FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

REEVALUATION OF
THE HUMAN HEALTH EFFECTS OF ATRAZINE:
REVIEW OF NON-CANCER EFFECTS AND
DRINKING WATER MONITORING FREQUENCY
AND CANCER EPIDEMIOLOGY

DOCKET NUMBER: EPA-HQ-OPP-2011-0399

FIFRA SAP WEB SITE http://www.epa.gov/scipoly/sap/

OPP Docket Telephone: (703) 305-5805

UNITED STATES ENVIRONMENTAL
PROTECTION AGENCY
CONFERENCE CENTER LOBBY LEVEL
ONE POTOMAC YARD (SOUTH BUILDING)
2777 SOUTH CRYSTAL DRIVE
ARLINGTON, VIRGINIA 22202

JULY 26 -28, 2011

July 26, 2011 - 8:30 a.m. Day 1

JOSEPH BAILEY: Hello, I'm Joseph Bailey, and I want to welcome everyone this morning to the FIFRA Scientific Advisory Panel Meeting. This meeting is a re-evaluation of the human health effects of Atrazine, review of non-cancer effects, drinking water monitoring frequency and cancer epidemiology.

I just want to take a few minutes and go over our standard comments before the meeting. This is a Federal Advisory Committee Act meeting, meaning that it is a public meeting, and part of my responsibility is to make sure that all the requirements of the Federal Advisory Committee Act is met.

The Committee provides advice to the panel. All final decisions and regulatory decisions are left up to the agency, but they seek advice from the panel in a peer review capacity and consider the advice the panel gives in reaching their regulatory decisions.

Part of our responsibility is to make sure that all of the ethics requirements are met for panel members. And to do that, we have asked the panel members to fill out disclosure information for us to review to make sure that there are no financial conflicts of interest or bias on behalf of any of the panel members.

We have agendas out on the table so you can take a look at it. It is a pretty full agenda. It does provide an

opportunity for public comment. The public comment opportunity is scheduled to begin this afternoon.

Hopefully we'll be on schedule but the agenda is floating. Things can shift a little bit, but we will begin the public comment opportunity this morning and wrap it up tomorrow morning, at which time when that ends we will go into the charge questions with the panel discussions.

If anyone has not let me know ahead of time they wish to present public comments, we have a little bit of time left in the agenda slot for that opportunity. Either let me know or any of the other people in the Scientific Advisory Panel staff know. And if you have not made prior arrangements, we ask that you limit your comment period to five minutes time.

As usual, we have a public docket established. All the material that has been provided to the panel so far is in that docket. It is available electronically unless it is sensitive in any manner. And those documents that are sensitive can be accessed at the docket by visiting on the 4th floor in this building.

EPA's presentations are in the docket. They should be available at some time today. And any public comments that are made, we will also put those in the docket if they are not already there. The docket number should be listed on the agenda. And as I mentioned, all of that information should be publically available unless it is sensitive information.

At this point, I want to introduce Dr. Daniel Schlenk, who is the Chair for this session of the SAP. And again, I want to welcome the public here and EPA, as well as the panel. We have some new panel members and some returning members here, so I want to thank you all. Thank you.

DR. DANIEL SCHLENK: Thanks, Joe. Good morning, everyone. My name is Daniel Schlenk. I am a professor of environmental toxicology from the University of California, Riverside. I will be serving as the session chair today, but in place of my esteemed colleague, Dr. Portier, who has been able to turn his attentions to some of the questions that have been asked, particularly with regard to statistics.

What I would like to do right now is -- we've got a fairly large panel -- I would like to go around to each panel member and have them introduce themselves, where they are from and state briefly what their area of expertise is so that you guys can get a feel for what we have throughout this week. So Ken, you want to go ahead?

DR. KENNETH PORTIER: Good morning. I am Dr. Kenneth Portier. I am Managing Director of the Statistics and Evaluation Center at the American Cancer Society, national office in Atlanta. I am a biostatistician and the expertise I bring today is statistics in some of the environmental modeling hydrology.

I should mention Dr. Chambers is going to be here a little later this morning. She is the third permanent panel member to make up our core for our meeting. She was delayed in Atlanta due to weather last night.

DR. STEPHEN KLAINE: I am Steve Klaine. I'm a permanent member of the panel and I am a Professor of Ecotoxicology at Clemson University.

DR. ELLEN GOLD: I am Ellen Gold and I am professor and chair of the Department of Public Health Sciences at the University of California, Davis and an Epidemiologist.

DR. FRANK BOVE: I am Frank Bove. I am with the Agency for Toxic Substances and Disease Registry in Atlanta. I am a Senior Epidemiologist in the Division of Health Studies.

DR. HEATHER YOUNG: Hi. I am Heather Young from George Washington University, Department of Epidemiology. I am a cancer epidemiologist and also focus on reproductive outcomes.

DR. NELSON HORSEMAN: Nelson Horseman. I am at the University of Cincinnati. I am a physiologist and endocrinologist, and my research areas of interest currently are in mammary gland development, lactation and breast cancer.

DR. JAMES MCMANAMAN: I am Jim McManaman. I am at the University of Colorado. I am Professor and Chief of the Division of Reproductive Sciences and my interests are mammary gland biology and reproductive mechanisms.

DR. DANIEL GRIFFITH: I am Daniel Griffith from University of Texas at Dallas. I am an Ashbel Smith Professor of Geospatial Information Sciences. My area of expertise is

spatial statistics and I am working on the monitoring part of the project.

DR. HERBERT LEE: My name is Herbie Lee. I am a professor of statistics at University of California, Santa Cruz where I am also the Vice Provost for Academic Affairs. And my research areas include spatial statistics and computer simulation modeling.

DR. ROBERT GILLIOM: Bob Gilliom, U.S. Geological Survey, and I direct our national studies of pesticides as part of the National Water Quality Assessment program, and I am here for the monitoring aspects, primarily.

DR. SUSAN AKANA: I am Susan Akana. I am in my second career as an instructor at City College of San Francisco. I am late of UCSF where I had a career in stress and its interaction with energy balance in rodent models.

DR. KEVIN O'BYRNE: My name is Kevin O'Byrne and I am from King's College London. I am a professor of reproductive neuroendocrinology and my research areas span the neurone control of reproduction; that is what I do.

DR. KATHERINE ROBY: Kathy Roby from the University of Kansas Medical Center and my expertise is reproductive endocrinology.

DR. BARRY TIMMS: Barry Timms, professor in the Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota. My area of specialty is in

developmental biology of the prostate glands with a secondary interest in endocrine disruption.

DR. TRAVIS JERDE: My name is Travis Jerde, Indiana University School of Medicine, Department of Pharmacology and Toxicology, and my area of expertise is prostate biology with an emphasis on prostatic inflammation and resulting adult disease.

DR. PENELOPE FENNER-CRISP: My name is Penny Fenner-Crisp. I am a private consultant from Charlottesville, Virginia and a member of the Commonwealth of Virginia's Pesticide Control Board. My area of expertise is toxicology and human health risk-assessment.

DR. BETTE MEEK: And my name is Bette Meek. I am Associate Director of Chemical Risk-Assessment at the McLaughlin Center, University of Ottawa. I have a background in toxicology and spent most of my career in regulatory risk-assessment at Health Canada.

DR. RICHARD GREENWOOD: I am Richard Greenwood. I am an Emeritus Professor at the University of Portsmouth, and I am here for my expertise in the area of pharmacokinetics and toxicology.

DR. WILLIAM HAYTON: I am William Hayton, a Professor Emeritus College of Pharmacy, Ohio State University, and my area of expertise is pharmacokinetics.

DR. DANIEL SCHLENK: Thanks, everyone. Just a point of administrative comment, Dr. Barry Delclos will not be

joining us. He is listed as a panel member. He could not make it today due to a family emergency, so he will not be providing comments today, for those of you that are interested in that.

Welcome, everybody. At this point in time, as we begin our agenda, it is our standard practice to introduce Dr. Steven Bradbury, who is the Director of the Office of Pesticide Programs, and he is going to give us our opening remarks. Dr. Bradbury?

DR. STEVEN BRADBURY: Thanks. I would like to welcome the panel to this week's meeting. I appreciate you all volunteering to help us as we explore the scientific issues before us this week.

The role of the Scientific Advisory Panel for the Pesticide Program is very important in the work that we do. We use this peer review body to help us evaluate both new methodologies that we want to bring to bear in our pesticide risk-assessment process, as well as bringing to the panel specific questions sometimes around specific chemicals to help inform our regulatory decisions.

The scientific foundation to the decision-making process we make in the pesticide program is really fundamental to everything we do in our work at EPA, and not just for the pesticide program but for the agency as a whole, and the administrators.

We emphasized that numerous times, since she has been running EPA, the importance of the best peer reviewed

available science to inform our decision-making process. What's also very important in our decision-making process is that it's open and transparent and that the public can participate in the process that we go through. So, not only having you all here and providing your scientific expertise, but also ensuring that the public has an opportunity to comment and the public has an opportunity to see all the documents that are coming before you and ultimately see your report, and see your report in a context of the deliberations we will be having during the course of the week.

So we are very much indebted to your time and service not only to us in the pesticide program, but more broadly to all the U.S. citizens, in terms of the kinds of decisions that we have to make, in terms of insuring pesticides that are affective for food and fiber production are also safe for human health and the environment. So your role is very instrumental in what we do.

I also want to thank the public as we embark on this week's activity for the comments they have already provided and the comments you will hear during the public comment period, and that input is very important for the deliberations that you will be making.

I also want to thank the Science Advisory Panel staff for helping us organize this meeting and the work it takes to reach out to all of you, and get you all lined up with all your paperwork so that you can be part of the panel and all the work it takes to put a meeting like this on and work towards the final report that will come out in the

coming months. So thanks, again, for all the effort that you have already done in getting ready for the meeting and the intense time you will have here, and then the intense time you will have in writing the report. We greatly appreciate it.

I thought it might be helpful if I could just spend a few minutes maybe reviewing where we have been, with regard to atrazine and what we are going to try to accomplish this week. And in that quick summary also try to weave in some of the broader issues and opportunities that we are looking at in terms of advancing our risk-assessment methodology in the pesticide program as we take a look at some of the new technologies and different approaches, evolving approaches as we go into the 21st Century.

So, I will talk a little bit about atrazine and try to weave in some of the other techniques and approaches that are coming to bear in the atrazine risk-assessment. So, as you probably recall from some of the background documents you got, that in 2003 we re-evaluated atrazine, and that was part of an effort that was ongoing for a number of years in which every pesticide that was registered before 1984 had to be re-registered, in other words, re-evaluated to ensure that it met human health protection standard as well as environmental standards.

And atrazine was re-evaluated in 2003, and in 2006 it was looked at again with other triazine herbicides to ensure that that group of herbicides together still met the safety standard associated with the Food Quality Protection Act, so looking at cumulative effects and

ensuring protection for the population in general, as well as looking as sensitive sub-populations, in particular, looking at children in terms of our safety finding.

The current re-evaluation program that we have that is dictated by statute is called registration review. And registration review requires that every pesticide be re-evaluated every 15 years. And so atrazine, like every other chemical, is on its schedule and it is set to be re-evaluated in 2013. But, just because a chemical is scheduled at a certain time in its re-evaluation schedule does not mean we have to wait before we take a look at a chemical if new information comes to bear that suggests we need to take a look, and just ensure that we are still meeting our safety findings.

In the case of atrazine, probably one of the most intensively studied chemicals in the scientific literature -- probably not the most, but among pesticides, probably one of the most heavily studied pesticides -- since 2003, there has probably been 150 or so papers that have been published with regard to atrazine. In addition, the registrant has submitted additional information over the course of the years as a part of the conditions of re-registration, and there has been a lot of water monitoring going on, both in drinking water sources as well as in headwater streams in terms of taking a look at potential ecological exposure and effects.

In 2009 the agency felt that, given the significant amount of information that has been published over the course of the last six to seven years, and the information coming in

through the monitoring program as a condition of re-registration -- which you can get detailed information on atrazine concentration and drinking water sources -- it made sense to sit back and take a look at the new information, take a look at that information in light of the monitoring data that we had received.

It was not suggesting any areas of concern in terms of our risk-assessment in 2003, but take a look at new information since 2003 and reaffirm or make adjustments if necessary, based on the new science that was coming in. So, the end of 2009 we had a consultation with the Science Advisory Panel just laying out the peer review plan that we were going to ultimately execute in 2010 and here in 2011.

And during 2010, we had three scientific advisory panels; one in February, one in April and then one in September. The February 2010 SAP was actually one that we had scheduled for some time and used that as an opportunity to look at atrazine as a case study, along with some other compounds.

And the major emphasis of the February SAP, Science Advisory Panel Review, was actually to take a look at a framework, a framework that was embracing the concepts that were coming out of the National Research Council's 2007 Report on toxicology testing in the 21st Century, and beginning to think about ways to integrate experimental toxicology data as well as epidemiology data in a risk-assessment process.

And also, looking at that NRC report in the context of what the NRC report called toxicity pathways, what we in the agency have been starting to call adverse outcome pathways, in which of more focused effort of looking at initiating events and thinking about the chain of events that happened in biological systems, as a way of organizing your thoughts and integrating information around the linkage between adverse outcomes and the various processes that could lead to adverse outcomes.

And how do you look at experimental toxicology data along with epidemiology data to try to integrate that information, and essentially, how you pull together a coherent story about what the potential of a chemical may be in terms of its effects, its exposure and ultimately how that can help inform a risk-assessment.

And so in that SAP of February 2010, trying to get some feedback on how to be thinking about these issues, which is not just toxicodynamics, but also toxicokinetics; how do we better understand what happens at one level of biological organization in terms of the magnitude of the effect, the duration of effect and how much of that has to happen in order to have another event happen further down or along the biological chain of events.

If you look at the NRC report, it talks about the fact that as we use these approaches, we will see perturbations in biological systems. Part of the challenge will be how much of a perturbation is necessary to elicit an adverse outcome, say a frank effect in the intact organism. And

so that February SAP was getting at some of the approaches, some of the things to think about as we go forward, and not just for atrazine, but more generally as we go forward with new risk-assessment methods.

But we also included in that SAP some case studies on epidemiology studies, ecological epidemiology study designs, primarily but not totally, and used that SAP to begin getting some feedback from you all on how to be looking at different kinds of epidemiology studies in terms of their design and their attributes, and how different types of epidemiology studies should be contemplated as you start to think about integrating epidemiology data with experimental toxicology data.

The studies that may be helpful in terms of formulating hypothesis versus the kinds of studies that may be very powerful in terms of establishing causation from an epidemiological perspective, and how to integrate that with toxicological information. So that really helped us to sort of get our framework together and get some of our thoughts together about how to approach this risk-assessment, as well as some initial thoughts on how to be taking a look at epidemiology studies. As you know, there are a lot of epidemiology studies associated with atrazine.

The April 2010 SAP then primarily focused on experimental toxicology data, looking at both in vitro information as well as in vivo information, and again, getting some feedback on the studies themselves and how to be thinking about those studies, and what are those studies telling us

in terms of what we knew in 2003, to the extent that there were some new insights coming since 2003.

That gave us some good feedback in terms of the reproductive and developmental outcomes that have been the focus of 2003 in the context of LH surge suppression being sort of a key event that could lead to changes in reproductive or developmental outcomes, and how that related to an other kinds of experimental toxicology data that had come out in the previous years in terms of immunological effects or neurological effects.

We also used that SAP to get some initial feedback on sensitivity across different life stages and things that we should be thinking about in that regard. We also had some discussions around dosimetry and how to take a look at what could be happening at a sub-organismal level and be thinking about oral uptake of atrazine in drinking water, and steady-state exposures versus pulse exposures and versus the kinds of patterns we would see in drinking water systems.

So that SAP also gave us some initial feedback on how to take a look at the monitoring data that already existed and how do you extrapolate across time and space, in terms of interpreting potential human exposure to atrazine.

The September SAP then had a heavy focus on noncancer effects, looking at both the experimental toxicology data as well as the epidemiological data that was available for noncancer effects, and again, also looking at drinking

water issues, in terms of monitoring designs, sampling frequency.

Because, one of the challenges with this risk-assessment -- it is a challenge for this risk-assessment, but through this risk-assessment, will give us insights for the future -- is that, as we understand what is happening in terms of the toxicodynamics and toxicokinetics clearly duration of exposure, timing of exposure is critical and it is highly variable in the real world.

We have nice steady-state exposures in the laboratories, typically, but that is not how real world exposures happen. And so, what is going on inside the organism in terms of differential exposure in time, and in the duration of those exposures, is really critical interpreting whether or not there is a perturbation in a biological system, and to extent those perturbations could be significant, in terms of eliciting adverse outcomes.

What is also challenging in the world of watersheds and the world out there in terms of the variability in timing of chemicals reaching drinking water sources. So the time and space in terms of chemicals being used in the environment, run off, getting into a drinking water system and realizing that in the real world, pesticide typically are not associated with nice quasi steady-state concentrations that you have seen from the reports; atrazine used in the spring, when there is a runoff event, it is usually happening after a rainfall event, not too far after it was applied. And so, we see very spiky, typically spiky exposures of atrazine, which may be in a

drinking water system for two, three, four, five, six days maybe, from beginning to end of a "spike".

And then you may not see any atrazine for days, weeks, months, maybe not for the rest of the growing season and into the next spring, or you may seen some periodic lower level spikes.

So how do we interpret those kinds of exposures in drinking water systems, relate that to human consumption and then get back to the experimental toxicology data, which probably had a different dosing regime than what we are seeing in the real world? And so, the toxicokinetics and the linkage of this in time and space is a critical part of the risk-assessment that we are doing. And you have all been experiencing it, some of you over the last year.

And as we come to this week's Science Advisory Panel, we hope to bring together many of these different threads that have been getting woven together over the course of the last year. So getting feedback again on the non-cancer effects in terms of the role of LH surge suppression, how that fits into an adverse outcome pathway interpretation; what's a dosimetry? Both a dose and duration of exposure that is important to consider in terms of perturbations and perturbations that may be significant enough to cause adverse outcomes, which leads us to the toxicokinetics and how to be thinking about how we deal with the factor's variable exposure to interpret those effects. And then how does that link into the drinking water monitoring designs, and how do we interpret

that information so it's toxicologically relevant to the risk-assessment that we need to do.

And the final, really important, part of this peer review is taking a look at the potential cancer affects associated with atrazine. As you know, as the scheduling was playing out, we were working with our colleagues in the National Cancer Institute and the worker health study cancer, a very intensive prospective epidemiology study.

The work associated with atrazine was finished a few months ago, so we will be able to bring the results of those studies to the table and take a look at the cancer issue then with that final epidemiological study completed; along with the other epidemiological studies as well as any experimental toxicology data and get your feedback on atrazine's potential with regard to cancer. Which today, has been concluded that it is not likely to be a human carcinogen, but we want to revisit that and see if that conclusion still holds, based on the most current information.

So my apologies for running a little long, but given the year and a half we have been working on this, I felt it probably made sense to spend a little bit of time just kind of reviewing where we have been, where we are, where we are heading, both in terms of atrazine and some of the underlying methods that we are hoping to bring to bear, not only for atrazine but for other risk-assessments in the future.

So with that, I will pause and just turn it over to Jack Fowle here in a second, if it is okay, and thank all the scientists and OPP. They have been working on this as well as our colleagues in the EPA's office, research and development, and the NCI for their assistance as well, as we have been putting this information together. Let us turn back to the Chair and thank you for your indulgence.

DR. DANIEL SCHLENK: Thank you, Dr. Bradbury, for that oversight and background. It is very useful for us, I think. Our next speaker is Jack Fowle. He is the Deputy Director of the Health Effects Division from OPP. Jack?

DR. JACK FOWLE: Thank you, Dr. Schlenk, and thank you distinguished members of the Science Advisory Panel. I would like to echo Dr. Bradbury's comments in terms of thanking the panel for all your hard work. We have a few new members here today; welcome, too. And for the folks that have been here over the last year and a half, we really do appreciate all that you have done to help us focus and winnow down our efforts.

Over the past year and a half, as you have heard Steve say, we have come to the panel on several occasions; three full-blown atrazine review panels and two meetings, one to sort of set the stage and one in the context of a case study for our epidemiology framework.

Because we are health protective with the Environmental Protection Agency, our mission is to ensure that, here in the pesticides program, pesticides use according to label, they are safe for human health and the environment. We

feel it is very important to take due diligence, and in essence, look at everything. So over the past year and a half, the topics we have come to have run the gamut from neuroendocrine mode of action to immunotoxicity, epidemiology, pharmacokinetics and various approaches for how we might analyze and integrate this information into drinking water monitoring data.

This meeting, in some ways, from a public health perspective -- not ecological effects, but from a human health effect -- is in some ways sort of an epilogue. We are trying to winnow things down and we are trying to bring things to a conclusion at this particular point in time.

So, basically what we will be presenting too, is sort of how we have built on your recommendations and how we have built on the guidance from the National Academy of Sciences and their report -- in particular, a 2007 report, Toxicity Testing at 21st Century -- to focus on what we understand about the adverse outcome pathway of atrazine and which of the biological changes that we are observing are leading to an adverse outcome, potentially, and which maybe are just bumps in the road due to normal homeostatic processes, would get us back to normal function.

So based on that, we are now focusing on suppression of LH surge and potential impacts on reproduction, and that's consistent with the report from your April Science Advisory Panel. You will hear that theme being woven in throughout the presentations today.

Having said that, as Steve pointed out, that atrazine is a major chemical from the pesticides program and we do continuously monitor new data, new information that come in on our pesticide products, and we will continue to do that as we go into the future.

At this particular point in time, I would also like to note that we are not really giving a risk-assessment at this particular point in time, but sharing with you our understanding of the potential impacts of atrazine on human health up to this state of the science, as we understand at this particular point in time, and also the impacts and implications for drinking water monitoring.

We will be, as Dr. Bradbury noted, coming back to you with a review of our understanding of the potential ecological impacts of atrazine in 2012, and the actual risk-assessment of atrazine will be conducted in 2013 when we conduct our registration review of the compound at that point in time.

There have been a few changes on the staff, at least for a temporary basis. You may recall in past meetings that Dr. Anna Lowitt has been leading the effort in terms of atrazine review. She, as you know, had a child that was born April 12th. She is on maternity leave and she may be coming today and perhaps -- oh, she is here. Oh, hi, Anna. I did not even know she was here. Welcome. She has a son. We miss her very much.

But in her absence, Dr. Elizabeth Mendez has stepped up to the plate and taken the lead for the effort, and she has just done an absolutely magnificent job in terms of leading the scientific team and all the various scientific challenges down to the nitty-gritty. You may note that you got the report a few days later. It is a 673, or whatever, page report, and it far exceeded the capacities of wonderful Bill Gates and his Microsoft products.

So, Liz led the team through two days, 17 hours going line by line on the report -- quality controlling it -- to get it into shape so we could mail it to you. So she has left no stone unturned. Liz, I just cannot thank you enough for what you have done in this.

I would like to thank the team that has conducted the analysis and pulled together the material that is presented on this topic for the past several years and also today. I will not mention by name because there are roughly 17 from the pesticide programs, about 13 from ORD and roughly a handful from the National Cancer Institute; but we really appreciate their help. And I will turn it back to you, Dr. Schlenk.

DR. DANIEL SCHLENK: Thank you, Dr. Fowle. Before we move on to Dr. Mendez, I would like to introduce Jan Chambers who is one of our permanent panel members who just arrived. Jan, if you could just introduce yourself?

DR. JANICE CHAMBERS: Thank you. Let me assure you I did not oversleep. I have actually been up since 3:30 after several hours of indecision. Last evening, Delta

cancelled my flight and I spent the night in Atlanta and just got in on a flight this morning.

I am Jan Chambers with the College of Veterinary Medicine at Mississippi State University. I am a pesticide toxicologist, specializing mostly in metabolism and neurotoxicity.

DR. DANIEL SCHLENK: Thanks, Jan. So with that, we will turn the microphone over to Dr. Mendez, if you could get us going and give us some intro and some status report here. Thank you.

DR. ELIZABETH MENDEZ: Good morning. Before I start with my presentation I want to reiterate what Dr. Fowle and Dr. Bradbury said before. As a member of the team, your input over the past year and a half has been absolutely invaluable to us as we move forward, and we really do appreciate your guidance throughout this process.

So, today's talks are going to be reflecting how we have gone about incorporating some of the recommendations into our evaluation that were feasible under the current timeline. But they are also built upon the previous atrazine evaluations, going back to the 1980s.

So, let's take a little trip down memory lane. In 1988 the agency sought the panel's input on the mammary gland tumors seen in the rat. At that point, the panel noted that a hormonal influence might be an important consideration in the development of these mammary gland

tumors in the adult rats, and so they guided us to look into that aspect of the tumor genesis that we were seeing.

So we returned to the SAP in 2000 for advice on atrazine's mode of action leading to the mammary gland tumors, the reproductive and developmental effects in the rats, as well as the human relevance of these findings.

The SAP agreed with the agency's proposal for atrazine's neuroendocrine mode of action and they concluded that it was highly unlikely that the mechanism by which atrazine induces mammary tumor in the adult female Sprague-Dawley rats could be operational in humans.

Based on the 2000 SAP guidance, the EPA reconsidered its position in atrazine and reclassified it from a possible carcinogen to not likely to be carcinogenic to humans. But another thing that the SAP did tell us at that point was that it was not unreasonable to expect that atrazine might cause adverse effects on the HPG axis if it was perturbed enough and that the effects that that could lead to in development and reproduction could indeed be relevant to human.

So, although it was not relevant for the mammary tumors, it was likely that it was relevant for other adverse outcomes. And that has sort of been the impetus of our research since, at that point. We sort of narrowed it down to that.

In 2003 we came back to the SAP, and that time was for the evaluation of the prostate cancer. There were a number of

studies, particularly one in an atrazine manufacturing plant, the St. Gabriel Atrazine Manufacturing Plant, and we wanted to look at what was happening there because there appeared to be an increase in the prostate cancer cases that we were seeing.

When we came back to the SAP, the conclusions of the panel, to a certain extent, was that those increases could, in part, be explained by PSA screening being more readily available to the staff at the plant, but that it could not entirely dismiss other contributing factors to the increase.

And their feedback to us was to maintain vigilant, keep looking at the data and look for the agricultural health study that was looking at the prostate cancer, as well. And that sort of brings us where we are today and why we have come back.

The goal of this re-evaluation is to determine if the risk-assessment for atrazine should be revised. And the basis and the genesis for that, as Dr. Bradbury and Fowle mentioned is, over the past seven years, since the 2003 IRED, the atrazine researchers have been a rather prolific group and have produced over 100 articles that we wanted to take a look at and make sure that we brought the state of the science to bear on this risk-assessment.

We wanted to evaluate that experimental toxicology data, both non-cancer and cancer effects. We have evaluated epidemiology data. There have been a few dozen

epidemiology studies that have been put forth since the 2003 IRED.

And the other thing that we wanted to do, which we had not done very clearly before was try to integrate the experimental toxicology and the Epi data, and that is what the February SAP was all about, how we were going to go about doing this in a systematic way. Another thing that we wanted to do was ascertain if the critical life-stages are adequately assessed.

Now, talking about a neuroendocrine mode of action, of course, makes us think about what is happening in the neuroendocrine system across different life-stages. So, we wanted to be very cognizant of the differences in the hormonal malaria at different life-stages and how it may be impacted by the atrazine exposure, also ascertain if the durations of exposure assessed are the most appropriate.

So in the case of atrazine, it is not only the life-stage that is important, but also how long during that life-stage the exposure occurs and trying to understand if what we were doing in terms of monitoring would help us get a good grip on that information. So, that brings us to identifying methods for analyzing the uncertainty in drinking water monitoring data.

So, I mean, ideally, we want to have the ideal dataset -- sometimes we do, sometimes we do not -- and we have to then understand and get a good handle on what those uncertainties may be and how we can address them in our

efforts to continue to produce a risk-assessment that is health protective.

So, I am just going to go over these very, very quickly because these are the atrazine 2010 SAPs. Some of you have been with us throughout this entire process and I can only imagine how very glad you are to hear us say that this may be the last one for human health.

In February 2010, the draft framework was brought to you incorporating the epidemiology studies and human health incident data in risk-assessment. That was actually intended to be a framework in general, not an atrazine-specific meeting, but we had two atrazine case studies as part of that and that helped to sort of set the stage, give us some ideas on what the panel was thinking so that we could start doing that as we came back for atrazine itself.

In April 2010 we had our preliminary evaluation of in vitro and in vivo lab studies, and at that point we concentrated on the non-cancer data. As I mentioned earlier, we have well over 100 studies that have been published in 2003, so we found ourselves in a situation of trying to apportion this in manageable sizes so that we could wrap our arms around it and not overwhelm you with 600-page documents, although it did happened at the end. And we also came back and talked to you about the frequency of atrazine monitoring in drinking water sources and there was a proposal for some approaches as to how we were thinking about going about doing that.

The September SAP in 2010 was the one that based on epidemiology studies; non-cancer, and was our first attempt to really start integrating the Epi and experimental Tox data into the hazard characterization for non-cancer. Based on some of the feedback that we got from the panel in April, we also had an update and analyses of the frequency of atrazine monitoring in drinking water sources.

So, those were the things that we brought to the panel and this is some of the feedback that we got from the panel. Obviously, the reports are rather extensive and lengthy so I have just sort of captured the highlights at this point.

But in February, SAP in general, the panel concurred with the agency's proposed approach for evaluating the Epi data. They told us to consider the likely contribution of human data and the scoping and problem formulation process, consider the tox and epidemiological databases to identify uncertainties and critical data gaps and consider overall quality of the Epi data; quality of exposure assessment, sample size, statistical power, careful definition of outcomes, source bias, et cetera.

Then in April when we came with a non-cancer data, from the experimental side we looked at the cancer data that had been generated since 2003. And the panel reaffirmed the conclusions of the previous SAP regarding the classification for atrazine; it is not likely to be carcinogenic to humans.

It concurred with the agency's conclusions that atrazine-induced effects on the neuroendocrine function remain as the most sensitive vaccine to date. And an important recommendation that they made to us was they started shifting our thinking from external dosimetry to internal dosimetry.

We had some PK data that would allow us to do that, which we do not often have in a lot of our chemicals. So, in this instance, let's make use of all the information that we have to the best of our abilities. And they also noted something that I just mentioned, that the toxicological duration of concern is key to determining the sampling frequency.

