deal of effort looking at the affects of aromatase, excuse me, of Atrazine on aromatase activity in a variety of tissues using both enzyme assays as well as message levels, and simply was not able to find affects of dosing up to 21 days in adult male rats at doses as high as 200 milligrams per kilogram.

And because of that we've come to the conclusion and that was looking at brain, adrenal, liver and testes we concluded that Atrazine was not affecting aromatase in animals.

And that's very consistent with a variety of livelong exposures to Atrazine in which no evidence of feminization of animals were seen.

Page 92

So once again as Glen has pointed out, looking or trying to determine a mechanism in mammals for a response that doesn't exist doesn't make a great deal of sense to us.

And those papers are all peer reviewed and published.

With regard to the Fan observations, you are correct, those studies are out there, they're reported and he describes what he believes, that is in the Fan papers is described a specific interaction of Atrazine with SF-1 transcriptional factor, those studies are being certainly reviewed. And secondly there are now attempts to replicate those studies.

Beyond that I would just comment that the affects of Atrazine on aromatase in vitro has been seen now in three cell types. Two of them are transformed cells, one is the transfected cell that Fan and colleagues used. It has not been seen in five other cell types that have been looked at.

The other observation I think is interesting is, over the entire dose range of Atrazine that's been tested, the magnitude of the aromatase response appears to be about a twofold increase which I find fairly remarkable in the lack of induction of aromatase in those cell types that respond.

Page 93

We currently are working on the hypothesis that the response seen in cell types that are capable of steroidogenesis may be a rather nonspecific response to the stress of Atrazine at or exceeding its solubility limits. We know well, at in the mammalian species that Atrazine is induced when cells are under stress and we think that may be what's being observed in some of those cell types.

DR. HEERINGA: Thank you, Doctor Simkins. Doctor Schlenk I believe had another question.

DR. SCHLENK: Yeah, it just occurred to me, I wonder why do people actually look at the gonadal aromatase and not the CNS if there's greater CNS activity? I mean it seems like all the studies that I've seen have focused on the gonadal aromatase, at least in this particular docket that we've seen.

Is there any relationship to the CNS levels or is that not even part of the equation? I'm just curious.

DR. SIMKINS: As to the relationship with CNS levels we know that at least ovarian steroids induce expression of brain aromatase. So there is a connection.

When it has been looked at, and agin I'll refer you to the Cooper papers, no affects of very

Page 94

high doses of Atrazine. These are doses that cause weight loss in rats. There was no affect on brain aromatase activity.

So again it's asking questions about mechanisms when no affect is seen.

DR. HEERINGA: Thank you, Doctor Simkins. Doctor Van Der Kraak?

DR. VAN DER KRAAK: There were, Glen Van Der Kraak, there were two papers that were published that deal with aromatase message and this was a paper by Parke, et al which described the methodology. And there was a second paper by Ecker, et al that looked at aromatase activity and that included evaluation in the brain.

And while I don't have in front of me the dose response relationship, as I recall there was no affect of Atrazine on the induction of aromatase in the message or expression, the messenger RNA expression.

DR. HEERINGA: Doctor Chambers?

DR. CHAMBERS: Doctor Solomon, with respect to the uptake in depuration studies that you reported that appears to be for adults, has something similar been done in tadpoles?

DR. SOLOMON: Just to clarify that, thank

Page 95

you, Keith Solomon, it was done in Stage 66 metamorphs. We wanted to, or one of the questions we were attempting to address there was not only the uptake in depuration in a size of aquatic organism that was reasonably easy to work with.

We also wanted to see if there was any accumulation in specific tissue such as the gonads and we needed to have animals that had at least differentiated gonads at that point. So we went for Stage 66. This was not adults.

DR. CHAMBERS: But still nothing in the younger tadpoles then?

DR. SOLOMON: No, there was no, we didn't do any work in the younger tadpoles.

DR. HEERINGA: Doctor Bucher?

DR. BUCHER: Doctor Solomon, it seems like based on the work done to distinguish the two populations of Xenopus and South Africa, the frogs that were chosen for the Syngenta studies were those from a lower background for testicular ovarian follicle populations.

Is that correct and would that have a did you think had any affect on the results?

DR. SOLOMON: I guess we can't both talk at the same, I apologize. We actually did not know the

Page 96

difference, the hapla typing was done after, long after the study had started at Wildlife International and IGB. So we did not know that at the time.

But at the current time those animals from the western Cape, the Cape sites are the source of exports of Xenopus laevis from South Africa to the rest of the world. And, but of course in cultures that have been in existence for many years such as is true in some laboratories, the actual provenance of those cultures is uncertain at this time.

Although this does offer a mechanism to ascertain what hapla type A might be.

MR. OSMER: Mr. Chairman, Alan Osmer, could I

DR. HEERINGA: Yes.

MR. OSMER: add to that that while those as Doctor Solomon said the hapla type, the genetic knowledge of the frogs that were used in the definitive studies was unknown at the time, and while they may be less sensitive to testicular oocyte, they certainly demonstrated sensitivity to feminization in the presence of estradiol.

DR. HEERINGA: Thank you. At this point I'd like, and we can return if there is another issue, but I'd like to return to Doctor Van Der Kraak's

Page 97

presentation specifically in terms of the description of the experimental design, experimental outcomes and statistical analysis of the two trials.

Members of the panel, yes, Doctor Patino?

DR. PATINO: I had a question if you could put your slide number 6, Doctor Van Der Kraak.

I thought there was an indication there on the sensitive period?

DR. VAN DER KRAAK: Sorry, Doctor Patino, this doesn't, I need to go to the slide show in order to show that. Yes?

DR. PATINO: Yes, the question I had or just a reaction or a comment, elicit a comment from you is, according to that sensitive period the sensitive stage or the period begins at Stage 42 of development and the experimental design has the exposure starting a little later than that somewhere between Stage 46 and 48 and I was just wondering if you had a comment on that?

DR. VAN DER KRAAK: I do have a comment on that and the, starting the experiment here in this Stage 46 is well within that window for which one could affect a 100 percent sex change, given the appropriate, you know, concentrations.

Page 98

In terms of how does this compare to the exposures that have been done in other labs, this falls right within what would be called the typical or normal exposure period that's been reported in the literature.

So the study group, and they may wish to comment more specifically on this, was well aware of this range of time for the sensitive window and they were confident that that was an appropriate to initiate the exposure.

DR. PATINO: And so you would expect a decline in sensitivity, say to a low dose as you wait during that window and start later?

DR. VAN DER KRAAK: Yes, we would expect that there would be a decline in sensitivity if we were to have extended this to have the exposure start at a later time interval.

And so if this declining component of this graph that I'm showing with the pointer here is saying that if you were to initiate the exposure here at Stage 54 or 55, you would have got a zero sex reversal. And if you were to start here you would have proportional up to 100 percent sex reversal.

DR. PATINO: Okay, so to make sure I understand, so if you start the exposure anywhere between 42 and probably 51

Page 99

DR. VAN DER KRAAK: 52.

DR. PATINO: it doesn't matter. I mean the sensitivity doesn't decline?

DR. VAN DER KRAAK: That's correct.

DR. HEERINGA: Presumably there's a Steve Heeringa presumably there's a distribution underlying this figure 2 in terms of actual individual exposure periods?

DR. VAN DER KRAAK: Yes, there would be a distribution. This is a cumulative figure that was prepared by Doctor Klaus who presented

DR. HEERINGA: Sure.

DR. VAN DER KRAAK: -- this at a recent conference on, you know, on aspects of sexual differentiation in amphibians.

DR. HEERINGA: Okay. Are there questions yes, Doctor Skelley?

DR. SKELLEY: This is David Skelley. Doctor Van Der Kraak, I wondered if you could show your slide number 20.

So it appears that your groups went to great lengths to conduct virtually interchangeable experiments in two locations.

And I have a two part question. First, I wondered if you could comment on what you think the

Page 100

origin of the distinction in influence of Atrazine on growth to metamorphosis might be?

And I'd just be interested in what your conclusion is with regard to the affect of Atrazine on growth?

DR. VAN DER KRAAK: I'd like to make a comment and then I'd like to also pass this to some of the investigators.

In terms of the response to Atrazine I would certainly say what these data show to me is that the response is not a very robust one, in the sense that the studies that were conducted at Wildlife International showed no significant difference across treatments over a very wide range of Atrazine concentrations.

The response in terms of the snout-vent length certainly in males did not show any significant differences with the treatment.

There were these affects that were seen in the Atrazine treated groups. And I guess that when I looked at these, one of the aspects that was struck by was how very tight the data points were.

And it occurred to me that I was having difficulty trying to understand what in a biological sense was a significant biological difference when some

Page 101

of these differences were measured in, you know, .1 or .2 of a gram. And similarly, when you looked at aspects of snout to vent length on the next slide they were also incredibly narrow in terms of differences that were in many cases much less than a millimeter, very much less than a millimeter.

So I wonder personally whether these magnitude of changes were ones that were, I would call, you know, great responses and question their, if you will, the global biological outcome that might result from these changes.

That's not withstanding that there are statistically significant differences within those groups.

But perhaps, and if you'd like an additional comment on that I could pass that to Doctor Springer perhaps.

DR. HEERINGA: Doctor Skelley, are you

DR. SKELLEY: David Skelley, an additional comment would be fine please.

DR. HEERINGA: Doctor Springer.

DR. SPRINGER: This is Tim Springer from Wildlife International. My interpretation of those figures is that there is probably a little bit of a variation between the groups that shows up here in the

Page 102

control group at IGB, it's a slight increase that occurred by chance.

And because the control is high you see that in comparison across all of the other groups, and the reason that I, I believe that is because you don't see that in the Wildlife International figure.

So if you consider that one group being one that varies, then that's the way I've interpreted those slides.

DR. HEERINGA: Doctor Skelley, please.

DR. SKELLEY: David Skelley, just one follow up question, I want to make sure I got this right.

The WLI control, you ended up with eight containers. Is it the case that the IGB treatment there is based on sixteen containers?

DR. SPRINGER: This is Tim Springer again. Yes, you're absolutely correct.

DR. HEERINGA: Doctor LeBlanc.

DR. LEBLANC: Gerry LeBlanc for Doctor Van Der Kraak. Just some clarification with respect to experimental design.

If I understand correctly, each treatment consisted of eight tanks divided into two clusters.

Page 103

DR. VAN DER KRAAK: Yes.

DR. LEBLANC: Were the clusters themselves treated as replicates in the analysis?

DR. VAN DER KRAAK: I have knowledge about that but I would really like to pass that on to Doctor Silken who has considered that very question and he may be better positioned to give you a specific answer to that question.

DR. HEERINGA: Doctor Silken.

DR. SILKEN: This is Robert Silken, I'm a statistician with Silken & Associates and we were responsible for the statistical analyses of all four aspects, the two gross and the two histo analyses.

With respect to the issue of the design of the experiment, we did follow a robust design based upon the estradiol studies and the earlier studies and we did allow for sixteen control tanks just in case anything should happen, and as it turns out it did happen.

I heard Doctor Skelley ask a question about whether this was due to the fact that IGB ended up with sixteen controls and Wildlife International ended up with only eight. The power of those two studies is very comparable. The impact of going from sixteen to eight did not affect the power of those

Page 104

studies, nor probably the outcomes here.

It was really the tight variability and you got a little bit of statistical significance, but not biological significance. That's not too surprising.

We did look at the idea of the grouping of four tanks together. This was something that we raised at the time of the design and said, well, why don't we have, you know, each tank separately. It just really wasn't practically possible to manage a separate pumping system for each of the individual tanks.

And we also looked at the issue of, well, if you're going to have eight feeds for the same concentration, the variability in getting those feeds all the same is probably a greater danger than having two sets.

We did nevertheless consider the question of whether there would be an affect between the two clusters, whether we needed to consider a cluster affect.

We did test for those affects and only on one occasion out of 160 tests did we find any evidence at all of a cluster affect. So we did look for one. We didn't find one. If there would have been a cluster affect it would have tended to increase the

Page 105

false positive rate and hence increase our chance of finding differences.

DR. LEBLANC: So just a clarification, typically when we're looking at statistical significance, tanks are the replicates, not the clusters, is that correct?

DR. SILKEN: Yes, throughout this analysis for Atrazine the tank was always the unit of observation. When there was extreme feminization which only occurred in the E2 portion, estradiol portion, for males there was only a very few males for the E2 treated tanks. Then we had to fall back to individual frog levels.

But everywhere else, and including all the Atrazine, it was all done at the tank level.

DR. LEBLANC: And I would go back to Doctor Van Der Kraak. Three of the clusters in one of the experiments was eliminated at some point in the course of the experiment due to problems.

And my question is, were they eliminated during the experiment or were they taken to completion and then the decision was made to not include them?

DR. VAN DER KRAAK: Glen Van Der Kraak, there were two clusters that were removed and one individual tank.

Page 106

DR. LEBLANC: Okay, so could you clarify which clusters and which individual tanks?
DR. VAN DER KRAAK: On the slide, this cluster was removed and this cluster was removed. And
DR. LEBLANC: Okay.
DR. VAN DER KRAAK: and it's tank number 6 in this situation here that was removed.
DR. LEBLANC: Okay. And these were never taken to completion?
DR. VAN DER KRAAK: I'll let Alan respond to that please.
MR. OSMER: And I was going to ask Doctor Springer, who was the principal investigator at that lab to responid.
DR. HEERINGA: Doctor Springer.
DR. SPRINGER: This is Tim Springer. The control tank cluster to the left and also the well, the two control tank clusters, the animals from those were actually processed and taken through histology and the information from those is available, okay?
The control tank, or rather the tank from the 1 microgram per liter Atrazine group was terminated at the time that that bloom was observed in that. We just terminated it immediately at that point.
We decided to keep those frogs in the

Page 107

process flow at that time, but we decided at that time that they would not be used in the value or excuse me, the statistical evaluation. So the decision to exclude them from the statistical evaluation was made when we discovered in the case of the control 2 tank that's circled, when we discovered the bloom in those we had to treat them differently and try to clean them up to try to stop the bloom.
So at that point they were no longer comparable and we made the decision at that time to exclude them from statistical analysis. But, we were afraid to not take the animals through because questions could come up about, well, what about those animals, you know?
So if you look in the report the raw data is there but they're not included in the statistical analysis.
DR. LEBLANC: Thank you.
DR. HEERINGA: Before we turn to Doctor Bailey, just a comment, my plan is to continue this discussion until a logical break for out noon lunch and then return to public comment, starting with the next public commenter after lunch.
So Doctor Bailey please.
DR. BAILEY: Ted Bailey. It was a

Page 108

cluster of four tanks that has the microbial bloom, is that correct?
DR. SPRINGER: Initially the bloom showed up in the tank number 6 in the 1 microgram per liter Atrazine group, that tank was terminated. And in a couple of days it showed up in the control 2 cluster that's circled there.
And so those five tanks were affected by it. It wasn't seen in any of the other tanks.
DR. BAILEY: It seems like the tanks are not acting independently, I mean as a cluster they were taken out of the study? Not independent tanks around the room?
DR. SPRINGER: You are correct, the observation was that that cluster of tanks, notice what we did for example, in the 1 microgram per liter tank it was detected because we walked into the room and the tank was cloudy, so it was obvious what was going on.
DR. BAILEY: Thank you.
DR. HEERINGA: Yes, Doctor Yeater.
DR. YEATER: This is Kathy Yeater. I think my question probably applies to Doctor Silken as far as the choice of statistical analysis of the data.
I was wondering if you could comment on the use of age to metamorphosis as a continuous

Page 109

variable as opposed to using it as a time to event response and perhaps applying, and being able to use the time dependent variable such as the snout length and body weight that were also recorded at the time of metamorphosis in terms of Kaplan-Meier estimation which would be more commonly used in the survival analysis, but it can still be used for a time to event data.
DR. SILKEN: This is Doctor Silken. Yes, well it's true the Kaplan-Meier is a standard procedure for analyzing a time to response event.
Here we did not of course a timed series of body weights or snout to vent length. We really only had one observation and not a timed series for those.
The only thing that we did have was one observation on the time to metamorphosis that a continuous variable as you point out, and it was treated in an analysis of variance context.
DR. YEATER: And so the body weights and measurements, were those taken at metamorphosis or at the end of the time frame of the study? Anyone?
MR. OSMER: This is Alan Osmer. All measurements of the frogs were taken at Stage 66, at termination of the individual frog.
DR. SILKEN: This is Doctor Silken, let

Page 110

me add on to my earlier comment.
We were also working with the tanks means, so we had tank means for age too, time to metamorphosis, or age at metamorphosis, so that was a tank means.
DR. HEERINGA: Doctor Chambers.
DR. CHAMBERS: Jan Chambers, I have two questions.
One is, was the same batch or lot number of Atrazine used throughout the entire experiment and at both locations?
MR. OSMER: This is Alan Osmer. Yes, the answer is yes for Atrazine, estradiol and any other parameter that we could harmonize between the two.
DR. CHAMBERS: And the second question to clarify, each of the four tanks in a cluster received the same solution out of the mixing tank, is that correct, so they were all the same water?
MR. OSMER: Alan Osmer again. That is correct. For each of the treatments there one stock solution that was then fed to a pump, went into a mixing cup that fed the four tanks in that cluster.
DR. HEERINGA: Steve Heeringa. A question maybe to Doctor Silken or Doctor Springer or Doctor Lutz with regard to the frogs themselves. There

Page 111

were a number of breeding pairs, I want to say sixteen.
How were they allocated to sites and then to tanks within, in terms of their prodigy, in terms of the tanks within sites?
MR. OSMER: This is Alan Osmer, let me begin an answer and then ask others to expand upon it.
All of the frogs originated in Michigan, Xenopus One.
DR. HEERINGA: About twelve miles from my home.
MR. OSMER: They were approximately ten breeding paid from that source. They were not the same pairs that went to Germany and Maryland. They were, many thousand were spawned and held at Xenopus One for a period of five days perhaps and then shipped.
And so they were essentially randomized at the supplier but the different spawn were kind of randomly mixed and then shipped.
DR. HEERINGA: So the spawn of the breeding pairs were randomized, there were different breeding pairs that were used at both sites, but the spawn was randomized within the site across tanks as best could be done?
MR. OSMER: Correct, yes. There was no process to try to randomize them but just in the

Page 112

procedure I think they were fairly random.
DR. SPRINGER: I think there were different levels of randomization that occurred. At Xenopus One they had their own procedures which, you know, I don't know the details of, but once they arrived at our laboratory, when they were allocated to tanks there was a randomization procedure occurring at that point in time too, which I can describe if you like.
DR. HEERINGA: I think the comment that I'll make, it appears that at least to the best of the ability there was no specific co-occurrence of breeding pairs with individual tanks or individual treatments. I figured that was the case but I just wanted to hear that.
Doctor Schlenk.
DR. SCHLENK: Yeah, Dan Schlenk. This is actually a twofold question.
The first relates I think to one of the biological aspects and the second relates more to the exposure chemistry, so I think there was somebody that you wanted to bring in with the analytical aspects.
But first of all I'll deal with the biological aspects. I notice in the report that was given to us that there, an affect was noted in males at

Page 113

the point 1 dose as gonadal hypoplasia and it was only seen at the WFI site if my notes are correct.
And I was just curious, the reason why that was not considered significant was because it was only seen at one location and not the other, would that be fair I guess?
MR. OSMER: Maybe I could make sort of a general comment on some of these other morphological features and
DR. SCHLENK: Sure.
MR. OSMER: and what the motivation was.
The EPA's white paper and the SAP's recommendations in 2003 were to examine the, what were referred to as apical end points, so sex ratio, inter-sex, mixed sex, the more overt findings.
The team met and decided that to be all inclusive, that if we were going in we would look for any and all morphological attributes that might be associated with estrogenic affects or Atrazine. And that is a question that I've had posed to me from the Syngenta folks for a long time, why are you looking there?
So it is the, our motivation was scientific in nature.

Page 114

And then I think over the course of the study we were reevaluating the significance or lack of significance of those other findings.

And I guess with that I might ask Jeff Wolfe to comment on his perception of some of the secondary end points. And then I believe the other question was really more to the statistical significance of it.

DR. SCHLENK: No, the chemistry, but that's

MR. OSMER: Okay, I will take that.

DR. SCHLENK: Yeah.

DR. HEERINGA: Doctor Wolfe.

DR. WOLFE: This is Jeff Wolfe, EPL out of Sterling, Virginia, I'm the study pathologist.

When I was asked to actually perform the histopathological evaluation of this study, I did not limit myself to any specific end points, even though we were aware of what those apical or primary end points were. I was not, I did not feel it appropriate and I was not asked by Syngenta to limit myself to only a few end points.

So I was looking for any possible abnormality that I could find.

Now, I'm not sure whether you were

Page 115

referring the gross finding of segmental hypoplasia or the histological finding of segmental hypoplasia.

DR. SCHLENK: It's table 7 on the report, whatever that is.

DR. WOLFE: Well just one comment I will make as I think it was stated before, that we considered a priority, the histopathological assessment to be the gold standard. As in most toxicological bioassays, the gross observations were more in terms of pointing out places to look in terms of histopathological evaluation.

And we did try to correlate all of the gross findings, or not correlate, but associate the gross findings with a histological diagnosis whenever I could.

So the biological relevance of some of these other findings such as segmental hypoplasia is really not very well known, not very well characterized and I think the important thing in my estimation is the fact that we had certain primary end points and secondary end points that were positive in the estradiol treated animals, and we just did not see any of that in the Atrazine treated animals.

DR. SCHLENK: Yeah, I just, not being a pathologist I just wonder what the relevance was of

Page 116

gonadal hypoplasia, if that was something that was biologically significant or not and maybe that was why is was not included, or not, you know, highlighted as an affect. And that wasn't an Atrazine treatment, it was in the .1 treatment.

And the second question I had was related to the chemistry. If you want to go to slide 15 on Glen's presentation. So the table that I have, at least in the report that I was given doesn't have the actual amounts that are listed as far as the concentrations, particularly in the two lower doses.

And what I have in the report is a graph that shows the percent nominal verus the study days. And one of the things I found when I was going through this, and this relates more to study question 4 I think we'll get to during the week, was the exposure regime and whether or not the concentrations were hitting the mark as far as nominal versus measured.

And one of the things that was shown in the report that was given was that if it was, I think your LOQ was 10 nanograms per liter at .01 which is your low dose. Is that correct?

MR. OSMER: Yeah, that is correct.

DR. SCHLENK: Yeah.

MR. OSMER: This is Alan Osmer.

Page 117

DR. SCHLENK: Yeah, and then your level of detection is half of that, basically it's .005?

MR. OSMER: Correct.

DR. SCHLENK: So, and anything that was below that was considered 50 percent?

MR. OSMER: If it was below the LOQ

DR. SCHLENK: It was condiered 50 percent.

MR. OSMER: 50 percent of that.

DR. SCHLENK: So when I went through the tables in the back to look at the actual amounts that were listed there was no measured value, it just had less than 10 nanograms per liter out of a majority actually of the water samples that were taken.

So I'm just wondering, is the figure here actually the one on the left or right, are those actual values then that were because that was, none of those actual values were actually in the report that I saw? They were all considered less than the ten.

MR. OSMER: That is, I believe that is correct and I believe this figure was, because of the software used we were creating values to plot on there. But the information in the report is correct.

DR. SCHLENK: Okay, so if it's just so that I know, the, so these values then, because if 5 is

Page 118

your MDL then you basically are above 50 percent on a lot of these then roughly? Because if that's 5 that would be half of that but does that make sense? Do you see what I'm saying?

MR. OSMER: I think I, I think I do but I guess at this point I should try to have a chemist come up here and give you the correct answer.

DR. SCHLENK: Well the point is, is that, and if you look at the table that was presented, I think it's the next slide maybe, if you go to the next slide, that basically you're only getting 50 percent at the IGB site which by the way is not where you didn't see the hypoplasia by the way, so that's kind of the relationship between the two, you're only seeing 50 percent of your official critical window mean.

Now that percentage is based on an arbitrary number, so you don't really know that that's not 50 percent of .01, that would be .09, .08, .07, right? Or it could be .01 or .02, .03, right? Because that's my, that's my question because in the table, if you go through the actual tabular things, and maybe I can show you this, you know, in the break or something, in the tabular break it only shows less than ten, it doesn't give you an actual number.

MR. OSMER: Uh-huh (nodding

Page 119

affirmatively).

DR. SCHLENK: And, you know, is it less than 10, is it 9, is it 8, is it 7 or is it 1 or 2? Because, do you understand what I'm saying? I mean because it could be less than your LOD but not less than your LOQ.

MR. OSMER: I do understand the question and I think there's an easy answer if I could ask Robert Yokeley to quickly join, just to

DR. HEERINGA: Okay.

MR. OSMER: clarify this.

DR. HEERINGA: The plan, I wanted to amend my statement before. We will go until 12 o'clock on this discussion. We can revisit some of these points but I want to get in another public commenter who is unable to be here later. So if you would like to do that, otherwise I know there are other questions on the panel, please go ahead though.

MR. OSMER: Okay, then I would

DR. HEERINGA: If it's just a matter of computation I'd rather have it worked out and then brought back to us for a statement.

MR. OSMER: That's fine, that's what we'll do.

DR. HEERINGA: If it's very clear,

Page 120

otherwise if we can take the time outside to work it out maybe Dan can speak with him and we can come back to the full group with the result.

MR. OSMER: Okay, we'll do that, we'll work this out and bring it back to you.

DR. HEERINGA: I think that's to everybody's benefit because then the question is clearly understood and the response is clearly understood too.

MR. OSMER: Yeah, that's fine, thank you.

DR. HEERINGA: Okay, panel members, Mr. Pauli, you had a question before?

MR. PAULI: I was actually, I was going to go back to something that Doctor Bucher it's Bruce Puali, Environment Canada we heard something that, I don't know if we have time for it, something that struck me during Doctor Van Der Kraak's talk was that there was no differential cell counts done in the gonads because of the immaturity of Stage 66 of Xenopus laevis gonads.

I have wondered, maybe with Doctor Wolfe here, if he might care to comment on whether or not he feels that might influence the overall judging of developmental abnormalities? If they're not differentiated at 66, would there be an advantage to

Page 121

take some older animals and look for affects?

DR. WOLFE: Yeah, this is Jeff Wolfe again. You are correct in that at Stage 66 the testis essentially is comprised of, the germ cell population is primordial germ cells and spermatagonia which really are very difficult to even differentiate histologically. And in the ovary there are primordial germ cells and oogonia and in occasional animals you'll see some oocyte, so it's correct that at Stage 66 it would be and this is really one of the challenge questions to come up it would be impractical and probably of very little value to do any type of differential type of counting.

I have actually done myself, differential counting of germ cells in fish and adult animals, in fact in minnows and it probably would be more appropriate for adult animals.

MR. PAULI: Bruce Pauli, Environment Canada, would that influence then do you think your ability to identify TOF's?

DR. WOLFE: Back to me again? This is Jeff Wolfe again. I think there might be a little bit of sometimes a confusion between mixes sex and testicular, TOF's, or testicular oocytes.

My interpretation, and this is not easy

Page 122

to find in the literature anywhere, is that a lot of the difference between whether you find mixed sex in testicular oocytes is one of age or stage of gonadal development.

In younger animals you're more likely I believe to find mixed sex whereas in older animals you're more likely to see testicular oocytes.

Now when I'm talking about older, I'm not talking so much about relative to stage of metamorphosis, I'm talking about chronological age and reproductive age, because that seems to be a little bit unhinged from metamorphosis and that's been shown in previous literature and also in some of our early estradiol work.

Does that answer your question?

MR. PAULI: Yeah, I think it does. I think Doctor Solomon wants to jump in.

DR. SOLOMON: We did, Ernest Smith did a study in South Africa where he looked at adults and testicular cell types in reference and Atrazine exposed sites, and found no difference between them in adults. So Paul, that is published in the literature.

DR. WOLFE: There is one more thing I can add. We did, even though we didn't do differential cell counting per se, we did some semi-quantitative

Page 123

work.

So for example in the females where I could differentiate between animals that had just oogonia and animals that had oogonia and perinuclear phase oocytes we did actually separate those two groups out and look at those.

So there was some semi-quantitative analysis done.

We also did semi-quantitative analysis of germ cell density in both males and females which I think was a lot more practical than doing differential cell counting in this case.

DR. HEERINGA: Thank you, Doctor Wolfe.

At this point what I'd like to do is to bring this period of the public comment to a close. We can revisit questions that arise from the panel with the Syngenta group during the public comment period if they come up.

I want to make sure we have a full discussion of all these issues and that the panel members have all of their questions answered. But I also want to keep the flow too of the public comment period.

So at this point in time I'd like to thank the public commenters representing Syngenta and

Page 124

I'd like to invite our second public commenter to the podium, and that is Doctor Jennifer Sass who is here on behalf of the Natural Resources Defense Council.

Doctor Sass, please. Doctor Sass has requested twenty minutes and please take that twenty minutes. Whatever you need. Doctor Sass has prepared written comments for the panel. They've been distributed to the panel and they will be part of the docket as will all public comments from this session.

DR. SASS: Thank you, Doctor Heeringa. And thanks for accommodating me. I am going to be rapid but my written comments should be distributed and they're more complete.

I'm Jennifer Sass, I'm a senior scientist with the Natural Resources Defense Council, which is an environmental nonprofit. I'm a senior scientist in the health program and I'm based here in Washington.

First of all, in summary, just to let you know, the reason why this meeting is happening is because after the '03 Scientific Advisory Panel, NRDC negotiated with EPA to have a re-review of this issue along with the cancer issue related to Atrazine when more data was available and when a full and informed review would be possible.

Page 125

So what happened in the intervening three years is the studies that you're going to look at now.

Unfortunately we are extremely disappointed that EPA chose to narrow the charge questions so severely. And not only that, but to limit the studies that are presented to you so severely that you're asked to provide expert advice on a very narrow charge question which is the affect of Atrazine on gonads in amphibians during development.

The question that we had wanted looked at, which is a more regulatory relevant question is data pertaining to Atrazine impacts on wildlife and human health, particularly its potential affects on endocrine destruction.

That was the issue that was left over in '03 and that's the issue that essentially EPA would be informed on in order to regulate Atrazine better according to its environmental statutes, to protect human health and the environment.

EPA is not statutorily authorized to protect gonads in amphibians from Atrazine during development specifically.

And some of the authorities that EPA uses to regulate pesticides that are relevant here are

Page 126

listed on the back of my comments.
So in summary, NRDC would look forward to a fair and complete review of all the available literature, with greatest consideration given to those studies that are robust and well designed and preferably published in the peer reviewed literature. We're disappointed by the narrow task that has been assigned to the experts of this panel.
NRDC also asks EPA that all scientific data relevant to Atrazine as an endocrine disruptor be evaluated, including mammalian, aquatic and mechanistic studies. And again, we're disappointed that the experts have been hamstrung by the arbitrarily narrow charge.
NRDC asks the Scientific Advisory Panel to either consider providing broader and more relevant advice to EPA or to ask EPA to convene a meeting in the future when it can answer a question that's more relevant to the regulation of Atrazine so as to protect wildlife and human health.
And in particular, to go to NRDC's response to the charge questions, which is how I've laid out my comments so hopefully it will be easy for you read when you skim it in your spare time, I know you have a lot to look at.

Page 127

Most relevant I think is what I consider to be a really glaring inconsistency in the way that EPA has developed criteria for evaluation, not a priori but in fact a posteriori to the studies. And then secondarily apply those criteria in its white paper.
So I'm only going to pick on one for these oral comments, but I have a few more in my written comments, which is that there are numerous occasions, and I, I think reference a table in the white paper on page 35 where EPA says that it was unable to determine or that there was not enough information provided in the report or that the report was not clear enough to somehow get the information that it needed to evaluate all of the other studies, except for the WLI/IGB studies, what it's calling the, what it had from the data call in in the last three years.
For that study, I'm going to call it one because it was two labs but they coordinated together, for that study EPA actually worked very closely with the labs during the study, while it was being carried out. And the white paper says they conducted inspections of each of the laboratories, including extensive review of the raw data, collection sheets and data summary tables by AWEEKA and OPP personnel as

Page 128

you've already heard, even going so far as to inspect the lab according to the white paper that actually conducted the histological analysis as we've heard here today.
So the idea that EPA is unable to get the information it needs in order to make a clear evaluation from all the other authors of all the other studies, because somehow it can't pick up the phone and talk to them, but in this particular study they were able to work so closely with the authors in order to get the information they needed, to me represents not only a glaring inconsistency, but I think biases the Agency towards unfairly considering what ends up being one study set which EPA then uses to hinge its conclusions on for the white paper.
Another charge question you're asked to look at is how EPA considered the open literature studies.
For this I am responding that EPA failed to consider many studies in the open literature that would have been relevant to a broader and more important question.
For example, EPA failed to include scientific evidence of neuroendocrine effects in amphibians associated with Atrazine. And I list some

Page 129

of those studies in my written comments.
EPA also failed to include scientific studies of long term or pertinent effects resulting from amphibians associated with Atrazine when they were exposed during early life stages. And that would the kinds of effects that would impact later life outcomes, including susceptibility to subsequent infection. And again in my written comments I list some of those studies.
EPA also failed to consider scientific evidence of nonlinear or nonmonotonic relationships. This is disastrous when one is considering an endocrine disruptor like Atrazine and again unfairly biases towards studies that fail to find an affect. And I list a number of studies in my written comments that are relevant to this issue, including some that are published by the registrant as well.
The severe limits placed on the SAP review are likely to bias the outcome in my opinion. EPA's scientific review failed to include studies that demonstrate adverse endocrine effects of Atrazine in mammals and evidence of hormone disruption activity in amphibians and reports of destructive normal progression of sexual development in rats. And I list a number of those in my public comments, effects on

Page 130

delayed puberty, effects on sperm count and motility, effects on testosterone production. And some of those are strain specific as you may know from the literature. And some of those are timing specific. But they're all relevant when wildlife and humans may be exposed.