So for an effect that -- maybe a critical window of exposure may be two days, you may need more frequent sampling than if the critical window of exposure is, say, 90 days or 30 days; and sort of got us thinking about how we would integrate those two aspects.

The September SAP, we brought in for the first time the mammary gland development data. There were some discrepancies between some studies and we wanted to get the panel's input on that. They agreed that, at this point in time the mammary gland development is not sufficiently robust, the dataset, for us to move in that direction. But again, we brought in our use of internal dosimetry and asked for your input on, were we on the right track; did we need to be redirected somehow? The panel concurred with us that the LH suppression is protective of the other effects and the use of Dr.

Cooper's data from 2010 to establish the point of departure.

One of the other critical things that we heard from the panel at that time was that, based on the available data that we have to date, there was no evidence of a higher sensitivity of the young relative to the adults. That is to say that when we are looking at LH suppression in the adult females, the point of departure for that is lower than any of the developmental reproductive effects that we see across different life-stages.

The non-cancer Epi findings were also brought at that time and we saw that they were helping us in certain terms of qualitatively ground-truthing our experimental findings because there was some consistency. And so, although we could not use it in terms of a quantitative assessment, we could use it as part of our weight of the evidence analyses.

And finally, there was a recommendation of a combination of statistical and modeling methods to quantify the uncertainty in exposure estimates from the monitoring data, which has been one of the things that we have been struggling mightily with over the past few years.

So what are we going to do over the next four days? We are going to review new experimental toxicology studies.

Since July of last year, which was when we closed the submissions to us for being included in the September SAP paper, up until April 29th of this year, there have been

approximately a dozen new experimental tox studies. Those studies have come in from industry from our own labs down in the park, as well as the open literature.

We are going to be looking at the species extrapolation from the rat to human and the duration of exposure and life-stage sensitivity analysis, approaches for analyzing monitoring data in the drinking water.

And one of the things that we wanted to bring to you -- and we are not going to be asking you questions or having a presentation on it -- is an update on the exposure assessment collaborative project with the AHS. It is part of your packet. We just wanted to give you an update on that but we have not, at this point in time, reached any conclusions in that project. So we felt that, given the voluminous amounts of data that we have to go through in other areas, we would just give you an update on paper.

The epidemiology studies; we are going to concentrate on cancer this time. And finally, we are going to try to integrate the weight of our evidence with Epi and experimental toxicology.

So in his opening remarks, Dr. Bradbury mentioned that we are trying to look at all of this in the context of the NRC's toxicity testing in the 21st century and how we go about this. So we have our compound; we have our metabolites. Dr. Cooper is going to be talking about the mode of action. And we are going to be assessing the biological perturbations, which is the GnRH pulsatile, pulse generator, the effected pathway which is the HPG

axis, and the dose response analyses for the perturbations of toxicity pathways.

This part is actually going to be a talk by Dr. Chester Rodriguez, so there is a little bit of an overlap there. Dr. Christensen is going to be talking about the epidemiology. The human exposure is going to be addressed by Nelson Thurman. And the human dosimetry and internal dosimetry is also going to be addressed by Dr. Chester.

Somewhere in here, although it is not explicitly here, we are going to start talking about the life-stage sensitivities, as well as the integration of the epidemiology and the toxicology data. So with that, I am just going to give you a brief run-down of the presentations. Dr. Cooper will be coming right after me talking about the adverse outcomes and mode of action.

Dr. Christensen will be reviewing the atrazine cancer epidemiology data. Then I am going to come back and talk about the integration of epidemiology and tox data into the health risk-assessment. Atrazine updates to the dose response assessment will be addressed by Dr. Rodriguez.

Mr. Nelson will be talking about the drinking water monitoring data. I will then come back, yet again, to talk about the potential sensitivity of infants and children, so that's the life-stage sensitivity. And finally, we are going to wrap up with a case study that attempts to overlay the water monitoring data and the water exposure with our dosimetry approach to try and bring it all together.

So, as you can see, we have a full agenda. The hope here is that, with this four days, we are going to wrap everything that we have gone through from February, April and September into a coherent story of what is atrazine doing. And with that, if there are any questions?

DR. DANIEL SCHLENK: Thanks, Dr. Mendez. What I would like to do is hold all our questions to perhaps after Dr. Cooper's presentation, if that is okay, just to keep this on time here. So with that, I would like to introduce Dr. Ralph Cooper who is with the National Health and Environmental Effects Research Laboratory, Office of Research and Development EPA. Dr. Cooper?

DR. RALPH COOPER: Thank you. And I would like to echo the remarks of my colleagues here at the front table about how much we appreciate the input that we have received from the panel on this arduous task of pulling all this stuff with atrazine together.

My presentation today really focuses on three things. A lot of it will reiterate what Liz just mentioned about the mode of action and why LH was selected as the key event or one of the key events in the evaluation of atrazine toxicity.

And then, there is two sort of related but separate components of the presentation where we are trying to respond to some of the earlier comments that this panel has made concerning some analysis of the mammary gland work that has been done, the development work that has

been done, and some presentation and data that was recently published by ORD, and then also talk a bit about one of the requests from the September panel about getting a better handle on exactly what happens with short-term dosing and this whole question about 1-day, 2-day, 3-day kind of thing.

So with that said, historically, as Liz Mentioned, the mode of action and hazard assessment of atrazine was focused around the fact that atrazine was found to cause premature development of mammary gland tumors in the female rat.

And to make a long story short and to avoid using a lot of slides, what was shown essentially is that these tumors develop earlier. There does not appear to be necessarily more of them later in life, but they come on earlier in life. And because of the nature of reproductive aging in the rat, the fact that when the rats go through an estro-pause or stops the normal reproductive cycle, the pattern of hormone secretion that develops in the aging female is just one of high estrogen and unopposed estrogen and prolactin secretion, and therefore, that is conducive to the growth of the tumors.

At the same time, the literature is pretty solid on the argument that one of the key moving factors in reproductive aging in the rodent is the disruption of the regulation of the gonadotropin, in particular, LH.

With advancing age, there is essentially a little bit of slippage in timing of the occurrence of the surge and the

amplitude gets progressively lower to the point where it can no longer sustain ovulation. The ovaries then develop persistent follicles, which secrete the estrogen and feedback onto the pituitary to increase prolactin secretion, as I just mentioned.

So with that background, it led to the studies where investigators looked more directly at the regulation of luteinizing hormone and what the dose response characteristics would be, and duration of exposure for the effects of atrazine on LH, and that was where they were almost at the 2000 SAP where, when that literature and that data was reviewed, they agreed that atrazine induced alterations of the secretion of LH.

Specifically, the ovulary surge of LH was the key event in the development of mammary gland tumors. It occurred earlier in the atrazine-exposed animals.

They presented essentially a toxicity pathway of sorts then. Some of it has been upheld. Some of the more molecular events have not been upheld. But essentially, it says that the brain, particularly the hypothalamus, seems to be a target site for this herbicide, and that through the effects on the brain, the regulation of the pulsatile release of gonadotropin releasing hormone out of the brain is disturbed. Therefore, the amplitude or the secretion of LH is altered.

Once that happens, you get the persistent estrous, as I said, the persistent secretion of estradiol and prolactin,

and these induce the proliferative effects on the mammary glands themselves.

Liz mentioned, and again I will reiterate -- and since I just mentioned the reproductive aging in the rat is driven by changes in the brain, or that is what is believed -- there are still some descending view points. But the primary changes take place in LH, and that is quite clear, but that is not the same thing that happens in humans.

When humans age it is the depletion of the follicles from the ovary that seems to be the driving factor. The hormonal environment present in the post-reproductive woman is quite different than that in the post-reproductive rat, and therefore, there was the basis for the conclusion that it would be highly unlikely that atrazine would have the same outcomes in humans.

At the same time, there were data available at that SAP that said, "Well, if you are going to look at LH and you are going to look at the regulation of this hormone and what the other physiological roles of this hormone plays in the male and female, what is happening there and is that relevant to the risk-assessment of atrazine?" And there were a number of studies that were done looking at the impact of changing LH secretion at different life-stages and in different sexes. What I have up there -- the first three bullets in black are outcomes that appear to be dependent upon alteration of the LH.

It is a regulation of luteinizing hormone, either during development where there were two studies from ORD -- and

they were replicated by other investigators -- that showed that this chemical will delay puberty in both the male and the female rat.

The data that I put there -- this is to add some numbers and to help get where I'm going in a slide or two -- is, the data I put there are the LOAELs for the adverse affect, that is the delays wherein the male 12 and a half milligrams per kilogram and the female 30, that the disruption of ovarian cyclicity which, again, appeared to be dependent on changes in LH, came in at about 75 milligrams per kilogram for a 21-day study.

There was a longer-term study where somewhere around 22 milligrams was the LOAEL. The lowest LOAEL up here is one that showed that the disruption of regular cycling in the female, the early reproductive senescence came in at about 3.65 milligrams per kilograms after a six-month exposure.

And then there was a study looking at comparing different strains of animals during pregnancy. Rat pregnancy has an LH-dependent phase. And in this study, the investigator dosed only during that stage of pregnancy and showed that there was a differential sensitivity cross-strains; the most sensitive strain being the Fischer 344, that when you dose with atrazine during that period it lowered the LH and you got full-litter resorption; that's what FLR stands for.

There have been two other adverse outcome types of studies that have been conducted that do not appear to be relevant to or dependent upon some change in luteinizing hormone,

per se. That was the work done by Dr. Tammy Stoker looking at the development of prostatitis after the dam was treated early in life, presumably was shown to knock down prolactin in the dam. And when you knock down prolactin in the dam, that impacts, based on the studies in the basic literature, the development of certain dopaminergic neurons in the brain which then alter, when that animal grows up, its ability to regulate prolactin and bad things happened in this case to the prostate where you saw prostate inflammation around the 120 days of age.

And there has been some similar work done following up with looking at the prostate where the animals were dosed during gestation. And then the last bullet up there, the altered memory gland development was one of the focuses of the September SAP. And mechanistically, or how that relates to LH, I am not clear if it does at all.

Let me just back up one minute. With the prostatitis, the LOAEL for that was 25 milligrams per kilograms. As I mentioned, full-litter resorptions came in at 50. And the mammary gland data, I don't have a number there. The majority of studies used 100 milligram per kilogram, but the work of Dr. Fenton, she has publications that shows that that dose could be run down considerably and you would see changes there. And again, we feel that might need to bear repeating before we can actually put something on those numbers.

What I show in this next slide though is -- and this is getting at the rationale why the agency feels that if we look at luteinizing hormone itself that we can actually --

since we are seeing changes in the physiology of animals that are dosed with atrazine at higher levels than the changes that we see in luteinizing hormone, that using this measure could be protective, or could be considered a centennial measure for the adverse outcomes.

What I have there is the top two lines show the studies looking at the pulsatile release of GnRH out of the brain using a surrogate measure that is luteinizing hormone pulses themselves in experimental models. Those have come in at 25 and 50 milligrams per kilogram. The duration of dosing there is four days.

And down, the next bigger block there, pituitary attenuation of LH surge. If you look on the left-hand side you will see the different durations and the doses that were considered LOAELs and NOAELs, and I highlighted the ones that seemed to come in at the lowest dose necessary to produce a change.

We had some one-day studies that demonstrated that you could identify a LOAEL, at least in the Long Evans animals, but it was a very high dose; 300 milligrams per kilograms. I will discuss in a moment some new data that we have taken a somewhat different approach, experimental design to look at the potential adverse effects or potential changes in LH secretion after one day of exposure; work by Jerome Goldman.

You have the data. You will see the study that he is conducting. There have been studies where the animals were exposed for three days. Prior to the SAP in 2010 was

a paper by Cooper et al. in 2007 where there was a LOAEL of 6.25 milligrams per kilogram after exposing the animals for four days.

And these are cycling animals, so they were dosed throughout one estrous cycle. That was discussed extensively in the September report or the panel meeting. And we came back upon request from OPP and finished out that dose response, and they performed a benchmark dose and found that the significant change occurred at 2.56 milligrams per kilogram per day. That is a 4-day exposure.

That is not too far different than the LOAEL that was reported or derived from the Morseth where she dosed for 28 weeks. And that study was also the one where the -- I mentioned the estrous cyclicity was effected. So in that one there was an effect that the same level as there was for LH.

And then the other ones there -- we have already mentioned the higher levels or higher concentrations that were required to perturb estrous cyclicity in the shorter durations; the one, the four and the 21-day exposures.

So again, the point being that if you want your most sensitive measure for perturbations of LH-dependent outcomes, look at LH, and look at it under the right circumstances and you will find those doses.

And again, this just going back to the rationale for that -- and I highlighted it in green -- that we see the

adverse outcomes are consistent with the LH or alter GnRH mode of action, and therefore, the proposed mode of action for development effects shares considerable overlap of the proposed mode of action for carcinogenicity.

Now that was to say that, okay, you saw your mammary glands that were dependent on LH. You see these other adverse outcomes. And that these neuroendocrin actions of atrazine are probably the dominant mechanism by which atrazine exerts its reproductive and developmental affects.

So again, if you protect for effects on the hormone itself you should be protecting for the effects of the chemical on the reproductive and development physiology.

I cannot get away without showing the slide of some LH data. These are the typical kinds of results that you see. This is actually a study from Fredis et al. where they had measured not only the parent compound, or looked at the effects of the parent compound and the dose response, but looked at also the different metabolites. And I put in there DIA as one of the ones, and we naively thought we could dose equimolar doses of atrazine and DIA based on what we applied. But the point is that, when you do that you do see dose responses that are not that different, where you see a suppression of the peak.

And again, this was coming in at about -- I think in that study our lowest dose was 6.25 or something -- but these were coming in about 12.5 for atrazine as being significant at the peak and then the equimolar dose of the

intermediate metabolite DIA came in at 10 milligrams per kilogram.

And then, to get back at the toxicity pathway and the mode of action -- but again, where we are with this, I think, after 15 years of looking at the effects of atrazine and how it influences the neuroendocrine function, it is sort of disappointing from the standpoint that, as you go across that adverse outcome pathway from where the toxicant interacts with the cells and disturbs or binds to a receptor or alters DNA or some protein and you see changes in activation of the genes or the production of different protein, we are really limited in our knowledge. There have been a number of papers, and that April SAP showed that there have been a number of papers that have been published, but there are no clear linkages and story that I think can be told.

There is somewhat of a consistency in that atrazine somehow disrupts the cyclic AMP-dependent cascade, but that's pretty diffuse effects that you can see. And to be able to link that to the particular alterations in the pulsatile release of GnRH; I think that is the key there, is that if you got back to the primary molecular or cellular change that is consistently seen in these studies by different labs, is that those GnRH neurons do not seem to be as active under atrazine as they are compared to the controls.

There is work done by Foradori where they looked at the cFOS staining and activity of those neurones, and you see it is down and it can be correlated roughly with the

decrease in pulse events as well as the decrease in LH. Where we have the stronger evidence though is highlighted in yellow. In my mind, the altered signalling is the GnRH pulses.

We have a lot of evidence that supports the altered physiology, the disruption of homeostasis tissue, changes in tissue development, as I mentioned -- not necessarily always with LH that prostatitis was that one study that was done and the other outcome.

So, still LH seems to be the key event for the majority of the adverse outcomes. It is quite strongly linked to some change in neural signaling coming out of the brain, but what is really taking place prior to that, we have limited knowledge.

Now I am going to switch a little bit and address one of the requests from the September 2010 SAP. And if I seem a little uncomfortable in this it is because this is really a little bit outside my life space mammary gland -- tumor evaluations -- but my colleague Jerome Goldman is here if questions come up for it.

There is a difference. The Fenton study shows consistently that there appears to be some type of developmental delay in the mammary glands of rats when the mother is treated gestationally, and you will look at the offspring.

In attempt to replicate the Fenton work -- that is the Rayner/Enoch papers up there -- Hovey did a rather

extensive study looking at the same strain of animals and used more objective techniques or quantitative techniques to evaluate development. He presented that data here in September and could find no difference across the different life-stages measured or doses that were used. Okay? You guys are very familiar with that.

One of the questions that came up was is it a technique issue. Is it the measurement issue as opposed to what you are seeing issue? So OPP requested that -- we were at the time running a gestational study where we were dosing dams with atrazine at different doses from 1 to 100 milligrams per kilogram, gestation day 14 to 21. And those animals -- we did it for a totally different purpose, but some of the offspring were available for looking at mammary glands and Jerome Goldman -- I want to say he agreed to, in his lab, evaluate those and try to use both the quantitative and qualitative measures that have been published.

So the animals that were available to him -- and this was an important note in even Sprague-Dawley rats -- the animals that were available to him were 45 days of age. The animals were available to him just before 45 days of age, and that happened to be the age at which the workshop on mammary gland tumors suggest it would be the best time where they were getting the most consistent data feedback when they did the round-robin evaluation of different ages.

So Jerome Goldman just said, "Yeah. I'll go ahead and look at those animals at postnatal day 45 using both the quantitative and the qualitative measures." And he worked

with different individuals to make sure that the Fenton data subjective scale that she used was the same scale that she used, with working with some of her former technical staff.

And also, with getting information on different sources on how the quantitative measures were to be made. And so he did that study and that study was published by Lori Davis. I think it jut came out about a month ago.

I just want to show the summary of the two different techniques so that you can see what is going on here, at least on postnatal day 45 in the Sprague-Dawley females. These are the quantitative evaluations. What Jerry looked at was the branching in a particular segment of the mammary gland 3 by a 3 millimeter square of the slide.

He looked at the number of terminal ends buds present in the animals and he looked at the distance from the lymph node to the most distal portion of the gland. These measures are familiar to some of you guys. I have seen them and they seem relatively straight-forward. But the important point here is he has his number of animals evaluated noted there in white in those bars -- and there does not seem to be a difference, or he could not find a difference in any of those measures across that wide range of dosing.

Now, if you notice up there, these are animals that were dosed twice a day -- but we also did animals that were dosed once a day; a higher dose hitting them up to a hundred -- and neither dataset showed a difference.

The subjective or the qualitative scale measures that were made are shown here, comparing just 100 doses in this case against the controls. And again, this is both for the one dose a day on your left and the two doses a day on your right. And again, they could not identify any difference.

And Jerry can go into the details of this study, but the way this study was run is there were three individuals who went through the scoring techniques, then they went through the same way to develop the range of scores that they could see. Once they agreed on that, they came back and blindly and independently looked at all the slides and then came back and broke the code and also looked at integrator reliability and those kinds of things, and they are all in the paper.

So, the bottom line here is that, again, this is a limited answer to your question. It is postnatal day 45. They are Sprague-Dawley rats, so these are some issues that may or may not be important. But in this kind of attempt to get at the question of whether or not there is a difference, no difference could be found with either or any of those type measures.

So to summarize, the reproductive and developmental effects in the rat, like we were looking at delayed puberty, ovarian cycling, full-litter resorption; they are all consistent with a primary mode of action of atrazine on the HPG and on LH secretion. And the alterations in serum LH provide the lowest LOAELs and NOAELs available. And these serum hormone measures serve as a sentinel for

two of the three adverse outcomes identified; that is puberty and ovarian function.

We are not a hundred percent certain about the FLR because of the limited number of doses that we chose and things, but I am pretty confident that we could say that also about the full-litter resorptions.

And although the proposed mode of action for prostatitis is quite different than the LOAELs and NOAELs for that effect, are below those that we see when we look at adverse outcomes or the actual measurements of luteinizing hormones. So, by default, the argument is, is that we are protecting against prostatitis, as well, if we use the LH measures as our point of departure.

Now, this is the final section of my presentation, and again, it is to go over some work that has been done to address one of the questions that was posed in the September SAP where the panel was struggling with the dosing durations and what is going on and how long you have to dose.

We had, if you will, limited data. At the time, four days was the shortest day that we had to complete dataset. And there was questions about what one single dose would do and what two doses or -- is do you need four doses and you were not seeing anything with one dose and those kinds of things.

So this is drawn right out of their comments. It says that it is clear that identifying that greater than one

pulse of exposure to atrazine is necessary for attenuation of the LH surge. For example, single doses of over 100 administered on the morning of proestrus did not alter the characteristics of the LH surge occurring the same day. And I will show you that dataset in a moment because it is kind of curious the way it happened, but it is true; there was no effect with single high dose in the study that we did in 2000.

Additionally, data clearly demonstrate a once daily dose for four days and beginning of the day of estrus can induce a significant inhibition of the LH surge peak, and that was the data that was scrutinized and scrutinized in September, and so, there is a big difference between no effect and four days later of LOAEL of about -- whatever that benchmark does was -- 2 point something.

In this instance, a dose response is observed. However, what is not clear -- and this is the key -- however, what is not clear is, if less than four days, but greater than one days' exposure is sufficient to alter the LH surge. Further complicating the matter, it is not clear if a four day exposure, beginning on a different day of the estrus cycle, and you start to note all these permutations could lead to differences.

So understanding the relationship between the duration of exposure in the phase of the cycle will be key in translating rodent data for humans for the risk-assessment purpose. So actually, there is a tremendous amount of work behind that paragraph, if you tried to answer all those questions in that paragraph.

And, again, we tried to address some of those issues, and in doing so, tried to incorporate some of the information that we had reviewed in the April SAP about the potential effects of atrazine on the adrenal access where we saw, and others have reported a significant increase in adrenal, progesterone and corticosterone after a single or three or four doses.

So, what we did is design a study where -- we did not do it in intact animals, again, because we had enough headaches just dealing with some of the changes in the ovarian hormone. So knowing that atrazine also increases progesterone from the adrenals, we decide what we would do is evaluate one, two and four days of exposure to atrazine in animals that were treated with estradiol or not. And the rationale behind this study is to understand the very, very basic observation about the role of estradiol and progesterone on regulation of LH.

An animals that is primed with estradiol first, you will see an LH surge three or four days later, but it is a very modest one. That is what is shown in blue in this figure here, and that is usually about six to 10 nanograms per mil at the peak. What I am showing here is a plot the way that -- you are going to see the data where you are looking at the peak on the fourth day of exposure, and the peaks are aligned based on the highest value that w see for LH. So zero is the peak and the surge curves on either side. And you can look at the area that it curve and do some other analyses with this.

If you give that animal three days of estradiol and on the fourth day you dose it with progesterone -- in this case, subcutaneously, you see it facilitates the LH surge or dramatically increases the amount of luteinizing hormone that is released.

Estradiol prepares the hypothalamic pituitary tissues. Estradiol produces progesterone receptors. Progesterone receptors are there and, boom; you get one of the only examples that I know of synergy, if you will, where you see this tremendous increase over estrogen alone if you add progesterone.

If you flip that order -- and this is where timing gets to be important -- where you put progesterone in the animal first, or concomitantly with estradiol then you are not going to have those preparatory changes take place in the hypothalamic and pituitary. And you will get nothing on the fourth day and that is what the green line is supposed to represent, where you hit P+E or progesterone followed by E.

So the hypothesis was this; that if we are going to evaluate the role of atrazine -- these one-day, two-day, three-day kinds of exposures -- we should take into account what it is doing not only to LH, per se, but also the other hormones that are involved in this. Even if we have ovariectomized animal, you had the adrenal hormones.

And our hypothesis was that atrazine should work like progesterone because one dose, we know, increases progesterone from the adrenals. And if we dosed once in

an estrogen-primed animal we should facilitate the surge; not knock it down; increase it. And if we dose consistently for three days with atrazine, and if it does induce that progesterone each day, then it should start to attenuate the surge. So that was sort of not really what you would glean from the literature, but we went back and we looked at our 2000 study -- actually OPP went back and looked at our 2000 study and said, "You didn't get any effects in one day but --"

When we did, this is actually what we had. And if you look at it -- this study was done where we dosed the animals with estradiol for the preceding three days and then gave atrazine once. And we killed the animals by decapitation. So we could not get area under the curve, we could not line up the peak heights and all that kind of thing.

And animals do not always have the same exact, precisely, the same peaks. But I was surprised, what is circled there is a tendency for there to be an increase as we increase the dose of atrazine, so that was curious. So maybe one day, if you did the study right you might see that a single dose does do something.

And this is the data that Jerry Goldman and his colleagues collected where they dosed animals with estradiol. They ovariectomized them and put the silicon estrogen-containing capsule subcutaneously for three days. On the fourth day, instead of dosing with progesterone, they dosed with atrazine at 12:00, 1:00, 1300, and low and behold, you see there was a significant increase in the

peak height and the area under the curve for LH, which tells us now that this is a single dose. We are having an effect on regulation of LH and that it is consistent with our prediction that this might have something to do with adrenal progesterone.

If you dose for two days, however -- and this is -- again, all these data are in the docket. We use zero and 100. These data were using zero and 100 milligrams per kilogram. The point is that there is no difference when you dose for two days. We saw no effect. There was a slight decrease in the area under the curve, but if you look on the right hand panel there, it is down 77 percent after 24 hours. After six hours it was up 181 percent, the amount of LH secretes.

So there is a big difference between day one and day two, but just the way we analyzed it did not really demonstrate that. And then again, if you dose for the four days, as we have in the past, you see that there is the suppression of the surge or the peak as well as a decrease in the area under the curve.

I do not know whether we are going to help answer that question about how long to dose and what the different durations would be, but you can see that there is nothing magical about that 4-day. The closer we get to understanding the basic physiology or interaction of the steroid hormones with this system, that maybe you can get at what is going on a little more clearly. So, we do have data now on one, two and four doses that there is a significant increase after one; two does of atrazine

revealed no real change in the LH from controlled animals, and four doses had the previously described decrease in LH.

Again, all changes in this case where we are substituting atrazine with changes in what might be imagined, if you will, from the changes that we see in adrenal progesterone. And it is important that we note at this point that these changes just speak to the duration of dosing. I do not think they really tell us anything or imply anything about the adverse effects of increased luteinizing hormone in these animals.

So back to our mode of action and toxicity pathway data. I think there is a wealth of data that talks about alterations and -- I think there has got to be, now, at least 20 studies looking at changes in LH after exposure to atrazine under a variety of different conditions and they all come from the ORD lab. Several different labs around the country have worked on this.

It is clear that you can see changes in reproduction and development. The linkage is back to the toxicity pathway, however, are still challenging and we do not really know what is taking place back there. But, again, the data that we have to date really, I think, supports the argument that if you use the LH measures that exist, and that they exist under, and the rationale that they are precursory changes to the changes in the physiology that using LH as the sentinel or the marker for the point of departure in the risk-assessment, it should be on safe grounds. Thank you.

DR. DANIEL SCHLENK: Thank you, Dr. Cooper. At this point, I would like to sort of group or questions or clarifications together for doctors Bradbury, Fowle, Mendez and Cooper, if you would. So anybody have any questions or clarification?

DR. DANIEL GRIFFITH: Numbers that you showed in the charts with the percentage changes, are they a factor of the smallness of the numbers or are they actually meaningful percentage changes?

DR. RALPH COOPER: Which slide?

DR. DANIEL GRIFFITH: The ones at the very end; these ones. So those percentage changes that you are showing, are they more a function of relatively small numbers or are they the meaningful percentage changes?

DR. RALPH COOPER: Well, it is just the percent. The number of animals is quite large. I think there were 10 animals in each one of those groups. So you have got a good dataset there. That percentage is just the percent -- you have the controls, and then what was the percentage of LH that was produced in the treated animals.

DR. NELSON HORSEMAN: I just have one question for clarification. The peak levels in the three LH experiments you showed us, in the controls, which are the blue bars, are a little different from experiment to experiment. Could you comment on whether those are meaningful?

DR. RALPH COOPER: One thing that is important, and we looked at this so the answer is no. But it is important. And this speaks to the way that you measure the hormone and what your control values look like. I just ran through them real quickly, but we are looking at about four and a half -- the question is whether or not our controlled animals varied in the different groups. We had the different days, okay? And they varied in this case, after four days of dosing they were approximately four and a half nanograms per mil at the peak. And then in this one they were up to six, which is -- these are typical numbers. I have seen as low as three in the published literature. They are usually less than 10. And in this case you, again, were at four and a half, so I do not put much stock in that. And as a matter of fact, if you compare those statistically, there was not a difference.

DR. NELSON HORSEMAN: These are not dose; these are control animals right?

DR. RALPH COOPER: Well they get the vehicle.

DR. NELSON HORSEMAN: The vehicle. The reason I ask is, because in the two-day exposure the treated animals are significantly lower, but they are not different from the controls in the other two experiments, so it is a question of whether --

DR. RALPH COOPER: No. The two days was not statistically significant.

DR. NELSON HORSEMAN: Oh, it is not statistically significant, it is just the difference between the 77 and 180?

DR. RALPH COOPER: Right. No, that is not significant either, neither one of those. I am sorry. The point I tried to make of that was not -- no. Two days was not different. If you did that study and killed the next day, you would say, "Oh, there is no effect at all of atrazine on the surge." What I pointed out there was -- I was struck, and it might just be my own weird way of thinking was that I was struck by a six-hour exposure having that much LH released, 181 percent of control and a peak height up to 10 -- that is pretty high for an estrogen-only treated animal -- and then dropping down within 24 hours to just the 77 percent. That is the only thing I pointed out. It's only statistically significant -- we did not do a three-day. The 4-day was down.

DR. KEVIN O'BYRNE: A very simple point of clarification. On the four days, you were giving atrazine on four days, starting from the time of the estradiol implant; is that correct?

DR. RALPH COOPER: Correct; one o'clock. It was rat time, 1:00 p.m. from the day of -- and that is what we typically did.

DR. KEVIN O'BYRNE: So on the two-day treatment, was that two days into that treatment? So is it two days relative to the peak of the surge? Okay. Thank you.

DR. RALPH COOPER: The day before, 20 whatever hours earlier.

DR. KEVIN O'BYRNE: 24 hours?

DR. RALPH COOPER: No, not 24 --

DR. KEVIN O'BYRNE: 48.

DR. RALPH COOPER: -- 28 hours, 28 or 30 or something like that.

DR. KEVIN O'BYRNE: Starting at 1300 hours.

DR. RALPH COOPER: Yeah.

DR. JAMES MCMANAMAN: Yes; just a point of clarification. There was some discussion in the September meeting about stress -- this is for the mammary gland, slide 11 -- having an effect on the outcome. And I am just wondering did you consult with Dr. Fenton's group? Because she made a big point about the way these animals are handled can affect the outcome. And since stress could affect the hypothalamus, I am just wondering is there any kind of connection and was that controlled for at all?

DR. RALPH COOPER: This study was run in four blocks, and one of the underlying reasons for running this study was to evaluate the role that gestational exposure to the dam may have on her pituitary adrenal access, and then what outcome that may have on the offspring. So in terms of stress, we were really quite fastidious, if you will, about the way we handled the animals. As a matter of fact, there was a lot of discussion about weighing them, even if we should weigh them, because we were concerned

whether or not that might compromise the potential effects that we were looking at from the gestational exposure.

So we took a lot of care in making sure that we did not have any stress, and we did not have the cannibal -- and these were Spragues. The cannibalism that was discussed there seems to be unique, I think -- this is my opinion -- to the Long-Evans rats. A lot of times the Long-Evans are just a little more fussier than the Spragues in terms of handling them during the perinatal period.

DR. DANIEL SCHLENK: All right. Any other questions on adverse outcomes or mode of action? Okay. At this point, we will go ahead and take a 15-minute break. We have lost our standard clock up here on the door here, so I am going to relegate to my watch, which I have 11 after. So let us come back at -- let's say 25 after 10. Look on your cell phones; I guess that is the best way.

DR. DANIEL SCHLENK: All right. Let's go ahead and get started, please. Our next presentation will be the review of atrazine cancer epidemiology, and it will be presented by Dr. Carol Christensen who is from HED/OPP. Dr. Christensen?

DR. CAROL CHRISTENSEN: Yes. Thank you and good morning. My name is Carol Christensen. I'm an epidemiologist with the pesticide program office. And over the next several slides I will be happy to review with you our assessment of the Atrazine Cancer Epidemiology literature.

So, as Dr. Mendez mentioned, EPA has presented its review of this database at two previous SAP panel sessions. In June of 2000, although other issues were discussed, EPA presented its assessment of epidemiological studies relating to breast cancer, ovarian cancer, prostate tumors as well as Hodgkin Lymphoma.

As other EPA presenters have mentioned, in July of 2003, the agency convened an SAP panel to discuss prostate cancer specifically, primarily studies among an occupational exposed cohort of Triazine manufacturing plant workers.

So, as a result of those external evaluations as well as other external evaluations and internal assessments, in October of 2003, EPA preliminarily concluded that the agency does not find any results among the available studies that would lead us to conclude that potential cancer risk is likely from exposure to atrazine.

However, the agency committed to revisit this issue when additional epidemiology studies became available, particularly those from the agricultural health study. As Dr. Bradbury noted earlier, within the last several months, researched with the Ag-Health Study have published an updated evaluation of the carcinogenic potential of atrazine in the human population. Those data are integrated within this presentation.

And I will just note for your information that Dr. Laura Beane-Freeman who is a co-principal investigator with that study with the National Cancer Institute, is here today

and available to address any clarifying questions you might have on the content of that research.

So, given that new information is available, the current evaluation updates in extents our assessment of the atrazine cancer epidemiology database.

So, what contrasts this review, perhaps, from previous EPA evaluations is our use of the draft framework for incorporating epidemiology into risk-assessment. As stated earlier, this framework was discussed with the SAP panel in February of last year. So using this draft framework, EPA described its methodology for identifying studies evaluating potential atrazine cancer risk in the human population. When looking at individual investigations, we evaluated the strengths and limitations, considering factors such as the consistent application of inclusion and exclusion criteria, use of valid and reliable exposure assessment methodologies, outcome ascertainment and so on, as well as an evaluation of the potential for systematic error or bias in the study. These factors are, again, reflected within our draft framework for incorporated epi into risk-assessment.

Looking across the cancer epidemiology database and moving to integrating with the experimental toxicology database, EPA utilized the framework considering the postulated mode of action, key events, as well as the, for example, observation of exposure response relations across the suite of epidemiology studies available on a particular cancer site, the strength of the measured association and the consistency of that association across the studies, in

addition to scientific judgments. So bringing together the experimental toxicology data and the observational epidemiology data, using the general principles described in this framework, today we are presenting our preliminary conclusions regarding atrazine cancer epidemiology literature.

So, EPA performed a comprehensive literature review purposely broad in nature. We search major biomedical databases, Pubmed, Web of Science, utilizing a search string developed with the aid of an EPA referenced librarian, for example. Our inclusion criteria, again, were broad. We considered studies that measured either atrazine or triazines in relation to any cancer outcome.

We did however exclude investigations for which no full text manuscript was available. Some of the initially identified investigations were exposure only. We excluded those that were editorial in nature.

So, having identified a suite of studies potentially meeting our inclusion and exclusion criteria, several EPA scientists from across the agency met to qualitatively adjudicate the relevance of those studies to the question of atrazine carcinogenicity among humans.

So, overall, we've identified 40 studies in the current epidemiology lit review. That span in publication date between the mid-1980s through the very recently published Atrazine cohort analysis by researchers with the Ag-Health Study. So these 40 studies are generally grouped in the following broad categories, several investigations of

endocrine and reproductive system. Tumors were evaluated, breast cancer, ovarian, prostate cancer and thyroid tumors.

In addition, several investigations of the potential relation between atrazine or triazines with multiple myeloma, non-Hodgkin lymphoma and other sub-types of lymphohematopoietic cancers, which is particularly relevant because it's understood that different sub-types may indeed have different etiologies.

In addition, we identified a few studies looking at other anatomical cancer sites, two studies on glioma published by researchers with NIOSH, and two on cancers in the paediatric population. And again, because research from the Ag-Health Study was deemed to be particularly important and relevant to the question of atrazine carcinogenicity in the human population, given the strength of the design and methods of that government-sponsored study, for the sake of completeness, we've delineated each of the atrazine cancer risk estimates made available over time since 2003, in either the two cohort evaluations, which we will discuss, and the sixth Nested case control evaluations on specific cancer outcomes. So these are also briefly summarized.

Regarding prostate cancer, as Dr. Mendez briefly alluded to, EPA had previously reviewed studies of an occupational cohort of Triazine manufacturing plant workers whereas there was an initial observation of an increased incidence of prostate cancer among men who were actively employed in this triazine manufacturing plant.

Additional analyses, including presentation to the SAP in July of 2003 concluded that the provision of a prostate cancer screening program, or the availability of the prostate specific antigen or PSA test among two men employed at this plant, was likely, at least, a partial explanation for this initial observation of an increased risk for prostate cancer. In fact, follow-up studies published subsequent to the last SAP review -- and that's the case control analysis within this occupational cohort in which authors were able to measure individual exposure levels -- seemed to support that conclusion when stratifying among men who received at least one PSA test over the course of their employment. No evidence of an association was observed between triazine and prostate cancer. There are other factors that support this conclusion delineated in the written material.

So moving on using data from the California State Cancer Registry, pesticide use information also reported to that state -- researchers reported evidence of a correlation between atrazine and prostate cancer among black men. This is based in an ecologic study in which atrazine exposure was measured at the group level, so it lacked individual exposure measures.

Using similar data sources, and that's the case control study among men who were part of a farm worker labor union, authors reported moderately elevated risk of prostate cancer in association with cyanazine. And I will just briefly note that this is the only study included in our review that did not actually meet our inclusion

criteria. It is the only one. We reflected it here because this study was brought to the SAP in 2003, and so its presented here for the sake of completeness.

So, within the Ag-Health Study, there are three point estimates to consider, or three evaluations, I suppose, to consider. In 2003, a preliminary case control study did not observe an association between ever use of atrazine over the lifetime in prostate cancer.

Similarly, in a preliminary, and in a very recent follow-up cohort analysis within that study, researchers did not observe evidence of an association with prostate cancer. Looking at quartiles and quintiles of atrazine exposure, the risk estimates do not significantly differ from the null, nor, obviously, was there a suggestion of a trend.

So, considering these available studies, those were the stronger design in methods, particularly the Ag-Health Study, I do not seem to suggest a positive association in those population studied. So this statement is supported by the fact that there is relatively consistent observation of essentially no association across study design and across target population, when you consider the role of detection bias in that early observation of an association. And I will note as well that both a preliminary cohort analysis published in 2004, as well as the resent follow-up study from the Ag-Health Cohort were performed with sufficient statistical power to detect a relationship with atrazine if one exists.

However, looking across this database, there are relatively few studies, 10 included, many of which the earlier ones reflect an aggregate exposure assessment methodology. The latter ones with stronger exposure assessment methods are primarily conducted among Caucasian male population. And we know that the initial correlation, noted in the ecologic study published in 1998, has not yet been replicated in other populations or in that population.

So, considering the breast cancer epidemiology database in relation to atrazine exposure, three ecologic studies provides some weak and somewhat inconsistent evidence of a possible relation, on initial study observed a significant 10 to 20 percent increased risk, however, this was not replicated in follow-up. Researchers attempted to use the same study design and the same population with very similar methods and did not repeat that initial finding. More recently, in an ecologic study in England, researchers observed potential association in only one of two of the geographic studies that's included in that study, so therefore, internally somewhat inconsistent.

And again, utilizing data reported to the California Cancer Registry, authors did not observe an association between atrazine and breast cancer among California Latinos, which is a target population. The study also utilized an ecologic-type exposure assessment method where atrazine was determined at a group level and assigned to individuals.

In a population-based case control study in the state of Wisconsin, authors measured atrazine in drinking water, actually in well water among residents in rural areas. These authors utilized a relatively robust exposure assessment method. They used an arcGIS method to interpolate atrazine exposure over time and across sampling sites. However, the range of exposure was somewhat narrow. So in this study, again, no observation evidence association was reported.

And within the Ag-Health Study, there are two investigations to consider. Among female spouses of enrolled pesticides applicators, authors did not observe evidence of a relation between atrazine and prostate cancer, and this is only using the ever/never metrics. So it has a woman ever been indirectly exposed to atrazine within this cohort.

And then, in the recent updated evaluation, authors were able to look at 36 cases of breast cancer among women who were directly exposed or who actually were licensed pesticide applicators. In this study authors, again, did you observe evidence of an association with breast cancer.

So across these data, although somewhat limit, they indicate that a strong positive association is unlikely. Consistent results; we observe consistent results across the available analytic studies which were performed in high use areas, which is the strength of the database. Each of these studies was able to measure and utilize breast cancer risk factor variables and its assessment of the association. And the range of exposure assessment

methods were significant in that, that one study utilizing the ArcGIS method for interpolation, and the measurement of both direct and indirect exposure in the Ag-Health Study is a strength of this database.

However, the limitations of exposure include the relatively narrow range reflected in the population-based study in Wisconsin in addition to the use of the ever/never metric and the ecologic exposure assessments in several of the available studies, in addition to a small number of exposed cases; so overall, strengths and limitations of this database.

Concerning ovarian cancer, two ecologic studies are not supportive of an association when observed an inverse risk; the latter observed no evidence of an association.

In a population-based study performed a couple of decades ago, authors observed three-fold elevated odds of ovarian cancer in relation to triazine exposure. This is a study previously reviewed by EPA and brought to the panel in June of 2000.

Critiques at that time included the lack of adjustment for other pesticides and the relatively small sample included in this study. Again, more recently utilizing information reported to the California State Cancer Registry and Pesticide Use Data supplemented by self-administered questionnaire, authors reported limited evidence of an association between triazine and ovarian cancer and little evidence of an association between atrazine and ovarian cancer. However, as noted, these assessments were based

upon a small number of atrazine or triazine-exposed ovarian cancer cases. Authors noted that fewer than 10 percent of that sample reported use of triazines.

And within the Ag-Health Study, again, the recent evaluation made available within the last several months observed three-fold elevated odds of ovarian cancer in relation to ever use of atrazine. So again, this is among women who are licensed pesticide applicators. However, due to the fact that there were only nine cases of ovarian cancer available for analysis, four of which were exposed to atrazine, authors were only able to evaluate the ever/never exposure metric in this study.

So, although available epidemiology database is limited, we note that positive associations were observed across these studies. However, at this time, alternative explanations may likely exist and cannot be excluded.

Strengths of this database include the observation of positive associations in high quality analytic studies, including the Ag-Health Study. A few of these investigations were hypothesis testing in nature and were conducted in a highly occupational exposed population of women. However, the small number of studies and the small number of exposed cases must be noted as a limitation.

So again, the recent Ag-Health evaluation was actually among the only point estimates that we identified in the database, looking at a potential association between atrazine and thyroid cancer. Within that study, authors observed evidence of a four-fold increased odds of thyroid

cancer in the middle and upper exposure range trend significant at the point (0.10) level. The Ag-Health Study, as I've alluded to over the course of this presentation, is a large scale prospective study with many strengths and design and methodology.

Authors, with respect to the thyroid cancer finding, note that there was only one case of thyroid cancer among women enrolled in the study, so therefore, this evaluation is among men only. Authors were able to adjust four body mass index, or BMI, which has quite recently been shown to be a risk factor for thyroid cancer. However, a limitation noted by EPA is the grouping of thyroid cancers together, and different sub-types of this tumor type may indeed have different etiologies.

So, based upon only one epidemiologic study or results in which a positive association was observed, the data are somewhat inclusive at this time and replication in other populations, perhaps with a larger number of exposed cases, is required before we can determine this causal nature of this potential association.

Strengths of the study are -- again, it is based within an epidemiology study that has relatively strong design and methods in which an exposure response was evaluated. But it is only one point estimate available at this time in which thyroid cancer sub-types were grouped together.

So there were actually a number of studies to consider regarding the potential association between atrazine or triazine and lymphohematopoietic cancers; leukemias,

lymphomas, multiple myeloma -- and they are listed briefly in the slides.

So, in the mid-1980s, the National Cancer Institute conducted a series of population-based case controlled studies in the Midwest in which they evaluated several pesticides, I think 38 specific herbicides, including atrazine, several dozen insecticides and other types of pesticides. So -- evaluated many different pesticides in association with these cancer outcomes.

So, looking across these three studies, authors did not observe an association between atrazine, specifically, in either multiple myeloma or leukemia. EPA notes that these studies are relatively strong in design; the low potential for systematic error. However, the studies were reflective of a relatively small number of exposed cases, in some instances.

In our literature search, we also identified two evaluations which are hospital-based case-controlled studies conducted in France. They are connected by the same group of researchers using similar methods, but at different points in time; so, again, looking at several different pesticides, including atrazine. So across these two studies, authors did report some evidence of non-significant elevated risk in association with triazine use. These authors evaluated triazines only.

A significant association with hairy cell leukemia -- that's the HCL acronym that I did not define for you -- were also noted by EPA. These studies, particularly the

latter study by Ochi in 2009 reflected good quality exposure assessment. I think it was self-reported exposure information administered by a trained interviewer with a small validation study included. However, the studies were, again, reflective of a few numbers of cases and did not adjust for the exposure to other pesticides over the relevant exposure period.

So, with respect to non-Hodgkin lymphoma, the same set of population-based studies conducted by the NCI in the mid-80s looked at atrazine in association with NHL. So, initially, some non-significant positive associations were reported. However, upon adjustment for the exposure to other pesticides, those associations attenuated significantly and became statistically non-significant.

In a pooled analysis using hierarchical regression techniques, so authors were able to co-adjust for exposure to the 47 other pesticides evaluated across these studies. Authors brought together the individual observations across the three case-controlled studies into kind of one big pool of the study, and again, looked at atrazine exposure, as well as exposure to other pesticides in relation to NHL and reported an odds ratio of 1.5, which was statically significant.

Within, again, the same group of studies, other investigators were able to measure the presence of the t(14:18) chromosomal translocation. This chromosomal anomaly is thought to be a risk factor for NHL in the human population.

So, among participants who were positive for the t(14:18) translocation, authors reported an elevated odds of non-Hodgkin lymphoma. However, this is the only study of the potential effect modifying role of this chromosomal translocation available.

Within the Agricultural Health Study cohort, while the initial cohort study on atrazine exposure, looking at various cancer outcomes, reported some suggestive, not significant, associations with non-Hodgkin lymphoma and multiple myeloma, this finding was not replicated in the recent follow-up study with over twice the number of non-Hodgkin Lymphoma cases.

So across NHL, multiple myeloma, leukemia and several different sub-types of lymphohematopoietic cancers for which these authors had the sufficient number of cases to evaluate -- including evaluation of an exposure-response trend -- across each of these risk estimates the odds ratios did not significantly differ from one or from the null; so a lack of evidence of an association.

So, among the available studies, those with stronger design and methods, again, do not suggest a positive association in the populations studied. The recent studies are prospective in nature. Again, the Ag-Health Study reflects a large sample with the ability to evaluate exposure response and control for the use of other pesticides.

Limitations of the database are the small number of cases for some of these sub-types reflected in a few of the

studies presented. The possible effect modifying role of the t(14:18) chromosomal translocation was observed in one, and only one, study with a relatively small number of cases and controls and has not yet been reproduced. And again, the target population across several of these studies is Caucasian male population.

So very briefly, we also identified epidemiological evaluations of other cancer sites. Research with NIOSH looked at the association between glioma, a major type of brain tumor, and pesticides including atrazine. These authors did not observe an association among either men or women.

In addition to an ecologic study on instance of cancers in the pediatric population, there is one hypothesis generating a case controlled study produced by the researchers with the Northern California Childhood Leukemia Cohort. In this analysis, authors observed four-fold elevated odds of acute lymphocytic leukemia in relation to triazine exposure.

Authors modeled pesticide exposure using maternal residence as a proxy for pesticide exposure. And we note that the elevated association was observed in the midrange group, but not in the upper ranged groups. So the data lacked an exposure-response trend -- and this is the only study available on this cancer endpoint in the pediatric population.

So, again, given the relative strengths of the design and conduct of the Ag-Health Study for the sake of

completeness, we have delineated each of the atrazine cancer-specific point estimates available across the two cohort studies, and the six Nested case-controlled studies made available since 2003. And overall, very briefly, authors did not report evidence of an association between atrazine in any of these cancer sites.

So, available preliminary studies concerning these other anatomical sites are not strongly suggestive of an association. Several of these studies were hypotheses-generating in nature among studies with relatively strong design, however they lacked a priori hypotheses.

The one observation of elevated odds of pediatric acute lymphocytic leukemia is just that, only one observation. It has not yet been replicated in this database at this time. It's inclusive as to whether or not this association is a true one.

So as we move toward integrating with the experimental toxicology database, I thought I would just take a moment to briefly summarize our view of the epidemiology datasets discussed. Concerning the lymphomas and leukemia, as well as prostate cancer, our preliminary conclusion is that the stronger studies are not suggestive of a positive association in those target populations evaluated; mainly white male pesticide applicators.

With reference to breast cancer, thyroid cancer and ovarian cancer, the available epidemiological database is limited. However, some positive associations are observed. But at this time, alternatives cannot be

excluded; namely, the relatively small sample size, a few number of exposed cases across several of these studies. With respect to the relatively heterogeneous grouping of other anatomical cancer sites, at this time these data are not strongly suggestive of a positive association.

So this slide summarizes information previously presented by other EPA presenters regarding the experimental database on the potential carcinogenic effect of atrazine. So the current cancer mode of action is mammary gland tumors in the rat due to the disruption of the hypothalamic, pituitary and gonadal axis. However, additional mechanistic data and the results of internal and external review determine that this mechanism is not operational in the human population. Therefore, the current cancer classification, as has been stated, is not likely to be a carcinogen.

EPA notes that there are no other tumors identified in the experimental toxicity data, and that the weight of the evidence does not support genotoxicity or mutagenicity of atrazine. So over all, considering these factors, the experimental evidence does not indicate a role for atrazine in the carcinogenic process in humans.

So integrating the experimental toxicology and observational epidemiology datasets, the two streams of evidence are consistent in our view, and are supportive of the conclusion of no association. There is no evidence of prostate hyperplasia or tumorigenesis in the rat although EPA acknowledges that the rodent bioassay is a poor predictor of human prostate cancer.

Across the observational studies, those available with stronger design and methods do not suggest a positive association. So, considering these streams of evidence, available data at this time do not support the association between atrazine exposure and prostate cancer. That is our preliminary conclusion upon which we are asking your feedback.

Regarding breast cancer, again, the experimental toxicology and available epi data are consistent. The mammary gland tumors are not relative to the human population, given the mode of action identified. And the available epidemiology database, however limited, is not indicative of strong positive associations. So, for these reasons, our preliminary conclusion is that available data from the toxicology and epidemiology literature do not support an association between atrazine exposure and breast cancer.

Concerning ovarian cancer, the tox and epi databases are somewhat inconsistent. This is mainly the observation of some significant risk in the human population or associations identified in studies in the human population.

Regarding experimental toxicology, there is no evidence of ovarian pathology or tumorigenesis in animal studies. EPA notes that tumors may indeed result from an altered endocrine environment however, the current mode of action is actually suggestive of a reduced risk of ovarian cancer.

And, again, the available epidemiological database is limited, but some positive associations were observed. However, at this time, given the relatively few number of studies and small number of exposed cases reflected in many of these studies, alternative explanations may exist.

So, for these unique reasons, considering the toxicology and epidemiology data, our preliminary conclusion is that available data do not support an association between atrazine exposure and ovarian cancer.

Concerning thyroid cancer, we only have that one point estimate made available through the recent updated atrazine cohort analysis within the Ag-Health Study cohort. Therefore, results are somewhat inconsistent. With respect to the toxicology database, there is no evidence of thyroid hyperplasia or tumor formation, nor is there evidence of altered thyroid hormone in rodents. We note, particularly, that the rat is sensitive to thyroid carcinogenesis, and no observation of tumor formation was identified in these studies. So again, within the observational database, based upon only one evaluation in which a positive association was observed, the data at this time are inconclusive and require replication. So given these specific reasons, our preliminary conclusions is that available data do not support an association between atrazine exposure and thyroid cancer.

Concerning the leukemias and lymphomas, these datasets are consistent. There is no evidence of lymphohematopoietic tumor formation in animal models. And in addition, there

is no evidence that disruption of the HPG axis or any other hormonal component is related to the etiology of lymphohematopoietic cancers.

And within the observational epidemiology database, those with stronger design and methods, at this time, are not suggestive of a positive association in the population studied. So for these reasons, again, our preliminary conclusion is that available data do not support an association.

Concerning the other cancer sites, again, we observed little evidence of an association in either the experimental tox or the epi database, and we find these two databases to be consistent in this way.

So as stated at the conclusion of chapter 3 of our draft issue paper, EPA believes that while epidemiology studies are weakly suggestive of an association across some of the anatomical cancer sites evaluated, considering the different lines of evidence from both experimental, toxicology and observational epidemiology studies, evidence does not strongly suggest a role for atrazine in human carcinogenesis.

So at this time, the weight of the evidence supports that atrazine is not likely to be a carcinogen in the human population. So that concludes my presentation and my review of these 40 observational studies and integration with the toxicology database and I would be happy to take any clarifying questions.

DR. ELLEN GOLD: So, I have a question about the data collection in the AHS regarding -- you know, they updated their analyses several times, and I am wondering if they did repeat administrations of a questionnaire to update the exposure information as well as the covariate information?

DR. CAROL CHRISTENSEN: Yeah. I can briefly address that, and maybe I will ask my colleague, Dr. Laura Beane-Freeman to elaborate. Yes, to my knowledge, reviewing these papers, the Ag-Health Study researchers have updated exposure. However, in the recent analysis made available earlier this year, that information was not included. Do you want to add anything to that?

DR. LAURA BEANE-FREEMAN: No. This is Laure Beane-Freeman from the National Cancer Institute. What Dr. Christensen said was correct. In the most recent analyses, they have all relied on exposure data that was collected at phase 1 or enrollment, which was collected from 1993 through 1997. However, they were asked about their lifetime use of pesticides or chemicals at that time.

The second questionnaire elicited information on their use. It took place about five years after the initial questionnaire was administered and elicited use about their use of chemicals within that intervening five years. So it would have only added an additional five years of exposure information.

Our cancer incidence data actually only goes through 2007, in that most recent publication, and so some of that exposure information, it would have only been pertaining to 1998 through about 2003.

DR. ELLEN GOLD: Covariate information?

DR. LAURA BEANE-FREEMAN: We used the same covariate information that was also included at phase 1.

DR. SUSAN AKANA: Just at point of clarification. On your weight of evidence, are there sites that includes -- I assume there is no evidence to support adrenal tumor formation in rodents?

DR. CAROL CHRISTENSON: That is correct. We did not identify any epidemiological evaluations of that cancer site; that's correct.

DR. KENNETH PORTIER: By the way, it is a very good summary. I really enjoyed reading it and it was very clear. The only thing that kind of caught my attention is the thyroid cancer study and the four-fold increase in two of the four exposure categories. Can you give me some insight as to why they choose those exposure categories? Because the ones that have very low odd ratios are the ones that have very few cases. So they have like three cases and there are 18 cases and then it is five cases and then 23 cases or something like that.

DR. CAROL CHRISTENSEN: Sure. I think I will defer to Dr. Beane-Freeman as that question relates to the conduct of the research.

DR. LAURA BEANE-FREEMAN: Sure. So, since we didn't a priori think that we did not have any evidence for thyroid cancer, we based our cut points on the distribution of all cancer cases. So what you are seeing is the reflection of a distribution of -- the cut points are based on the distribution of all cancer sites combined.

We did not change them specifically to be thyroid cancer cases. Certainly, we could have made those quartiles specific to thyroid cancer cases, but we had made the decision up front to base cut points on all cancer sites combined.

DR. FRANK BOVE: Yes. On the slides on ovarian cancer, it mentions that alternative explanations may exist. Have you thought of what those are?

DR. CAROL CHRISTENSEN: Yes. Again, there were only a few studies available to consider in our synthesis. Among those studies available, the relatively small number of exposed cases included just sample size, increasing sample size and possibly issues with method of exposure. I think, for each of the ovarian cancer studies, authors were only able to look at ever/never exposure, whether additional clarity would be provided through review of exposure response is a question.

DR. BARRY TIMMS: I have some questions about the prostate cancer study. In that study, did they --

DR. CAROL CHRISTENSEN: Are you referring to the study of Triazine manufacturing plant workers or which --

DR. BARRY TIMMS: Yes, the agricultural study on atrazine exposure to the workers. Did they show a difference in the timeframe from the initial diagnosis to clarification of prostate cancer or a change in the Gleason score in those workers that were exposed compared to the controlled group?

DR. CAROL CHRISTENSEN: I will ask one more time if you could clarify which set of studies you are referring to. Let me try to go back to that slide. The studies produced by the Agricultural Health Study or the Triazine manufacturing plant studies?

DR. BARRY TIMMS: The manufacturing plant studies.

DR. CAROL CHRISTENSEN: Oh, manufacturing plant studies. And again, your question is whether there is information regarding Gleason score?

DR. BARRY TIMMS: Gleason score and/or the timeframe from the initial diagnosis to the actual confirmation of prostate cancer through prostatectomy or biopsy.

DR. CAROL CHRISTENSEN: So, I believe across these studies, these were all PSA -- the cancers identified were indicative of PSA era prostate cancers in which they were

identified among men who were generally younger, and only among men who were currently employed in the plant during the time period of the PSA testing program.

Tumors were generally of low-grade and low-stage, although I do not recall Gleason score being reported within that study by Hessel et al. in 2004, and I do not recall information regarding the time period between employment and diagnosis.

DR. HEATHER YOUNG: Just a minor clarification on your slide 11 on the ovarian cancer. For the Italian study, I think that is actually a 90 percent confidence interval. You have that correct in your appendix B, but on the slide you have it as a 95 percent. So, if people have not read the Appendix B, it is a 90 percent confidence interval.

DR. CAROL CHRISTENSEN: Thank you very much for that clarification. I apologize for my error.

DR. TRAVIS JERDE: I have just a follow-up on Dr. Timms' question regarding the Gleason score. So, I looked around and I see about 50 men in the room. Twenty-five of us will get prostate cancer in our life, but only one or two of us will probably die from it. So, this is a disease about strength progression, what type of cancer. I see from your chart that you say relatively few studies available, and you have got database limitations. Do you know if those data are extractable from those studies that one could go and look at Gleason grade biopsy and things like that from those studies? To get that answer, would a new study have to be done?

DR. CAROL CHRISTENSEN: You are considering the totality of that database or considering the manufacturing plant workers? So with regard to Gleason score, that information, I believe -- and I will defer to Dr. Bean-Freeman -- that information is available through the Agricultural Health Study, and I believe have been used in other analysis, although not in the recent cohort evaluation. That information is collected and reported to the respected state cancer registries. Presumably, the cancers reported in the states in which the triazine manufacturing plant employees resided is also theoretically available, although I would have to look more into that question.

DR. DANIEL SCHLENK: Any other questions or clarification? Okay. Thank you, Dr. Christensen. Let me just point out, too, just for the agency; when questions come to you, can you please state your name for the record when you make the answer? I know it sounds repetitive but we need that for the auditory record, so I just want to highlight that a bit. Okay?

Our next presentation will be by Dr. Mendez again, I think two or three; got another couple to go here. And she is from HED/OPP.

DR. ELIZABETH MENDEZ: All right. As Dr. Schlenk just mentioned, this is the second of my third presentation. I will be coming in periodically to sort of into the nodes to start tying things up together as we progress during today's talks.

So, the second presentation is the integration of epidemiology and toxicity data into the health risk-assessment. And Dr. Christensen just went through the integration of these two disciplines with respect to cancer in great detail, so I am just going to go very quickly over that. And I am going to concentrate primarily on the integration of the noncancer effects and the epi data.

Now, typically, the agency's human health risk-assessment relies heavily on experimental toxicity data. But realistically, we are not trying to protect the rodent, we are trying to protect the human population. So it is important for us to -- our ultimate goal is to evaluate the potential impact of the toxicant on the human population.

And this instills an understanding of the mode of action as well as what is happening within the epidemiology data. Because that way we can start to extrapolate what we are seeing in the rodent or what we are seeing in the test species to see if that is relevant to the human and how good our model might be.

So, we start out our evaluation with a draft framework for integrating these two streams of evidence, and the concept in the draft 2010 framework are based on peer-reviewed robust principles and tools.

The Bradford Hill criteria has been used for many, many years in helping us organize data to look at data when we

have multiple lines of evidence. And to that effect we have used modified, Bradford Hill criteria to look at all of these datasets.

But in addition, it is integrating new approaches based on the recommendations from the National Academy of Sciences in their reports of 2007 and 2009; Toxicity testing in the 21st Century: Science and Decisions and Advancing Risk-assessment.

So what we are trying really to do is move beyond just looking at adverse outcomes and trying to understand the underlying biology that is leading us to these adverse outcomes.

But using these processes, it allows us the flexibility to incorporate information from different sources, and it also provides as a transparent tool for organizing, reviewing and interpreting the complex information that is also not only useful to us, but to the general public who is looking at our work so they can see how we are looking at these data.