In my final point as far as the published literature goes is that EPA failed to consider evidence of impacts of mixtures and co-contaminants on Atrazine. This is sort of a failure of the regulatory system in general, but it is not a failure that EPA needs to accept when it's regulating pesticides. There's a lot of published literature showing that the effects of multiple pesticides together may have more than additive effects. And in addition we have USGS data showing that streams are contaminated with more than one pesticide at any given time.

So it's both relevant from an exposure perspective and from a toxicology perspective. And I list some of the information in my written comments.

Concerning the data call in studies I have no specific comments on them at this time.

And so finally we believe that the agency has intentionally and unfairly hamstrung the

Page 131

Scientific Advisory Panel by developing a series of charge questions that clumsily avoid asking relevant regulatory questions about whether or not Atrazine poses a risk to human health and wildlife, in particular through its activity as an endocrine disruptor.

And NRDC asks the experts on this panel to move beyond this limited set of charge questions and request that a meeting be reconvened in the future to review the more relevant questions related to Atrazine as an endocrine disruptor and its potential impacts on wildlife and human health.

Thank you.

DR. HEERINGA: Thank you very much, Doctor Sass. Are there any questions from members of the panel for Doctor Sass?

Doctor Isom.

DR. ISOM: Doctor Sass, I was wondering if perhaps you could provide us with the full citations on those papers. They just list the names.

DR. SASS: Would it be okay if I emailed those this afternoon to Joe Bailey and he could distribute them?

DR. HEERINGA: That would be just fine.

DR. SASS: Okay.

Page 132

DR. HEERINGA: I think throughout this three or four day period there are going to be pieces of information which will be requested and I think we would handle that way, that they would be supplied to Joe Bailey and they would be provided to the panel and put on the docket.

DR. SASS: That's fine, thank you.

DR. HEERINGA: Additional questions from the panel? Not seeing any at this point I'm going to thank Doctor Sass for her comments.

And we are at 12 noon. I would like to call a break for an hour and fifteen minutes. Experience has shown that sixty minutes doesn't allow everybody to get back here.

So let's plan to reconvene at 1:15 and we will continue with the period of public comment at that point in time.

My intent would be to take that commenters who have registered with Joe Bailey first. We may return to additional questions from the panel for the Syngenta group because of the complexity of that presentation.

But any other public commenters who have had an interest in making a short, five minutes or less, comment at this point, we encourage to please see

Page 133

Joe Bailey during the break to arrange to be added to the agenda.

Thank you very much. See everyone at 1:15. I'll tell you what, let's make it 1:20, you'll get a little extra time.

(WHEREUPON, the morning session was adjourned.)

DR. HEERINGA: I'd like to welcome everyone back to the afternoon session for the first day of our multi-day meeting of the FIFRA Science Advisory Panel on the topic of the Potential for Atrazine to Affect Amphibian Gonadal Development.

We are in the middle of our public comment period for this meeting and we've heard this morning from representatives of Syngenta Crop Protection. Also from Jennifer Sass or the Natural Resources Defense Council.

And we're ready now to move on to our third public commenter. And that would be Rebecca Adcock of the American Farm Bureau Federation.

And Rebecca, are you here?

MS. ADCOCK: Good afternoon and thank you to the members of the SAP here today. The members that I represent are glad that you're here and seeking the review and looking into these matters that are very

Page 134

important to farmers, to agriculture and to the environment.

My name is Rebecca Adcock and I am the Congressional Relations and Government Relations Director for the American Farm Bureau and I'm here today to speak to you on behalf of our members, farming and otherwise who believe that the registration of safety and understanding the environmental effects of all pesticides and all the chemicals we use are very important.

The American Farm Bureau Federation is the nation's largest general farm organization. It represents farm families across this country and for Atrazine it's the most important herbicide used in soil saving conservation tillage and non-till farming. Farmers depend on the safe and effective use of Atrazine to control weeks on about two-thirds of the country's corn and soy acreage, and 90 percent of its sugarcane.

Atrazine is effective against the toughest weeks. It's cost effective and it improves crop yields.

Benefits it achieves for an estimated $28 per acreage advantage over other herbicides and that is an EPA quote.

Page 135

AFBF is pleased that for more than 12 years or review EPA has completed another milestone in establishing the safety of this important crop protection product. As a result of this most review EPA has determined that Atrazine does not adversely affect amphibian gonadal development and believes that there is no compelling reason to pursue additional testing of Atrazine for amphibian gonadal affects.

AFBF does recognize that uncertainties were identified in 2003 by the EPA SAP and that a need to examine both field and laboratory studies on the purported affects of Atrazine on amphibians was needed. Because of these uncertainties EPA did require that the registrant, Syngenta, conduct these studies and test the potential for Atrazine to affect amphibian development.

EPA has now reviewed 19 laboratory and field studies, including the registrant's studies and the research available in the public literature. And according to EPA only two studies, the two that you've heard from, submitted by the registrant incorporated all of the necessary design elements and fully accounted for experimental and environmental conditions that could influence the results.

These two identical studies were

Page 136

performed separately by independent laboratories with a third laboratory doing the pathology evaluations. Both showed that Atrazine does not have an affect on the development of the sexual organs in frogs at ranges from very high to very low.

AFBF believes that this objective research clearly reinforces the safety and supports the continued availability of Atrazine for American farmers.

AFBF and our counterparts in the crop protection industry support extensive thorough research and testing of the products relied upon to protect the world's food and fiber production.

However there are some people who are still critical and continue to condemn studies that are sponsored by anyone other than the government or perhaps themselves.

Relevant to all stakeholders is the fact that no federal rules or policies suggest or should suggest or require that quality controlled objective scientific work be ignored or given lesser weight based solely on who may have paid for it.

The simple truth is, studies conducted to support registration of pesticides must and should meet the extremely stringent standards of GLP audits in

Page 137

submission for all raw data so that EPA can reconstruct the study from the ground up.

Atrazine has undergone the most extensive safety testing, both in time and volume, ever conducted on an herbicide. Our farming members appreciate and support EPA's extensive review and continue to support the safety of Atrazine in crop production.

Thank you.

DR. HEERINGA: Thank you very much, Rebecca. Any questions of this particular public comment? Not seeing any, I'd like to thank Rebecca for her comments and invite up the next scheduled public commenter and that is Scott Slaughter and he's representing the Center for Regulatory Effectiveness.

Mr. Slaughter has submitted comments in writing in advance and those will be posted on the docket.

MR. SLAUGHTER: Hi, I'm Scott Slaughter and I'm presenting the comments on behalf of the Center for Regulatory Effectiveness and I want to thank you for this opportunity. The mike's now on.

CRE agrees with EPA's recommendation that the higher tiers of testing proposed in the 2003 white paper are not needed at this time that's a

Page 138

quote from EPA.
The data call in tests are dispositive, Atrazine does not harm frogs.
CRE commented in the 2003 amphibian SAP that there were no test for Atrazine gonadal affects that were accurate, reliable and reproducible.
CRE recommended that EPA develop a valid test before reaching a conclusion on this issue. EPA and the SAP agreed with CRE. They rejected all prior tests as unreliable and following guidance from the 2003 SAP, EPA and the Atrazine registrant developed a new laboratory test that is accurate, reliable and reproducible.
The DCI tests show, and I quote EPA, "no affects of Atrazine on amphibian gonadal development". We do not believe there is any need for EPA to explore this issue further.
CRE does wish to comment on charge question 8B which asks about the potential value of having the gross morphology and histopatholotical sections from studies published in the open literature, to potentially be volunteered by the authors for a pathologist's review.
CRE does not believe that any data considered in this manner should be CRE believes that

Page 139

any data considered in this manner should be documented with raw data and with regard to the chain of custody and audited and verified by good laboratory practice standards as have the DCI studies that you're reviewing now.
Additionally, any study submitted for this purpose, the open literature pathology review, must meet the standards of the Information Quality Act as does the DCI test which you're reviewing now.
CRE commends the 2003 SAP, EPA and the registrant for their integrity, effort and commitment to answering the questions of Atrazine's affects on amphibians. The 2003 SAP and the DCI test developed and performed pursuant to the 2003 SAP, are a model for how government regulatory science should be conducted.
CRE is confident that this SAP will be conducted in accordance with the same high ethical and scientific standards.
Once again, thank you for the opportunity to submit these comments and we thank the members of this SAP and the members of the 2003 SAP for their service.
DR. HEERINGA: Thank you very much, Mr. Slaughter. Comments or questions from the members of the panel for Mr. Slaughter and his comments?

Page 140

MR. SLAUGHTER: Thank you.
DR. HEERINGA: Thank you very much. I should also mention that Rebecca Adcock's written comments are also available for panel members and will be posted on the docket too later. I neglected to say that before.
I'm consulting with Doctor Portier here. Mr. Slaughter, you had introduced a few additional comments on the I think charge question number 8 and Doctor Portier was suggesting you may want to amend your written comments to reflect that.
MR. SLAUGHTER: Okay, so you me to just add it? Okay.
DR. HEERINGA: Just add that and send it in to Joe Bailey. We appreciate it.
MR. SLAUGHTER: Can I send it tomorrow?
DR. HEERINGA: Anytime.
MR. SLAUGHTER: Thank you.
DR. HEERINGA: Thank you very much. Our next public commenter is Doctor Richard Fossett who is here on behalf of the Triazine Network. Doctor Fossett.
(WHEREUPON, there was a discussion off the record.)
DR. FOSSETT: Sorry I took a little time. My name is Richard Fossett with Fossett Consulting and

Page 141

I am appearing on behalf of the Triazine Network. And I appreciate the opportunity this afternoon to meet with the panel and very briefly share some background and perspectives on the use of Atrazine and some changes the farmers have made in management to try to reduce the chances of Atrazine entering surface and to protect aquatic environments. Next slide please.
Atrazine remains the most widely used corn and soy herbicide in the U.S. and it's the most widely used herbicide because it provides farmers with value, effective weed control at low cost. And that effective weed control results in increased yields.
An analysis of 20 years of university weed control trials across the midwest, almost 250 different trials, treatments that contained Atrazine yielded on average 5.7 bushels per acre more than comparable treatments of combinations of herbicides that lacked Atrazine.
What's interesting is that in recent years that yield benefit from Atrazine remained very similar to what it was 10 or 15 or 20 years ago, despite the introduction of many new compounds, many of those used in combination with Atrazine, there still is that yield benefit.
One of the attributes of Atrazine is it

Page 142

very much facilitates farmers in converting to what we call conservation tillage where farmers make fewer trips across the field to till and may make no tillage at all in order to protect the soil from soil erosion and to produce other environmental benefits.

Atrazine is used more frequently by conservation tillage farmers than conventional farmers. It was used on 84 percent of conservation tillage corn compared to 61 percent of conventional tillage corn. And there's a number of reasons for that. I won't go into detail but it's just ideally suited to conservation tillage.

Because more farmers have converted to conservation tillage there have been a number of environmental benefits. Soil erosion reduction, the USAD's national resources inventory whoops, if we can go back one showed that between 1982 and 2001 there was a 33 percent decline in soil erosion across the U.S.

Conservation tillage also reduces the runoff of sediment into streams of nutrients and pesticides, all these things that can affect aquatic habitats. For example, no till on the average in controlled studies has reduced pesticide runoff by 70 percent.

Page 143

Because farmers are making fewer trips, especially those high intensity tillage trips, they're using much less fuel today. Very few industries can say that they use less fuel today than they used 10 or 15 years ago, but agriculture can, largely because of conservation tillage. Just in corn alone, conservation tillage corn alone is making a savings of 89 million gallons of fuel annually in the United States. If farmers were to revert back to conventional tillage they would be using 89 million gallons of fuel more a year. Next slide.

So Atrazine remains the most widely used corn herbicide and yet what is interesting is that Atrazine concentrations in surface water have declined over the last decade and they continue to decline. Next slide.

Why has this happened? Well, the actions that growers have taken have succeeded. And I think probably farmers feel sometimes they don't get enough credit for it. But there have been a lot of management changes. There were label changes in 1990 and in '92 there were changes in the Atrazine label designed to try to protect water quality. Rates were reduced, maximum allowed rates reduced, and setbacks or untreated areas required more surface runoff in the

Page 144

streams. They farmers have abided by those label changes and they're having an impact.

They've also adopted a number of voluntary BMP's or what we call best management practices, the conservation tillage I just talked about. Post emergency applications, there have been controlled studies that show when Atrazine is applied after the corn and weeds emerge, that runoff of Atrazine is 70 percent less than when applied to a bare soil surface like we've traditionally done.

We can also use lower rates when you apply post emergence so it further reduces runoff. And that's been a great trend, a change in how Atrazine is used.

Conservation buffers, by planting vegetation adjacent to streams, that buffer acts in entrapping anything that may be in the runoff from sediment and nutrients by the pesticides like Atrazine.

On my own farm we seeded out several miles of conservation buffers along streams. Other farmers have as well and they're having an impact. Next slide.

Monitoring studies have confirmed these declines in Atrazine concentration in surface water. The U.S. Geological Survey found about a 50 percent

Page 145

decline in median Atrazine concentrations in the early growing season when you expect the highest concentrations. This was over the period of 1989 to 1995.

And more recently the National Water Quality Assessment or NAWQA, also has shown significant reductions in Atrazine concentrations in streams over the period of '92 to 2001.

States have conducted thorough evaluations of their databases. In Iowa the Department of Natural Resources did a statistical analysis of a very large database of pesticide in the water and concluded that there had been a significant decline in Atrazine, both in surface water and in ground water.

More recently it's useful to look at some of the intensive monitoring that's been done on drinking water reservoirs. Some of these are small watersheds. I've worked personally on a number of these across the midwest and with some educational efforts we've seen the Atrazine concentrations decline in these reservoirs, a lot of times by 50 percent or more and really are stable and declining. We've had the reductions over many years in different kinds of weather conditions. Next, please.

So in conclusion, Atrazine remains a

Page 146

valuable tool for farmers. It's used on more acreage than any other corn herbicide. And it facilitates the adoption of conservation tillage. Farmers have abided by the water protective label changes. They have adopted voluntary surface water best management practices which have resulted in reductions in the concentrations we find in surface water and those levels continue to decline.

Atrazine does provide many benefits including increased yield and the adoption of conservation, reduction of fuel use, reduction of pesticide and nutrient runoff in the surface water.

And farmers realize that they have to have good stewardship of products like Atrazine to keep it available in the future so they have a vested interest in using these practices to try to reduce runoff as much as possible.

I appreciate the chance here to speak to you and I'd be glad to answer any questions.

DR. HEERINGA: Thank you, Doctor Fossett. Any questions for Doctor Fossett? Thank you very much for your comments. Can you see that your PowerPoint is forwarded to Joe Bailey for inclusion, thank you.

Our next public commenter is going to be Jerry White who is also here representing the Triazine

Page 147

Network and also the Kansas Corn Growers Association and the Kansas Grain Sorghum Producers Association. Jerry White.

MR. WHITE: Thank you Mr. Chairman and members of the committee. My name is Jerry White. I'm the Executive Director of the Kansas Corn Growers Association and also the Kansas Grain Sorghum Producers and serve as Chairman, such as that is, of a coalition under the Triazine Network. And my expenses here today are covered by the Kansas farmers.

The Triazine Network was formed in 1995 as a response by thousands of growers of over 30 commodities and from over 40 states to provide input to the EPA special review of the Triazine herbicides.

Our objective is to ensure that the EPA has and uses the best science available. And it's probably no surprise, I'm not a scientist and we don't have a Holiday Express in Garnett, Kansas so I'm just here representing the farmers.

I have participated in every SAP concerning Atrazine since the beginning of the special review in 1994 and so I do recognize some of the faces here today.

Network membership encompasses farm groups from border to border and sea to sea. Our

Page 148

executive committee is composed of farm organizations from Kansas, Missouri, Florida, California and Hawaii. So you can see we are a very diverse group, focused on a single outcome and that's the science based review of the Triazines and in this case, Atrazine.

As Doctor Fossett commented earlier, Atrazine has been the foundation of midwest wheat control programs since the 1950's. It's been around for a long time and we know this product well. Even today it is associated with the best yields and many of the best practices, like conservation tillage as Doctor Fossett commented.

We know how to store Atrazine in a way that provides safety for ourselves and the environment in which we live and farm. And I think that's important to know. We're not talking about a philosophical situation, this is the land where we live and we farm and where our kids grow up.