So, in February 2010, the SAP reviewed the agency's proposed draft framework, and in general, they were in agreement with our proposal. And so, you have seen this image before when Dr. Cooper spoke about the mode of action, and Dr. Christensen just spoke about the human epidemiology data. So my job, at this point, is to try to bring those two disciplines together.

The NRC report had two very important statements for us. One is that we are trying to shift away from a focus on adverse health effects and experimental outcomes towards a deeper understanding of biologic perturbations in the key toxicity pathways.

But the other thing that the committee cautioned us is that, virtually, all environmental agents will perturb signalling pathways to some degree. And the challenge for us to determine when is that perturbation meaningful in terms of leading to a toxic effect, and when it is just -- as Dr. Fowle usually refers to it -- a blip in the road.

So, let's just go into a little bit of detail in the adverse outcomes pathway. So the organism is going along and it has its biological inputs and its got its normal biological function. And in comes and exposure, some sort of insult to the system. And there is a delivery to the target tissue and there is a perturbation.

Now, when we have a toxicity pathway, what happens is that that perturbation is of either such a magnitude or such duration that it can cause an early cellular change. The question then is, can there be adaptive responses or mechanisms that can lead us back to the normal biological function, or is the insult of such degree that it actually causes a cell injury and an adverse outcome? And our goal here is to identify the difference between this and this, and trying to make sure that what we are regulating on is letting us prevent going from this path on.

So then, to the mode of action framework, the modified Bradford Hill criteria; we have a postulated mode of action which is the neuroendocrine disruption of the HPG axis. As Dr. Cooper mentioned in his presentation, we have identified sequence of key events on the path to the health outcome.

We have a rather robust dataset with experimental support that has concordance of dose response for key events as well as temporal relationships for the key events. The data shows that all of these events are biologically plausible.

As Dr. Christensen mentioned, we have strength and consistency not only within the experimental tox data, but also when we look across the epi data. We keep our eye out for other modes of action, but we have not seen one as of yet. And we have tried to the best of our abilities to identify the uncertainties and tried to reach some preliminary conclusions based on the data that are available to us today.

But the goal here is to promote the maximal use of the relevant information that is available to us. So the organization of the draft framework for integrating epi and tox data -- we are reviewing the epidemiology studies for using pesticide risk-assessment.

And as Dr. Christensen elaborated in her presentation, we have looked at a variety of types of studies. We have looked at the scientific factors that need to be

considered in reviewing. What is the exposure? It is an ever/never? How are we doing the odds ratios?

The benefits and uses of epidemiology and risk-assessment cannot be denied. Ultimately, that is the population we are trying to protect.

The way that we have gone about this is looking at it from a proposed weight of evidence analysis. We consider the mode of action information that we have in the test species. We consider the experimental toxicity data and we consider the epidemiology data all wrapped together; so, the weight of evidence analysis; the non-caner effects. The mode of action of is neuroendocrine perturbation of the HPG axis, and we see ovarian cyclicity disruption, reproductive senescence and other reproductive effects in the animal tox dataset.

We also see low birth weight or small for gestational age, but those are not really related directly to the HPG axis perturbation, are more likely indicative of just general toxicity.

In September 2010 of SAP, the SAP concurred with the agency's conclusion that the epidemiology data is useful for hazard ID but not robust for dose-response assessment or risk characterization.

So, we do see some disruptions in the menstrual cycle in women. We do see, when it comes to menstrual cycles and estrous cyclicity, it is actually going in opposite directions in the rodent. We see an early reproductive

senescence in the epidemiology data. It appears that there may be a little bit of a delay, and either reproductive effects are generally testosterone levels in semen quality, and they are kind of tracking with the animal data.

Dr. Christensen just went through the cancer effect, so I'm just going to go through this very quickly. The mammary gland tumors in the rats are due to the HPG axis disruption; a mode of action that has been established not to be operative in humans. We do not see any other tumors identified in experimental toxicity data. And to address Dr. Akana's question earlier, we do not see any adrenal either in the rats.

And at the April 2010 SAP, the panel concluded that they felt comfortable with retaining the classification of atrazine as not likely to be carcinogenic to humans, based on the information available.

The epidemiology data, we have varying degrees of study quality and limitations that Dr. Christensen went through in detail ranging from inconsistency in findings to method of exposures assessment and the small number of exposed cases. But the weight of evidence does not support an association between atrazine exposure and cancers in general.

So when we are trying to bring the two lines of evidence together, what we see is that, for the non-cancer effects, based on the experimental toxicity data, LH suppression in the rats appears to be protective of other effects. And

we have a benchmark dose of 2.56 milligrams per kilogram per day coming from the Cooper et al. 2010 dataset. The epidemiology data is useful for hazard ID but not robust for dose-response or risk characterization. And as Dr. Christensen mentioned, in the cancer effects, the available data do not appear to support an association between atrazine exposure and cancer. And with that, I will conclude.

DR. DANIEL SCHLENK: Thanks, Dr. Mendez. Any quick questions on the integration of the Epi and tox data? Yes, Dr. O'Byrne?

DR. KEVIN O'BYRNE: I just have one point of clarification. I must have missed it in the literature. I was not aware that there was any evidence of menstrual cycle disturbance.

DR. ELIZABETH MENDEZ: That actually comes from the epidemiology data. There are one or two studies, and I will defer to Dr. Christensen for that.

DR. CAROL CHRISTENSEN: Yes, hello. In September of last year we brought forward to the panel the epidemiology data on the non-cancer health effects of atrazine and that included two evaluations, again, within the Agricultural Health Study; this time on the non-cancer side in which they looked at the relationship between several different pesticides including atrazine, and two specific outcomes: Menstrual cycle characteristics -- and I think, if I recall correctly, there were five outcomes from long

cycle, short cycle or regular cycle, that type of thing -- and then a separate evaluation on timing of menopause.

And so those were the only two evaluations that we identified looking at menstrual cycle characteristics. They were epidemiology studies in the human population and, again, among women in the Ag-Health Study who are among the more highly exposed in the population.

DR. DANIEL SCHLENK: Okay. Any other questions before we move on? Okay. Thanks. Our final presentation before lunch; no pressure, is going to be made by Dr. Chester Rodriguez who is also at HED and OPP. Thanks.

DR. CHESTER RODRIGUEZ: Thank you very much. So the title of the talk is Updates to the Dose-Response Assessment with Implications for Water Monitoring Frequency.

Just to put the presentation in context, this is what the outline is. I am going to start with a recap of the September 2010 SAP meeting. The agency proposed using internal measures of exposure based on radiolabeled atrazine studies. And then, I am going to move on to analysis of additional data based on radiolabeled atrazine studies. Then I'm going to cover some information that is available on the pharmacokinetics of atrazine in human. That is not a radiolabeled study. That is a co-study, if you will.

Based on those analyses, I am going to go over a pharmacokinetic modeling approach that the agency is proposing based on a simplified one-compartment linear

model. And I am going to go over some model evaluation exercises.

So based on that, then, I am going to walk you through a current understanding of pharmacokinetic behavior, internal dosimetry and the endpoint of concern, LH attenuation in rats.

And lastly, I am going to go over how we plan to estimate the human intake of the dose rate through an area-under-the-curve analysis of water chemographs. And the application of these methodologies will follow in a later presentation when we apply them to case studies, so stay tuned for that.

So, I am going to start with a recap then of the September 2010 SAP regarding international measures of exposure. Just as a historical context, in the previous risk-assessment to support the RED, the risk-assessments were actually based on a NOAEL and LOAEL approach. The critical study -- it was a six-month study, and the key event is actually attenuation of LH.

At the September 2010 meeting we actually introduced more sophisticated approaches for doing those response analyses, and those include benchmark of the dose modeling, as well as internal measures of exposure.

Now, for an old chemical like atrazine, there is enough information, pharmacokinetic information, to depart from the traditional external dosimetry to an internal dose

that can help refine the linkage between atrazine exposure and the endpoint of concern, LH attenuation.

This is a quote from the National Research Council regarding the use of internal does, and it says; "The does at the target site, the internal dose is the ultimate determinant of risk." So that is a good motivation of moving towards an internal dose-response analysis.

In the case of atrazine, the use of internal measure of exposure is even more relevant because atrazine as a parent chemical is short-lived in the body due primarily to being metabolized to other species, which at least some of them tend to also be active in the endpoint attenuation of LH.

At the September 2010 meeting we actually proposed to use the area under the plasma concentration-time curve as internal measure of exposure. And the rationale for that actually follows the endpoint of concern, LH attenuation which does not seem to be a single dose effect.

This is a figure from the Cooper et al. 2000 report that shows that a single does -- and it has to be as high as 300 mg per kg to what you see a decrease in LH -- whereas, a much lower does, as low as 3.12 mg per kg per day given to rats over four days, once daily, leads to a nice dose-response relationship. So based on these findings then, duration seems to be a critical parameter for the endpoint in rats, LH attenuation.

So plasma AUC as an internal dose metric does not only take into account internal dose levels, but also duration. And a simplified way of thinking about it is that it is basically the product of how much the exposure is and for how long. So the AUC that was selected, by the way, was for plasma triazines based on radiolabeled atrazine studies. And the reasons for that is as follows: There is a lack of detailed pharmacokinetic information for atrazine and its metabolites as it relates to the endpoint.

Radiolabeled atrazine studies are available in rats. The same species where the endpoint has been characterized, LH attenuation -- and another reason for selecting radiolabeled atrazine studies is that these studies, for the most part, achieve a high degree of mass balance so you know where the dose is going. Is it getting excreted; is it getting retained; et cetera?

Radiolabeled pharmacokinetic studies with atrazine have been carried now with a **14C** radiolabeled triazine ring. And it is usually labelled at the all the carbons on the ring. This ring is metabolically stable, will now be degradable by metabolism.

So the radiolabeled atrazine study that was selected was by Thede 1987, and we selected this study for two reasons, actually. We wanted to use it to estimate the plasma AUC for radiolabeled triazines and as well as to learn about the temporal relationship between atrazine exposure and LH attenuation in rats. This study was very similar to

the 4-day LH attenuation study carried by Cooper et al. in that he actually used intake young female rats.

It involved repeated once daily dosing with atrazine for at least four days, and it covered a wide range of atrazine doses from one to 100 mg per kg per day. And he also provided repeat plasma measurements which included the elimination phase.

This is what the plasma profile looks like and I just want to make two points from this slide. Number 1 is that when you start dosing with atrazine, you get accumulation of plasma triazines. So the dosing is being done once daily. And as you can see, by the fourth day of dosing you start to get a plateau or what I called pseudo steady state plasma triazine levels. And what I mean by that is that, the plasma levels stay within a specific range as long as dosing continues at the same level and frequency.

This is a hypothetical figure showing how three studies with different durations can have the same pseudo steady state plasma levels. So as you can see, we have four days, we have 14 days and then we even have six months. These studies, even though they differ drastically in duration, they will have the same pseudo steady state level, because as soon as you reach this level and you continue dosing at the same level and frequency, plasma levels will remain within that range.

Plasma triazines will decrease only when dosing actually stopped, and that is what the elimination phase is. So if the endpoint of concern is related to pseudo steady-state

plasma levels then studies of different durations should have the same LOAEL; and that is what the next slide shows.

This is the critical study from the 2003 risk-assessment. This is the new study by Cooper et al. Two studies are of different durations. You know, they were performed in different labs, but the one thing that they have in common is that the frequency in dosing that was involved were very comparable.

Now, as you can see from the LOAELs, they are very, very similar. So the bioaccumulative profile of plasma triazines then is consistent with the similar LOAELs for studies across different durations of atrazine exposures in rats. So based on these observations, then, we actually proposed a daily steady state AUC for internal dose response analysis.

Other features that were noted in the behavior of plasma triazine from the Thede 1987 study is that there is linear pharmacokinetic behavior. Now, what I mean by that is that the internal dose metric that we are proposing in a steady state AUC, it scales directly with atrazine does. Also, the elimination kinetics that is exhibited by radiolabeled plasma triazines follows linear behavior. And I would like to spend some time trying to define what that is in a conceptual way. Linear elimination kinetics, it actually means that a change in plasma concentration or, a change in time, equals -- now, there should be a minus sign here, by the way.

So the change in plasma concentration then will be directly proportional to the plasma concentration. This means that the body can adjust to eliminate more or less without changing anything. But actually, more importantly though, this is elimination rate constant, kel.

When you solve this simple differential equation, what you get is an integrated form of this expression that relates plasma concentration as a function of time. Now, as you can see from this, this is the equation of a line with slop; kel, the elimination rate constant. So the linear expression of plasma triazines will have kel as a slope.

Now, this should say fractional rate of elimination. The fractional rate of elimination of plasma triazine will proceed at a rate that is independent of plasma levels. The fraction or rate will be determined solely by the elimination rate constant, which is a constant. That translates to a plasma half-life, a constant plasma half-life that is predictive for extrapolation across dose levels.

This is pretty important because this is one of the most attractive features of having linear kinetics in that you will have a constant plasma half-life that is independent of dose levels.

So when we analyzed the elimination phase from the different groups of the Thede Study, we actually noted two things; number 1, linear behavior across different dose levels. But actually, more importantly though, the elimination rate constant was very consistent for the one,

three, seven and ten dose groups. And that elimination rate constant translates into a plasma half-life between two and three days.

So now I am going to go over the analysis of additional animal pharmacokinetic studies with radiolabeled atrazine. Two of the limitations from the Thede Study that were noted by the panel at the September meeting was that only two animals were used per dose group, and the elimination phase was estimated with only three data points from a single animal.

And so, the panel urged caution in actually analyzing this dataset. So one of the things we wanted to do right away is to try to find additional support that will support our findings about plasma clearance from the Thede Study. We were able to find two additional rats studies with radiolabeled atrazine; one by Paul et al. in 1993 and the other one by Simoneaux in 1985. These studies are not quite ideal to actually compare to the 4-day LH attenuation study.

For one thing, these two studies actually involved male rats. The 4-day study use female rats. But regardless, we wanted to analyse the plasma clearance from these two studies to see how they compare from our findings from the Thede Study.

In the first study by Paul et al., plasma measurements resulting from single oral gavage doses of 1 or 100 mg/kg, radiolabeled atrazine was given to groups of three male rats. In the Simoneaux 1985 study, plasma measurements

were taken at various days post-dosing resulting from doses of either .4 or 4 mg/kg radiolabeled atrazine given to 36 male rats, 3 per time point for seven days.

This is the analysis from the Paul et al. Study. This is what the plasma profile looks like either at 1 or 100 mg/kg radiolabeled atrazine. This is the plasma elimination analysis from these two dose levels. And as you can see, the slope of the line, which is elimination rate constant, is about .01, which is very consistent from the study from the Thede 1997 report. We did the same thing for the Simoneaux 1985 study, to see what the plasma profile looks like. When we analysis the elimination phase this is what we get.

Now, it should be noted that the plasma levels were actually reported as days post-dosing. This slope that you have on the first line is elimination rate constant in per-day units. But when you convert that to per hour, as the other studies, you again see a very similar elimination rate constant of about .01.

So, when you put all these studies together, what you have is a wide range of atrazine doses that goes from .4 all the way to 100 mg/kg per day, and they all exhibit a very similar elimination rate constant. So what we are seeing is that there is a half-life that is from two to three days that is exhibited across different studies in oral doses of radiolabeled atrazine.

So, all the studies that are discussed thus far have been in rats. There is a new study that was recently published

by Hui et al. in which they examine the pharmacokinetics or radiolabeled atrazine in non-human primates; monkeys.

Rhesus monkeys were actually dosed by oral gavage with 1, 10 or 100 milligrams of radiolabeled atrazine. Plasma was sampled for radioactivity at various time points. But the most important observations from this study is that the internal dosimetry that we are purporting to use also are scaled in the linear fashion with atrazine dose. And the elimination of plasma triazines, of radiolabeled triazines, was also linear. And these are what the pharmacokinetic parameters that were reported.

So now I am going to go over some human pharmacokinetic information that is available for atrazine. So as for most environmental chemicals, human information is very limited, like especially controlled human studies.

There is a report from 1985 by Davison where six human volunteers received a single dose of atrazine at a level of .1 mg/kg. Whole blood from a single subject was analyzed for atrazine and its chlorotriazine metabolites DEA, DIA and DACT. And urine from all subjects was analyzed for DEA, DIA and DACT. So the results from the study suggest that only DIA and DACT, two of the chlorotriazine metabolites for atrazine, were detected in whole blood. Only DEA, DIA and DACT were detected in human urine.

So it should be noted that the parent atrazine could not be detected in either blood or urine. But the most

important thing here is that the three metabolites that were detected, they all exhibited linear elimination kinetics, and I cannot emphasize that enough.

I should also note that in the study, there was a lack of mass balance. That when they monitored DEA, DIA and DACT, those three metabolites only accounted for 14.5 percent of the atrazine dose that was given.

So in summary, I can say that chlorotriazine -- whether it's atrazine DEA, DIA or DACT -- when you monitor those individually, they tend to exhibit linear elimination kinetics, which is the same when you monitor radiolabeled atrazine. So we are seeing very consistent pharmacokinetic behavior in this.

The human pharmacokinetic parameters that were reported by Davidson -- and as you can see, there is a linear elimination very constant, along with elimination of half-life. Whole blood; like I said before, atrazine was not detected, and DEA was not detected either. In urine; DEA, DIA and again, DACT were detected, and those are the half-lives that were reported. I should also say that these half-lives were obtained using a one-compartment linear model for urinary excretion. So this is a quick summary of the pharmacokinetic information that I just presented.

The use of radiolabeled plasma equivalents will include atrazine in all of its metabolites. We feel that this is a very conservative approach, given that we do not have detailed pharmacokinetic information of this species as it relates to the endpoint.

The proposal of use in the area under the curve for plasma as internal measure of exposure accounts for duration which seems to be important for the endpoint, LH attenuation.

Plasma triazines, they tend to accumulate upon repeated dosing with atrazine to reach a plateau or pseudo steady state by the fourth day of dosing. And I should also note that the endpoint in the 4-day study was also measured by 4th day or once daily dosing.

So the proposed internal dose metric then that we are moving forward with is the daily steady state plasma AUC for triazines. And I should emphasize once again that linear behavior of plasma triazines is the most consistent feature that is observed across different doses of atrazine in species, including humans.

There is additional pharmacokinetic information that is actually emerging. There is a new in vivo PK study that was carried out in rats that was recently submitted to the agency by the Registrant. A new PBPK modeling effort based partly on this new dataset that was also submitted by the Registrant in collaboration with the Hamner Institutes. I should note that the agency has not completed a thorough review and evaluation of this new PBPK modeling effort, but it is something that we plan to do in the future.

So now I am going to go over the pharmacokinetic modeling approach that the agency is proposing. Pharmacokinetic

modeling can be very useful in the case of atrazine because it is a tool that you can use to get an estimate of an internal dose metric for your endpoint of concern. It can also be used in the extrapolation of an internal dose associated with the endpoint to different species, including humans, life-stages, different exposure conditions, et cetera. And actually, from the exposure side you can use the model to relate the human ingested dose through drinking water to human plasma levels that can then be compared to a rat plasma point of departure for the endpoint LH attenuation.

So the ideal approach for doing all these fancy things is a PBPK model, okay? Given that we do not have one at this time; the agency as such is considering other options that will inform on pharmacokinetic behavior of internal dose and water monitoring.

So this is the proposed pharmacokinetic modeling approach, based on a one-compartment linear model that I should relate atrazine dose to plasma triazine levels, and is a single elimination rate constant. This model is actually based, like I said before, on linear behavior. That is a very consistent feature of plasma triazines across different studies, doses and species. This model is also based on the internal dose that scales directly with atrazine dose. And the elimination rate constant, which is a process that is linear that results in a constant plasma elimination half-life.

So this one-compartment linear model is consistent with previous efforts that have been reported in the past by

Timchalk et al., by McMullin et al. and Hui et al. Again, modeling chlorotriazine equivalents; so we are actually using a similar approach, but based on radiolabeled atrazine studies. So just a few things about the one-compartment linear model that is being proposed; the good thing about having a simplified model like this is that you only need two parameters, the volume of distribution and the elimination rate constant.

I have been talking a lot about elimination rate constant and the volume of distribution. These two parameters have to be reflective of the conditions for the endpoint which is in steady state. So we were able to estimate the two parameters, the volume of distribution of plasma triazines as steady state and the elimination rate constant, from single dose plasma data from the Paul et al. Study.

The only nice thing about having a simple one-compartment model is that you actually have an analytical expression that relate those rates to plasma levels, and this is what the expression is. So plasma levels at steady state is actually equal to the dose rate in mg per kg per day, or with the volume of distribution or the elimination rate constant in actually 24 hours.

So, one of the things that we wanted to do is to see how the model will behave in predicting pseudo steady state plasma levels from the Thede Study. As I mentioned before, this study only use two animals per dose group. These are the average values that were reported for each animal.

So we wanted to use the model to see how close we can come to the values that were measured for plasma radiolabeled equivalence. And the model does a pretty good job of actually estimating those levels. In some case, the model predicts at the halfway, actually, between the two numbers. That is all very consistent for all of those groups. So this builds confidence in using the model to inform a PK behavior of internal dosimetry.

So now I am going to talk about what our current understanding is of pharmacokinetic behavior, internal dosimetry and the endpoint of concern, LH attenuation.

So this is our understanding of internal dosimetry and LH attenuation is rats. So we know that an oral dose of atrazine is actually given to rats. It is a good assumption to say that you will get very close to 100 percent absorption, so you can assume complete absorption, so this will be your input. That absorbed dose then will get distributed in a body volume of distribution to result in plasma triazines. At this point, these plasma triazines can get eliminated with eliminate rate constant. That is your plasma clearance. So this will be your input and this will be your output.

When the input equals the output is when you have the condition of pseudo steady state, if you will. So upon repeated dosing with atrazine, you will get bioaccumulation until you get to what we call pseudo steady state, and that will result in your steady state plasma levels.

You add duration to that and you have your internal dose metric that we are proposing to relate to the endpoint of concern, LH attenuation.

So what the model is doing, pretty much, is to relate the atrazine dose to this, okay? And that is what that is. This is the expression of the one-compartment model for the adult rats. Like I said, the dose rate to the internal dose metric of concern, AUC, daily steady state AUC.

Now, so the human situation is different. It always is, right? So, in the case of animal studies, you know, they have been carried out at a constant dose of atrazine as well as a constant frequency of dosing. From here you will get then an internal dose that can be related to the endpoint of concern, LH attenuation.

The human situation is different in that the dose level, nor the frequency, is likely to be constant. But we think that this would also lead to an internal dose that can be associated with the endpoint that has been observed in rats. So this is how we are relating the two of them. So this is our current understanding of the human situation.

The intake rate is going to be determined by the water at consumption rate in the chlorotriazine levels in water. The product of these two will give us the absorbed dose just like we did with animal studies. That will get distributed in body volume of distribution to result in the plasma triazine concentration, which can get

eliminated at this point. And then, once again, you have the input and the output. When those two are equal, then you have the condition of pseudo steady state.

Upon repeated exposures you will also get bioaccumulation of plasma triazines to reach what we are calling an equivalent pseudo steady state level. Then, if you add duration to that, you will have a human-equivalent of an average daily AUC.

And the reason why I do not say pseudo steady state here is because humans are not likely to reach such conditions in the same way as rats, basically, because we have a very variable intake dose rate that is determined by the chlorotriazine in water and in the water consumption rate. But we think that we can still use the one-compartment model to relate what we think is the intake dose rate to the internal dosimetry of concern, which will be the human average daily plasma AUC.

So this will be the expression for the one-compartment model. Once again, so you have an expression that relates the dose rate to the internal dosimetry for plasma triazines that will include all the metabolites as well as atrazine. And in the two parameters that we are proposing for humans have been allometrically scaled from the rat values.

Now I am going to go over how we plan to estimate the human dose rate. Okay. The way we are proposing to do it is that we are following a recommendation directly from this panel. So we are not inventing the wheel here.

At the September 2010 meeting the panel recommended the use of an integrated or daily average internal measure along with a related drinking water monitoring approach based on the area and the concentration time curve.

So, the way we feel that we have addressed this is that the internal dose, or daily average is our plasma AUC. And the proposed approached for water exposure would involve what we are calling a water schemograph AUC, AUC for water.

Now, the way we are proposing to do this is that -- let's take a typical schemograph, a water schemograph, showing how all triazine levels vary as a function of time. But the way we are proposing to use this is that we can estimate the area of this curve for a given duration. So we can use the old-fashioned sort of rule, so you will be dividing the duration of concern into trapezoids, which will be dictated by the sampling frequency.

So the area of the trapezoid will basically be the area, at the average level, water times duration. And the AUC for water then will be the sum of the area of all the trapezoids.

And the way that area is used, is that it actually provides us to actually come up with an estimate for the average atrazine level in water. You will take the AUC for water for a given duration and you will divide it by the number of days, whether it is 4, 14 or 28, and then by 24 hours.

So this expression four here gives a time-weighted average level of atrazine in water which, along with water consumption information, can result in an estimate for the human dose rate, and this is the way it works.

So after you come up with the average value for atrazine in water, then that can be incorporated into the one-compartment linear model expression that will result in an estimate of the human equivalent average daily plasma AUC.

So this is the approach that we are proposing. And here, I am just going to give you a quick summary. So plasma AUC seems to be a reasonable internal dose metric since the duration appears to be important for the endpoint of concern in rats.

So the accumulation of plasma triazines to pseudo steady state is consistent with a temporality of LH attenuation. Linear pharmacokinetic behavior of plasma triazines is a feature that is very consistent across different doses of atrazine, studies and even species, including humans, and they all support the use of a one-compartment linear model.

The extrapolation to adult humans is actually possible with two parameters, the volume of distribution and the elimination rate constant being allometrically scaled. And the human dose rate -- we are proposing that we can estimate this through an AUC analysis of water chemograph.

And the application of these methodologies will follow in a presentation that we will do later on today. Thanks for your attention.

DR. DANIEL SCHLENK: Thank you, Dr. Rodriguez. Questions or clarification? Dr. Greenwood?

DR. RICHARD GREENWOOD: I was unable to get hold of either the Paul report or the Simoneaux report because they were internal Ciba-Geigy reports, so I have not got all of the information I need to evaluate those. Can you tell me what the limits of quantification in these studies were?

DR. CHESTER RODRIGUEZ: I do not quite recall exactly, but I believe they were in the range of .01 ppm, but I will have to double check on that.

DR. KEVIN O'BYRNE: It was quite difficult for me to follow the science behind your talk because it is just so foreign to me. But I was astonished that the plasma levels and the elimination rates of atrazine and its metabolites were sort of constant, irrespective of dose.

So, this only implies to me that there is no toxicity to the kidney, even up to 100 milligrams per kilo per day, which is astonishing. Is this hopeless?

DR. CHESTER RODIGUEZ: That is very interesting, and it tells you that the body can adjust up to a point. But if you get something like kidney toxicity, then you have other issues. But for the dose range that has been studied, you see linear kinetics all the way.

DR. JAMES MCMANAMAN: Yes. I have a question about the assumption that it is a single state elimination, because if you go to slide 22 for humans you will see that the DACTs get the rate constant for elimination of that is -- lets just look at the urine is .060 versus the DIA's .3.

So, that suggest that both are two elimination rates, one for each compound, which is still linear but that is fine, but there are still two elimination rates. So does that mean that there is a single compartment or -- you would have to postulate that the rate of elimination of one would have -- the mechanism would be different from the other then, right? So it suggests that there may be more than one compartment.

DR. CHESTER RODRIGUEZ: Yes. And that is actually one of the reasons why we are proposing to use a radiolabeled study. When you look at the radiolabeled, it does not matter what the metabolite is, just follow the radiolabeled and that is the elimination rate constant that is actually estimated here.

DR. JAMES MCMANAMAN: Well, if you do that, you would have to know what percentage of metabolite the radiolabeled represents. So lets say that the majority of your radiolabeled compound is eliminated as metabolite A, and a very small minority is eliminated as metabolite B -- so if you are just looking at radiolabeled, you are going to pick up the totality, which is going to be mainly metabolite A. Whereas, the active component may be

metabolite B and you will not be able to distinguish between those two using the radiolabeled.

DR. CHESTER RODRIGUEZ: That is correct. And we feel that this is very conservative, but in the absence of specific information about what the contribution of the metabolites are, I think this is our best option and it is conservative.

DR. DANIEL GRIFFITH: When you talk about water consumption, is it oral consumption? Is there any evidence that there is absorption through skin?

DR. CHESTER RODRIGUEZ: The information we have is that the main route of exposure is drinking water.

DR. WILLIAM HAYTON: Now, as far as scaling the rate constant to human, I just found it a little curious that, as you go from rat to monkey, the half-life actually, for the radioactivity, gets quite a bit shorter, whereas you would expect on a body weight, you know, three-quarter par scaling, expectation that it would get longer.

DR. CHESTER RODRIGUEZ: Yes. And you are correct, but you have to go by weight of evidence. You know. You have multiple radiolabeled studies that give you a very consistent kel. Also, we did a minor exercise with DACT. We took the half-life that was estimated from rats and we allometrically scaled -- so the half-life of DACT in rats is about seven to eight hours. So we allometrically scaled that to see if it could predict the measured value in humans. And I can tell you that the estimate was in

two-fold. It is in the issue paper. But that is the best we can do, I think, with the information we have, and you have to go by weight of evidence. You know. You have a single monkey study. You have multiple rat studies, so we do the best we can.

DR. JAMES MCMANAMAN: Yes. So do you know how the metabolism of atrazine differs between rats and primates? I mean, the P-450 system of the rat is pretty good. I am not sure that the same system is equivalent for the monkeys or other primates.

DR. CHESTER RODRIGUEZ: I do not think that information is known.

DR. DANIEL SCHLENK: Any other questions before we head to lunch? I did not want to shut everything off by saying that, but since it is time -- okay. Let's adjourn for lunch. And since we are a bit early, let's try to get back by 1:20, okay?

DR. DANIEL SCHLENK: Let's go ahead and get started. Welcome back to our atrazine SAP re-evaluation of the human health effects of atrazine; review of non-cancer effects, drinking water monitoring frequency and cancer epidemiology.

So, we spent the first half of the day talking about the latter. We are about to talk about some of the former now, in terms of drinking water monitoring data. And giving that presentation is going to be Nelson Thurman,

who is with the Environmental Fate and Effects Division of OPP.

NELSON THURMAN: Okay. Good afternoon. Just to try to give you a very, very brief recap; as part of the 2003 condition of re-registration for atrazine, Registrant Syngenta began taking weekly samples of both the source water and treated drinking water at a selected number of community water systems to ensure the concentrations did not exceed the toxicological level of concern for a 90-day exposure period, which is what we had at that time.

And as we started re-evaluating based on some of the additional studies that came in and started looking at potentially shorter durations of exposure for the toxin points, we started asking how well those weekly samples were characterized as shorter duration of exposure. And that led to why we are sitting here with Liz at the table in that regard.

We have focused on methods for analyzing the uncertainty and monitoring for atrazine. And, in actuality, for the drinking water they are looking at total chlorotriazine. So, it is not just atrazine that is being measured; it is the chlorodegradates as well as simazine.

What we are going to present to you is not an analysis of drinking water monitoring data itself, but we are taking the recommendations of the previous SAPs. The methods that we have been evaluating -- and we are flushing those out. So we are focusing on the methods and not the assessment itself, but the methods we had used to analyze

that data. What we have done is based on the recommendations from the SAP. We have developed some proof of concept approaches of various methods. We are not saying we are zooming in on one as ideal; we actually are looking at a handful of methods. And when it comes down to the ultimate assessment, it may depend on which duration of exposure we end up with and which method we ultimately use, but we at least want the flexibility of the tools to get there.

So, we have looked at a number of approaches and there are a lot of people who put effort into developing these approaches. I get to talk, but I want to make sure that my colleagues who have worked on these are up here and be ready to answer the questions in some of those.

Mary Frankenberry had conducted a comparative statistical analysis looking at the effect of different monitoring frequencies on estimating concentrations of different durations. Drs. Jim Hetrick and Jim Wolf evaluated some of the Geostatistical and stochastic methods that were used to conduct the time series distribution. It can be used to fill-in between sampling measurements. A lot of that is detailed in the Appendix D-2. They went into a lot more detail than we summarized in the background paper.

Dr. Stephen Wente also looked at a simple watershed balance model similar to something he worked on when he was with USGS. That is presented in Appendix G-3. He had some issues with the flow data available for doing that, and so we did not fully develop that assessment. We are

not going to be really talking about it, but if you have some questions, Dr. Wente is here to respond to those.

So the first part of this presentation is going to provide a brief summary of the monitoring issues as they have evolved as a result of the feedback we have had from the, primarily, the April and September 2010 SAPs. The bulk of the presentation is going to be looking at approaches we are proposing to characterize the uncertainties and estimates of the atrazine concentrations in water, addressing both the day-to-day or short-term variability as well as year-to-year patterns and the spatial patterns.

And so, I will begin with a recap. I think, as I pointed out earlier, some of the questions related to monitoring between the April SAP and now kind of evolved from; is the existing weekly monitoring program adequate for the shorter term duration of exposure to how do we best characterize the uncertainties in exposure estimates based on existing monitoring. So, instead of determining do you need to monitor more, it is what can we get out of the available monitoring that we have?

So we are focusing on approaches to characterize the uncertainties in a way that takes into account the short-term variability and atrazine concentrations in water that you expect to be able to address with monitoring. But we also need to take into account the year-to-year variability that we will see, as well as spatial patterns.

So the complexity of the spatial patterns and the temporal patterns in pesticide concentration are driven by a number

of factors, some we understand and can predict better than others, but there are some tools out there that help us take into account the impact of those factors on pesticide exposure.

I will say that they do make it very challenging to design a monitoring study that takes those into account, and so as a result, we are often looking at not just the monitoring data but what models or tools can we bring in to help better characterize that monitoring data so that we can use that.

As we go into this presentation, the background paper, we have broken the variability down to the components, and actually we have separated the temporal variability into your short-term day-to-day patterns as well as year-to-year patterns. But we are also mindful that the uncertainties are not necessarily additive in that there is a lot of integration that needs to go into the final result. So while I am presenting approaches separately, what we are going to look at, at the end, is making sure we do not compound uncertainties where they do not exist.

So this is going to be a recap of some of the preliminary monitoring evaluations we did at the last SAP. One of the emphasis of the SAP is that you really need datasets with more intensive sampling to evaluate the potential uncertainty that we have seen in the weekly samples.

And as we have presented in previous SAPs, we do have some data available, but they are not community water systems. We are aware that Syngenta began this year monitoring six

community water systems on a daily basis, and we look forward to being able to analyze that data as additional data to help us as we develop these and evaluate these.

But in this case, we are looking at a chemograph, which is the dark blue line which is based on daily monitoring. There is a dashed blue line there that represents your 4-day rolling average. For community water system that have weekly samples, you might have a sampling date that hits that peak.

Honestly, with the seven-day sampling intervals, you would probably have about a 1 in 7 shot of hitting that. You may not hit that peak. The problem we have is giving weekly samples we do not know, and that is part of what we are trying to take a look at.

One of the reasons we are looking at the daily monitoring is it helps us develop methods and gives us some feedback so that when we do apply these methods to the community water systems we have some confidence in terms of what they are able to provide for us.

We have built on the recommendations of those April and September SAPs. We are not really trying to open up new ground here, but to more or less tie together the sets of tools that have been discussed in those previous SAPs and try to follow up on the recommendations that the panel have made.

To evaluate the monitoring data, the April of 2010 SAP recommends that we consider, first of all, the tox

exposure duration of concern because that is going to define how important capturing the peak concentrations are; the shorter that duration, the more important those peak concentrations are in there. They also recommended using intensive monitoring that cover a representative range of sites. You want monitoring that is sampled more intensively than what you are evaluating.

And one of the comments they made is, when you are looking at monitoring, you really need to look at methods that can predict values that are greater than what was measured, because with some sampling of any duration, you cannot expect to have captured the highest concentration in every case.

As we came back and explored a number of approaches, the September SAP had recommended taking USGS's watershed regression for pesticides, combining that model -- which is regression-based model -- with a deterministic model, such as the pesticide root zone model that we use in our drinking water exposures, or SEAWAVE-Q, which is another USGS model that they have used to evaluate pesticide trends over time.

I also noted that most of these models are fairly data-intensive. And one of the comments was well, it may be easier to use WARP in combination with some statistical approach such as kriging.

So with that background, I am going to begin by talking about looking at some of the approaches we have evaluated

for characterizing the uncertainty monitoring in capturing day-to-day patterns.

As I pointed out, the SAP recommended using intensively sampled monitoring data. We are aware of, in general, a couple of monitoring datasets that monitor atrazine either daily or near daily during the periods of time when you are expected to find atrazine in the water, which roughly corresponds from April to August/September timeframe, from the time the corn is planted to well into the season when you are likely to find the high runoff periods.

One of these is from Heidelberg University, the National Center at Water Quality Research. They have collected data on a number of watersheds of various size in Ohio. We looked at the Maumee River, which is roughly a 17,000 square kilometre catchment in Ohio.

Another dataset is the monitoring Syngenta has done on atrazine for the ecological exposure monitoring. This was a separate monitoring program that focused on, primarily, headwater watersheds and looking at the impacts on aquatic plant communities.

It is a much smaller watershed, but it gives us an opportunity to look at a range of exposures. They do have monitoring that covers more than just the spreads from Ohio well into Missouri and Nebraska, so it gives us a broader geographic range.

As we have developed the approaches, we picked two particular datasets as an evaluation approach. One was

Maumee River from 1995 and the other one was Missouri-01 from 2007. Missouri-01 had a fairly high peak. Maumee River had much lower concentrations. It gives us a way of bracketing that information.

So one of the first approaches we did was -- let's take a look at how well we can characterize the uncertainty of different sampling frequencies in estimating the exposure varying durations.

Essentially, we are asking the question; how well do estimates that are based on sampling at different intervals compare to the true value, and can we develop a multiplication factor of some sort based on sampling frequency and duration of concern?

To do this, we defined a sampling window across the datasets. And the sampling window range from 4-day intervals up to 28 days. Then we used a bootstrapping simulation to select a random day within each of those sampling windows to reconstruct a monitoring dataset for those. And we did that 10,000 times for each of these four windows that we looked at, ranging from 4-day intervals to 28-day.

The community water system is represented by the 7-day intervals. Then for each of those sampling simulations, we derived the 1-day maximum, or peak, as well as a maximum for 7-, 14-, 28- and 90-day rolling average for each of those.

And then what we did is we took those 10,000 simulations for each of the sampling intervals and compared the true maximum concentrations against the 5th percentile of those estimates.

That ratio, we have described in the paper as a bias factor, which we look at as a potential multiplicative factor that could be applied to exposure estimate, depending on the sampling frequency and the duration of exposure.

I want to emphasize that this is more of a proof of a concept based on two examples. It is not an exhaustive analysis. For instance; we have only sampled one. We only looked at one year in each of these sites. We have multiple years on those.

To develop this further, we would obviously be looking across the years. We would also probably be looking at sites that represent not just flowing water -- and both of these, by the way, are flowing water bodies, but we look at reservoirs, looking at water bodies of different sizes. So this is more of an illustration purpose of how this might be applied.

The two trends I want to point out, and first off, is that the multiplicative bias factor or the uncertainty increases as your sampling interval gets wider, which means that it gives you obviously less data to work with. The other point is that, as your duration of concern expands, becomes larger, then your uncertainty factor decreases.

And I want to point out, I would caution too much against saying, "All right. This is a small watershed; this is a large watershed and the bias factors are greater for the small than the large." We are not sure if that is the case of the watershed or if it is the case of the magnitude of the concentration.

And for example, Maumee River -- we used the 1995 data for this -- in the previous SAP we did some analysis with 2008 data where the maximum concentration was around 50 parts per billion rather than 14. We would like to do a little further analysis using wider range of data to see exactly how that bias factor would play out. And that kind of leads us to the next approach we were considering.

Kind of following up on the recommendation of the SAP is that, when you are not sure what that exposure period is, you may be better off investing your resources in trying to capture the pattern of atrazine concentrations, and we started looking at ways that we might be able to do that.

The SAP had recommended combining a regression based model such as WARP with either a deterministic model or a geostatistical approach. We operated on the philosophy of lets go simpler -- the simpler, the better -- in terms of turning things around, and we started looking at WARP in combination with the geostatistical approach.

But as we started building on that, what we found is that -- we found some promising results in constructing the shape of that time series using the geostatistical

approaches and conditional simulations with some of the more intensive sampling.

And so, this may be a little bit of departure of what the SAP recommended, and that is one of the reasons why we want to bring this back to get your reaction to this, because is it a little bit -- in some cases, we think we might be able to construct fairly reliable time series just with a geostatistical analysis. In other cases, it looks like we would need to move on to add WARP in there.

So I want to present a little bit about how we went about approaching this. This is kind of the analysis strategy that we developed after working around with the data for a while. This particular figure is in -- I think it is Figure 6.1 in the background paper. It outlines the approach we are considering.

And we began by looking at the time series, the frequency of sampling. In this case, we basically looked at are there 15 or more data points in the time series, and that roughly equates to a sampling frequency of seven days or more often.

And what we found in there is that, if you have sufficient samples, we can provide reasonable time series estimates by going through a variogram analysis, doing some kriging of that, and then doing statistic simulations.

With less frequent sampling, then you need to do something to fill-in in the dataset. We presented a couple of options in the Appendix D-2. One was looking at covariate

to estimate concentration either with something like another pesticide or with flow.

We also looked at -- and what we will highlight here is using WARP to provide a percentile of the time series, and then using it in relation with flow percentiles to create the time series. So, to begin with, because we are looking at weekly samples for the community water systems, I am going to focus on the approach we did for our dataset, but would be that robust.

Now, before we apply these geostatistical methods, we needed to consider stationarity. And essentially, stationarity is the assumption that there is an equal probability of occurrence, in this case, regardless of the time.

This is a typical chemograph over a year for atrazine. If you were to apply this on an annual basis, then as stationarity, it is probably not going to hold. There are times outside the use period where you are not likely to find atrazine in the waters.

However, as we took a closer look, if you narrow that window to what we describe as the runoff period -- and that basically coincides from the time the pesticides are going to be applied to the field and after that. You can define a runoff period within the chemograph where we can at least make a contention that stationarity will hold well enough for this particular analysis. For atrazine, we can come up with a reliably predictable period, depending on the corn planting season.

Now, as we did some of the exploration with the Heidelberg data with some other pesticides, what we did find is that is probably going to be more difficult for some of the insecticides that have a less predictable or less consistent application period. And so, this may work well for atrazine or other corn herbicides, but it may not necessarily work for some of these other pesticides.

For that runoff season, what we did is we analyzed the monitoring points for a covariate structure in time. We could have used the correlogram and we chose to use a variogram, which is what you see here.

Essentially, what we did is we took the 1995 Maumee River data and simulated a 4-day sampling in that, and we also simulated a 7-, 14- and 28-day samplings, so I wanted to show you a couple of examples. A couple of terms for those who may not be familiar; the Nugget describes the amount of the variance that results from random processes that do not have a temporal correlation, and there would be such things as measurement error.

The Sill represents the variance value where the variogram levels off. And the Range is that time to where you reach the Sill. Within that Range, the monitoring values are auto-correlated. So, for Maumee River, that Range of autocorrelation is roughly 50 to 60 days.

When we pulled together this analysis we used a visual process to define the model rather than the least squares method. And because of that, as we did the kriging, we

put more emphasis on fitting the nearer points in the variogram. We also had more data pairs in the nearer points than the farther points. But that was the approach we used to fill in between the time series.

And what you see here are realizations of the atrazine time series using the variogram models to fill in the data points. The red line, which you can sort of see on these is the actual time series from the daily measurements. The black points are the realizations of the time series using the variogram models, and the bars around each of those points represent the standard deviation.

A couple of things I want to point out here is that when you look at -- this is based on 4-day sampling intervals. This is 7-day sampling intervals. When you look at these two, what you do see is that the variogram models using conditional simulations provide a reasonable characterization of the time series.

What happens when you started going to 14-day sampling intervals, your variogram structure started falling apart; looking fairly messy. Your standard deviations got a lot larger. We missed the highest sample peak.

At 28 days, we really could not construct a variogram. What you see here is for illustration purposes and that was a variogram constructed based on daily flow. And this is one reason why if we go back to that figure we use that cut-off, in terms of the amount of data, the approach we took.

Now, if we take a look at the Missouri dataset, what we did find is that the variogram models do not fit as well as they did for the Maumee River dataset. And your range is a lot shorter and it may very well reflect the spiky nature of the smaller watersheds.

But as you go from here, these are the variograms from the 4-day sampling intervals and for the 7-day sampling intervals. I want to show the realizations of the time series from those variograms.

And a couple of points; we still had a fairly good -- except for the fact that we did miss the peak -- we did do a fairly decent recreation of the time series for four and seven days, and once again, it started falling apart at the 14- and 28-day intervals.

And I do want to point out is that, the sampling -- and it was a 4- and 7-day intervals we simulated -- did miss that peak concentration. But what we wanted to see -- this provided us an opportunity to take a look, as we go through with the assessment, is how much that impact has from missing that peak concentration as it carries through in analysis.

Okay. I am going to jump back to the Maumee River. For the case study you are going to hear about later this afternoon, what we did was we generated a thousand conditional simulations of the time series -- and in this case for the Maumee River, as well as for the Missouri-01 dataset -- using the variogram model that was constructed

from the 7-day sampling intervals, which represented what we are seeing in the community water system.

The black line you see here, which you can see a lot better in the previous, is the known concentration. It is based on most intensive sampling. So this is the daily time series. This is the 4-day rolling average that is constructed from the daily time series; the 14-day and 28-day rolling averages that we constructed from the time series.

So what we did is, for each of those thousand simulations, we took at 95th and a 5th percentile, which is what the upper red line represents; the 95th percentile, those thousand simulations, and the lower red line represents the 5th percentile, those thousand simulations. And so, we did this for the daily time series and we looked at the constructive 4-, 14-, 28-day rolling averages as well.

If we take a look at the Missouri-01, as you can see, we did miss the actual maximum peak and we started looking at those simulations. In the 95th, we were still underestimating the maximum peak. But when you move to something as short as a 4-day rolling average, the 95th percentile did provide a reasonable upper bound on the estimate of that exposure.

We want to examine this in more cases to see whether it holds true or this one just happened to be a fortunate circumstance. We are also looking at, ultimately -- what we are interested in is how well the estimates that we provide here, based on monitoring, represent what we are

going to see in the internal dose study to what you will see in the case study later.

So let's go back to those 14- or 28-day averages. This is not an issue with the community water system monitoring that is going on now because they have done weekly samplings, but it may be an issue if we wanted to pull in other monitoring datasets that are likely to be sampled less frequently, or if ultimately we want to try to apply this to other pesticides. But typically, if you get sampling every two weeks or monthly, you are doing pretty good on a lot of the monitoring.

As I pointed out, we evaluated a couple approaches, and one is a covariate approach using other monitoring data or flow data. And one of the things I wanted to point out here -- because this played into how we went from a percentile time series to a time series distribution -- if you trying to do covariate analysis between flow and monitoring data over a long time period, you really do not find a correlation.

However, if you narrow that window primarily to the time whenever your pesticide is applied and shortly after, we did see some correlations between flow and monitoring data. So we took advantage of that in the approach that we did use, and we are going to present to you.

So we go back to something like the sampling every two weeks or monthly. We found that the data was too sparse to really go directly into variogram analysis, so we

needed some way of filling in that time series in between those data points.

So, what we did is we used WARP, pulling together the characteristics for the monitoring site. We used that to estimate a time series and that gave us a percentile distribution of your monitoring time series. Then we could place the existing monitoring in context with that percentile distribution.

Then we took advantage of what we knew about those percentile distributions and we ranged the flow percentiles and matched them up with the percentiles from WARP, and then we could rearrange that based on the actual time series. So we could reconstruct the time series from that percentile distribution. And once we did that, we could proceed through the variogram and kriging and stochastic simulations.

I am going to just show you an example that we presented in Appendix D-2. When we are looking at the Maumee River data, we used a 28-day sampling intervals. If you remember I pointed out, we really could not even construct a variogram with those 28-day intervals because there was too little data in between.

But once we merged that with WARP estimates -- and what we ended up doing, if you read through that appendix, we did not have all the WARP parameters we needed for the Maumee River watershed at the time. We did have a couple of the atrazine eco-monitoring sites located nearby and we use

those WARP data parameters to bracket this just as a proof of concept to see how well this might work.

I am showing you one that we did with the Ohio '03 monitoring from the WARP site. We used that and related it back to the flow to reconstruct a time series, and what you see is that we were able to construct a variogram with that merged dataset, and we went from that variogram to the conditional simulations.

We came up with the realization that we felt was not too bad of a fit for the time series. You can see there is the actual peak concentration so we were not too far off on that. We did miss a little bit there. I think we are likely to see greater uncertainty bounds around the estimates from the less robust monitoring data but it does give us an option for providing a time series estimate from that dataset.

So we do have a couple of questions that were related to that approach. What I would like to do, if we are going to address this, is to move on to year-to-year patterns, because if we are going to address the uncertainties in estimating pesticide concentrations from monitoring data, we do need to account for that year-to-year variability as well.

And once again, we are looking at this separately, but in the end we are going to have to integrate that to show that we are not compounding uncertainty that may not be compounded.

We did a quick look at the atrazine monitoring program. There are more than a hundred community water systems included in this program that have at least six years of monitoring data.

And what we did, as a quick look at those, is that we looked at the highest maximum annual detection and compared that to the lowest maximum annual detection for each of the community water systems that had at least six years of data. And what we found is that the difference between the highest measured concentration and the lowest measured concentration in a given year might be an order of magnitude or more.

The issue for atrazine comes down to how many years of monitoring are really necessary to characterize that expected range in concentrations from year to year. And alternately, can we use modeling in addition to monitoring to provide a characterization to that year-to-year variability?

We have presented a few options in the background paper. One is to take a look at monitoring in spans of longer periods of time. And a couple are looking at modeling type approaches that we might be able to tie in with the existing monitoring to provide some characterization of the range and year-to-year variability.

One is a PRZM Hybrid model. I think Syngenta has put a couple of papers in the docket related to their approach to the hybrid model. In effect, what you are going to do is you run PRZM with the watershed-specific inputs, the

rainfall data for that particular year. I think, in their analysis they were evaluating the utility of using this to fill in between monitoring datasets. What we were looking at is, this may be a way to -- by calibrating this to the concentration monitoring data for a particular year, then running that model for additional years, and we might be able to use this to characterize a range of concentrations over time.

With PRZM, we do have weather datasets that run at least 30 years of data that we could use to characterize that. That is data-intensive in that it does require pulling together a lot of site watershed-specific data to work with.

One other option is to use WARP to provide a way of characterizing the range concentrations by varying the temporal patterns. In particular, you can look at a range of your May/June precip and the atrazine use intensity over a likely range of values. And by running that, it may give a way of characterizing that range.

One thing I will point out is, one of the biggest uncertainties in input data that we have is atrazine use intensity, which is a major driver in WARP. And it is possible that the uncertainty in estimating the use intensity may override the uncertainty in all the other parameters. So that is something we are wrestling with. We laid those options out in the background paper and we have asked for the panel to provide us some recommendations and feedback on that approach.

And finally, I am going to wrap up with the spatial patterns, and you will be glad to know that we are not asking you a question on this. We are actually punting this to a future SAP that -- since I have got to be involved in another one, we might as well go with that. What we are asking is what can we say about pesticide exposures from one site? How much can we apply that to another site? Can we link the results from the monitored data sites to other sites either based on some type of statistical design or similarities in site characteristics?

Now, it is important to point out that the community water systems that are included in the atrazine monitoring program are based on compliance monitoring data. In other words, these were quarterly samples that were taken, and essentially, Syngenta did an analysis and any community water system that had annual average atrazine concentrations from the quarterly samples of 1.6 parts per billion or greater were included in the monitoring program.

As it turns out, this map -- the background you see on that map -- the dark represents a watershed vulnerability assessment we did based on WARP and WARP monitored.

The darkest blue that you see there are the watershed areas that we identified as being the most vulnerable, likely to have the highest atrazine concentrations based on WARP. The lighter blue is the next highest here. As it turns out, the community water systems that were included in the monitoring program all fell within these -

- almost all fell within the highest two vulnerability tiers based on WARP.

While this helps corroborate the vulnerability approach we used, community water systems do not represent a statistical sampling across the vulnerability tiers. And we may not necessarily be able to infer results of these to other community water systems that occur in the same vulnerable areas, not without some additional work.

Now, fortunately, we have had a separate analysis looking at vulnerable watershed properties for the eco-exposure assessment, and we have identified characteristics in those watersheds that are associated with sustained elevated atrazine concentrations.

Essentially, you are looking at watersheds that have a fairly high atrazine use intensity. They are dominated by soils that have a shallow soil layer that restricts drainage. And in fact, what you are doing is you are enhancing runoff and spreading the runoff over a larger period of time. And related to that, when you set it back on a national scale, there is a rainfall component that contributes to that.

We will be, in 2012, coming back and saying, "All right. Based on our analysis, based on the monitoring, these are what we have determined to be the watershed characteristics and threshold values in those watersheds that would drive high exposure for the eco-exposure."

It would be simple enough, looking at these community water systems, to take a look at what the characteristics are within those to determine whether we can make a similar relationship in that regard.

So that is our planned approach for the spatial component, to put those in context. It is important just to keep in mind we are confident that what we are dealing with are community water systems that are in vulnerable tiers, just making sure we can make inferences to others that may also reoccur in that tier.

So I am about to wrap up. This is a simple overview. The focus of what we are bringing on monitoring to the SAP is looking at addressing temporal variability. And we have questions for the panel regarding the methods we use to address both short-term variability -- in other words, how well can we address the variability in day-to-day variations given the sampling interval frequencies we have -- as well as to look at year-to-year variability.

So we have looked at potential of taking a multiplicative bias factor to whatever exposure estimate we have and using that as a way of characterising the variability, or alternately, generating, creating a time series analysis, time series distribution that could be used in that regard.

And honestly, we may use both. We may, in the end when it comes down to it -- depending on the duration of concern and the tox threshold concentration -- may find that one

works better than the other, but we would like to have that option to look at.

And we have also asked questions about how best to address that year-to-year variability in terms of how long do we need to keep this monitoring going? How long do we need to look at it? And with that, I am going to quit talking and open this up for questions.

DR. DANIEL SCHLENK: Thanks a lot. Dr. Coupe. Glad you could make it. Would you mind just briefly introducing yourself and where you are from and your area of expertise for the panel just so that everyone knows who you are?

DR. RICHARD COUPE: Sure. I am Richard Coupe. I am with the U.S. Geological Survey. I am working out of the Mississippi Water Science Center and spent most of the research time on the fate and transport of agricultural chemicals.

DR. DANIEL SCHLENK: Thanks a lot. Again, let me just remind speakers to please introduce yourself before speaking into the microphone so that we know who is talking. So do we have any questions about water sampling strategies? Yes, Dr. Lee?

DR. HERBERT LEE: When you say you used ordinary kriging, does that mean you estimated an unknown mean?

DR. NELSON THURMAN: I am actually going to punt this over to Jim Hetrick who did the detail kriging analysis.

DR. HERBERT LEE: So when you say you used ordinary kriging, does that mean you estimated an unknown mean level for the function?

DR. JAMES HETRICK: All I know is that we used the ordinary kriging that was in GEOEAS. All right?

DR. HERBERT LEE: Okay. So then my follow-up questions is, because normally ordinary kriging you are estimating mean. On slide 16, the Maumee river, the 28-day intervals, they do not look anything like the real data, and so I am wondering if you think that may be because it's mean reverting to the wrong level as opposed to mean reverting to zero or something like that, or if you have an alternate explanation for why that does not look anything like it?

DR. JAMES HETRICK: First off, if you look at those 28-day intervals on the -- when we went back and looked at the variograms for that particular sampling interval, we essentially had a Nugget. There was no real temporal correlation.

I was trying to squeeze something out of nothing, to be quite honest with you. So what I did is I went back and took the flow data that we actually had for that site, fit a variogram to it and then used that variogram in the stochastic conditional simulation. So that is probably one of the reasons why it does not fit, I would guess. I don't know.

DR. NELSON THURMAN: And this is one reason why we knew needed to do something else for the 14- and 28-day intervals.

DR. JAMES HETRICK: Actually, Nelson, can you put up the Missouri site, the conditional simulations? Actually, that is a representation, a 28-day is essentially a Nugget for that 28-day, and that is what we would have probably seen in that 28-day for the Maumee River if we had put a Nugget model simply in the stochastic simulation.

DR. KENNETH PORTIER: On that same graph, how does the program handle potential negative values? I mean, are you just truncating at zero; are you censoring at zero?

DR. JAMES HETRICK: Yes. You can truncate at zero. It does truncate at zero. And in addition to that, which is kind of interesting -- I do not know that it would be interesting to get SAP's input on -- you can also extrapolate to a maximum value and it can be higher than the value that is in your dataset.

So one idea, at least in my mind, was to take the bias factors that we have for a certain sampling interval and we could multiply that out from the peak and be able to put in a maximum value to make sure that we are not underestimating.

DR. RICHARD COUPE: I am sorry if you have already discussed this. Let me know and we could talk about it later; but explain to me how the bias works, how you plan on applying it.

DR. NELSON THURMAN: What we were looking at is exploring whether we could take a bias factor and -- let's say a 28-day tox window of exposure window. And so, we come up with -- based on weekly sampling, we estimate here is your maximum 28-day rolling average concentration. The idea would be we'd take that bias factor where you'd go to the table and look up where you have 28-day duration window and your 7-day sampling intervals and multiply that exposure by that factor.

Now, I do want to point out that if we took that approach, it would be difficult to do what we are doing in a case study, which is folding that time series into the case study to get an internal dose. So that is one of the reasons why we were looking at that as one approach, but also looking at it in terms of creating that time series; filling in between.

DR. KENNETH PORTIER: If you look at the 4-day interval and you mentioned that you use common kriging, and I was sitting there thinking, you know, this is really just time series modeling, right? In time series modeling; you have a mean pattern and you have variability. It is structured variability in this case. You are putting on a variogram on autocorrelation function. You are saying that the residuals -- so in my thinking on common kriging, that mean is usually just a mean. It is not a function of time. It is just a constant. Am I correct?

I was thinking in what they call generalized kriging, you are actually smoothing, so your mean is a function of time. You are pulling a little bit of the pattern out and

then you are looking at residual variability. And if you were doing generalized kriging, I would think that's what you would get because you're really following pretty much the general pattern in your simulations. They are all kind of following the pattern over time with some noise. So your simulations create a bunch of noisy patterns that are about that general pattern. So maybe I am kind of confused between common and generalized kriging.

DR. JAMES HETRICK: Well, let me go back and tell you how we did the analysis and then I may straighten -- it may either cloud up the issue or may clear up the issue; I don't know. But the bottom line is, what we did is essentially -- the data that we used to build the variogram off of is the same data that we used in the conditional simulation without any type of in-filling. So the missing data points there are strictly estimated using a stochastic approach, okay? We did not use the best linear unbiased estimate for estimating those missing values.

DR. DANIEL GRIFFITH: When you did the variogram analysis, you did not use the log transformation? You did it on the original?

DR. JAMES HETRICK: No. I did a log transformation in the variogram analysis.

DR. DANIEL GRIFFITH: Okay. So the back transformations would not have negative values; is that right?

DR. JAMES HETRICK: That is probably true; yes. By the way, the conditional simulation was done using a z-score approach, so those were normalized through that program.

DR. KENNETH PORTIER: I want to remind Nelson that we talked at lunch, on the next slide -- I think it is the next slide -- where we talked about the five percent and 95 percentile, and I was going to ask you to kind of remind us because it is not clear in the write-up exactly what the five percent and 95th percentiles really mean.

DR. NELSON THURMAN: Okay. What we did is, we did those conditional simulations, a thousand simulations. And essentially what we did is we went across each day of the simulation to come up with the 95th and 5th percentile. So, in effect -- we were discussing -- you are going to end up with a wider range because you are looking at that percentile for each day.

DR. KENNETH PORTIER: So they did a thousand simulations, then you take one day and you look across a thousand simulated values for that one day and you get the five percentile and the 95th percentile. And what that tells me is that it is 95th percentile simulated curve there is not any one of the patterns that you simulated; it is really the worst or the worst, right? And the 5th percentile is kind of the fifth best of the best.

DR. HERBERT LEE: So, I want to clarify now because I am a little confused. When you did the conditional simulations, what scale did you do them on; the log scale or the original scale, the z-scores?