We have seen the product's continued use challenge based on a number of different allegations over the years, and certainly since 1994. Yet we have seen science successfully sort out those allegations through the EPA process, including those like this week's SAP.

And I must say, diverting from my

Page 149

comments I'm a little taken aback at the challenge that was I think put forth to this panel and to EPA for maybe not doing the right thing in what they're attempting to do today when according to my recollection of the process, the reason that you're here today is because the same people raised that issue in court and got a consent decree to have you do what you're doing today. It just seems a little bit ironic.

We do not take the allegations of harm from the use of Atrazine lightly. But when the value of agriculture is so high, the science must be sound. When the activist community has made Atrazine their post child and they stall out on one front, they simply go after another one. Or, as in the case of this morning, several new ones.

We welcome the scrutiny but insist that science prevail.

And with Atrazine it always seems to be something. First it was cancer if you go back to the origination of the special review. And now it's frogs. Certainly we care about both. Regulatory bodies around the world from the U.S. and the E.U. have concluded that Atrazine is not likely to cause cancer.

On a personal note, two months ago I buried my father, a farmer who lost his fight with

Page 150

cancer and I understand full well the implications of the disease.

But it's important for me to know that the tools my family and friends use on our farms are safe. And the fact is they do need tools. Farming is pretty simple when your field is a desk and your plow is a pen. But where I come from it's a business that requires real solutions to real problems.

We believe the scientific weight of the evidence shows Atrazine to be both safe and effective and that is the best kind of tool that farmers can have.

As for frogs, contrary to the sensational reports on their demise, they seem to be doing quite well in Kansas. Apparently they haven't read the reports.

I take personal pleasure in doing local biological assessments from time to time which my wife calls fishing. And I can tell you in the farm ponds and reservoirs that I frequent when given the chance, fish, turtles, minnows, algae and yes, frogs are having a banner year. These are locations surrounded by corn production as you can imagine. And based on what some were stating as fact during the public comments in the '03 SAP, this would seem illogical, this simply could

Page 151

not exist. But they are there, not the sad frogs from the other three PowerPoint presentations, but frogs that seem to be living the good life in the environment that I observe them.

Mark Twain philosophized in his writings on life on the Mississippi, that there is something fascinating about science, one gets such wholesale returns of conjecture out of such a trifling investment in fact.

Now I would not suggest that conjecture concerning gonadal development in frogs in '03 was absent some scientific merit for further review. But the overall weight of the evidence suggests more conjecture than fact.

Subsequent studies performed under the direction of EPA have been sufficiently robust and have resolved the questions set out by the previous SAP and by the Agency.

The fact that they are industry funded is irrelevant because that is a function of the system that requires a registrant to pay for them, it's just that simple.

Certainly the activist funded studies paraded in front of the '03 SAP were done with minimum guidance and quality control and with little

Page 152

transparency of process and data.

EPA has now completed yet another extensive review. My growers appreciate this thorough review and look forward to a science based conclusion concerning the use of Atrazine on their farms, it's important to them. Not because of their uncertainty with the product, but because the product has been the target of those who would have us farm 40 acres with a mule. And that might sound romantic until you figure out it takes 15 acres to feed the mule and the resultant greenhouse gases and soil erosion would probably require at least two more SAP's to sort out.

We appreciate the work of this panel and I don't mean to be facetious, the EPA has done a fantastic job over the years, there have been a lot of challenges, but science has risen to the challenge. And certainly we appreciate from a grower's standpoint, not only the work of the Agency, but those of you that contribute your time to help sort out some of the bigger and the tougher issues.

And I must say, the growers appreciate the work of the registrant in stepping forward and supplying the science that lets everyone else do their work.

Thank you.

Page 153

DR. HEERINGA: Thank you, Mr. White. Comments from the panel on Mr. White's comments representing the Corn Growers and Sorghum Growers Associations of Kansas and the Triazine Network? Thank you very much for your comments.

We have one additional public commenter who has registered with our Designated Federal Official, and that's Rick Robinson representing the Iowa Farm Bureau. Rick, please step forward.

MR. ROBINSON: Good afternoon. First of all let me say thank you as well to the panel for their due diligence in the review of these issues. It's very important, the work that you're doing and we don't take it lightly at the Iowa Farm Bureau.

The Iowa Farm Bureau is Iowa's largest general farm organization and my written comments today reflect a lot of the benefits of the use of Atrazine by Iowa corn farmers.

But I'm compelled to visit with you a little bit more today about some other aspects and some general reactions and kind of a 30,000 foot view to this process and also an on the ground reaction to what some of the water quality issues are in Iowa that we're working on.

Let me also say that I was born on an

Page 154

Iowa farm, grew up on an Iowa farm, I've been involved in agriculture all my life, some 47 years now. And over those years I've seen significant changes in agriculture, significant improvements in water quality and conservation efforts which sometimes aren't fully accounted for and they're hard to account for.

But my perspective is, with that in the background, looking at this process, at that 30,000 foot view, Iowa farmers need an effective tool to deal with soil erosion. Believe it or not, sediment in water is our biggest issue in Iowa. It's not pesticides, it's not Atrazine. And Atrazine is an important tool, an effective tool in the no till systems, the conservation tillage systems that Doctor Fossett talked about. If Iowa farmers don't have this as a tool it will negatively impact water quality in the state of Iowa in ways that are hard to imagine.

So I want you to keep that in mind. That's why it's so important to Iowa farmers. It's been around for some 50 years and it's been around because it's effective, it's cost effective and it's safe for farmers and for the environment.

And I think this process just further reinforces how safe of a product this really is. I can't imagine other products having this degree of

Page 155

scrutiny over the years.

I've been working on these issues for the Iowa Farm Bureau for 13 years now and this special review process has been going on for that period of time. The month that I started, 13 years ago this month, this special review process started and here we are today, 13 years later.

So, Iowa farmers are interested in resolving and answering these questions just as you are. And they're also interested in, once it is resolved, in the EPA communicating to the public what are the facts and what are the science. Because when I go out and, you know, do a Google search on Atrazine in frogs and pull up these websites that are out there that have bad science, inaccuracies, it's imperative, it's important to Iowa farmers that EPA also communicates to the public and to these folks what the facts are and what the science is so the public understands it and they also understand then what the water quality issues are and how Atrazine fits in to protecting water quality and aquatic life.

Thank you.

DR. HEERINGA: Thank you very much, Mr. Robinson. Questions for Mr. Robinson on his comments?

MR. ROBINSON: Thank you.

Page 156

DR. HEERINGA: Thank you very much. And again the set of written comments submitted by Rick Robinson is available to the panel and will be included in the docket for this meeting.

So thank you very much to the public commenters.

Now, I'd like to put out one last call. This is the period of public comment. It is really the only official period of public comment during these meetings. If there is anyone who has not had a chance to speak but feels they would like a chance to speak in this period, just indicate so.

Okay, not seeing any additional interest, there are some written comments that have been submitted to the panel. Those will be included on the docket in response to the proceedings today and the next three days, will be available to everybody on the docket as well.

I want to, there is some interest on the part of the panel to return to a couple of questions related to the public comment and presentation by the Syngenta Crop Protection team and I wonder if they would be willing to come forward again to entertain a few more questions from the panel.

Thank you very much. Again to the panel

Page 157

members, I think we have an opportunity at this point to continue this morning's discussion.

There were two additional points I wanted to follow up on. Doctor Bailey had a question with regard to the error bars, including on the figure on page 30. I think that was just a question related to the width of those bars and his concern that they should have a similar size or a range, given the underlying structure.

And I think that was something that, Mr. Osmer, you were going to come back with a response at some point. It doesn't need to be now by any means but just to remind everybody.

And there was also a conversation which Doctor Schlenk was going to have with the representative from the team on the percentage basis on actual versus nominal levels of concentrations of Atrazine.

Have you addressed that?

DR. SCHLENK: Yeah, we met with, actually Peter and I have met with Tim to discuss that.

DR. HEERINGA: And you've reached a finding?

DR. SCHLENK: Yeah, well basically the idea was that concentrations in the, on page 15 of the

Page 158

presentation, it wasn't clear to Peter or myself whether or not those values were actually between the level of detection and the level of quantificaiton. And apparently they are between those values provided on that particular slide.

DR. HEERINGA: Additional questions from the panel for the Syngenta Crop Protection team?

Yes, Doctor Miller.

DR. MILLER: I have one for Doctor Wolfe. Could you explain, did you score the histologic changes or did you do a presence/absence?

DR. WOLFE: Yeah, this is Doctor Wolfe from EPL. Yes, they were scored on a grading scale from 1 to 4 for almost all the findings. There were certain findings that were scored as present or absent I believe, but that was a very few things like mixed sex was scored as present rather than as given a severity grade. But they were all severity grade scored.

DR. HEERINGA: Additional questions? Doctor Portier.

DR. PORTIER: I'm not quite sure how to ask this question but I'll attempt it.

I guess I'm bothered by the loss of that control that had the low levels of Atrazine. And in

Page 159

the paper it was mentioned and you gave us a little bit more insight as to what you observed.

But my question is, how did that happen with the high level of quality control that you indicated you have, and to still lose it this way, I just wondered, you know, can you give me a little bit more insight into what you think happened to produce that level of contamination of the control?

MR. OSMER: This is Alan Osmer, Syngenta. We, the short answer is we don't have a complete explanation of how that happened.

What we do know is that because it was randomized in the fashion, that you ended up with a ten thousandfold difference between the LOQ for that dose level and those tanks that were adjacent to it.

We did consider some possibilities, some explanations, and were never able to resolve it totally.

Fortunately the study was designed in a robust fashion that allowed us to anticipate such problems and continue with the study.

DR. PORTIER: You know, the thing for me is that it raises additional questions that I worry about.

For example, were all of the clusters

Page 160

I know they were tested for the nominal level of chemical that you added. Were they also tested for the estradiol, potential cross contamination with estradiol? For example in whatever it is, next to control one you have an estradiol treatment. Do we know whether any of that went the other way? Were those things tested for that kind of thing?

MR. OSMER: We tested all of the controls for both the presence of Atrazine and estradiol and it never had an occurrence in the controls.

I mean I think it's not coincidental, the proximity of the control tanks to those high levels, and it was not a singular event. There wasn't a human error involved because we saw it chronically over the long term through the study.

So there was some transfer in some fashion systematically at low levels. I think it's significant that we were able to detect it. The fact that it was just barely above detection gives me more confidence that the others were clear.

And as I said the, both the negative controls, the other negative controls were sampled for estradiol and Atrazine and never found to be present.

DR. HEERINGA: Doctor Isom.

DR. ISOM: Gary Isom. Just to follow up

Page 161

on that then. Your control group 2 at the top, the blue one, was Atrazine detected in that also at any levels? And that would be the second to highest level of concentration.

MR. OSMER: No, there was never any Atrazine detected in that cluster of you're referring to the blue control 2?

DR. ISOM: Right.

MR. OSMER: Those were lost because of a microbial bloom that was described earlier.

DR. HEERINGA: Yes, Peter Delorme.

DR. DELORME: Peter Delorme. Just another question, Alan. Were those tanks covered?

MR. OSMER: Yes.

DR. DELORME: They were all covered?

MR. OSMER: Yes.

DR. DELORME: So you thought about possible airborne contamination as a source?

MR. OSMER: We did, but what you're reviewing there is a diagram of an environmental chamber.

DR. DELORME: Right.

MR. OSMER: A large environmental chamber. I think if it were airborne contamination you would have expected to see it in more than just that

Page 162

one cluster. And yes, they were covered.
DR. DELORME: Thank you.
DR. HEERINGA: Any additional questions? Yes, Doctor Bucher.
DR. BUCHER: John Bucher. This is a follow up to a question that I think Doctor Wolfe was answering this morning and I've been thinking about it over lunch.
I'm still a little confused about the relationship between the diagnosis of mixed sex, inter-sex, the testicular ovarian follicle and how these various things are affected by the actual time it takes these different populations of frogs to get through to metamorphosis.
Could you expand on that just a little bit so that I could get a little clearer on it?
DR. WOLFE: Okay, sure, this is Doctor Wolfe again. The point I was trying to make is that I think sometimes people look at testicular oocytes and mixed sex as being somewhat apples and oranges and wonder why didn't we see mixed sex why didn't we see testicular oocytes in such and such a study and why did we see mixed sex in another study.
And I think a lot of it has to do with in my observations, in my opinion, is the reproductive

Page 163

age of the animals in that specific test. And a lot of times that doesn't come out when you read the literature on a specific study. They say they used Stage 66 animals, but I think in some cases somebody's Stage 66 animals are not the same reproductive age as somebody else's Stage 66 animals.
So if you have animals that are more reproductively mature my hypothesis is that they're more likely to develop testicular oocytes because the females at that particular reproductive age would also be more likely to have perinuclear oocytes rather than gonial cells, okay?
And that whereas at a younger age you're more likely to get a mixed sex consisting of less developed immature tissue.
Does that help?
DR. BUCHER: Yeah, that helps a lot, thank you.
DR. HEERINGA: Okay, I think that we'll be hearing more about the actual studies with the EPA presentations and I think if any additional questions come up which could be answered by the Syngenta Crop Protection group that's conducted those studies, I'll leave it to the EPA to call on them appropriately if that makes sense.

Page 164

So I want to thank you all for your contribution here. And at this point in time yes, Mr. Osmer.
MR. OSMER: Thank you, Mr. Chairman. Just for the record, we will correct that figure, figure 15 and indicate the actual values that were plotted and resubmit that with that figure.
DR. HEERINGA: Okay, we appreciate it, thank you.
MR. OSMER: And we appreciate your time.
DR. HEERINGA: Thank you very much. At this point in time I'd like to call the period of public comment to a close and we are at 2:00 p.m. where we were anticipating to be at, at 5:00 p.m.
And I think the EPA scientific staff I understand through Joe Bailey is willing and able to proceed at this point with the presentations that were on the agenda for tomorrow morning.
Is that in fact the case? Okay. Let's have Doctor Steeger and your team come forward.
While we're waiting to get set up here I want to thank all of the participants in the public comment period for not only the presentation of data and experimental study results, but also comments and views on this particular scientific question.

Page 165

Doctor Steeger, anytime you're ready, please feel free to proceed.
DR. STEEGER: Thank you again for this opportunity to present to the FIFRA SAP.
In this presentation I would like to provide a brief overview of the open literature published since the 2003 SAP.
In addition to the two studies submitted by Syngenta in response to the data call in, a total of 18 laboratory and field studies combined were reviewed by the Agency.
Although several of those studies were published subsequent to the 2003 SAP which discussed the affect of Atrazine on amphibian gonadal development, they were not reviewed because they did not contain primary data or they reported on affects other than amphibian gonadal development.
As mentioned in the Agency's introductory remarks the current focus concerns the potential for Atrazine alone to act on gonadal developmental affects.
Of the nine laboratory studies published in the open literature, two, both by Cody, et al had been previously reviewed as interim reports in the 2003 white paper.

Page 166

As was the case in 2003, laboratory studies reported on a variety of measurement end points such as survival, time and size at metamorphosis, shifts in sex ratio, laryngeal muscle area, gonadal abnormalities, plasma steroid levels and aromatase activity.

All of the laboratory studies relied on static renewal exposures. Atrazine exposures ranged from a single concentration tested up to five concentrations tested. Few of the laboratory studies verified Atrazine and/or degrative concentrations.

The open literature studies generally failed to account for potential sources of variability that confound the interpretation of the data. For example, loading rates, that is the number of animals per volume of treated solution exceeded the ASTM recommended rate of one tadpole per liter per day.

Using static renewal conditions the majority of the laboratory studies had both incomplete and infrequent exposures to solution changes which in previous studies markedly decreased the water quality conditions.

In some cases exposure chambers were constructed of materials such as plastic that could have influenced measurement end points. In general,

Page 167

environmental factors such as dissolved oxygen and ammonia were not well controlled.

Similar to the laboratory studies reviewed in 2003, the most recent laboratory studies lacked consistent dose response. In some cases adult rather than larval frogs were evaluated and survival gonadal development was not measured.

In some of the studies there was a poor response to positive controls, typically estradiol, indicating that the test or assay was not sensitive at means of measurement. High mortality was problematic in some of the studies as well.

Although the Agency and the FIFRA SAP had made recommendations for study designs to address potential sources of variability and uncertainty, none of the laboratory studies incorporated these design elements.

With respect to the field studies, all of the most recently reviewed field studies had previously been reviewed in some capacity as interim reports in the 2003 SAP. The field studies contained that limitations that were identified in 2003.

These include the Atrazine or Atrazine and Triazine and their degratives in reference sites, poor characterization of environmental conditions, poor

Page 168

characterization of larval exposure conditions and unusual weather events that may have compromised the study.

The study designs did not address potential sources of variability. In general the field studies provided a very limited opportunity to correlate Atrazine exposure to measurement end points.