DR. JAMES HETRICK: We used the z-score for the -- that's in the 6M.

DR. DANIEL SCHLENK: Any other questions? Clarifications? Okay. Let's move on to our next presentation. Dr. Mendez, your third of three there.

DR. ELIZABETH MENDEZ: Good afternoon. Yes, three of three. Now I can sit back and enjoy the discussions later on. So throughout the day, you have heard a lot of discussion about mode of action, about epidemiology, about pharmacokinetics, water monitoring now.

In addition to all of those things that we typically have to evaluate for all chemicals, for pesticides, we have a unique situation that we have a statute that speaks to us about the sensitivity of infants and children. So in this presentation, what I am going to do is to describe the state of the science with respect to the potential for pre- and/or post-natal toxicity and the completeness of data with respect to toxicity in infants and children.

So as I just said, we have a statute that governs pesticide regulation, and perhaps we would start with that so that we are all on the same page.

The Federal Food, Drug and Cosmetic Act, as amended by the Food Quality Protection Act in 1996, requires the agency to give special attention to the potential risk to infants and children. Specifically, FQPA instructs EPA in making its "reasonable certainty of no harm" finding that in "the

case of threshold effects, an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of data with respect to exposure and toxicity to infants and children."

Section 408(b)(2)(C) further states that "the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children."

This additional margin of safety is referred to as the "FQPA Safety Factor." So in essence, all these words, what they mean is, when we start looking at a pesticide, we start out with a 10x FQPA factor that is automatically on, and then we have to, based on the data that we have in front of us, we may reduce it; we may change it but it has to be, from the get-go, we start with a 10x.

Now, this talk about both exposure and toxicity, but as Nelson just finished with his presentation, we are still dealing with the exposure uncertainty so I am not going to really address that in this talk. I am going to concentrate on hazard and toxicity. The hazard considerations; the important issue is do we have available data to assess critical life-stages? And if you remember from my talk earlier this morning, I said that when we have a neuroendocrine mode of action like the one we have for atrazine, this becomes a particular critical issue because the hormonal environment changes

significantly between life-stages, so that is something that we really have to be very cognizant of as we move through our process.

We have to consider all the relevant information, mode of action obviously, the animal database of toxicity studies, the dose response relationships, the human relevance of the animals findings and, last but not least, epidemiology findings.

So back in 2003 when we came to the SAP, in 2000 actually, the neuroendocrine mode of action was determined to be relevant for reproductive and development effects, even though it is not for the mammary gland tumor development. And during the epidemiology findings that were discussed in the September SAP meeting, what we heard from the panel and what the agency concluded was that there is qualitative information on human relevance of the animal findings, but that they were not sufficiently robust to establish a causal associations.

So, we were seeing things that were somewhat similar in epidemiology data to what we were seeing in the toxicity data. So we felt that we could still believe our premise that these findings were relevant for human risk-assessment.

So let's talk about what we do have in terms of experimental toxicity studies. We have core guideline toxicity studies. These are a developmental toxicity studies in two species; rat and rabbit. And for those who don't know how our guideline studies are done, those

studies usually start with exposure during the implementation time, usually around gestation day 6 through gestation day 21 when the animals are sacrificed and their fetus' are examined.

And we have a multi-generation reproduction study. That study actually -- exposure starts 10 weeks prior to mating of the animals. It continues throughout mating, gestation and lactation, and so the pups are exposed in utero and also through lactational exposure postnatally; and that is what we typically get from most pesticides.

But in addition to that, in the case of atrazine, we have a rather robust dataset of specific and special studies that concentrate on specific life-stages, mainly gestation, perinatal, peripubertal and reproductive age.

And when we last visited the SAP before this re-evaluation, one of the things that we heard from the panel was we need a little bit more information on the peripubertal period.

Now, now we have all this data, and thus far, none of the available studies have provided us an endpoint lower or more sensitive than the studies on LH attention in the adult female rat. So I am just going to go quickly through this.

Now, we have a lot more studies than these, but I have just selected the ones that are the most sensitive within the datasets. And as you can see, we have for gestational exposure, the lowest NOAEL that we have is 10, with a

LOAEL of 50, and that is delayed preputial separation after exposure from gestation day 14 through parturition. And from Fraites et al., data that was in Davis et al., data that came in around April of this year, the NOAEL is 20 and the LOAEL is 100.

And we have the Fraites et al. is looking at males. The Davis et al. is looking at females and their siblings. The endpoints are decreased pup weight and pup viability and delay in VO, vaginal open in the Davis et al. dataset; and the exposures were from gestation day 14 through 21.

Perinatal exposure we have data from Stoker et al. in 1999, and this is postnatal day 1 through 4 and postnatal day 6 through 9. And the lowest NOAEL in there is at 12.5 and a LOAEL of 25 and the effect is prostatitis.

Peripubertal exposure, which was the period that initially we needed more information on, we got more information and then some. But you can see that the lowest that we have is a delay in preputial separation with a NOAEL of 6.15 and a LOAEL of 12.5.

Now what I want to remind you is that, when we do a benchmark dose analysis for the LH attention after four days of exposure with Cooper et al. data, the BMDL is 2.56.

In addition to these studies, we recently received the completed multiple life-stage study from Syngenta. This is an exposure encompassing prenatal, postnatal,

peripubertal and adult-life-stages. It evaluated female offspring only.

The doses were 0, 6.5, 25 and 50, and no effects on LH were seen at doses of 50 milligrams per kilogram or less, and that is kind of inconsistent with a preponderance of the peer review literature where we are seeing things starting to happen with LH around 6-ish or even lower, depending on the duration, more like 3.

To be honest, we have looked at that data and we can't quite figure out why that inconsistency is happening. It appears to be a well conducted study over all, so we are still puzzled by that.

What they do see is a slight delay in vaginal opening or delay of 1.4 to 2.3 days at 50 milligrams per kilograms per day. And what is interesting about that is that this delay is seeing if the exposure started in utero, but not if it started postnatally. So let me just kind of quickly flash up the study design so that you can see how that goes.

Cohort 1 in Subset A; you have exposure from conception gestation day 0 through lactation day 0 of the dams, then from postnatal day 21 through the time of blood collection and necropsy. So for B you have exposure again from the day of conception through postnatal day 133, C from conception to postnatal day 133 and then you have a recovery period, and then for Cohort 2; D, E and F, exposure starts on a post-weaning on postnatal day 21, and you have a similar paradigm.

What is interesting here is that some of these exposure paradigms are similar to others that have been seen in the literature where we do see the LH attenuation.

So the FQPA safety analysis, the summary; what we have is the data available spanning life-stages from conception to adulthood. There does not appear to be any evidence of a unique quantitative susceptibility in the developing organism. The reproductive and development effects that we are seeing are consistent with the perturbations of the HPG axis and the decreases in LH that we are proposing to use as a sentinel key event in atrazine's neuroendocrine mode of action that leads to these adverse outcomes in the rat.

The effects that we see, none of the doses are lower than those eliciting the LH surge attenuation in the adult female rats with BMDL of 2.56; and with that, just a quick overview.

I did not go into a great deal of detail about the experimental data because we discussed that quite a bit during the September SAP meeting, so I just wanted to give a general broad overview. With that, I will stop.

DR. DANIEL SCHLENK: Thank you, Dr. Mendez. Any questions on safety factor evaluations? Wow.

DR. ROBERT GILLIOM: So what was the conclusion about whether the safety factor was appropriate or not?

DR. ELIZABETH MENDEZ: Well, at this point in time, we have not finished our evaluation and exposure, and the FQPA safety factor looks at both things. But from a hazard standpoint, it seems that we don't have any sensitivity in the young.

DR. TRAVIS JERDE: So you repeated again the results of the Stoker paper from '99, but last year in December the EPA group had put out a group, lead author was Stanko. Have you seen that? Because it looked like the atrazine metabolites were effective in inducing prostatitis semen as low .87. Are you familiar with that work?

DR. ELIZABETH MENDEZ: Yes. We are familiar with that. When you discussed that paper back in September, the agency had some concerns regarding the conduct of this study and how reliable it could be for selection of a point of departure. And during the September SAP meeting the panel agreed that there were some significant issues with the paper at that point.

DR. TRAVIS JERDE: Okay. And yet they published it and it came out in December. Okay.

DR. DANIEL SCHLENK: Okay. Are there any other questions for Dr. Mendez? Okay. Due to the massive consumption of caffeinated beverages, I would suggest that we take a break right now. I have got 2:32 according to my watch, so let's try to get back at maybe 2:45. Okay? We will re-adjourn at 2:45.

DR. DANIEL SCHLENK: Let's go in with our last presentation by the agency. This will be made by Dr. Rodriguez who has not elevated his self to Dr. Mendez's status in terms of three, but is doing number two -- still up there though -- and he is going to be talking about some case studies to sort of put some of these applications in better light there. Okay? Dr. Rodriguez?

DR. CHESTER RODRIGUEZ: Thanks very much. I just wanted to mention that this is going to be a tac-team effort. Nelson Thurman is going to be speaking with me here.

So the title of the presentation is Case Studies, and this is where we actually apply the one linear compartment model to inform water monitoring.

So this is what the outline is. I am just going to give a brief summary of how did we get here. And then we are going to go over the proposed water monitoring durations; that is going to be done by me. And then I am going to turn that over to Nelson who is going to be going over the water exposure estimates based on two datasets that he previously talked about.

Then he is going to turn it back to me and I am going to be presenting how we take that information and try to come up with estimates of human plasma area under the concentration time curve for plasma triazines from his drinking water exposure estimates. And then I am just going to end with a quick summary.

So how did we get here? Well, got here from the four-day study in the endpoint LH attention, which does not seem to

be a single dose effect? Okay? It seems to be a repeated dosing effect. And this is the same slide that I showed you before with the 4-day study with a LOAEL of 3.12 that is based on repeated daily dosing for four days.

We tried to understand what the pharmacokinetics of atrazine that would correspond to this study would be, and for that we turned our attention to the Thede 1987 study because it actually resembled the 4-day study by Cooper et al. in that it involved repeated daily dosing for at least 4-days. It used intact young female rats, and you also had plasma measurements that were taken daily. And the dosing frequency, I should say, was the same. So these animals were dosed daily with atrazine, once daily at different dose levels.

So one of the things that we noted from the plasma profile from the Thede study is that we saw linear pharmacokinetics. And actually, what I mean by that is that the internal dose metric, plasma AUC, is scaled in a linear fashion with atrazine dose at all dose levels tested. And also, we see elimination kinetics for plasma triazines. And as I said before, we were able to analyze two additional studies that actually support our findings for the elimination rate constant or the plasma half life for plasma triazines.

So based on these findings, then, we proposed a one-compartment linear model. It is very simple. It is only based on two parameters; the elimination rat constant and the volume of distribution. But we think that it is

informative because it takes into account information on body distribution and elimination rate.

We show in my other presentation that we can predict plasma levels that will correspond to the rat study using the one model linear expression. And we also want to extrapolate this one compartment model to humans using allometrically scaled volume of distribution elimination rate constant.

Now, we are going to walk you through this. And the critical part more than anything is the estimation of the human dose rate which, like I showed before, actually depends on the water levels as well as the consumption rate. So the product of these two will give us an estimate of a human dose rate for a given duration.

In terms of the range of windows of exposure, we are proposing three durations. We are proposing 4, 14 and 28 days. And the rationale for these is as follows. The 4-day duration, that duration is based on the accumulation of plasma triazines to a plateau, or pseudo steady state level, which seems to be related to optimal LH attention. It should be noted that this 4-day duration is based on a constant dose level and frequency.

So, based on this condition, the time to steady state in the rat is four days. Four days just happens to be also the length of the rat estrous cycle. Twenty-eight days is within the, at the allometrically scaled time range, for adult humans of 60 kilograms to reach steady state based on a constant dose level and frequency. And actually, 28

days also happens to be the average length of the human menstrual cycle. So we have four and we have 28 days.

We also are proposing to include 14 days just to serve as a mid-point, given that the other two durations are on the extremes. And it also seems to be reasonable to include, given that human exposure is neither going to be at a constant dose level nor constant frequency. So, those are our proposed durations and I am going to turn this over now to Nelson.

DR. NELSON THURMAN: Okay. I should be able to do this pretty quickly since we just got finished talking about what we did here.

We took the two monitoring datasets that we talked about earlier, the Maumee River, the 1995 monitoring dataset, and the Missouri-01 from 2007. We simulated the 7-day sampling intervals for this data, then we used the geostatistical methods to develop a variogram model that captures the covariance of monitoring data based on the 7-day sampling intervals.

We ran the 1,000 conditional simulations of that variogram model to estimate the daily time series from the 7-day sampling. We bracketed it with the 5th and 95th percentiles. As Dr. Portier pointed out is that we calculated those percentiles and we looked each day, so they may be a wider range than you may have if you looked at the overall simulation, but that was one thing we wanted to take a look at.

Once again, you just saw this in the last present, the Missouri-01. We want to point out, we did the daily time series. We derived the 4-day rolling averages, which is what we used for representing the 4-day window. Those would be used for representing the 14- and 28-day windows in that regard.

So there's the Missouri sites, there is the Maumee River sites and those are in your slides so you do not have to leap back through mine for a reference. And at this point, I am going to turn this back over to Chester and let him carry it the rest of the way through.

DR. CHESTER RODRIGUEZ: Thanks. So this is how we come up with estimates of human equivalent plasma AUC from atrazine concentration in drinking water. We basically applied the expression that I showed before, based on a one-compartment linear model.

So what you have here is the average atrazine level for a given duration, whether it is 4, 14 or 28. We have a water consumption rate which, in this case, we are assuming two liters per day for an adult human, a body weight of 60 kilograms. And we have the elimination rate constant in the volume of distribution, both of which have been allometrically scaled from the adult rat values.

It should be noted that when you take this estimate of the human plasma average daily AUC and you compare that to the rat plasma, point of departure value for the endpoint LH attention, you will get an estimate of the margin of exposure that will be based on internal measures now.

So this is similar to what we are used to doing when we have a NOAEL here and we compare that to human exposure. It is actually very similar, but in this case it is going to be based on internal measure of exposure.

So what you have on the Y axis here are the human plasma AUC estimates based on the water exposure values that Nelson showed. It is not surprising that the pattern is actually the same, because what you have is a linear relationship between water exposure and human plasma values. It is a one compartment linear model, so it is not surprising then that you have a similar pattern.

Just for reference, the dash lines here represent the BMDL rat plasma AUC with a combining certain factor of either 300x or 100x. And as you can see, that the general trend here for the 2007 Missouri-01 dataset is that, as a duration of exposure gets shorter from 28 to 14 to 4, then you have a higher probability of exceeding this reference values based on this dataset.

For the Maumee 1995, this is the results that we get. Basically, the water levels that Nelson actually estimated were actually very low and the human plasma values are actually correspondingly very low, so they will not exceed the rat point of departure value. There is a linear relationship.

So this is just a quick summary. This is a short presentation. I wanted to summarize just a few points about the approach that we are using. It is actually

based on an internal dose metric, plasma AUC, which is consistent with temporality of the endpoint, LH attention.

One of the reasons why we selected this internal dose metric is that it accounts for magnitude of exposure as well as duration. Duration seems to be critical for the endpoint and we feel that the one compartment model is actually based on the best available science in the absence of a fully calibrated and evaluated PBPK model.

We think that this model is simple and incorporates an average dose rate for a given duration, it incorporates body distribution information as well as plasma clearance. So we think this is the best science right now that we can use.

And it is not surprising that the water levels, you know, the higher the predicted human plasma levels for a given duration, because what you have is a linear relationship, is a one compartment linear model. And with that, I am going to stop talking and try to address questions.

DR. DANIEL SCHLENK: Thanks, Dr. Rodriguez and Mr. Nelson; appreciate it. Any questions or clarification? Yes, Dr. Hayton?

DR. WILLIAM HAYTON: The human volume of distribution, you scaled that but did you use the rat? -- liter per kilogram, you assumed it to be the same, right?

DR. CHESTER RODRIGUEZ: Yes, you are correct. Yes. So we took the rat volume of distribution value on a liter per

kilogram body weight basis, and we scaled that to the corresponding human body weight.

DR. WILLIAM HAYTON: Okay. And then the elimination rate constant value was about 1/4th of the rat value in the human, something like that. That is about how it is scaled out. But the monkey -- you know we talked about that this morning. The monkey actually went the other way. It got bigger; not smaller, but I guess you do not use that information in this scaling.

DR. CHESTER RODRIGUEZ: But the monkey study; I mean, you just have to go by what the science gives you. And what it gives you in the monkey is that the internal dose metric is like it scales directly with atrazine dose and the elimination is linear.

We cannot explain why it goes the other way, but you have to go by weight of evidence. We have multiple rat studies. Rats are a species where the endpoint has been characterized, not in monkeys. So based on that, we do the best we can.

DR. KEVIN O'BYRNE: I mean, I like the simplicity of your one compartment model but do we have some idea as to where atrazine and its metabolites sit within that? I mean, does it sit in fat? Does it sit in some other tissue?

DR. CHESTER RODRIGUEZ: Yes, indeed. So let me just say that the condition of pseudo steady state also supports the one compartment model which, by definition, assumes that all

the other compartments, all the tissues are at equilibrium. So do you follow that?

DR. KEVIN O'BYRNE: But is that realistic? I mean, I don't know. Does it have a tensity to sit in fat, for example?

DR. CHESTER RODRIGUEZ: Well no, actually. But the site of action is in the brain and we think the plasma is a good surrogate because that is how chemicals travel in the body.

DR. KEVIN O'BYRNE: The brain is full of fat.

DR. CHESTER RODRIGUEZ: Yes, but there is no accumulation in fat. There is no significant accumulation in fat.

DR. KEVIN O'BYRNE: So that is known?

DR. CHESTER RODRIGUEZ: Yes. Let me just say that there is accumulation in red blood cells, you know, apparently up to 1.5 percent of the dose actually accumulates in red blood cells, but there is no red blood cell toxicity that we can talk about.

So, it is like we think that, at this point in time, this is what the science has actually given us. This feature of linear kinetics I think we should take advantage of because it simplifies everything. You know. What you have is a constant half-life that is independent on those.

DR. DANIEL GRIFFITH: If you go to slide number 9. Clearly in the 1-day, and even in the 4-day, you see that there is a

breaking of the threshold line. So why wouldn't you be adjusting that threshold line as you do increasing smoothing to try and make an adjustment for what is happening with the smoothing of the data?

DR. CHESTER RODRIGUEZ: If I understand your question correctly, you are asking about the reference values that we are showing, correct?

DR. DANIEL GRIFFITH: Right.

DR. CHESTER RODRIGUEZ: Okay. That is just for a reference value just to compare how values will become relative to the rat point of departure.

DR. ELIZABETH MENDEZ: Let me just clarify a little bit about the BMDL and the 300. The BMDL comes from the point of departure from the study, so that is standard throughout. The 300 factor comes from the fact that, in the current risk-assessment from 2003, the uncertainty factor is 300, 10x for intraspecies, 10x for interspecies and the 3x that was retained as an FQPA factor showed where those reference values come from.

DR. NELSON HORSEMAN: On that point there; you said you are using a 3x FQPA, not the 10x?

DR. ELIZABETH MENDEZ: Correct.

DR. JAMES MCMANAMAN: So I have concerns about using the rat data as a model, and I understand what you are trying to do. At least, I think I understand what you are trying to

do, is trying to see how well you can model both the water modeling and the animal pharmacodynamics. But maybe the point of departure and dose for a human is not the same as it would be for a rat, but it might be closer if you chose a monkey model.

And so, if you are going to use this as a way of setting policy, I am a little concerned about using those values. And I am also concerned about the fact that you really do not know very much about how the atrazine is metabolized.

I mean, you know a lot about it in the rat but not so much in a primate model. So, all those things are going to go into helping you set standards, but it seems to me you are lacking critical information and, from my perspective, I would urge you to try another animal model, in addition to the rat, to see if you can get a more closely modeled what is going to be going on in the humans.

And then, in regards to the water, there is a huge variation in the Maumee data and the Missouri data in terms of the Y axis. I mean, it looks like there was a four- or five-fold difference. Presumably, that was because of where the catchment area was, so wouldn't that also have to be factored into your model as having a standard catchment area at the headwaters or wherever? And maybe I just did not understand that. But I think that all that would have to go into whether you would break the bar in terms of being in a critical range to potentially have some adverse outcome.

DR. NELSON THURMAN: Let me clarify. I mean, first of all, these are not drinking water monitoring. Those take a look at how well the simulations would play into this. And I think what we would be doing is we'd be looking at each individual community water system in terms of providing those estimates for each of the community water systems, so it wouldn't be breaking down a catchment size in that regard. We would actually apply this approach to the weekly sampling for each system.

DR. ROBERT GILLIOM: So you helped a lot explaining where the benchmarks came from for illustration and I might've just missed something in what is written, but how are you going to actually determine what the actual threshold from all this information is? Because you kind of were right on the brink of just saying, based on all this stuff, this is what the threshold is and therefore here is what the water level, but you are not quite saying it. So I'm just curious where that fits in.

DR. ELIZABETH MENDEZ: All right. Let me try and see if I can explain this a little bit more clearly. So at this point in time, we have our point of departure that is pretty solid. Now, in regards to the uncertainty factor, what you saw today from Nelson and from Chester is a proposal in a case study. We have an enormous amount of data that we need to go through before we can make a final call on what the factor is.

Now, in this particular instance, what you are seeing in this slide for instance is, if your critical window of exposure is four days -- let's just say that -- and you

are sampling every seven days, then there are some instances, not very many, when you are going to be exceeding that benchmark dose divided by a factor if the factor were to be retained at 300 as it is right now.

If once we have looked and done the full-blown analyses on the exposure, from the hazard standpoint, it appears that we may not need to factor. But that could change if new data were to become available. But at this point in time, we are just going with the 300 that is currently, so to speak, on the books, but we will make that determination once we have fully analyzed all of the data.

DR. CHESTER RODRIGUEZ: I just want to go back to the monkey study question that you raised. You know, this is state if the science right now, but there is more data coming out for atrazine. My understanding is that the Syngenta is actually doing monkey study with radiolabeled atrazine. So in the future we should be able to get more information. This is all we have right now.

DR. RICHARD GREENWOOD: On this slide that is on the screen at the moment, is my interpretation correct that where the red line, which is the area under the curve, breaches the limit, then that is when you have exceeded the critical exposure for the effect?

So when you've managed to get the peaks fairly well defined when you do daily sampling, then it is approximately 10 days out of that whole sampling period that it has exceeded the critical exposure, but only for 10 days. So if the critical exposure for humans is longer

than 10 days, you have not actually exceeded it. Is my interpretation correct?

DR. CHESTER RODRIGUEZ: Yes. Let me try to make sense out of this. So all the lines here are actually human plasma AUC estimates, okay? This line here is a 95 percent confidence interval. That is the worse case scenario: someone said worst of the worst, you know.

This lower line here is a 5th percentile and the black line is the actual data based on 4-day rolling averages, 14, 28, et cetera. So the trend that we are seeing here is that the shorter the duration of concern -- whatever that is -- the higher the likelihood of exceeding a given reference value. That is a take-home message.

DR. ROBERT GILLIOM: Sorry, one last thing to make sure I have it right. So because it is a simple linear model linking plasma to water, if you translate that graph into water concentrations, which is on the previous slide there, you are talking about roughly 50 micrograms per liter as the 4-day moving average, something like that. It does not matter the exact amount.

So where this would end up going, whether the 300x stays the same or whatever you do to policy-wise change that, we could just visualize that being translated back into a particular rolling average duration level like 50 or 40 or 30 or whatever you come up with.

DR. NELSON THURMAN: Yes, that is correct. And once again, we are just pointing out that this is not community water

system monitoring. The concentration that we have here are -- except for a couple of sites where the monitoring was in a source that does not necessarily feed into -- these concentrations are higher than what we have seen in the community water system.

It was a scoping exercise in that regard to see what happens if we push something that we think may be at or around a duration concern. By doing this analysis, would be able to catch that if it did indeed occur? So I look at it more of a scoping exercise.

From my point of view, looking at it a different way, is that if the duration of concern -- the window tox exposure is four days, then I need to pay more attention to the frequency of sampling than if it is 28 days. So that is another way for us to look at it in that regard as well.

DR. DANIEL SCHLENK: Any other questions, clarification? At this point, what I will state to the panel is what we would like to do is, maybe tomorrow after we have had a night to stew on the plethora of data, that you guys would be available for a final questioning period before we actually begin the charge questions, if that is okay. All right, with that I guess we will conclude the agency's presentation and move on to the public comment period. Our first presenter is the Syngenta Group, which I believe Dr. McFarland is going to sort of lead off with an intro, I believe.

DR. DANIEL SCHLENK: Just for the panel's knowledge, you are going to get copies of part of the presentations,

initially, and more will be flowing in as the presentation goes on, so we are getting hardcopies of the presentations. Dr. McFarland, are you going to introduce everybody or -- just make sure that they introduce themselves as they go through; that'd be great.

DR. JANIS MCFARLAND: Yes. Thank you. Thank you, Dr. Schlenk and thank you very much to the panel. I am Janis McFarland, head of Regulatory Affairs North America for Syngenta Crop Protection. Syngenta Crop Protection is a research and development company that discovers and develops herbicides, fungicides and insecticides.

We are also a world's producer and breeder of seeds. Many people don't know we are the number one Pansy flower producers around the world, and we also produce sweet corn seeds to make tomato seeds. And we introduce about a hundred new ornamental flower varieties every year.

We would like to thank the panel for the opportunity to be here. We greatly appreciate, and we'd like to express our sincere thanks to both the panel for their work as well as to all the scientists at EPA for the amazing amount of work that has gone into the atrazine assessment over the past year and a half, and then also prior to that.

Syngenta has listened closely through the SAP process to the questions, the suggestions, and also to the recommendations that the science advisory panels have provided. And we have responded by conducting many new studies and assessments that we provided to EPA and through the docket process of the Science Advisory Panel.

The basic research that has been conducted on atrazine both recently and over the last two decades have led to several improvements in methodologies, databases, study designs and also risk-assessments.

As part of the SAP process in 2010 and 2011, Syngenta, with the help of several university experts as well as external scientific experts, have submitted 15 new toxicology and mode of action studies, more than two dozen reports with different statistical analysis and water monitoring, and we have also developed at new PBPK model that we will be discussing later on today and that was spoken about earlier by EPA.

We have developed the framework and published an assessment of how to assess epidemiology and toxicology data, and we have provided several summary reports on a broad range of the key aspects of toxicology and exposure.

At the same time, we are continuing to monitor 88 different community water systems with weekly monitoring data, and we have continued a second year of an extensive and comprehensive ecological monitoring in smaller streams, first- and second-order streams for the ecological assessment which involves daily monitoring, and some of that you also saw earlier in the EPA presentation.

We have several stewardship projects that are going on in both environmental, putting in buffers. One of our projects, we have planted a million and a half trees along the side banks of streams in Iowa and Illinois

working with a non-profit to reduce runoff of pesticides, fertilizers and segment into water ways and improve water quality.

And in additional, we have developed many different environmental databases and modelling that you will hear about soon from Dr. Hendley and his team. We really look forward to the next several days and listening to the advice and suggestions on the risk-assessment of atrazine.

We are excited about the progress that has been made both by the EPA studies and our research in the various areas of mode of action, risk and exposure. And with the comprehensive database we have, we look forward to any recommendations on how to statistically analyze that.

We did start six new water systems, daily monitoring in community drinking water systems this year voluntarily, in order to aid in the modeling and statistical analyses.
The conclusion that we see when looking at this comprehensive database is, with the toxicology and exposure, there are wide margins of safety. And the current regulatory standards are protective. And we look forward to advice on how to advance its overall area of science from the panel. And with that, I will go through what we are going to cover for Syngenta in the next slide, please.

And I am going to introduce Dr. Paul Hendley. He is our senior research science fellow with Syngenta and he will be introducing his team to discuss the atrazine occurrence in drinking water and the statistical analyses.

We decided to actually start with the water area in line with the first batch of questions, and then we will follow up with the various toxicology and modeling of the risk-assessments; so with that -- Paul?

DR. PAUL HENDLEY: Okay. Thank you, Janis. Thank you, Mr. Chairman. Paul Hendley, a senior fellow at Syngenta. I would like to introduce the two gentlemen on my right; Dr. Chris Harbourt from Waterborne Environmental, Dr. Wenlin Chen from Syngenta, and on my left, Dr. Paul Mosquin from RTI International and they will be answering questions.

We are looking forward to this presentation and I am very pleased to say it is highly complementary to the presentation you've heard from Nelson Thurman, and I think we've got some exciting things to show you.

So the overall statement -- and we have talked about this before -- is that atrazine exposure is exceptionally well characterized due to the database. The database sample numbers provide high confidence on the exposure. In that, we mean they help us understand what the peak shapes look like. They help us understand the upper percentiles of a distribution because of the magnitude of the number of samples in the database. They help us understand and differentiate between community water systems.

On the community water system side we are going to talk about how we have learned more about them and differentiated between them, in terms of their watersheds and their sources. And that is important because that

leads on to how you use these bias factors. And the bias factors, we have seen two examples in the earlier presentation.

We are going to show you bias factors from a large number of additional case to give us a better sense of the distributions, et cetera. And that is going to show how we have actually developed a synthetic chemograph for a margin of exposure assessment for TCT that Dr. Breckenridge will be finishing the presentation with later on this afternoon.

Now, I am particularly excited to be able to talk to you about a modeling development, PRZM-Hybrid which is showing great promise at being able to help supplement 7-day monitoring data. And then we will talk a little bit about how the year-to-year variation is defined well for atrazine by the database and how there are opportunities. Obviously for atrazine we've got a lot of measured data, but there are opportunities to learn a lot from that database for understanding monitoring questions, in general.

So let's turn to a database which is, of course, extensive. And when I say that, what I mean is, there is approximately 340,000 surface water samples in the database; that's not talking about the 200,000 additional ground water, safe drinking water, samples that are in there. 140,000 of those samples approximately come from drinking water related programs, and 200,000 also from non-drinking water.

The biggest contributor for non-drinking water is actually NAWQA program with USGS which covers enormous range of years, grounds and is very valuable for helping to calibrate.

We are actually going to focus most of our attention today on the three highlighted groups, and let me just sort of explain what these initials mean. The AEMP is the Ecological Monitoring Program that the Missouri-01 site is from. This was probably now 60 or more sites that we have investigated, 180 plus site-years of data.

Some of these -- Missouri-01, for instance -- they are very small. They are 11 square miles. You can drive through them very quickly and they are largely agricultural. And they are, of course, not drinking water related. It is about 15,000 samples, and the last two or three years have been daily, as Dr. McFarland mentioned.

The next one of importance is the dataset from which the Maumee came from, which is the NCWQR, as Nelson explained, the National Center for Water Quality Research, again about 15,000. That is interesting because the AEMP -- the eco-program has a lot of sites with not as many years in between three and six or seven, whereas the NCWQR has fewer sites but far more years. So we have got a temporal and spatial match or complementation there.

The other dataset that we are going to talk about, as Dr. McFarland mentioned, we started daily monitoring at six community water systems, so we have actually gone back and found out what the variation is at six real community

water systems just to build some confidence that our models are appropriate.

However, all of that is based on what I call the fundamentals which is; the monitoring we have from the Safe Drinking Water Act which started in 1993, about 55,000 finished water samples from about 4,000 community water systems -- quarterly samples -- which is served as a screen to identity community water systems that move into higher frequency programs. Initially, that was the voluntarily monitoring program, which operated between '94 and 2003. That's got about 22,000, maybe a few more, finished water samples, and then that transition to the atrazine monitoring.

Nelson gave the perfect description of that. All samples that have exceeded 1.6 ppb annual average atrazine concentration, from 1997 on, were put into that program in 2003. Any subsequent community water systems that have exceeded that have moved into the frequent monitoring program. And so, that's why the AMP program is looking at those community water systems, if you like, at the pentacle of a pyramid, which is a model I have shown you before.

In addition to that, there is a couple more additions. One is the database of environmental vulnerability factors, which is pulling together the information we have been gathering because of the eco-program to understand watershed behavior, and in addition, a database of community water system characteristics and watershed

information. So those are the new tools we have to play with.

When we looked at the challenge before we panel, it was interesting because there's two types of question: One is retrospective and one is prospective. The retrospective is sort of, what can we say based on the data about the specific atrazine question?

For example, from the magnitude of the data we can say the 95th upper confidence interval of a 99.9th centile for finished water from the frequent monitoring is 41.6 ppb. We can be very precise because of the number of samples involved.

We can also use 7-day data to look at bias factors to understand shorter endpoints by the same approach that Nelson explained very clearly. And we can use that to help us understand modeling. We can look at that for 14-, 20-day, 28-day intervals as well.

We also have used a database year-to-year variation, residue patterns and trends, but there is a prospective element of how might we understand bias factors for other sorts of water bodies? What about future assessments presumably for molecules with less frequent monitoring, and how would you go about setting upper bounds on the year-to-year variation?

Nelson made the point clearly that those were exciting questions if you liked the scientists, but for atrazine, the database already actually tells us. We have answered

a lot of these questions by sheer volume of data that are measured.

And for example, 14-, 28-day in tools, while interesting, philosophically, when you've got a great database of 7-day data; those are the ones we need to focus on when we look at bias factor, et cetera. The existing database also gives us the modeling clues. Okay.

Moving on to differentiating community water systems; we've set up a new database, 200 plus community water systems. We've been looking at their source water types, their watersheds. Interestingly, there are 375 intakes associated with that. Quite a lot of community water systems have multiple intakes. I'll show you an example.

The database is dominated by static water bodies. 224 of those intakes are static compared to 100 flowing. And most of the static intakes are on on-channel reservoirs with the stream running through it, but some of them are off-channel where the water is stored, and it has to be pumped to get in. They don't have a watershed of their own that is storage units.

Once you have characterized the watershed you can use that environmental database I mentioned in order to find the environmental parameters, soils data, cropping data, vulnerability. And equally, the community water system characteristics like the atrazine monitoring data, summarized, is all pulled into one convenient location, and that allows categorization.

Now that sounds nice and fun, but why are we doing it? It is the help us understand how to use things like bias factor, because community water systems are individual and you need to target your thinking and your learning appropriately to different community water systems.

So here, for example, is a community water system that the red line, which is a little hard to see coming down here, is a rather tiny creek. It is a two square mile watershed. But the water they store -- and you can see five ponds here -- there is actually another one off to the side we cannot see in this image, so five intakes from five separate units collecting from one watershed. That is the level of complexity the database has to cope with to be useful to understand what might be going on when we are looking at the residues from this database.

We talked about dividing up community water systems by source and watershed size last time. We have taken a simple approach; small, medium and large, and basically 50-squre mile cut off for small. And the for the statics, a medium to large cut off of a 1000 square miles for flowing 800 square miles. And you can see that the database is dominated by small static water bodies. 143 which is half of all the intakes we are looking at here with small static water bodies with watersheds less than 50 square miles.

When you look at the flowing you see, actually, the majority of them come from large watersheds, which when you think about the hydrology immediately makes sense.

I'm not going to dwell on this, but once you have categorized them you can start looking at the statistics and the environmental parameters associated with the different groups. That is the reason why we have been doing this categorization and spending the time developing the database.

And example is shown here, and I'm coming back. The royal blue color here, this is a distribution of areas for the eco-watershed. And you can see that there is overlap in terms of watershed areas with the small flowing community water systems, and there are 16 of those, but there is almost no overlap with the rest of the medium and large community water systems on flowing watersheds. So the eco-datasets are very useful for understanding the hydrology and temporal behavior but they are not relevant to the vast majority of community water systems on a scale basis.

So moving on to bias factors and supplementing what you've learned from Nelson, we have looked at many locations and years. And using exactly the same nomenclature that the 95th centile of the error ratios -- and the error ratios were the ratios between the true from the daily data and the simulated value from the 4 of a 7-day simulation, and the 95th centile of those ratios makes up the bias factor.

Syngenta use systematic sampling and I am going to spend a minute on that because it is a question that has been asked of the panel. Systematic sampling means if it is four days, you've just got four ways of doing it. You can do it on day 1, day 2, day 3, day 4; seven days just the

same, Sunday, Monday, Tuesday, Wednesday. And we use that rather than stratified approach which will be a random selection from period 1 and then period 2 and could come up with a range of different intervals.

However, we have gone back and looked at what the actual guys taking samples in the community water systems have done, and 66 percent of all those samples were taken spot-on the 7-day intervals. They got used to a weekly routine.

Twenty-four percent of those were actually taken with one day of that; Thanksgiving falls July the 4th, or whatever. And, so 90 percent of those samples were being taken within one day of the systematic point. And what my statistical colleagues are showing me is when you've got systematic sampling in the field, systematic simulated samplings is an appropriate way to look at it.

The downside of using systematic sampling is if you've got four or seven measurements, error ratios, it is difficult to come up with a 95th centile. So that is why Syngenta calculated a 95th centile on the group. And when I say a group, this is not a random group. This is all the eco sites pulled together, or six community water systems with daily monitoring or all the years for an NCWQR site, so it is grouped by logic.

Moving on; the data we see is that -- here is 34 eco-sites with one to two years. So that is our spatial information. There are four NCWQR sites with 15 or 16 years, so that is our temporal dimension. And then St.

Louis, which is finished water from a real but not very flashy community water system.

Just using 7-day simulated sampling and looking at 4-day average for, let's say Honey Creek, the bias factor will be 2.73. If you look at it as you move across, as you move -- as Nelson pointed out -- from shorter durations to longer durations, the bias factor drops; the amount by which it drops decreases.

I would stress here, this is adding to what Nelson showed us for two examples. This is giving us a good sense from a distribution of bias factors. And you are going to see the individual ratios in a minute.

We have also done the monitoring at six community water systems. Those are flowing water; they've got a wide range of areas. And the preliminary data that I'm reporting here goes up to the end of June. It covers the peak atrazine season and, as many of you know, it's been quite a high runoff season.

We did, again, the same 7-day and 4-day simulated sampling. Bias factors estimated in just the same way and we will see some of the individual error ratios. The bias factor that results and you can see in the table, very similar between raw and finished water, and the bias factor is actually four within the range that we saw from the temporal dimension NCWQR sites.

Here's an example where, for clarity, we've spread out 15 years of Honey Creek and of Maumee from the NCWQR so we

can see the individual variations of the seven error points when we are using the 7-day simulated sampling to look at 4-day rolling averages.

You can see there that the red lines are the group means for all those years for bias factor, the 95th centile. The open circle is those points above the 95th centile. And you can see the larger watershed, Maumee, which is 6300 square miles, has a lower bias factor, as you might expect, than the smaller watershed.

Here, what we've done is something slightly different and the X axis is logged area. So we are looking at the distributions across the whole range of things we've looked at, CWS error ratios, for cross area.

What you can see is St. Louis, which it has the whole Missouri which is 500,000 square miles and is finished water. You've got Maumee, Sandusky which is 1200 square miles, Honey, Rock Creek, and the gold ones are the six community water systems with daily monitoring, and the gold line is their combined bias factor, and the gray dots are the ones from the eco-programs, from these very small non-drinking water watersheds.

What you can see here is a difference as you have an area with larger watershed areas, has generally lower bias factors. So what we've drawn from our look at bias factors is that, from 110, 116 sets of error ratios, systematic sampling is an appropriate way because of the way the AMP samplers did it. Raw and finished is showing similar ratios. Bias factors decrease as the averaging

period increases, and they also decreases the watershed area increases.

There is a simple way, perhaps, we could look at this. For smaller watersheds you might want to pick a larger bias factor than for larger watersheds. And just something simple; not some regression equation but just pick a couple of categories that match perhaps the categories for dividing up community water systems.

Once more, there was another point, and I'll show you an example. With what we know about the atrazine database, we can put firm figures on some of these upper centiles. So, to use a bias factor and create numbers that fall into highly improbable areas wouldn't make a lot of sense. That is another factor to consider when you apply bias factor to a chemograph.

We've got a good sense from this wide range of years and sites about flowing waters. What about static? And I think the panel agreed last time that static is generally less flashy than flowing water systems.

The one thing we do know is the flowing water bias factors would be conservative if we were looking at some static water bodies. This is a way of applying factors to come up with a TCT margin of exposure synthetic chemograph, a worse case chemograph. And to do this, we took the 149 community water systems between 2006 and 2010. And the reason why we chose that was because every sample was analyzed for the components of TCT. And the 4-day rolling averages were calculated and ranked, and the 17 community

water systems that had the highest 4-day rolling was selected as an indicated dataset of the uppermost tier of 4-day rolling averages.

What we did was, we took a worse case assumption, and atrazine runoff event happened between every pair of 7-day samplings. And that will generate an unrealistic number of atrazine events and it probably distorts the peak shapes.

What we chose was to use a three-fold magnifying factor that's greater than the maximum of two adjacent residues. Now, what I mean by that; you can see the red points here are describing the measured values. The linearly interpolated chemograph that would describe that site for that year is shown there.

Now we put in these three x factors between each of those pairs of points and we create the new chemograph, and you can see the new daily points marked on that. And so, that's what has gone into the margin of exposure assessment that Dr. Breckenridge will discuss.

Because that was meant to be a worse case assessment, we did not apply the upper bound cap I just mentioned. But what you would normally think about doing is using what you know about statistics of an enormous database to remove highly improbable values, which would have the effect of capping those synthetic peaks at some number justified by the sampling statistics.

Moving to PRZM-Hybrid -- and I would like to give credit to Dr. Miller of Waterborne who's actually driven this piece of work resourcefully and relentlessly, I think is the phrase. And I think it's a particularly clever piece of work he's done.

The phrase to remember here is PRZM-Hybrid is site and local event specific. It uses PRZM code, which EPA and the industry are familiar with, atrazine Efate data. The only regional information used is crop reporting data which comes in groups of 10 counties from the NASS service.

But if you are simulating a monitoring year, you have watershed and year-specific data for soils, for local rainfall of the year from the radar maps we see every night on the TV.

So actually, the rainfall that was falling across the whole area of the watershed is taken into account and the cropping of the year from the NASS mapping, which maps all the fields by crops across the nation now. The output you get is a watershed scaled concentration time series. It uses available data.

There is no model-specific calibration for each site. This is applied in the same way to all sites using code. And it uses conservative edge-of-field concentrations.
What I am trying to say here is it is driven by rainfall events, which is what happens in the field. You can see here, rainfall events coming down from the upper line in

the graph, flow figuratively below. There are uniformly sampled points. That is our linear interpolation.

PRZM-Hybrid runs; every time there's a rainfall big enough to cause a runoff, we get a PRZM-Hybrid estimated concentration. What we do then is say, "Is that concentration higher than the linear interpolated value?" If so, you use it in a schemograph; if it isn't, like point D, you don't use it. So again, you are retaining a worse case element in this assessment.

Just for the record, we did look. When we were trying to do this, we thought flow would be an attractive way of getting into trying to fill in the gaps. We didn't find useful correlations during the period of high atrazine runoff when we did it, but the PRZM-Hybrid seems to be a better way from the way we are looking at things.

The reason why things have improved -- the report that you have in your docket uses a growing degree day approach. The simulation was good but it wasn't great.

There is a new algorithm that accounts for land workability; can I get a tractor on the land, and distribution across time; how many people apply even if it's a day they could apply. And what that takes account of is the local response of a soils to rainfall. Is it too wet to go out? And the wariness of some farmers, frost concerns -- if it's a field that isn't no till, to create seedbeds before you can go out and spray. And the equipment capacity; in some watersheds you simply can't spray all the fields in a day even if you'd like to. And,

of course, the possibilities of post-emergent treatments -- there are quite a number of atrazine treatments applied post-emergent.

So PRZM-Hybrid chose considerable promise for supplementing 7-day data. It reflects reality. The peaks are only predicted when runoff events are likely to occur, unlike some of the models we've seen. And it does tend to over-predict at bit at greater than 20 ppb. There is no need anymore because of its algorithm improvement from distribution matching we thought we might have to do.

Here is a before picture. This is the growing degree day approach. This is a site. The black marks are the PRZM-Hybrid predicting concentrations. The opened triangles are the measured values; not a bad fit but could do better.

Here you see what we have with the workability approach. And what I'd like to do is go back to this slide and just talk about the way we simulated the application across the watershed. These green bars show that we applied a chunk, about 15 percent of the chemical that was going to apply on four occasions, and we filled in in-between with daily add-in loads of atrazine. And that made sure there was always a little bit of fresh atrazine present if it was a runoff event.

Using the workability approach, you find all the workable days in that period and you divide the application equally as a fraction between those. And so, you can see here it identified a whole bunch of workable days. The chemical

went down and we caught that first peak rather well. We also, because of those days, managed to catch the second peak quite well, so we are matching reality with this technology.

There's a couple more here. The top one is one of the less good ones. We thought we really ought to put some less good ones in as well as the better ones, but it's still pretty good. I'm still quite pleased with it. And of course, here's another one where we're picking the peaks up in time. And as the modelers will tell you, the problem is modeling and catching this in time on a watershed scale.

We realize this is sort of rabbit out of a hat data coming in at the last minute because it is hot off the press. We want to get this out in the open literature and as a report into an EPA very soon. There are still some more tweaks on the workability approach, but the challenge is perhaps on larger watersheds because I don't think we do quite a well for that.

There are a number of ways we could do it. We do not need to dwell on it, but I actually think that is where moving towards the regression models may be, as Dr. Nelson suggested, an attractive way of tackling larger watersheds.

So moving on to the last key point, year-to-year variation is well defined, and the database is useful for general purposes.

Atrazine is a near-ideal case to look at the year-to-year-variation. It is applied to a high fraction of a major crop nearly every year at uniform rates. It has a great length of monitoring to understand the results and look at modeling and it covers a wide range of scenarios for all of the data in NorAqua and our programs.

We have a direct answer for year-to-year measurement of variation for atrazine, but the data offer an opportunity to answer questions about monitoring for other compounds. And in simple summary -- and there is actually, the back on the handout I think you'll find a few examples showing the variation -- but there is high variation of residues and error ratios across years.

The primary driver for that is the interrelationship between rainfall inducing runoff timing and application. And so, basically, even if you don't have the extensive database we have for atrazine, even medium term prospective monitoring is probably not going to answer the question of putting bounds around year-to-year variations. And that's why, for years, for ecological modelling, we've used probabilistic approaches of many weather years.

So we think that where monitoring is required, which isn't always, one approach would be exactly as Nelson suggested, using PRZM-Hybrid calibrating for local monitoring data and then extending that to a probabilistic environment. And when I say that, I mean this schematic will show the PRZM-Hybrid approach.

We run that for a site for two or three years of monitoring maybe. We check that the model is behaving itself. Then we move into a probabilistic environment. And that environment link here, the key link, is taking the watershed parameters that we know we are fitting and then playing the year-to-year variation of rainfall data in a probabilistic sense on that watershed shown to work dataset.

You could also chose to variate the crop treated percent, the crop rotation and perhaps the rate. But the key driver here will be the weather, and that will give you a host of PRZM weather years of 365 days of predicted environmental concentrations which can be looked at in terms of whatever you want in variation; magnitude duration, peak shape; that all comes out the PRZM daily record. So, we think that's quite an attractive approach.

In summing up, atrazine exposure is exceptionally well-characterized. It is more than sufficient for analyses of exposure, magnitude and variation.

The database numbers give us high confidence. We know the high centiles, shapes, trends. The community water system differentiation allows us to know what's out there and think what would be the appropriate way of dealing with filling in the 7-day record for that community water system.

The daily or near daily data validate analyses. We've looked at the wide range of bias factors so now we've got a sense for how those vary across area and across time.

That's been backed up with daily work at six true community water systems. And we've used this for synthetic chemographs for 7-day TCT record, as Dr. Breckenridge will show you.

The database has provided a test bed for looking at modeling and monitoring. PRZM-Hybrid is promising. The application algorithm has moved us forward, and that approach is actually giving us a reality-based way of coming up with simulated peaks that depend on rainfall, which is the driver.

Year-to-year variation is characterized by the atrazine monitoring data. Probabilistic modelling is an attractive way of looking at other questions beyond atrazine. And we think it is a smart way of doing it using the new tools. So that's the summary. I thank you for your attention.

DR. DANIEL SCHLENK: Thank you, Dr. Hendley. Let's go ahead and have some questions for he and his group right now for the panel if you have questions for clarification. Yes, Dr. Coupe?

DR. RICHARD COUPE: On the PRZM model, does that generate hydrology also? So, like, you have a point on a stream you'll get flow through that stream?

DR. PAUL HENDLEY: Simply put, no. PRZM is an edge of field model. And particularly, in this case, we are using it as an edge-of-field sense. In the EPA normal methodology, it's linked to the exams model to simulate entry into a pond system. And there are various implementations that

can link it to a river system, but because we in PRZM-Hybrid simulate the watershed in terms of area waiting, the different soil runoffs run under PRZM, the edge-of-field numbers is simulating edge-of-watershed, if you like. And it is quite surprising that the fit is as good as it is without having that dilution factor from a stream, but very satisfying when it does that. Does that answer the question?

DR. RICHARD COUPE: Yes. Thank you. But did you show real data versus your simulated data?

DR. PAUL HENDLEY: Yes. The open purple triangles were the real data, daily data coming from the ecological monitoring program from those sites, and it's been run for the 34 sites that have been in the program, so probably 48 to 50 site years.

DR. RICHARD COUPE: I like the PRZM, it's really good. I read the paper on PRZM and how we triggered planting under the growing degree day, so how did we trigger planting with the new workability algorithm?

DR. PAUL HENLEY: Can I pass that one to Dr. Harbourt?

DR. CHRIS HARBOURT: There is a couple of different ways that we're doing it, and right now, the main characteristic of workability is soil moisture, so we are modeling soil moisture and figuring out when is the soil trafficable. You know, when can a tractor enter the field; defining that as the workable days. We are using temperature windows to set the time. And then we can also, if we

choose to, use some of the characteristics of the GDD model and use the growing degree days as an end characteristic to kind of limit the window in time to where the corn is at a height where it's no longer feasible to apply herbicide or where it's off label.

DR. PAUL HENDLEY: And there is actually a description of that at the back of the slide set.

DR. KENNETH PORTIER: Dr. Hendley, this was all pretty quick and I followed most of it. I just wanted to double check. When you do the PRZM in-fill, you're not filling everyday though. You're filling event days between the 7-day sampling, is that right? So you'd have a rainfall event or something that triggers PRZM to compute an estimate that would go into between two sample points to pick a peak; is that correct?

DR. PAUL HENDLEY: That's absolutely correct. And rather like those simulated peaks, it creates a new schemograph.

DR. KENNETH PORTIER: Well it supplements the schemograph by adding higher peaks that hopefully reflects some aspect of variability between the sample points. Have you looked at other statistics, the maximum, in terms of duration at a concentration? One of the other things we were talking about -- we'd been talking a lot about peak, but actually for the AUC you are more interested in duration at a level of concentration. And I wondered if kind of the 7-day supplemented graphs, how they would compare to the everyday picture in terms of that kind of statistic? You

know, is it five days at 10 parts per million? You understand what I'm asking?

DR. PAUL HENDLEY: Yes. You've got it dead right. And what we've actually got -- and the report is in draft -- is an analysis of the eco-sites, the NCWQR and a lot of NorAqua sites, as well, in terms of looking at the peak wits at different concentration ranges for exactly that reason. My suspicion -- and I don't have the data to back it up at the moment -- is if in fact there is one event between seven days -- well, I'm certain, for sites like Missour-01, which are very small, very flashy sites, that we are making the peaks must broader than they would be in reality. But if you went to Maumee, which is a very big watershed, the peaks are broad. So it's a watershed scaled dependent thing and that is another factor we are looking at in this analysis of the database.

DR. KENNETH PORTIER: So when you're looking at those periods, statistics, are you thinking to relate them to water site characteristics so that you'd be able to say, well, a large water slope might have a distribution of durations that look different than a flat small -- I don't know if you follow what I'm saying.

DR. PAUL HENDLEY: Yes. However, let's not expect -- I think we are looking for simple relationships here because there are so many things that sort of complicate -- one of the biggest ones is the duration of rainfall events because you can have very flashy watershed, but if you get two days of solid rain it is going to look like a broad peak,

so there are some confounders in there that you really have to think hard about.

DR. ROBERT GILLIOM: Staying on the PRZM theme first. So in terms of size of watershed limits that you are thinking about from what you see so far, I mean how big are you thinking it's comfortable to go, given no adjustments for the flow system?

DR. CHRIS HARBOURT: We looked at some base flow calculations and looking at break points in different streams to try and see is there a scale at which routing is necessary. And one of the thoughts -- and Paul had it in one of the slides -- about 800 square miles as being a break point; we are fairly confident that between 800 to 1000 square miles or smaller. When we're talking about time of concentration in watersheds on the order on the scale of a day, and our monitoring data is on the scale of a day, it will do fairly well with PRZM, a daily time-step model. I would think much larger than that, we are going to need to do some sort of routine to be accurate in the short-term. At some point you will catch the peaks, but we'll mimic what happened in reality, potentially not, depending on routing, and then depending on the complexity of the scale of storms.

When you get to a scale of 800 or 1000 or 5000 or 50,000 square miles, it can rain in part of the watershed and not the other. And if we are diluting that across the watershed average, as an example, you may not see the response that you would see in a local portion of that, and that somehow needs to be reflected in the modeling

scheme as you go up in scale with some of these drinking water supplies that are much, much larger, potentially.

DR. ROBERT GILLIOM: I just got a couple more just to follow-up. In terms of the readiness of the method to be applied geographically, am I understanding right that the basic method can now be applied to any watershed in the US? Or is it only for the drinking water intakes you have looked at?

DR. CHRIS HARBOURT: The underlying data -- and that's one of the strengths of PRZM-Hybrid. I mean, in traditional modeling, setting up a model, parameterizing it and calibrating it is a challenging exercise. What we have done here instead is process data nationally. We have set up datasets. The underlying SSURGO soils, the weather, all that stuff. It's setup and ready to go in a way that can run everywhere.

One of the challenges though with the PRZM-Hybrid is that it corrects itself back to a real time series with measured points. The hybrid concept of them using it in a probabilistic fashion, the thought there is using a few years where you have sampled monitoring data -- you know the crop rotation history of the watershed from national land cover data, and we are able to expand that or extrapolate that to multiple years, 50 years of rainfall record and do some estimates.

So there are opportunities, I would think, outside of areas where there is monitored data looking at that because it's an un-calibrated model, you know, comparing

one where you have monitored data and you check it, but it's realistic and its behaving property and you could compare that side-by-side to one where you have no monitored data; maybe not in a realistic sense but side-by-side within the model perhaps in comparison.

DR. ROBERT GILLIOM: And I have one last non-PRZM one. On the earlier part of the presentation we were talking about the bias factors for every year from the AMP data, and basically, when you do the weekly sampling frequency you have the seven values because you used the systematic sampling. I know, you made the argument for pooling those because you only have one year from each. Did you look at how those pooled values compared to assuming a reasonable frequency distribution for the individual years? I know you only have seven values and you may have to estimate a 95th, but using something like a Weibull or a lognormal -- you know where I'm going -- is some way to get at the individual site.

DR. PAUL MOSQUIN: No. We did not do that but we provided the plot so you can see what the underlying sampled values are.

DR. WENLIN CHEN: I just want to add to what Paul said. We did some comparisons, not to the normal distributions, but actually compared to the sort of stratify it or random something. Actually it generate fairly small differences; not a whole lot.

DR. DANIEL SCHLENK: Okay. Any other questions, Dr. Hendley? Okay. Thanks, Dr. Hendley. We'll move on to the next group.

DR. JANIS MCFARLAND: Thank you. And we'll now bring on our toxicology and pharmacokinetic modeling team. Dr. Breckenridge will be leading that discussion. I am introducing Dr. Charles Breckenridge. He is a senior science fellow with Syngenta Crop Protection and has been intimately involved in several years of the development of the mode of action and toxicology of atrazine as well as basic research on many of our other products. And Dr. Breckenridge will be introducing his other experts that will be discussing the biology and the modeling of atrazine.

DR. CHARLES BRECKENRIDGE: Good afternoon, ladies and gentlemen. Thank you again for having us and listening to our long presentations. We greatly appreciate the opportunity to discuss our data and concepts relevant to EPA processes on atrazine.

I would like, while I'm introducing the people that are available at the table and in the room, if we could advance that slide set to slide 39. I'm sorry I just did not have the linkage slide at the beginning of the presentation. We'll come back to this after.

The people that we have here today are experts in the endocrine effects of atrazine are Dr. James Simpkins from the University of Texas, Dr. Tony Plant from Pittsburgh, Bob Handa from Arizona.

And we've complemented that team with a group that are involved with the pharmaco PBPK modeling, principally, the folks at Hamner Institute. Harvey Clewell will be marking the presentation but Mel Andersen is also in the room and he was intimately involved in the creation of the first atrazine PBPK model while he was at the university in Colorado. And we also have Jerry Campbell who implemented the code for the PBPK model which we will illustrate today.

Finally, as part of the PBPK modelling exercise, we coupled it to a simulation program, and Dr. Bob Silken did all of that work. And so, he's in the room as well to answer detailed technical questions about how that simulation was conducted.

I'm going to start with a simple schematic that tries to capture the essence of what we are going to be discussing today. On the one hand, we want to have a brief discussion of some key factors relating to the selection of the point of departure, and for that, Dr. Simpkins and Dr. Plant will lead that discussion.

Largely speaking, we want to bring up the point, in fact, that we believe that pulsatile GnRH release is the more relevant endpoint for human risk-assessment. And we acknowledge and we recognize the difficulty of dong studies in that arena, and so the LH surge stands as a convenient endpoint for point of departure consideration. Dr. Plant will have that discussion with you.

The PBPK modelling, such as we've implemented it, permits us to take into account distributed dose versus bolus dose such as has been done in the rodent studies, age-dependent sensitivity, animal model sensitivity, functional outcomes; those are the topics we'll cover under point of departure discussions.

We then go backward and pick up the information that Dr. Hendley introduced the concept of residues in water are fluctuating dynamically over the time, and coupling that with a water intake record that permits one to enter those factors into a PBPK model.

Dr. Harvey Clewell will introduce the work we have done to develop that model and to characterize it for you so that you can see then when we go to apply the model for margins of exposure calculations, you will have some understanding of where that's coming from.

And I should just say that, in fact, we did take an initiative to commence cynomolgus study, a pharmacokinetics study. Effectively, that has begun in April and is still going on. We are not administering **14C**-atrazine. We're administering cold material and we are doing a rather comprehensive characterization of metabolites in plasma, urine, feces, cage-wash. We are trying to achieve the mass balance that people were envious of with the **14C**, and also some postulated metabolites that were introduced into the discussion just recently.

So our intent is to actually take the rodent model that we've developed, scale it to humans, take analogous data from the cynomolgus monkey and scale it to humans and see how we do so that there will be continuous development on this front.

I think, with that, I will stop and I'll turn the topic over to Dr. James Simpkins. Thank you.

DR. JAMES SIMPKINS: Okay. We need to go back to the first slide, please. I'd like to thank you for the opportunity to present -- Tony Plant and I will be very brief. We've identified five issues that we think are key for consideration in decisions about point of departure concentrations of atrazine. Those five features are listed here.

We will discuss four of those features. The fourth one that is developing animal less sensitive than the adult to atrazine. We fully agree with the EPA's position that the developing animal is not more sensitive than the adult and we'll have no more to say about that.

We will provide a summary of the presentation that Dr. Plant did in September of 2010, relative to the role of pulsatile LH secretion across species in comparison to the LH surge, the mechanism of which does not cross species well.

In addition to that, we will discuss every so briefly the concept of distributed dose, and that being more relevant to the manner in which humans are exposed to atrazine.

And we will show you pharmacological data, and then later in the presentation Dr. Clewell will show you kinetic data showing that distributed dose produces a remarkably different response as well as exposure than does bolus dosing with atrazine.

We will show you data that we have that functional endpoints do not appear to be affected by the atrazine-induced modest decline in LH surge suppression, and finally show you data that supports our opinion that the Long-Evans animal may not be the appropriate animal to look at because of the instability of its estrous cycle. With that, I will quickly turn this over to Dr. Plant and he will talk about comparison of pulsatile and surge LH secretion

DR. TONY PLANT: Thank you, Jim. So in September I did present some data to this panel, and basically, the conclusion from that talk was, understanding the way atrazine interacts with pulsatile LH secretion is more relevant to understanding or translating data on the rodent to the human.

Those slides are in your docket. I'm not going to go through those slides again. I'm just going to sort of hit the bullets for sake of time, and so I won't have time to go through any caveats which, of course, there are to any scientific discussion.