In general, since 2003 a total of 35 documents have been reviewed and none of these study reports have experimental designs or data sets sufficiently robust to assess whether or not Atrazine alone can affect gonadal development.

In the next three slides I depict all of the studies reviewed for the 2007 white paper and it represents Table 24 from the white paper itself. The table provides the lead author and the test species and the developmental stage used, the Atrazine concentrations tested and the major results of the study and the studies' limitations.

Only two of the ten laboratory studies reported affects on gonadal development. Three of the ten laboratory studies reported affects on time to metamorphosis. None of the laboratory studies report a consistent dose response and where affects were noted, there were conflicting results for the same species,

Page 169

for example Northern Leopard frogs across labs.

Where Hayes, et al in 2006 reports no gonadal abnormalities nor affects on time or size at metamorphosis at Atrazine concentrations from 0 to .1 to 10 micrograms per liter, Orden, et al 2006 reports testicular oocytes at 10 micrograms per liter.

Of the nine field studies, only one of the studies showed an increased incidence of affects, and that's Bidder's organ development in male Cane toads collected from various sugarcane production sites where measured concentrations of Atrazine were highest. Also, in one of the two years in which Murphy, et al 2006 collected green frogs, the incidence of testicular oocytes was correlated with Atrazine concentrations. However, the affect was not reproducible across the entire study period.

The open literature, taken as a whole again suggests that Atrazine does not consistently affect amphibian gonadal development.

In the presentations that follow, Doctor Diggitts will provide an overview of the scientific approach to the design of the DCI studies, the data call in studies. Doctor Diggitts is a research aquatic biologist with the Ecology Division of the EPA Office of Research and Development in Duluth. And Doctor

Page 170

Diggitts is kind enough to pinch hit for Mr. Joe Tugi who has been called away on a family emergency. Doctor Diggitts however has participated in the 2003 SAP and is familiar with the DCI study protocols and he has extensive experience conducting laboratory studies with samples.

DR. HEERINGA: Thank you very much, Doctor Steeger. Before we move on to Doctor Diggitts' presentation, are there any comments on the summary here of the open literature review?

Yes, Doctor Bucher.

DR. BUCHER: John Bucher. Given the fact that the aromatase theory has sort of fallen by the wayside according to some of the data we've seen, the utilization of the estradiol positive control and the failure to produce affects in some studies, was that taken into consideration in the selection of the positive control in the evaluation of the value of some of those studies where the positive control may not have worked?

DR. STEEGER: Estradiol was chosen as a positive control, not because it was intended to mimic the action, or the presumed action of Atrazine on amphibians, it was selected because it's known to produce gonadal developmental affects.

Page 171

And it was necessary to demonstrate that the protocols that were being followed were capable of detecting a change in gonadal development. That was the only reason that estradiol was chosen as a positive control. Because we do know that it can result in affects on the sex ratio and on the incidence of mixed sex ovarian tissue in the testes of males.

The studies that had been conducted and reported in the open literature, where we were privy to some of the details concerning those studies it became clear that in many cases the animals, because of husbandry conditions were so poorly developed that they weren't even responding to a strong estrogenic chemical.

DR. HEERINGA: Doctor Skelley.

DR. SKELLEY: Doctor Steeger, I just want to make sure I understand clearly in particular how field studies were evaluated.

Is it the case that a couple of your slides, the way that you were reviewing these studies, they needed to be able to show a clear unambiguous association with Atrazine alone in order for you to conclude that there was evidence?

DR. STEEGER: No, I don't think we were looking for just a clear indication from Atrazine

Page 172

alone. I think that whenever we look at field studies for ecological risk assessment we're trying to identify whether the study had proper reference sites that you could determine whether in any way the Atrazine could be related or the chemical in question could be related to any of the affects being measured.

In this case most of the field studies had such profound compromising affects in the way that the data were being collected, that it was difficult to even get to the point where we could ask that question.

In a lot of cases the animals were collected over protracted periods of time, were being held together in collection buckets for up to eight hours, and then they go out and they measure steroidal concentrations in the plasma. Well, you know, if you hold animals together for that long it's a little difficult to believe that males and females are not going to react to one another and that could potentially influence the parameter that's being measured.

In many of the studies concentrations, not only of Atrazine, but of chemicals, other triazine herbicides, the degratives, a large variety of pesticides were found in the reference sites as well as the, what were supposed to be the treatment sites.

Page 173

So there was no really, there was no way to distinguish what was the treatment and what was the control.

DR. SKELLEY: This is David Skelley again. So I'd like to ask you specifically about the Cane toad sugarcane study. Could you summarize just very briefly what you see as the major compromising issues there?

DR. STEEGER: To be candid, the Florida study of the Cane toad was one of the most compromised studies I've ever read in my career here at EPA.

The reference to the Atrazine levels that were reported in the plasma of the animals, those concentrations were on an order of magnitude below the level of detection of the assay that was being used to measure them. The animals were held for protracted periods of time in the collection vessels. That was the eight hours in the collection, in the field collection. They were sampled by cardiac puncture and then weighed and so the weights of the animal depended on the volume of blood that was sampled. It would have been difficult to believe that it did not influence that measurement end point.

The animals were collected over several seasons and they were combined. So looking at the

Page 174

differences in the you have a mixed, potentially mixed developmental stages for all of the animals that were collected.

Even though the study stated that adults would be sampled and that only animals beyond a certain range in size would be included in that sample, in actuality when you go to the data, at least 40 percent of the animals were lower than that and many of them appeared to be juvenile animals.

The study author failed to measure other pesticides at the treatment sites and I think that and those are only the major difficulties with the study. In general there was not much utility that could be gleaned from the study.

Also the authors state that the Bidder's organ can develop in males depending on the state of sexual development. So given that the animals could have been at various stages of development it would have been difficult to know whether that was a natural process or whether that was somehow chemically induced.

DR. HEERINGA: Yes, okay, Doctor Furlow.

DR. FURLOW: David Furlow, UC Davis, so as a bench scientist I'm just kind of curious and maybe some of the other folks who work in wildlife and doing field studies so in your opinion none of the wildlife

Page 175

or the field studies represent anything that indicates or that you can't make any conclusions from it.

It's not one way or the other, you can't draw any conclusions? That's the first question.

The second question is, based on what you're saying though, and maybe some of the other experts can weigh in, can an adequate field study be proactively designed to say, look, you know, this is what we need, these are the criteria that we need to meet, let's do the experiment and decide one way or the other?

DR. STEEGER: I think that a field study can be properly designed. The intent of our review wasn't to preclude the use of field studies.

The fact is that none of the field studies that we were presented with had the proper design elements to address a question as to whether chemical exposure could be correlated with any affects that are being measured.

When we went to the 2003 SAP the question was put forth that there are a lot of uncertainties surrounding the data that we had and most of them had to do with sources of variability and how we could better control them. And the Agency proposed a process for trying to do that very thing.

Page 176

What would you really need to do in order to answer the question as to whether Atrazine is affecting amphibian development?

And we had proposed that first of all you'd want before you moved into the field, let's first establish in the lab whether you can get cause/effect relationship. Because if you can't do that in the lab there's no point in going out in the field to determine whether that relationship exists when there are many more confounding factors that would limit your ability to draw that cause/effect relationship.

DR. HEERINGA: Doctor Denver.

DR. DENVER: Bob Denver. I have a related question to the one that Dave Furlow just asked. And that is, in reading the white paper it wasn't clear to me that EPA considered any of the published literature to be sufficiently robust that it would tell us anything for or against the effects of Atrazine.

Is that fair to say?

DR. STEEGER: I think it's fair to say that the open literature as it was in 2003 is as it is 2007.

There are excuse me, there are

Page 177

uncertainties regarding how the data were collected which limited the Agency's understanding of how to interpret them.

I think that we could continue to draw on lines of evidence and state that there are indications that Atrazine may be affecting amphibian gonadal development such as in the case of the development of Bidder's organ, as in the case of delayed metamorphosis in the Freeman study.

But in terms of looking across, has anyone replicated those results? When you try and do the study similar to what the authors did, do they come up with the same results? Is there any consistency in the information that's being presented?

There's a smattering, you get a result, there you don't. The Agency identified a way of getting around that and we still see that back and forth and that's why the DCI studies were critical to our analysis.

DR. DENVER: Can I just have a follow up? Right, so you get variable results depending on the study, but I'm curious, are any of the published papers, do you consider any of those to be sufficiently well done that you would put them in the same category as the two DCI studies?

Page 178

DR. STEEGER: No.
DR. HEERINGA: Any additional questions for Doctor Steeger?
DR. STEEGER: I'd like to make a follow up statement to
DR. HEERINGA: You may, absolutely.
DR. STEEGER: The tool that the Agency uses to assure that a study is conducted in accordance with the guidelines or the process that we've identified need to be followed, is good laboratory practice.
It isn't possible for most researchers to maintain the rigor that's required under a GLP study in terms of tracking, keeping track of data, having standard operating procedures, having protocols that are being followed where the Agency then has the luxury of going through and analyzing the data ourselves as though we had done the experiment ourselves.
The open literature can't hope to compete against that standard.
DR. HEERINGA: We'll have an opportunity if additional questions come up but at this point I'd like to move to Doctor Diggitts and his presentation and again he is stepping in for Joseph Tugi who is away. The panel should have copies of this

Page 179

presentation. It's labeled with Joe Tugi's name.
Please proceed, Doctor Tugi Diggitts.
DR. DIGGITTS: In this presentation I would like to accomplish the following three objectives.
First I will recap the conclusions of the 2003 white paper, particularly as they apply to the scientific approach developed to assess the risk of Atrazine using a tiered analysis plan.
Second, I will summarize the comments of the 2003 SAP which are pertinent to the analysis plan that was proposed in 2003.
And third, I will review the rationale and details of the study plan, including a time line activities from 2003 to 2007.
The EPA's analysis of data available in 2003 suggests that aneurine reproductive fitness may be adversely affected by exposure to Atrazine. However the data were insufficient to conclude that Atrazine adversely affect aneurine reproduction through affects on gonadal development.
Therefore, further studies were proposed following the guidelines of ecological risk assessment to reduce the uncertainties and permit eventual risk characterization if warranted.

Page 180

The major uncertainties about Atrazine's potential affects on amphibian gonadal development based on the data available in 2003 were characterized as a small number of affirmative studies. In 2003, three studies conducted under laboratory conditions demonstrated gonadal abnormalities in males indicative of mixed sex gonads, that is, ovarian tissues present in predominantly testicular tissues.
There was limited evidence of repeatability. When comparable laboratory studies were evaluated some demonstrated an affect while others did not. The dose response relationship was undefined.
The studies which demonstrated an affect on Atrazine on gonad development did not provide convincing reproducible evidence of a dose response or exposure response relationship, whether it be linear or nonlinear.
Understanding the dose response relationship is a necessary component of the risk assessment process because it forms the basis for determining the risk associated with environmental concentration of the chemicals in question.
The mechanistic plausibility was unsupported. The hypothesis presented at the time regarding excuse me the affect was not supported by

Page 181

appropriate experimentation and data, specifically the working hypothesis or risk hypothesis was that aromatase led to increased estradiol levels which were of sufficient magnitude and duration to feminize male gonads resulting in individuals of mixed sex, sometimes referred to as hermaphrodites.
To date this mechanism has not been demonstrated in the species tested.
The ecological relevance was undetermined. The assessment end points used in evaluating ecological risk is at the population level, that is adverse affects are considered important in the ecological risk assessment process if there is evidence that the affects result in population reductions or loss.
Obviously successful reproduction is necessary for the maintenance of population, however none of the reports demonstrated impaired reproduction in either laboratory experiments or in field studies.
So the EPA proposed to the SAP in 2003 that a tiered approach be used to examine the cause/effect, dose response, mechanistic plausibility and ecological relevance of Atrazine exposure to amphibians.
Such a systematic approach would reduce

Page 182

these major uncertainties and permit a more thorough analysis of risk.

In this very simplified diagram of the ecological risk assessment paradigm one can see the three major phases, namely problem formulation, analysis and risk characterization.

As Doctor Steeger has pointed out in his earlier presentation in more detail, the conceptual model or risk hypothesis is defined in the problem formulation phase. The conceptual model is based on the currently available information and sets the stage for developing an analysis plan.

The analysis plan is a set of studies that determines the affects of the chemical on specific end points and the extent and likelihood of exposure. Risk characterization follows, and is the phase in which the effects and exposure data are integrated.

The next two slides will present the conceptual model which originates in the problem formulation phase and the tiered analysis plan which originates in the analysis phase as they were proposed in 2003.

The proposed tiered analysis plan was designed to run a conceptual model of risk hypothesis on Atrazine action. The hypothesized affects are

Page 183

initiated by an undefined molecular interaction. This interaction results in a hypothetical increase in aromatase which results in a hypothetical elevation of indogenous estradiol which affects changes in male gonads.

If this affect impairs the fertility of the male, then reduced reproductive fitness could result, leading to impaired population maintenance and recruitment which is the assessment end point.

This slide depicts the proposed tiered analysis plan essentially as presented to the SAP in 2003. The steps outlined in the conceptual model shown in the previous slides can be systematically tested using a tiered approach. Beginning at the organismal level the affects of Atrazine on gonad development, particularly in males should be the entry point of this analysis.

If these tests are affirmative, then affects on Atrazine exposure on sex steroids could be evaluated if feasible. If estrogen levels are elevated in the Atrazine treated organisms, then evaluating the affects of Atrazine exposure on aromatase could be indicated.

Although sex steroids and aromatase measurements are necessary to test of working

Page 184

hypothesis and inform the mechanistic plausibility, they do not necessarily provide meaningful information on the ecological relevance of a potential gonadal affect.

Therefore if gonad affects are observed at the organismal level, the studies which evaluate fertility and reproduction end points that are relevant to maintenance of population which is indicated in this slide as an ecological relevance should be pursued.

If the working hypothesis is ordered by the organism and sub-organismal studies, then it may be possible to confirm the mode of action by conducting confirmatory studies which utilize known aromatase inhibitors. Rescue of the normal male phenotype by an aromatase inhibitor co-administered with Atrazine could provide substantial support for the working hypothesis.

If no affects are observed at the organismal level then there may be no need to continue with any further testing above or below the organismal level. If the organismal level tests are affirmative and any of the sub-organismal studies are negative, then an alternative hypothesis could be considered.

So if the tier one test for gonad affects is pivotal to the implementation of the tiered analysis plan, I would like to provide some more detail

Page 185

about the objectives, the recommended experimental approach and the use of study quality indicators as performance criteria by which the quality of the study can be judged.

The objective of the tier one studies were to determine Atrazine exposure results on gonadal affects in males under controlled laboratory conditions and determine the shape of the dose response relationship, if any.

So as many of the studies reviewed in 2003 had a variety of experimental problems, we proposed an approach that would follow current standards in aquatic toxicology and we specified several parameters.

The species recommended was Xenopus laevis. The recommendation was based on the fact that some previously conducted studies had suggested that this species was sensitive to the affect of Atrazine. Furthermore, from a practical standpoint this is the most widely available and most robust experimental model species among aneurines.

The tests should be conducted such that the stage known to be sensitive to the affects of estradiol on gonad development are included. The study should terminate at Stage 66 which is completion of

Page 186

metamorphosis. The tests should be conducted using a flow through conditions and should conform to the ASTM standards for organismal loading. Atrazine concentrations should bracket those used in other studies, particularly those which demonstrate an affect on gonad development, and Atrazine concentrations must be verified analytically.

A positive control, 17 beta estradiol for E2 should be included to demonstrate the sensitivity of the species under the test conditions used.

Sample size should be sufficient to test the hypothesis to determine a priority by power analysis. Minimal replication was set at two tanks per treatment. All organisms on tests were to be sampled.

The principal end points were to include growth, survival, development, gross gonadal morphology and gonadal histopathology.

Again, based on experimental problems observed in the studies available for review in 2003 several quality indicators were proposed to ensure that a quality tier one study was conducted.

Proposed test conditions required that the organism loading did not exceed eight STM standards and a minimum pH ammonia and dissolved oxygen were

Page 187

monitored regularly and did not exceed acceptable levels. The required survival of test organisms should meet or exceed 90 percent.

Growth as determined by body weight should approach a maximum of approximately 1.5 grams at Stage 60 and the terminal body weight at Stage 66 at the end of the test should be approximately 50 percent maximal.

This recommendation was based on the fact that the maximal body weights are typically achieved between Stage 58 and 60, followed by a period of weight loss through metamorphic climax.

And finally metamorphosis should be completed in less than 10 weeks.

To summarize our recommendations for the 2003 analysis, in order to reduce the major uncertainties associated with the potential risk of Atrazine to amphibians, the Agency recommended that additional studies be conducted that followed a tiered sequence of laboratory investigations that focus on the critical components of the risk hypothesis. The currently available, high quality methods which are standard for aquatic toxicology establish and adhere to study quality indicators.

In response to the 2003 white paper the

Page 188

SAP's major responses to the Agency's analysis were that EPA's reviews and conclusions were thorough, appropriate and valid, that significant data existed to formulate a hypothesis that Atrazine exposure causes gonadal abnormalities, but existing data were insufficient to test the hypothesis, and that additional studies were warranted.

The SAP endorsed the tiered analysis plan as logical and recommended that tier one studies should proceed immediately. The SAP also suggested the ecological relevance of the studies should be initiated as early as possible within the framework of an analysis plan.

Finally the SAP agreed with the Agency that standard methods needed to be used which conform to ASTM standards, including the use of flow through exposure conditions.

Given the Agency's recommendations for a tiered analysis plan and an endorsement of that plan by the SAP, how was the tier one study approached?

As I mentioned earlier, we recommended that estradiol be used as a positive control in the tier one study as an indicator of species sensitivity towards estradiol affects under the test conditions used. However there was insufficient information to

Page 189

establish an estradiol test concentration. So the registrant decided to develop estradiol dose response data in a preliminary study to ensure the appropriate test concentrations would be used in the tier one study.

Once that was accomplished the tier one Atrazine study could be conducted which was the subject of the Agency data call in.

So what was the rationale for the estradiol study?

By way of review the risk hypothesis that I presented earlier assumed that elevated endogenous E2 estradiol levels were responsible for gonadal affects in males although it should be pointed out that other mechanisms could be operative as well.

Therefore, to reinforce the validity of the tier one Atrazine study an E2 positive control was included by the registrants, primarily to test the sensitivity of the species using a specified experimental protocol.

The preliminary work was also used to establish histological sampling technique and develop diagnostic histopathology terminology. The recommendation was that estradiol concentrations could be set at the EC50 concentration based on complete sex

Page 190

reversal, basically altered male/female sex ratios.

The preliminary estradiol study results demonstrate the EC50 to be .2 micrograms per liter.

In November of 2004 a data call in was issued to the registrant to conduct the tier one study as recommended by EPA and endorsed by the SAP. Earlier I showed the recommended approach. Here I would like to show how the actual study was conducted compared to the original recommendation.

Xenopus laevis was used as the test species and the developmental stage utilized in the study included most of the estrogen sensitive period. The test was terminated when organisms attained Stage 66. Flow through conditions were used and loading rates were below the 1 gram per liter per day ASTM recommendation.

The Atrazine concentrations were .01, .1, 1, 25 and 100 micrograms per liter which bracketed the range of concentrations used in the previous studies. These concentrations were analytically verified by LCMSMS periodically throughout the study.

As discussed earlier an estradiol positive control at 2 micrograms per liter was incorporated into the experimental design. The number of organisms on test in each tank was 25. Tank

Page 191

replication was 8 for each of the Atrazine and estradiol treatments and 16 for the clean water controls. All organisms on test were sampled.

Gross survival, development, gross gonadal morphology and gonadal histopathology were evaluated as recommended.

In terms of the quality indicators the study met the ASTM standards for loading. As I previously mentioned, the water quality parameters of pH, ammonia, dissolved oxygen were within acceptable ranges. Several exceeded 90 percent with the minimum survival survival exceeded 90 percent with a minimal survival of 93.5 percent for one of the replicate tanks.

Although originally recommended maximal body weights were not measured due to the excessive handling that would be required in the middle of the test, terminal body weights at Stage 66 were approximately 500 milligrams which suggested the protocol was sufficient to promote acceptable growth.

And finally metamorphosis was complete within seven weeks, well below the ten weeks maximum, indicating that the test conditions were adequate to promote normal metamorphic development.

Although the conduct of the DCI study

Page 192

followed the recommended approach, there were experimental deviations. Atrazine contamination was discovered in a block of four control tanks in one of the laboratories and in the other laboratory Atrazine contamination was found in the estradiol positive control. This contamination events were discovered through the routine chemical analyses conducted periodically throughout the studies. The magnitude and time and duration of the contamination will be discussed in more detail in the analysis presentation which follows.

And in addition, two replicate tanks for the 1 microgram per liter Atrazine treatment group in one laboratory were lost due to mortalities, explained by the registrant as the result of an algae bloom. Again, the implications of these lost replicates will be addressed in the analysis presentation.

To provide you with a sense of how this work unfolded over time, the critical events are mapped onto a time line which spans an interval from the previous SAP meeting in 2003 to the current meeting of the SAP.

Starting on the left I will walk you through the time line. In February 2003 all of the data from the relevant studies were collected and the

Page 193

original Agency white paper was developed and submitted to the SAP which met in June 2003.

Following the SAP meeting the registrant submitted a preliminary study design based on the outcome of the SAP meeting and began additional preparative work, such as coordinating the power analysis and establishing facilities that could accommodate these relatively large studies, which the registrant at two independent laboratories, one in the U.S. and the other in Germany.

In May of 2004 the E2 positive control study was submitted to the Agency for comment and those studies were initiated in 2004.

The Agency data call in was issued in November of 2004 and the E2 positive control study exposures were completed in December of 2004.

The tier one study design was submitted to the Agency for comment in April of 2005. The tier study exposures were initiated in September of 2005 and were completed in December of 2005.

The pathology was submitted in April of was completed in April of 2007 and the final report in response to the data call in was submitted to the Agency in June of 2007 which brings us to the current SAP meeting.

Page 194

Through this presentation I have detailed the approach taken to develop and implement the scientifically sound analysis plan which would provide additional data to aid in the assessment of Atrazine risk as it pertains to the affects of Atrazine on gonad development in Xenopus laevis.

First I summarized the conclusions and recommendations of the original Agency white paper presented to the SAP in 2003 which indicated that there were significant weaknesses in the existing data at the time that prevented one from clear assessing the hypothesis that Atrazine exposure resulted in gonad affects in aneurines.

The Agency proposed a tiered analysis plan which addressed the uncertainties at various levels in the risk hypothesis. The SAP agreed with the Agency's analysis and endorsed the tiered experimental strategy embodied in the analysis plan.

The registrant submitted a tier one study plan in response to the Agency's data call in and finally a time line was presented that details the implementation of the tier one study into 2007.

Including the conduct of the preliminary E2 studies and the tier one Atrazine studies, in general the registrant met the intent of the data call

Page 195

in for these studies and though these studies generally met the study quality requirements, there were deviations in the actual conduct of the study. These deviations will be addressed in the analysis of the study results which will be covered in the next two presentatons.

Thank you.

DR. HEERINGA: Thank you very much, Doctor Diggitts. At this point I'd like to open it to the panel for any questions of clarification by Doctor Diggitts, the speaker on this particular presentation.

Yes, Doctor Bailey.

DR. BAILEY: Ted Bailey. On page 6, your side number 18 you indicate 8 replications for Atrazine treatments. And I think in lieu of our discussion this morning that that's not going to be accepted because the four tanks that received the flow were treated as one unit. They were not randomized. I mean they weren't filled independently, the tanks.

So each time the four tanks were filled that would correspond to one application, the way the flow of the treatment came.

DR. DIGGITTS: The flow goes into clusters of four tanks and there's two cluster per treatment, yes. We did not analyze the data by

Page 196

cluster.

DR. FRANKENBERRY: This is Mary Frankenberry, I'm giving some of the stat analysis but that was probably detailed in it. The company did do a test the cluster differences on every end point and with every two tanks and I think as Doctor Silken said they found 1 out of 160 or so.

We noted that the test was not very powerful but we didn't go beyond that and we did collapse and use the tanks as replicates, not clusters and I think the company did as well.

DR. BAILEY: Okay, Ted Bailey again. I wasn't talking about the analysis, I was talking about the application of the treatments. And when you applied the flow to those four containers, that essentially is one replication, not eight, because you didn't do the complete containers independently. It was not randomized across those four, let alone those eight containers.

So the experimental unit was a group of four tanks and those four tanks all received the same treatment. So that would not be eight replications or four replications even.

DR. DIGGITTS: Well it would still be because they used tank splitters so that the flow to

Page 197

the group of tanks was split four equal ways.

DR. BAILEY: But there was one mixing cup that provided that flow.

DR. DIGGITTS: So you're suggesting that the mixing cup is the source of replication and conventionally it's the tank.

DR. BAILEY: You would have had, on one of those clusters it would have been necessary to have four mixing cups, not one. Four, one for each of them, mix it up four times for the four tanks for that to be an experimental unit.

DR. HEERINGA: I think at this point we'll return to this in the presentation, the discussion of the statistical analysis and then in our comments. I take the point but is everybody clear on the actual mechanics of the delivery of the flow, one mixing vial with a mixing gauge essentially through four separate routes to the four separate tanks?

Yes, Doctor Delorme.

DR. DELORME: Peter Delorme. I was just wondering if you could go to slide 20 and comment on a disparity.

You say that there was contamination in the eight replicate tanks, yet the presentation this morning said four.

Page 198

DR. STEEGER: It's four, it's a typo on the slide.
DR. DELORME: Okay. And the same for the loss of two replicate tanks and the .1 would be the bloom effect?
DR. STEEGER: Yes.
DR. DELORME: Okay, thank you.
DR. HEERINGA: Yes.
MR. PAULI: This is Bruce Pauli, Environment Canada. Can we go to 17 please?
I was just wondering, something that I've been thinking about and this comes back to a question Doctor Patino asked this morning. The protocol that was settled on was to go from 42 to 54, right? And that didn't happen in the end, it's just the developmental stage when the exposures happened.
I'm interested in guidelines as I think you know and I wondered if this is a deviation that we might be addressing shortly or is this, did that happen basically because of logistics that the animals had to get to Berlin?
DR. STEEGER: It happened because of logistics and because our experience in the pilot studies indicated that starting at an earlier stage there was a higher rate of mortality in the treatments.

Page 199

But just to get the animals to Berlin required that both studies start at developmental Stage 46 as opposed to 42.
MR. PAULI: It's Bruce Pauli, so the pre-studies with E2 were full window, 42 to 54?
DR. STEEGER: I don't recall, I'd have to ask Mr. Osmer.
DR. HEERINGA: Mr. Osmer, if you want to come on up please.
MR. OSMER: Alan Osmer, Syngenta. I don't recall if they were at 42 or 43, what that stage was. We did attempt earlier staging. The commercial supplier, Xenopus Express, had, or Xenopus One had advised against it. You know, these people ship Xenopus around the world. They advised us against it.
We tried several times, and as Doctor Steeger mentioned, just the physical handling, whether they were going to Maryland or Berlin, just that handling led to high mortalities and we essentially reverted back to what most researchers were using, the Stage 46, 48 as the starting point.
DR. HEERINGA: Doctor Skelley.
DR. SKELLEY: Doctor Steeger, I'm not sure if this question's for you but, and if you're going to address it later that's fine, one of the

Page 200

points that you kind of brushed up on was a decision not to pursue studies of North American species and I wondered if you can flesh that out?
If you're going to do it later that's fine as well.
DR. STEEGER: The decision not to pursue testing with indigenous species came about for two reasons. One, because we were unable to demonstrate an affect with Xenopus in the previous SAP. It indicated that there was no difference between Xenopus in terms of biochemical pathways, physiological responses compared to indigenous species.
The second reason is that it took roughly two years to develop the protocols for conducting the definitive Atrazine study using a regularly tested amphibian species that could be induced to spawn, that would have a reasonable amount of time to complete metamorphosis within the study period.
Using indigenous species the husbandry, coming up with the proper husbandry and standards for the conduct of that study seems to be a daunting task at this point.
DR. SKELLEY: Well as someone who works with this is Dave Skelley again as someone who

Page 201

works with North American species I'll take that as a compliment.
So as you know the 2003 SAP report actually strongly encouraged that on the basis that, I guess the short way to put this is Xenopus is strange, it's kind of an outlier among amphibians, you name it, everything is different.
So what is the basis of your confidence that this wouldn't have turned out differently with a different, say a North American species?
DR. STEEGER: We do not have any information to substantiate that claim. Our confidence is only based on the fact that the SAP in 2003 could not identify a reason that Xenopus would not serve as a reasonable model for representing amphibian species, nor could they identify any process in Xenopus that would be different than an indigenous species.
You are correct, they are very strange animals, they're purely aquatic and have a lot of baggage to support the fact that they are strange.
DR. HEERINGA: Doctor Schlenk.
DR. SCHLENK: Just a question of curiosity and life history, I'm curious, does Xenopus actually live under flow through conditions or is I mean I realize you have to do the flow through

Page 202

conditions for the water quality issues, but in the wild are they actually under flow through conditions or are they more of a, you know, stagnant water type of life history stage for where they survive?

DR. STEEGER: My understanding, and this is based on personal opinion, is that the Xenopus appear to be able to live just about anywhere. They live in static conditions as well as flowing, but it appears as though much of their habitat is static.

We do have in the audience Louis Dupree who is very well versed on Xenopus. If Louis Dupree wants to comment on that.

DR. HEERINGA: Doctor Dupree, if you'd come forward please.

DR. DUPREE: This is Louis Dupree, Northwest University of South Africa.

The way you described it is very accurate. Xenopus is a very opportunistic frog. You will find it from roadside pools to bigger dams and you do find them in rivers and streams. But primarily in static water. But they do very well in any water body. And the best place to find them is in sewage ponds.

DR. HEERINGA: Thank you, Doctor Dupree. Doctor Delorme and then Doctor Portier.

DR. DELORME: Just an additional comment

Page 203

to what David was saying earlier. I was looking at our response in 2003, I was part of that panel so I actually have my copy here with me, I believe it's e of question let me get the number here anyways, it's one of the later questions that we were asked and I can read out the question for the record.

In this regard are there important differences between species to conclude that any affected developmental processes observed in Xenopus would not occur in Rana?

Several panel members stated there are little or no evidence to demonstrate that there are significant differences in development processes that would preclude the Agency from using Xenopus as a model in future studies. However some panel members noted that there are significant differences between the two groups of species in timing of life cycle events such that concerns about differences in developmental pathways cannot be eliminated.

So I think our conclusion as a panel was they're not one for one and you cannot totally eliminate the differences between them. Just for clarificatiion.

DR. HEERINGA: Doctor Portier.

DR. PORTIER: One of the benefits of

Page 204

being a statistician is that I don't have to go in sewage ponds looking for frogs.

Twice today there has been mentioned to a power study or a power analysis that was done. And I wondered if somebody could give a little bit more information on what outcomes were used as the basis of the power and whether there was really discussion on what affect sizes were we looking for when you settled on sample sizes? And that may be covered in the analysis phase, but we're talking design right now and for me design is power.

DR. FRANKENBERRY: Yes, actually EPA did not do an after the fact power analysis. In the protocol there was one done and I think the protocol then subsequently changed. I just learned this morning that Syngenta has done an extensive one I guess in the past few weeks. And there is maybe much more definitive than anything done before.

At some point maybe they could discuss it. I'll be able to tell you what we've done after the fact but it's not as extensive.

DR. PORTIER: And it just, something I haven't seen to indicate that power might have been based on the male/female ratio which would have been a simple outcome. And I just wondered if that was kind

Page 205

of what they were originally thinking.

DR. HEERINGA: I think Doctor Silken's motioning. Are you willing to have him come forward and discuss the Syngenta power

DR. PORTIER: Yes.

DR. HEERINGA: Doctor Silken, please.

DR. SILKEN: This is Doctor Silken and I really don't want to interrupt EPA's flow, so I'd like to come back to this a little bit later when we can do this a little more extensively.

But we did for Syngenta as the statistical people on the study, we did do both an early pre-study evaluation of the power looking at what affect sizes we could detect, depending upon the numbers of tanks and the number of animals within the tank and assuming a different correlation structure within the tank.

So there was a pre-power analysis. There was also a post or after the fact power analysis which was done by simulation to take into account the whole statistical analysis regime. And that was done for both measurement end points such as age, body weight, time to metamorphosis, those continuous measures.

There was also a power analysis done for

Page 206

incidence based, the percentage, the counts data. And we did, we do have slides that we can show about the different affect sizes and how those affect sizes were affected by 8 versus 16 controls. And we can show that the power for E2 versus Atrazine were comparable.

So at an appropriate time we can give a quantitative as well a qualitative discussion of power at your convenience.

DR. HEERINGA: Doctor Silken, if you wouldn't mind what I would prefer to do is coordinate with the EPA scientific staff and we'll try to find a way. I appreciate that way you've handled that and I want to make sure that anything that's presented at this point comes with their approval and at their request. So we'll do that.

What I'd like to do, any other immediate questions, and I think the power analysis issue too as long as we consider it in conjunction with the statistical analysis discussion, I think would be appropriate, even though it is a design stage issue, it bears on the question of interpretation of the data too. So we'll consider it there.

Are there any other questions at this point? In that case I would like to take a fifteen minute break and return at 3:20.

Page 207

Panel members, if we could just meet briefly in the breakout room just for a short administrative note, and I'd like to speak I guess with Doctor Frankenberry and Doctor Steeger as to how they might want to handle this supplemental discussion.

(WHEREUPON, there was a recess.)

DR. PORTIER: Okay, we're moving quite fast on our program here and talking with Doctor Heeringa, we're going to attempt to go through the overview of the DCI studies and then the overview of the statistical analysis. And that'll probably be the end of day today and we'll come back tomorrow morning with a discussion of the power analysis which gives the EPA staff time to look at Doctor Silken's material.

And then at that point we'll have the Agency conclusions and that'll start our panel deliberations at that point.

So I'm figuring we have about another hour or a little less or a little more.

And before we move into the next presentation I'm going to continue to see if the panel has any additional questions for the overview of the open literature or the scientific approach to the design of the data call in studies. Do we have any additional questions or comments? Yes, Doctor Leblanc.

Page 208

DR. LEBLANC: Gerry LeBlanc, just sort of a general question. It seems that one of the criticisms EPA has received in this whole process is basically not putting a lot of weight in the open literature. And I understand why. It's perfectly logical to me.

But I'm just wondering if in the intent for openness and inclusiveness, did you ever look at the contract study results which were done under GLP's and we had good control over, look at the results and then go back to the open literature and say, now does it support it, does it contradict it? Is there any benefit, anything added if we look at the open literature now in comparison to that data?

DR. STEEGER: I think we tried this is Tom Steeger I think that the Agency always tries to go back and look at how studies that have been conducted according to Agency guidance compares to what's showing up in open literature in terms of affects and at what concentrations.

There were as I pointed out in the slide, looking at the 19 studies that have been conducted since the 2003 SAP, some concordance with what the DCI, the data call in studies have indicated, that although a majority of studies that were

Page 209

available, there is no affect that has been produced by Atrazine on amphibian gonadal development.

I did not want to give the impression that we have discarded open literature as a source of information. Clearly in 2003 we raised they hypothesis that Atrazine could potentially affect amphibian gonadal development and we're here today because of the open literature that was available to us at that time.

So there were a lot of lessons learned and much of the information in terms of putting this very detailed study together and actually being able to accomplish it was a result of the open literature and what we learned from it.

DR. LEBLANC: Just as a follow up I agree. I think the value of the open literature is enabling the Agency to establish the hypothesis in the first place.

But it just, I guess when, in reading the white paper it just wasn't clear to me as to whether or not the Agency ever then looked at that data and the additional open literature data a second time to see if there's any value there now to contribute to the more definitive questions that are being asked, or whether it simply was used simply to establish the hypothesis and nothing further?

Page 210

DR. STEEGER: No, we did use the open literature subsequent to 2003 as lines of evidence to confirm that in addition to the DCI studies, that it does not appear that Atrazine exposure results in consistent affects on amphibian gonadal development.

There does not appear to be a dose response, there doesn't even appear to be a cause/effect relationship across most of the studies that are available in the open literature.

DR. LEBLANC: And that's important. Again I don't want to belabor the point but I think that the, it's important to make it clear that you embrace the open literature in the decision making, rather than just simply excluding the open literature because of a variety of problems.

DR. STEEGER: Yes. We do make use of the open literature.

DR. PORTIER: Any additional questions? Well, seeing none I guess we'll move on with the next presentation by Doctor Steeger on overview of the Atrazine DCI studies.

DR. STEEGER: In this presentation I'm going to continue to build on what Doctor Diggitts just discussed regarding the DCI study design. I will provide an overview of the study conducted by the

Page 211

registrant in response to the data call in that was issued in November of 2004.

In response to both the EPA and the FIFRA SAP in 2003 which recommended a tiered study approach that initially focused on laboratory studies, Syngenta developed a tier one study protocol.

In November of 2004 the Agency notified the technical registrants that they were required to conduct a study to address the uncertainties identified during the 2003 SAP.

Consistent with the recommendations made by both EPA and the SAP, the tier one studies were laboratory based and used Xenopus laevis larva.

The Agency reviewed the registrant's proposed study protocol and its associated standard operating procedures throughout the development of these documents.

The registrant conducted pilot studies using 17 beta estradiol to ensure that the protocols were adequate for measuring the potential affects of chemicals on amphibian gonadal development. During the pilot studies EPA inspected both in live phase laboratories to ensure that the protocols were being followed.

Based on the pilot studies and EPA's

Page 212

inspections, the Agency made additional recommendations on the proposed study protocols and the registrant incorporated the necessary changes.

Feeding regimes and algae blooms that resulted from too much food being provided was the predominant component of the modified protocols.

The registrant conducted two independent laboratory studies with Atrazine. One of the studies was conducted with Wildlife International in Easton, Maryland and the other study was conducted by the Leibniz Institute for Freshwater Biology and Ecology in Berlin, Germany.

All aspects of the definitive Atrazine studies were conducted to follow good laboratory practice, procedures for quality assurance and quality control.

EPA staff from the Office of Pesticides Programs and the Office of Enforcement, Compliance and Assurance conducted inspections of each of the laboratories involved in the DCI studies and as part of the inspections reviewed data and the quality assurance processes in place.

The German GLP Federal Bureau of the Federal Institute for Rick Assessment also conducted inspections of the IGB facility during the conduct of

Page 213

the study.

For the in life phase of the tier one Atrazine studies, each study consisted of five Atrazine treatment groups with nominal concentrations of .01, .1, 1, 25 and 100 micrograms per liter that were intended to bracket the concentrations reported in previous studies to cause gonadal affects in amphibians. Nominal concentrations were verified through HPLC and tandem mass spectroscopy. Exposure solutions were delivered through a continuous flow through system at a rate sufficient to maintain a loading rate of less than 1 gram per liter per day.

The positive estradiol control relied on a concentration of 0.2 micrograms per liter of estradio, representing the median effect concentration of estradiol for the feminization of males. And that is the increased frequency of males resulting in a shift in the sex ratio of males to females.

Negative controls were also run. All treatment tanks were color coded to ensure that the study was suitably blinded to prevent bias in the data measurements.

The study was initiated using Xenopus laevis larva, eight days post fertilization or six days post hatch, developmental Stages 46 to 48. And

Page 214

exposure continued through metamorphosis which is at the complete tail resorption or Stage 66, or after 75 days, whichever came first.

Flow rates through each of the study units was adjusted to yield a loading rate of less than 1 gram per liter per day and flow rates resulted in seven complete volume changes in each tank per day.

Each study unit consisted of a nine liter glass aquarium consisting of 25 larvae and a seven liter treatment solution. Each treatment was replicated each time. Negative controls consisted of 16 replicates. With 25 animals per tank, 8 8tanks per treatment, 16 for negative controls, each study utilized a total of 64 tanks and 1,600 animals. Each tank was treated as a replicate.

Again, all the tanks were color coded by treatment to limit potential biases.

Based on recommendations from EPA test animals were fed Sera Micron two times per day beginning on day 21 of exposure. Supplemental variation was provided to each of the treatment tanks to prevent dissolved oxygen from dropping since one of the performance criteria was that dissolved oxygen levels would remain greater than 60 percent of saturation.

Page 215

At completion of metamorphosis or after 75 days of exposure, whichever came first, the test animals were sacrifice by immersion in tricaine methyl sulphonate. Animals were then weighed and measured, dissected and gross morphology recorded. Digital images were taken of each of the frogs and gonadal surface area was measured with a digital image.

Afterwards the animals were fixed in solution for histology. Fixed tissues from both Wildlife International and the Leibniz Institute were forwarded to and processed by Experimental Pathology Lab, Incorporated in Sterling, Virginia. Once specimens were embedded in paraffin, longitudinal sections were made of the gonads and kidneys. Sectioning continued until the vertebral column was reached. Step sections of 4 to 5 microns in thickness were cut at 12 micron intervals and sections were affixed to glass slides.

Only slides with gonadal tissues were read. Generally 20 to 30 sections per animal were read by the pathologist.

For both study laboratories a minimum of 57,340 sections were reviewed.

All of the statistical analysis for data collected during the in life portion of the study and

Page 216

the histology portion were analyzed by Silken & Associates Consulting, Incorporated in Texas. Protocols for these analysis in terms of hypotheses tested and the statistical approaches used were reviewed by the Agency prior to the analysis.

Most of the statistical analyses were conducted using statistical analysis systems software or SASS software and standard statistical tests.

The next presentation will provide greater detail on the statistical analysis.

Although relatively vigorous study conditions were maintained throughout the course of the study, some of the protocol considerations were identified. Issues included the contamination of 4 out of 16 negative controls with Atrazine at 0.1 micrograms per liter at Wildlife International. However because of the frequent weekly analytical measurements it was determined that the contamination was limited to a specific cluster of control tanks and those tanks were discarded from further analysis.

Also at Wildlife an algae bloom occurred in four additional tanks and these tanks were also discarded.

Due to a combination of algae blooms and Atrazine contamination a total of 8 out of 16 tanks or

Page 217

replicates were dropped from the study. Thus at Wildlife the number of control tanks used in later analysis was the same as in the Atrazine treatments, that's 8 replicates.

Early in the study algae blooms were observed in 1 out of the 8 tanks in one of the Atrazine treatment groups at IGB, resulting in high mortality. This tank was also dropped from the analysis.

Additionally, Atrazine was inadvertently added to the positive estradiol controls in one week of the entire exposure at IGB.

Atrazine degradates were not measured in any of the treatment tanks. Given the measured concentrations deviated from nominal concentrations it would have been helpful to know the extent to which Atrazine was being degraded.

However, the flow through delivery system was intended to reduce the accumulation of metabolites in the water column and plus the study was not specifically designed to assess the toxicity of Atrazine degradants. Water samples from the study are archived and could be analyzed if necessary.

Subsequent to the completion of the white paper, Syngenta has provided preliminary analysis of the archived exposure solutions. These preliminary

Page 218

data indicate that out of the three primary Atrazine degratives diammino chloroatrazine or DACt, deisopropylatrazine, DIA and deethylatrazine, DEA, only DIA and DEA were measured above the level of detection.

The maximum measured concentrations in the two degradates, DIA and DEA were around .1 part per billion in the highest, that is the 100 microgram per liter Atrazine treatment solution.

Measured concentrations in the stock solutions were consistent with great than 90 percent, although measured concentrations in treatment units deviated from nominal actual concentrations verified on a weekly basis throughout the course of the study. Reductions in Atrazine concentrations may have been due to uptake by the test organisms or other biological processes. However the actual concentrations did span the intended four orders of magnitude and did not overlap.

This table reports the mean measured concentrations and their associated standard errors for each of the Atrazine treatment groups by laboratory. The range and percent of nominal is also presented.

Across the entire study period, measured concentrations averaged between 87 to 112 percent of nominal at Wildlife International, and between 55 to 88

Page 219

percent at Leibniz Institute. The highest amount of variability was associated with the lower treatment concentrations.

Study results, although there were limitations in the DCI studies, the rigor with which the studies were conducted rendered the studies of use in addressing the hypothesis that Atrazine exposure causes affects on amphibian gonadal development.

The estradiol positive control demonstrated that the study protocol was sufficient to measure affects on amphibian gonadal development. Sex ratio in the estradiol control was 75/25 female to male and is consistent with the target EC50 for estradiol under flow through conditions.

The most relevant end points in the study to assess the hypothesis were the extent of inter-sex or mixed sex, sex ratio and time to, and size at metamorphosis.

The study demonstrated that Atrazine concentrations ranging over four orders of magnitude from .01 to 100 micrograms per liter did not result in an affect on time or size at metamorphosis, sex ratio or the incidence of inter-sex or mixed sex.

The histological analysis of the gonadal tissue in Atrazine treated frogs only revealed a single

Page 220

animal with testicular oocytes in the 0.1 microgram per liter treatment. No other occurrence of mixed gonadal tissue was observed in the Atrazine treated animals.

This study did provide a broad range of histological end points, some of which were statistically significant. However, the biological and mechanistic relevance of those end points in gonadal development is unclear.

As mentioned earlier, fused kidneys and renal mineralization were statistically significant in both laboratories. However, there is not an apparent relationship with these measurement end points to gonadal development.

With respect to some of the end point there is uncertainty regarding their interpretation. The relevance of some of the histological end points of the hypothesis in not clear. Observations such as fused kidneys and renal mineralization are two such observations.

In defense of the researchers though, the Agency requested that the report include any abnormalities or lesions observed in the renal tissue as well as gonads.

With respect to the histological analysis conducted by Experimental Pathology

Page 221

Laboratories where the severity of the measurement end point was rated, it is unclear what serves as a reference. Since all the sections that were reviewed by the pathologist color coded the reader would not have known which animals represented controls and which represented treated.

There is uncertainty regarding whether some of the comparisons such as the number of gonad oocytes were made relative to amphibian or fish histomorphology.

There is uncertainty regarding the relevance of gross morphological end points. The terms used as descriptors of some of those morphological features implied an understanding of the underlying cause that would not have bee apparent based on the gross morphology and could be determined only through histology. Therefore the histomorphology is considered more definitive than the gross morphology.

In the next presentation Mary Frankenberry, a Senior Statistician in the Environmental Fate and Effects Division, and coauthor of the 2003 white paper will provide an overview of the statistical analysis of the DCI studies.

DR. PORTIER: Okay, before we go on do we any questions on the DCI study? I think we've covered

Page 222

a lot of those questions already. I think we'll go on.
Doctor Frankenberry.
DR. FRANKENBERRY: Thank you. What I have is an overview of the analysis plan and the analysis of the study and then some summary slides of the results that I hope will organize what both the DCI study and EPA's evaluation found in them. Next slide, thank you.
The study design employed multiple levels with replicated tanks and controls. As you've heard, we did treat the tank level as the level of replication in the study. There were five Atrazine treatment levels, one positive control and at the beginning of the study, two negative controls and subsequently one at the end with 8 tanks in each group. 25 animals per tank developed into approximately 10 to 15 males and females, although that ratio was more skewed in some of the tanks. So when the individual sexes were analyzed there were some where the numbers were fairly small per tank.
The data were analyzed using one-way analysis of variance followed by comparisons and trend tests. The Kruskal-Wallce and Wilcoxon and Mann-Whitney were the nonparametric equivalent tests used. Many of the major apical end points were represented by

Page 223

continuous variables, but most of the secondary gross of histology affects were categorical variables.
Now a protocol for the statistical analysis was submitted by the registrant to the Agency and reviewed before the pilot studies began. It was subsequently changed, partly by the estradiol, results of the estradiol study and partly in response to comments that EPA made that, some recommendations that were consistent with Agency study evaluation protocols.
And then both the study authors and the Agency and its evaluation followed the final analysis plan for evaluating the data.
The scope of EPA's review of the studies, over 330 SASS files were submitted to the Agency as part of the studies analysis of the data. They contained data sets and output files as well as program files for running the tests.
EPA reviewed all of these, performed quality checks, verified the data sets and outputs and then ran the programs for all end points with a few minor modifications that I'll mention in the upcoming slides.
Also for the major, the primary end points EPA ran our own independent Agency programs and

Page 224

One major difference between the DCI study and EPA's evaluation is that EPA tested most of the categorical variables for a one-sided increase in affect across the Atrazine treatment while the DCI study employed more two-sided testing, many not for many more variables, but more than we did.
The overall outcome of the analyses were really the same except that EPA found two additional effects as statistically significant in pairwise comparisons and those were fused kidneys and renal mineralization. That was as a result of looking at the one-sided testing rather than the two-sided.
Just summing up the differences again, more two-sided testing in the DCI study and mostly one-sided testing with EPA. Also the DCI study assumed that there would be no differences for any pairwise comparisons that followed a non-significant F test. EPA ran those comparisons for the major end points since in some percentage of tests we have found differences. In this case however there were no differences.
And then finally EPA required that the contaminated controls not be used in the analyses.
Actually one further difference was, I think we mentioned severity codes this morning, EPA did

Page 225

not use the severity codes in our analysis but grouped all of the levels into one measure for affect. The higher level severity codes were so infrequent that we though this made sense and was probably more reliable to do.
Now for the major affects or the apical affects, starting out showing no difference as you have seen in many slides this morning. Mortality, failure to complete metamorphosis, age at completion, percent of males as a measurement for sex ratio and mixed sex, and I think as was noted also this morning, there was only one animal in all of the Atrazine treated groups with a strictly defined, the definition of mixed sex.
For the apical affects where we did see differences, and this was in pairwise comparisons as well as others, length and weight differences were significant at the same three levels in the IGB lab, both at IGB, both in females and there was no dose response apparent, relationship apparent to us. These were not significant at the Wildlife lab.
And if we look at, these are our graphs, they're similar to what you've seen this morning.
I did over lunch calculate the affect size which we probably should have had in the white paper that wasn't there.

Page 226

At IGB the decrease for females was 7 percent and that was significant. At Wildlife the largest decrease at all, and this was strictly in males also, was six and a half and it was not significant.

For snout-vent length the significant affect size at IGB was 2.2 to 3.3 depending on which dose you looked at, somewhere in that range. At Wildlife the largest difference was 1.6 and it was not significant. This may be a case where the 8 extra control tanks would have helped. We don't know but maybe Doctor Silken can helps tomorrow morning.

For the histology end points where we found a difference, fused kidneys and renal mineralization, they were both in males. This was in pairwise comparisons again. They were at both labs, one lab had one, one at the other, of course both at the 1 part per billion treatment level. This I think was the result of one-sided testing and we don't see a dose response, just at that one treatment level.

The secondary gross morphological affects, again in pairwise comparison, these are the significant end points. They cross, include both sexes and both labs, probably about equal numbers. I guess there were a few more at Wildlife here in these, than at IGB. No dose response again and for a gonadal image

Page 227

area there was a significant increase in pairwise comparisons at the Wildlife lab. But if you look at the data from IGB that was significant for a decreasing trend in the data. I point that out. Next slide.

This is just a summary of the trend tests that were significant at two to four treatment levels. For the first three affects of decreased ovarian cavity size and pigmentation, also mineralization. The trend includes three levels of treatment, were significant at three levels. All at the IGB lab. The gonadal segmental translucence is down at the bottom. It covers four of the five levels.

And summary slides similar to what you've seen in the registrant's report, but combined with, put alongside the Atrazine data and the positive controls. The major affects among the Atrazine treated animals, again only the length and weight in the one lab and in the females showed significant differences, pairwise differences, these are shaded in red.

Estradiol animals consistently in both labs showed significant affects for the time to complete metamorphosis, the sex ratio and mixed sex.

For the histology end points, fused kidneys end point showed significant tests at both laboratories, but in pairwise comparisons, only at

Page 228

Wildlife. It was significant in the trend test at IGB, renal mineralization was significant in pairwise comparisons at IGB and those are the two main effects.

The Atrazine treated, you'll see consistency between the two labs in the positive control for dilated testis tubules dividing gonad oocytes and internal melanophores. Other effects for the Atrazine treated animals vary between the labs. For Atrazine treated several end points showed a significant overall difference among all levels tested, but no significant differences between any of the, a pairwise comparison between any treatment and control, and these are highlighted with pink but marked as non-significant. Also for these end point, often a major contributing factor to the significance of this test was the difference between two treatment levels, but was greater than the difference between any treatment and control.

And finally for the secondary gross effects, hypoplasia was detected in the Atrazine treated animals at one lab and in the positive control at both. Other effects were significant at one lab or the other, including segmental translucence. I think that was significant for both Atrazine treated males and females at Wildlife along with the positive

Page 229

controls at Wildlife.

The gonadal image area again was significant at Wildlife but decreasing in a significant trend at IGB. The positive controls at both labs showed an increase similar to the increase at Wildlife.

Again we have a lighter shading for the end points that showed the significance in the overall tests but were not significant in any pairwise comparison.

Finally in conclusion, for many of the categorical end points, a moderate frequency variability in the data was such that results were not reproduced in both labs. For the apical end points in general there appeared to be a sufficient power to detect small to moderate differences, particularly for those represented by continuous end points. But finally the reproductive relevance of a number of these other effects still remains in question.

DR. PORTIER: Okay. Doctor Handwerger.

DR. HANDWERGER: I'm sorry, I should have asked this question this morning. What do you mean by renal mineralization? Are you talking about calcium deposits? And if you're talking about calcium deposits, where are they, are they tubular, what are we talking about by the term renal mineralization here?

Page 230

DR. WOLFE: Yes, this is Doctor Wolfe. We did not assay them to find out exactly what mineral they were made of. The diagnosis is based purely on a histomorphological conclusion based on my experience in many species of animals.

Renal mineralization, I do a lot of work with fish, very, very common in fish. Many species, both, I see it in wild fish, I see it in cultured fish, especially in cultured fish it may have something to do with the way we raise them.

But I don't want to get off on a tangent here. In this particular case you asked where in the kidneys they were found. They were often found in tubules. We also had gonadal mineralization for that matter. We had mineralization occurring just at random sites within the gonads.

So again this may be part of the fact that our husbandry, while it's good it's not 100 percent, or it may be just something that just tends too happen in certain species of animals.

DR. HANDWERGER: I don't know much about fish but if it were a human with renal calcium deposits I'd really be very concerned.

DR. WOLFE: No, it is extremely common in many species of fish that you look at, from salmonids

Page 231

to small aquarium species, we see it all the time.

DR. PORTIER: A lot of statistical questions. I'll hold mine and start with Doctor Bailey I guess.

DR. BAILEY: Yeah, Ted Bailey. You have the same experiment conducted at two different locations. Did you consider a joint or a combined analysis of the data?

DR. FRANKENBERRY: I think in the original protocol there was a discussion of that. We were not in favor of it and I think the experimenters weren't either toward the end.

My personal feeling is that seeing effects in one lab that are not repeated in the other does not negate the finding in the one lab. And I don't think they were controlled well enough to do that in my mind, although we could have tested for them.

DR. PORTIER: Kind of associated with that Ken Portier did you see really differences in underlying variability between the two lab studies? I mean I know you did a lot of homogeneity tests within the studies and I wondered if there was a comparison between the study?

DR. FRANKENBERRY: We did look at the end points that were significant across, in one lab or the

Page 232

other and there really was little overlap in terms of numbers. We at first expected that there would be fewer end points that showed up significant at Wildlife and that wasn't the case, at least not in the secondary gross effects.

That doesn't mean that some weren't missed of course.

We looked at, for the primary end points we did look at the affect sizes that were seen and declared significant. And in general they ranged between about 2 percent and 8 percent. For two of the, for failure to complete metamorphosis and mixed sex those effects were nearly nonexistent across both labs. There was nothing to work with there.

The frequency of males between the two labs, there were some tanks that were higher and some were lower, there was variability there but they averaged at most 10 percent in both labs.

We didn't go on beyond that.

DR. PORTIER: I was struck by the number of zeros in the data set. Doctor Yeater?

DR. YEATER: I was wondering if you could clarify by tank because when you were speaking it sounded like you were talking about both labs but then on the slide this is just data from the IGB lab?

Page 233

DR. FRANKENBERRY: It's only IGB, I'm sorry

DR. YEATER: Okay.

DR. FRANKENBERRY: yes.

DR. YEATER: And then what are the asterisks for?

DR. FRANKENBERRY: Those are the significant

DR. YEATER: Thank you.

DR. FRANKENBERRY: levels.

DR. PORTIER: Doctor LeBlanc, did you have a

DR. LEBLANC: No.

DR. PORTIER: Okay, Doctor Miller.

DR. MILLER: And just to clarify, for the histopath, when they were scored did you say that you did not include those scorings or you actually did on the present/absents?

DR. FRANKENBERRY: We combined any level of affect as affect or no affect. I think the company's analysis, even though there were four severity levels put out at the outset, I think they looked at only two, well three, they are no affect and then I think affect at the high any affect greater than severity level one. And the numbers in those

Page 234

categories at greater than one up through four were fairly small for most variables.

And I think we had a little bit of question about how easy it was to reproduce that.

DR. STEEGER: This is Tom Steeger. To add to Mary's response, as I indicated in my presentation, EPA conducted a number of inspections on the labs and during one of the inspections to EPL I requested that Doctor Wolfe reread several of his slides. I had his original diagnoses in front of me and my intent was to see how well he would replicate his readings.

So I chose the slides at random plus I had a few in there that I knew had some marked pathologies. And while Doctor Wolfe was able to very well replicate the different lesions, his scorings of the severity tended to deviate from what is original reads were.

And so based on what appeared to me to be somewhat a subjective interpretation by the pathologist, it moved us towards not sticking with the original severity ratings.

DR. PORTIER: Doctor Patino.

DR. PATINO: Reynaldo Patino. I think there was a discussion earlier about what the unit of

Page 235

replication is or should be, but assuming it is a tank, in slide 2 you show that there was 25 total animals and when you did the analysis by sex there were as little as 10 of one or the other sex per tank.

And I was wondering, for the categorical variables, this indicates that the ability to find an affect is not the limit of detection, and I don't know if that's the right term. Was it like 10 percent, that anything lower than 10 percent, assuming that the tank is the unit of replication, you would not be able to detect it?

Is that what you took?

DR. FRANKENBERRY: Yeah, some, that could explain why we saw a lot of zeros and there may have, out of 8 tanks there may have 6 tanks with zero frequency and then perhaps 2 that did have 5 or 6 animals that resulted in a higher frequency overall.

And yes, we could miss the low frequency numbers. On the other hand we had 8 chances of seeing them.

DR. PORTIER: I think the phrase you were looking for is the affect resolution.

DR. PATINO: Yes.

DR. PORTIER: Was like 10 percent or even less in some, or higher in some cases, 12 percent.

Page 236

You talked about pairwise comparisons and can you tell me kind of exactly what you did? I'm trying to decide whether what you did was conservative or liberal. And I couldn't quite get that by and that's for statisticians, you know, whether it's conservative or liberal, it has nothing to do with politics.

DR. FRANKENBERRY: Yes, actually we can look at the slide. For the analysis of variance, anything with a continuous end point we followed up with pairwise contrast comparisons. For the Kruskal-Wallace we use the Wilcoxon and Mann-Whitney.

DR. PORTIER: And so the contrasts were all done on the 5 percent level, .05 --

DR. FRANKENBERRY: Yes.

DR. PORTIER: for significance level. So there were no Bunn-Ferroni adjustments in here, nothing that would actually --

DR. FRANKENBERRY: I'm sorry, I think we did use Dunnance.

DR. PORTIER: Okay, you did a Dunnance procedure?

DR. FRANKENBERRY: I can look to be sure though.

DR. PORTIER: As a different meaning.

Page 237

Doctor Bailey.

DR. BAILEY: I think I remember reading that you did this only after the F test was, will begin?

DR. FRANKENBERRY: Yes.

DR. BAILEY: Because this would help put it on the considered us test.

DR. SCHLENK: Dan Schlenk. Just a quick question, it's more for Doctor Steeger I think. But again going back I just want to ask you the same question that I asked the Syngenta folks.

On slide 16 there's the summary for all the secondary gross morphological affects I guess. That's what I was getting at this morning, was the hypoplasia in the male Atrazine dose, which I think was at the .1, and I hate to do this but if you had seen a significant affect in both labs, would that have raised any concerns at all?

I realize that there was a discrepancy with another indicators that showed the opposite affect, but I'm just curious what your evaluation of that particular data set says?

DR. STEEGER: This is Tom Steeger. If there was an affect noted in both labs, yes, we would have been concerned.

Page 238

But in general I did not know what to make of the gross morphological affects because hypoplasia, hyperplasia, those terms to me imply that you have an understanding of the causality in terms of there's too few cells or there's too many cells. You can't tell that from a gross morphological basis. You can only tell that the organ was smaller.

And so it's only the histological end points that we grade, that I gave greater import to.

DR. SCHLENK: And basically what you're saying is that didn't match the histological analysis?

DR. STEEGER: In many cases the histology did not match the gross morphological, right.

DR. PORTIER: Doctor Handwerger?

DR. HANDWERGER: Stuart Handwerger, I'd like to go back to the point that Doctor Miller made about the pathology. I mean I'm not surprised that you would go through the same pathologist and get two different, differences in severity. I see that a lot of time clinically and I'm not surprised by that.

But what I am surprised, is that you abandon then with an attempt to quantitate things and I'm wondering why you chose to negate the quantitation by not getting perhaps the import from only one pathologist to handle that.

Page 239

And I think in many studies that I read and review where you really want to grade things like that, you have more than one pathologist doing the reading, recognizing the fact that there is this inconsistency, going back and reading the same slide, two people looking at the same slide may come up with different interpretations.

So I think if the pathology is really critical to this study and you see that there is this variation, I'm wondering why you didn't have more than one pathologist analyzing some of the critical data.

I'd like to just hear what you can say about that.

DR. STEEGER: It's our understanding that it is a common practice for a single pathologist to review slides. And it is a charge to the panel whether that is sufficient in this case.

DR. PORTIER: I think Doctor Wolfe had a comment on the previous question.

DR. WOLFE: Yes. This is Doctor Wolfe. One thing I wanted to follow up on the hypoplasia, I believe again that the hypoplasia was, is supposed to be an indication of the general size of the gonad based upon the gross morphological features.

But there's several considerations here.

Page 240

One is that if you look, take any one of these gross morphological features or many of them, when you look at these features histologically, you actually could come up with multiple different types of histologic diagnoses for any one of these gross findings.

So for example you said take something like translucence. Translucence on a gross basis, on a microscopic basis that could be dilated tubules, it could be decreased germ cells, one might not be able to find anything histologically to correlate it.

So that's one issue about gross findings that is kind of important, is why in gross findings there's a hazard with that.

The second thing is I think hypoplasia again was only one treatment group or one dose group, so there wasn't any kind of dose response, it wasn't common between the two different laboratories. And everybody should also remember that another exercise that was done in this study that really wasn't emphasized very much was, we did actually do gonad areas, we did morphometric measurements of gonad areas among the various animals and these were not different among those groups.

And to me that's a lot more sensitive measurement than actually estimating and saying, well,

Page 241

that gonad looks like it's a little smaller than I expect it to be.

DR. PORTIER: Is this the same thing as a GSI, would that be a comparable measurement, the hypoplasia measurement, is that the same type of end point?

DR. WOLFE: I think that probably is a similar type of calculation. I think the actual gonad are in this case is a lot better than a GSI would be. When you talk about, you know, when we flash these gonads up there on the screen, they look humongous. Okay, we're talking about something that are, you know, a millimeter or less actually when you're looking at them, even under a dissecting microscope, these things are tiny.

There would be no way to do a GSI in this particular case. But yeah, you're getting I think similar types of information.

DR. PORTIER: And what's a GSI?

DR. WOLFE: I'm sorry, this is Doctor Wolfe again, gonadal somatic index, which is a fancy term for, you weight the gonads, you weigh the animal and you can get a ratio.

DR. STEEGER: Just as a follow up the GSI was not measured in this study because the organs were

Page 242

not weighed.
DR. PORTIER: Any additional questions?
MR. PAULI: Sorry, Bruce Pauli here, can we just go over gonadal image area. You were just talking about gonad size and I'm just looking at the table up there with the image area.
Is that a can you just explain that measurement and whether or not this is a is this a one tail?
DR. STEEGER: The measurement is recorded off the digital image and it's just digital analysis software that's being used. Mary, do you want to talk about this?
DR. FRANKENBERRY: This was a two tail test I'm sure. We looked for an increase or decrease and we did see both.
DR. PORTIER: It looks like the panel has run out of questions for the day. And it usually happens the first day anyway, we kind of run out of steam. It's a lot of material for us to process at one time even though we've all read.
Oh, we've got one more on the end here. Doctor Bucher.
DR. BUCHER: I can't let you get by John Bucher. So I've been sitting here looking at the

Page 243

estradiol measurements and you've got a dose here I think that was used that created 50 percent, it was an EC50 dose, right?
DR. FRANKENBERRY: That's right.
DR. BUCHER: And looking back of some the Hayes work, he's used a dose of half of this and has gotten 100 percent sex reversals in his work.
Have you, have you actually compared what he's recorded in some of his papers with what has been reported here to try to get a sense of the sensitivity of these different studies?
DR. STEEGER: No, we did not go back and do a comparison between the histology of this study and that of his.
DR. PORTIER: This is Ken Portier. Did his frogs come from the same source?
DR. STEEGER: My understanding this is Tom Steeger Doctor Hayes' research animals are from an in-house culture.
DR. PORTIER: Someone was just asking me whether we were going to do the conclusions, but the ground rule is we're going to start tomorrow morning with a discussion of the power and then have the conclusions and any additional comments that the EPA staff want to make to the panel.

Page 244

And our expectation is that probably after the morning break we'll start with the charge questions to the panel.
I think at this point we're going to call today's meeting to an end. We will start again tomorrow morning at 8:20 sharp and hope to see you all here.
I'd like to see the panel for a few minutes in the break room once you get your stuff together and I'll turn it over to Joe Bailey for some final comments.
MR. BAILEY: Just very briefly I just wanted to thank the public for attending today and thank those who did present public comments during the comment opportunity.
I want to thank EPA presenters for giving their presentations today and I want to especially thank the panel for their discussions and asking questions of the presenters.
And thank Doctor Portier and Doctor Heeringa who will join us back tomorrow.
So thank you all for being here.
(WHEREUPON, the meeting was adjourned for the day.)

Page 245

CAPTION

The foregoing matter was taken on the date, and at the time and place set out on the Title page hereof.
It was requested that the matter be taken by the reporter and that the same be reduced to typewritten form.
Further, as relates to depositions, it was agreed by and between counsel and the parties that the reading and signing of the transcript, be and the same is hereby waived.

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

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

MARK REIF, COURT REPORTER / NOTARY

SUBMITTED ON

October 9, 2007

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