The first point I want to make is that, the pulsatile mode of secretion is a distinct mode of secretion versus the surge mode of secretion. So I think you are all familiar

with pulsatile LH secretion in the human female. This result from brief episodes of secretion of LH from the pituitary, results in a small increase in circulating LH levels, and these decay exponentially over a matter of 15, 30 minutes, 60 minutes.

Surge secretion, on the other hand, gives you this massive discharge of LH, which spans in the human female maybe two or three days. And these modes are regulated by different hypothalamic mechanisms.

If we look first at pulsatile LH secretion -- as we've talked -- and I think it's well recognized that this is driven by a corresponding pulsatile patent of GnRH release from the hypothalamus, and this occurs in both rats and humans. A neuro-mechanism, which we call a hypothalamic GnRH pulse generator, which even in 2011 is still somewhat of a black box that is responsible for this pulsatile GnRH release, that of course is present in both rat and human hypothalamus.

In both species there is an increase in the activity of the GnRH pulse generator as puberty is entered. However, I know that you're interested in lifestyle effects. And one difference I do want to point out between the rodent and the human is that, GnRH pulse generator activity in the human infant is robust, and this is again, a species difference with the rat. And that leads to gonadotropin secretion. In fact, in the infantile human male you have testicular testosterone secretion and elevated blood levels during human infancy. So that is a very different

endocrine environment from childhood and juvenile development in the human.

Now from what we know, the neuromechanisms that are responsible for GnRH pulse generator appear to be similar across mammalian species. So what you learn probably in a rat, what you learn in a sheep about the GnRH pulse generator is probably translatable to the human.

The other two points I want to make is that you all focus on ovulation in the LH surge, but pulsatile LH secretion together with that of FSH is absolutely critical for folliculogenesis. And if you don't have health follicle or health follicles you may have deficits in ovulation. You may have deficits in the corpus luteum. And pulsatile LH secretion also plays a major role in maintaining the corpus luteum and progesterone production in the human female.

So now, what about the surge mode of LH secretion? So, in the rat the LH surge is short. It's entrained by the light/dark cycle. It has a critical period. It fires on the afternoon of pro-estrous and it is sensitive to barbiturate.

In the human and primate on the other hand, as I mentioned, this is a protracted event. There is no critical period and it's photoperiod and barbiturate insensitive.

In both the human female and the rat, the LH surge is initiated by a positive feedback of estradiol, which is

produced by the Graafian follicle. It matures and results in increasing blood levels of estradiol, which talks to the hypothalamus in the pituitary. So, again, the ovarian signal is the same.

Now, a key side of this estradiol action in the rodent, in the rat, is in the rostral hypothalamus, and this is a major difference. In the human female, this side of the positive feedback action occurs at the level of pituitary. Now, the positive feedback action in the rat, essentially it opens the gate to the circadian signal in the rat, so this can now be relayed to the GnRH surge generator, which then in turn triggers the LH surge.

And interestingly, it appears the GnRH pulse generator during this LH surge is actually decelerated or arrested, and that's an important point. And again, it emphasizes the difference underlying the hypothalamic control of these two modes.

In the human female, on the other hand, the LH surge results by an interaction of GnRH pulses, pulsatile stimulation of the pituitary and this action of estradiol we call a positive feedback action to amplify the response to the pulsatile stimulation. And in the human female, there is no evidence for a GnRH surge. On the other hand, GnRH pulse generator activity is maintained throughout the surge in the human female.

And so, this difference in the hypothalamic control of the LH surge in the rat it involves a GnRH surge generator and a suppression, it appears, or a block of the GnRH pulse

generator; whereas in the human female there is no GnRH surge generator. All you have is a GnRH pulse generator, which is maintained, and the action of estradiol is at the pituitary.

Because of this key hypothalamic difference, I think if you want to understand the mechanism of action of atrazine or anything else using a rodent model, it's okay if you are translating the effects on GnRH pulse generator. But I think you're going to run into trouble if you're going to translate effects that you study in the rat on LH surges to the human female.

DR. JAMES SIMPKINS: Despite our opinion that the pre-ovulatory LH surge in rodents is not relevant to non-cancer endpoints in humans, the pre-ovulatory LH surge can and has been used to gain information about mode of administration of atrazine, duration of administration and functional outcomes of presumed reductions in LH.

I would like to point out to you that the mode of administration of atrazine has pharmacological consequences when looking at the LH surge. A 4-day atrazine administration by a distributed dose in Sprague-Dawley rats does not reduce pre-ovulatory LH surge if you administer the same or equivalent doses by bolus. You do get a high dose of atrazine induced reduction in LH secretion, so there is a remarkable pharmacological difference there.

If one does chronic distributed dose administration in Sprague-Dawley rats, six months exposure to atrazine, in

the neuroendocrine aging animal results in prolonged estrous, and it has been agreed that that is the mechanism by which tumors in Sprague-Dawley animals are induced. In Fischer 344 rats, the same distributed dose of atrazine has no effect on LH secretion. I will quickly show you datasets relative to that.

This is a 4-day bolus dosing with atrazine in Sprague-Dawley rats looking at the pre-ovulatory LH surge, so this is the afternoon of the day that the animal shows an LH peak. These are the doses of atrazine that were administered by bolus (gavage) once per day, and these are the resulting LH secretions. As you can see, we can dose up to 50 milligrams per kilograms, which causes an approximate 50 percent reduction in peak LH secretion with bolus dosing. In contrast to that, if we do feeding at roughly equivalent atrazine doses for the same 4-day period in Sprague-Dawley animals and then monitor their pre-ovulatory LH surge, we see no effect of atrazine treatment on the pre-ovulatory LH surge. And again, Harvey Clewell will present pharmacokinetics data that we think is relevant to this difference.

This is six months distributed dose atrazine in Sprague-Dawley and Fischer 344 rats. In Sprague-Dawley rats, high-dose atrazine feeding four/six months markedly blunts the pre-ovulatory LH surge; whereas in Fischer 344 rats, a similar feeding paradigm is without effect. So there's strained difference in the response to feeding.

In addition to that, we asked the question whether or not the modest LH suppression achieved with bolus (gavage)

dosing -- this is in Sprague-Dawley or Long-Evans animals over broad dose ranges -- or distributed dose feeding of atrazine over these dose ranges, which are equivalent to the higher doses given by bolus administration, had effects on outcomes that would be expected if LH is suppressed.

So we looked at percent of animals that became pregnant when exposed to a male number of corpora lutea that were formed, number of implantation sites, number of fetuses. And the data here are expressed with the zero at 100 percent. As you can see in both Sprague-Dawley and Long-Evans animals, there is no effect on any of these outcomes parameters of gavaging or feeding any of the doses of atrazine. So we are not entirely sure there is a functional outcome of the modest LH suppression that is achieved.

I will not present the development data because we fully agree with EPA's position. And then the final issue we addressed was the animal that's been proposed for the point of departure determination, and that is the young adult female Long-Evans rat, and we have a bit of a problem with that for a couple of reasons.

In a CODAR study recently submitted to the EPA, greater than half of the Long-Evans animals in the study, before they were treated with atrazine, failed to show normal 4- to 5-day estrous cycles. And it has been known for some time that treatments with high doses of atrazine by gavage results in a further disruption of estrous cycles in Long-Evans animals.

So we have an animal we think that is not stable for doing these kinds of studies. These are the data -- and I apologize for the small size. I'll point to what I think is the relevant issues. These are the Long-Evans animals, and again, these are prior to exposure to atrazine. 38 percent of the Long-Evans animals; 306 total animals, 38 percent had normal 4-day estrous cycles. 62 percent had abnormalities of one or another type. In contrast to that, the Sprague-Dawley animals, about 60 percent of the animals had normal estrous cycles. So we think the Long-Evans rat has an unstable estrous cycle.

So to summarize, we do believe that Pulsatile LH secretion is more relevant to do non-cancer endpoint assessments than the LH surge, more relevant to humans. Distributed dosing is more like the exposure to which the vast majority of people are exposed to atrazine and it has not been routinely studies. In fact, distributed dosing is not being used in setting the point of departure. We are failing to find the functional outcome of the suppression of LH that occurs with bolus dosing of atrazine. Developing animals are indeed not more sensitive than adults to atrazine. And finally, Long-Evans animals, we think, have a very unstable estrous cycle. And with that, I'll stop.

DR. DANIEL SCHLENK: Okay. Any questions for the panel? Jan, Dr. Chambers?

DR. JANICE CHAMBERS: Dr. Simpkins, do you have a reason to suggest for the difference between the Fischers and the Sprague-Dawley rats in their responses?

DR. JAMES SIMPKINS: Responses to functional outcomes or estrous cycle?

DR. JANICE CHAMBERS: You showed a graph where the Sprague-Dawleys responded and the Fischer 344 did not. Is there any reason that you have for the difference there?

DR. JAMES SIMPKINS: Well, what we know, this study is essentially a combination of chronic exposure to atrazine while animals are aging. And so, we know for example, in Sprague-Dawleys that by the time of this LH observation, many of those animals have gone from normal estrous cycles to persistent estrous. That is, they are not able to mount a pre-ovulatory type LH surge probably because the neuromechanism is no longer working sufficiently.

We also know that in the Fischer 344 rats, those animals can age to as long as two years, and in our hands, have absolutely normally regulation of LH secretion. The problem they experienced during their aging is they cannot suppress prolactin secretion so they suffer from a hyperprolactinemia, that causes retention of corpus luteum, and they go into a persistent diestrus state.

But their regulation of LH secretion is preserved even into very late life, and we think that's the explanation for why atrazine is not adversely affecting LH secretion in the Fischer. They just have a more robust regulatory

system for LH secretion, whereas the Sprague-Dawley animal is actually breaking down over their regulation of LH secretion, is aging, and then you superimpose the atrazine insult on that, and that happens earlier in their life and so we detect it by the six months of feeding.

DR. JAMES MCMANAMAN: So let me get this straight. You believe that the LH surge in the rat is totally due to a hypothalamic effect, and the human, it would be due to a pituitary effect, and that atrazine, you believe, acts solely at the level of the hypothalamus, therefore would not effect the LH surge in the human because that is through the pituitary; is that correct?

DR. TONY PLANT: Yeah. In the rat, clearly it's a hypothalamic action of estradiol which, together with the circadian cycle, triggers the GnRH surge.

In the human, you have a hypothalamic component. You have to have GnRH pulsatility. Without that, you will have amenorary cyclicity. So it's a combination of a pulsatile hypothalamic input and an effect of estrogen at the level of the pituitary.

Now, we are told though -- or I'm told -- I mean, I'm not an expert on the rat estrous cycle, but that the effect of atrazine is hypothalamic, is a brain effect. I think that was quoted recently. And what effects does atrazine or any of these compounds have on the GnRH pulse generator is much less clear, but there is no reason why it shouldn't have an action. That's what should be investigated for relevance to the human.

DR. JAMES SIMPKINS: If I can add, Dr. Handa, do you want to respond to that question?

DR. ROBERT HANDA: We've done studies looking at the pulse generator in the rat and atrazine has an effect at very high doses in lengthening the pulse period, but that doesn't occur until 200 milligrams per kilograms in our hands. We have also looked at pituitary effects of atrazine and we've found no effects of atrazine on pituitary sensitivity to GnRH even at very high levels.

DR. JAMES MCMANAMAN: I just want to clarify why it's the pulsatility versus the surge component that you thought was the most relevant, and I think that you've answered the question but I just want to double check on that.

DR. TONY PLANT: Well, all the evidence for the human female suggests there is not a GnRH surge. Now that's indirect. But if you don't have a GnRH surge then you can't translate the findings in the rodent where the LH surge is driven by a GnRH surge to the human female.

DR. KEVIN O'BYRNE: I'm delighted to see the distribution dosing. It took a long time for that to come. But why wasn't it put in the water, the atrazine?

DR. CHARLES BRECKENRIDGE: The problem of achieving a high dose in water is the water solubility limit of the compound, so it can put approximately 20 part per million. And so, you cannot actually get to an effective dose at a solubility limit. So that is essentially the reason that we needed

to go higher. And by incorporating it in the feed, you can actually get a distributed dose and have the animal consume considerably more.

DR. KEVIN O'BYRNE: This separates toxicology from physiology.

DR. SUSAN AKANA: Could you remind me with the distributed drug dosing whether all the animals voluntarily ate the diet with the atrazine?

DR. CHARLES BRECKENRIDGE: We've done many studies with feeding of atrazine to animals, and there is an initial body weight suppressive effect of the compound and that's coupled with a food intake reduction. There is a reasonably rapid recovery of that food intake reduction that happens, and it stays stable throughout. There is a possibility that those effects on body weight and food intake are actually mediated through some direct action in the hypothalamus, but it seems like the principle interest was on the endocrine response. We don't understand that transient change, but we note it does occur regularly, and that happens even if you give it in bolus dose administration.

DR. KATHERINE ROBY: And what will be known about a change or differential absorption given in feed versus either water or a bolus?

DR. CHARLES BRECKENRIDGE: We're exploring that question in our monkey study, effectively. We're interested not, obviously, in feed, so we're trying to move away from that. Typically in the bolus dose scenario, people use

five percent or one percent CMC suspension, and effectively, one would anticipate at high concentrations that could modify absorptions. We have evidence that that actually is occurring.

In a second phase of our primate study, we took atrazine and put it in five percent ethanol water and we were able to achieve a hundred part per million under those circumstances and be able to compare the pharmacokinetics of aqueous mediated formulation as apposed to a CMC. It's our intent to try to do that same study in the monkey with only 20 part per million in water.

You start to get to limits of detection issues in terms of picking up metabolites in plasma, but we think we can actually achieve that by virtue of the volume of water we might administer to those animals, and the highest achievable water concentration. And by that way, we can hope to get around the question of absorption and presumably what we observe when atrazine is in water, is that kinetics of absorption are rapid. And we suspect that we eliminate the potential for gut metabolism, which can further confound the question, which I think I had come up at some of the previous SAPs. So that's our strategy to try to understand the impact of the vehicle on kinetics of absorption.

DR. JAMES MCMANAMAN: So just let me clarify because I think I'm hearing maybe different things. Syngenta's position is that atrazine does have an effect on the hypothalamus or does not have an effect on the hypothalamus?

DR. CHARLES BRECKENRIDGE: You know, you're asking a very difficult question as to exactly where the molecular target is, and we don't know where the molecular target is. We believe it is in the hypothalamus for probably the pulse generator and the LH surge, but we have been pursuing that over the years and we still do not have that knowledge.

DR. JAMES MCMANAMAN: A follow-up on that then: So the weight loss effects, you wouldn't attribute that to an LH effect, would you?

DR. CHARLES BRECKENRIDGE: No, I wouldn't, but I wouldn't discount the possibility that it is somehow affecting food-intake centers in the brain. I mean, that's always a conceivable outcome. It could also reflect just general toxicity.

DR. JAMES MCMANAMAN: So, if it is affecting the food intake, the food regulatory centers and the hypothalamus, then perhaps by focusing solely on the LH, we might be missing an important effect of atrazine.

DR. CHARLES BRECKENRIDGE: I imagine the simple answer to that is the dose is whereby the food intake effect disappear or are substantially higher than the doses, were the LH effects are observed. So to that extent, the agency's choice of the LH surge is protected.

DR. JAMES MCMANAMAN: So in regards to the neonates whereas the LH may not be anymore sensitive or less sensitive -- any difference in sensitivity than the adults -- that might

not be true for food intake. For instance, it's known that the food regulatory mechanisms develop, at least in rodents, earlier in life, and it develops through the hypothalamus, so that might be a key thing to be concerned about.

DR. CHARLES BRECKENRIDGE: We have probably extensive data on reproductive measurements of food intake in neonatal pups. And again, I think then the point of reference becomes evidence of pulse generation change in those developing animals. And most people believe that vaginal opening and preputial separation are coupled to the operation of a pulse generator. We see effects on that pulse generator in the range of 10 to 15 milligrams per kilograms. Food intake effects, I believe, tend to occur at much higher doses. So I think that could be looked at carefully, just to explore that question. Thank you.

DR. TONY PLANT: Just to comment on the infant and the maturity of the hypothalamus; I mean, the infant primate, the GnRH pulse generator is robust, it's functioning. And this is a fundamental difference in the ontogeny of the reproductive axis between a rodent and a primate.

DR. KEVIN O'BYRNE: Could you possibly comment on the distribution of atrazine in the various tissues in rodents and primates?

DR. CHARLES BRECKENRIDGE: Thanks for that question. I think it's going to be answered by the next speaker, and so, perhaps that would be a nice segway into our next speaker.

DR. DANIEL SCHLENK: Thanks. We'll move ahead with Dr. Clewell, is it?

DR. HARVEY CLEWELL: Okay. Thank you. In the September meeting, David Kim who was with Syngenta at the time presented a number of pharmacokinetics studies that had been performed by Syngenta and promised that at the next meeting he would present a PBPK model based on those data. However, David is now in Africa. He lives and works in Africa doing completely different work, and so I'll be presenting in his place.

It is actually an effort that has been carried out between several of us at the Hamner, Mel Andersen, Jerry Campbell and myself along with David and Charles and others at Syngenta to try to design studies that are informative for a model and then develop the model on that basis.

So, I'm going to briefly talk about the questions relating to the nature of a pharmacokinetic model that could be used to help inform the relationship between animal and human dosimetry for atrazine in support of risk characterizations, and then I'll actually go through the steps in the development of the refined model.

It's called a refined model because an initial atrazine model was developed in Colorado State by Tammy McMullin and Mel Andersen and others, and we began with that as our starting point. It was a rat model. We have expanded it to be also a model for monkey and human, and I'll just take you through those steps. And then at the end, I'll very briefly mention how this model can be used and has

been used by others to evaluate margins of internal exposure.

So first of all, comparing what seems to be called EPA's simplified model; the total radioactivity-based estimates of pharmacokinetics with a PBPK approach. I've spent many years trying to justify why I go through the trouble of developing PBPK models when you could use a compartmental model instead, so I'll try to kind of take you along on that.

This slide shows the known metabolites of atrazine. I've put red boxes around atrazine and the metabolites that are considered to be active in the sense they still have the chlorine in the key position so that they are part of the common mechanism group, if you will.

Those are the desethyl, desisopropyl, initial metabolites which are subsequently metabolised to DACT. All the other unboxed metabolites are subsequent to glutathione conjugation which replaces the chlorine with the glutathione conjugate and are considered, generally, considered inactive.

The box in the top on the left, which is called other oxidative metabolites? -- is because Ernie Hodgson's lab recently looked at atrazine metabolism in microsomes and identified a couple of oxidative metabolites of atrazine apart from the three that had been known historically. But those have not yet been identified in vivo. So we do not actually know if, quantitatively, they are produced in

vivo in amounts that are significant, and I'll show you later why I don't think so.

Finally, I want to emphasize this dotted arrow over here from DACT to protein adducts. One of the things that we were reminded of by Mel Andersen, who remembers well because he was involved at the time, is that Colorado State looked carefully at the impact of protein adducts of DACT on the concentration of radioactivity in the blood and plasmas, in the red cell in plasmas, so I'll show you that data.

This shows studies that were done in actually Tammy Mullin's first paper, looking at red cell binding, and they determined that -- and I think it's been mentioned earlier that as much as two percent of the oral dose binds to the hemoglobin in the red cells of the rat. The red cells in the rat are different from those of other species in having a cysteine that is very open to adduction.

DACT does react with that cysteine-125 and this is, as I said, an example, as dimethyl arsenic also binds in this way to rat red cells. And so, that is a consideration for the disposition. But as long as you are measuring plasma samples, then it doesn't really matter in terms of the dose metric measurements. But a similar effect occurs in the plasma. DACT was also shown by Colorado State to react with cysteine and albumin and in similar fashion to that with hemoglobin. And they actually showed nice proof of it. It turns out that the half-life of the adduct is consistent with the turnover of albumin in rat plasma, so that's undoubtedly what you're actually seeing.

Tammy McMullin did a nice simulation when she took Timchalk's data on the total **14C** in the plasma and then ran the model to show what the total chlorotriazine concentrations would be. And you can see that the tail is due to the bound material that DACT bound to the plasma element.

You can see that is responsible for the terminal half-life, which is on the order of 60 hours, as I recall. And that terminal half-life does not occur for the free compounds. DACT has got the longest half-life that's on the order of six or eight hours, as I recall.

So that's kind of important as it turns out for understanding what would be a good dose metric. And so, looking at the **14C** atrazine-equivalence in plasma, it's been pointed out that they account for total mass of atrazine derived metabolites, atrazine derived metabolites. So that's a blessing and a curse.

The problem is that ^{14}C includes compounds that are active and compounds that are inactive. It's not necessarily conservative to lump everything in to a measurement. In fact, in order for it to be an accurate basis for cross-species extrapolation, it would have to be true that there was a similar fraction of relationship of active to inactive metabolites in both species. And so, it could be conservative or anti-conservative depending on whether the glutathione pathway versus the oxidative pathways had different relationships, which they very often do.

The terminal half-life has the disadvantage that it is driven actually by the covalent binding in the plasma, which means that it's really reflecting albumin turnover in the plasma, and so it is not a good metric for target tissue exposure to active compounds.

Another disadvantage is that there is no human **14C** data. I like to say that risk-assessment is a ratio business. If you want to relate human toxicity to animal studies, you got to have information in both. So if you don't have human **14C** data which, of course, is hard to collect, then you have to use default allometric scaling. And I myself am not against default allometric scaling. This is what I would've done in this situation.

I like to use the term chemical-specific adjustment factor which is what IPCS likes to call these kinds of cross-species relationship factors. And so the animal to human kinetic relationship using default allometric scaling, which is body weight to the three-quarters, is on the order of three, and that's what EPA used.

And they say in their report that, basically, for the same applied dose, then you would have roughly a three-fold higher steady state plasma level predicted in a human compared to the rat.

We developed the PBPK model basically to get away from the problem of a dose measure where you don't know to what extent it's based on active materials. And so, what we worried about doing was accounting for the majority or the active species. And one of the things we tried to do is

determine are the four that have traditionally been listed as the active species, do they seem to actually account for the metabolism of atrazine.

It's difficult to assure that kind of mass balance because of the complexity of the glutathione conjugates and down-stream metabolites were captured at 16 conjugates. I mean it is very messy, so it's hard to look for all those things.

But actually, Syngenta is very aggressive in trying to do that study in the monkey and Mel and I are both really pleased that -- you know, we basically have asked for an awful lot here and they are going to try to do a very, very thorough study to identify metabolites in the monkey.

There is data in both rat and human. And one of the things we in risk-assessment have to deal with more and more is that the chemicals that were studied to death in the human are being taken care of and we are stuck now with the chemicals where there is not a lot of human data. And so you cannot calibrate a human model based on a number of in vivo studies; you actually have to use more indirect ways of doing it.

But there is actually a human study where the half-life for DACT, which is almost all of the exposure was measured, and we have used the model that I'll describe to you to look at the cross-species equivalence. What we found is that it indicates similar internal exposures at roughly the same ingested dose rate. So the CSAF is about

1.5. In other words, the human has about 50 percent greater plasma level at the same dose.

So what we included in the model were, of course, atrazine and its oxidative chlorometabolites, so that's desethyl, desisopropyl and DACT. Then we are now trying to improve our description of the conjugation metabolites, the glutathione conjugates and downstream metabolites in order to be able to use the model to evaluate mass balance. And we are adding description of the adduction to plasma proteins so that we can actually do some simulations to compare **14C**-based dosimetry with active compound-based dosimetry under different exposure situations.

So now I'm going to go through a description of the model that we've developed. We had a hepatic metabolism from in vitro studies in the rat, human, and now the monkey. We had Jeff Fisher when he was at Georgia determine the partition coefficients in vitro. There is not a very high distribution of atrazine of its metabolites into tissues. It's a fairly even distribution around the body.

We have beautiful datasets on the kinetic differences of bolus dose and distributed dietary dosing in the rat. There are also studies in the monkey that are going to look at different vehicles, comparing water and ethanol and CMC slurry, as Charles mentioned. The monkey data, in particular, is going to, as I said, have very extensive characterization of the various metabolites.

This shows the in vitro data that was a study that David Kim designed. You can see atrazine, DEA, DIA and then

DACT. The red lines are the rat. The blue curves are the human. There is a difference in the relative split between the two intermediate metabolites across the species. Two different concentrations were used. We used this data to estimate the metabolic parameters, a nice spacing of the data across the four-hour period of the study.

So the description that we used, we actually had to model that in vitro data, so we modelled the in vitro system, including competitive metabolic inhibition, which had been described earlier at Colorado State. And Jerry Campbell did this work and he actually had to account for the loss in viability of the rat hepatocytes during the assay because four hours is really kind of long to have a hepatocyte functioning.

So, by adjusting for the viability of the hepatocytes, we were able to actually obtain good data even on the DACT, which takes a while to appear. And so, then we used affinity constants from Tammy McMullin's study which were at higher concentrations and so were better for determining a KM. And then we re-estimated the Vmax's for the conversion of atrazine and the metabolites.

This shows the model simulation of the rat hepatocyte data. You can see it correctly describes the loss of atrazine, the production of the DIA and DEA and their subsequent conversion into DACT.

I really want to emphasize the fact that -- so the model has no compartment per other metabolites. So this

demonstrates that there is a mass balance between the loss of atrazine and the production of DIA, DEA and DACT in this in vitro system. That's really all that was going on. There's not a mystery metabolite that everybody has missed.

This shows the same analysis with the human data and, again, you can see that we were able to coherently describe the conversion of atrazine to DIA, DEA and their subsequent conversion to DACT quantitatively with the model.

So then, that gave us our in vitro metabolism. We had our partition coefficient physiological parameters, and then this is the study that was performed for actually evaluating the model's prediction of in vivo kinetics. There was three different dietary concentrations, three different gavage doses and a very, very high rate of sampling that actually required parallel groups because you cannot actually sample animals that frequently, so two groups were used to go back and forth and get a very dense spacing for the data.

Four-day study; you can see here a pulse each day of atrazine for the gavage in red, and it has broaden peaks for DACT because there is longer half-life. You can see for the distributed dietary dosing is the blue lines, and it's a much flatter profile. You can see here the DACT comes up to about half of the peak value.

You can't see the Y axis values, but I can tell you that DACT accounts for almost all of the exposure. It is very

rapid metabolism of atrazine and the intermediate metabolite is 2 DACTs and then DACT has a longer half-life.

You saw this LH surge data before. What I wanted to point out was that it is interesting to see that you have an apparently greater potency for gavage than for diet. And if you look at the kinetic time courses for those two administrations, you can see very high peaks exposures, particularly looking at the DACT which, as I say, is most of the exposure. And Colorado State shows the DACT is roughly equal potent with atrazine for the LH effects.

So the peak values with the gavage study are roughly a factor of two greater than for the dietary study, even though the area under the curves, are similar. So this suggests a nonlinear relationship between the kinetic of target tissue dosimetry and the nature of the response; actually suggest perhaps time above a critical concentration might be a good metric. But one of the things we like to do with the kinetic data is to try to get a good idea of the nature of the dynamic relationship.

So, just to review, we took the PBPK model of Tammy McMullin and added physiological parameters for the monkey and the human from the literature, added additional target tissue compartments, used the metabolic rates from our in vitro modelling and partition coefficients from Jeff Fisher, and then we simplified the description of oral uptake because we were modelling lower doses of administration where the kinetics of oral uptake was not as complicated and it makes for a simpler description.

This is the model. It looks complicated because you have four different compounds you are tracking simultaneously, which are interconverted by metabolism in the liver. The oral uptake was modeled as a slurry portion and a soluble portion. Below about 20 milligrams per kilogram, I think it is, that it all ends up being just in the soluble dose. So for human exposures, for example, it's all in the soluble compartment. And then there's also glutathione conjugation in the liver.

This shows the predictions of the in vivo data. In this case, this is the single dose data. We used this data to estimate the oral uptake parameters. So we had the metabolism petitioning disposition, was already determined from in vitro studies. The only thing we could not do in vitro was oral uptake. So we estimated the rate of oral uptake for atrazine and its DIA and DEA. And we were able to get a nice reproduction with the model of the time course for the four materials. So this is in vivo then for a single gavage.

Now, what we did then was to use that model for the 4-day administration data, a much richer dataset. And you can see DACT, as I said, is the major contributor, and the model does a beautiful job of reproducing the time course for DACT in the rats over the 4-day exposure period.

What I was really impressed by was, however, it was the dietary administration. When they did the study they actually measured the food ingestion during the dark and light cycle so that we could input that into the model

which produces the diurnal cycles of DACT concentration which perfectly mimics the data. As you can see, that wave effect is because each day the animals are eating more at night than they are during the day.

So this is extrapolation; it's not root to root, I guess. It's administration form to administration form from gavage to a dietary. No parameters in the model were changed in order to predict the dietary as opposed to the gavage. We are not done with the model. In particular, we're really focusing on the monkey now, but as I had mentioned, we are going to incorporate plasma protein binding. We are doing more work on the formation of glutathione conjugate in vitro and verifying partition coefficients that were done in vitro with in vivo tissue disposition data just to confirm them.

Then with the monkey study, we will be looking particularly at the glutathione conjugates and mercapturates, and the developing data by which we can estimate with the mild urinary and fecal elimination rates and try to calculate with the model an in vivo metabolite mass balance.

In particular, we will be -- we -- I won't be doing any of the work on this study but I'll be modeling it later. We will be looking for the newly postulated oxidative metabolites from Ernie Hodgson's lab to see whether they are produced in any quantity in vivo.

And then, the major work that we have ahead of us once we finish with this monkey elaboration is to do a model-sensitivity uncertainty analysis to characterize the

propagation of the uncertainty from the model inputs to the prediction of dose metrics for the animal and the human.

Finally, what we're going to do with this model -- so we have a model for the rat, monkey and human. The monkey really is to help us define the human model with a more appropriate surrogate than the rat, since you cannot collect as much data in the human as you can in the monkey or rat.

We have already had an initial evaluation of this model by Battelle, Pacific Northwest Laboratories. We asked them to follow the new World Health Organization's guidance on evaluation of PBPK models for risk assessment. They actually are the contractor for EPA NCEA for evaluation of PBPK models so they know what they're doing. They concluded that the model was credible, reliable and applicable for this. That it is "fit for purpose" is another way of putting it. Everybody has their favorite terms for just saying it works.

We are still improving the model, but I think that right now I am comfortable that the model accounts for the active forms of atrazine and its metabolites and that that is a better way to do the cross-species dosimetry. What you'll hear about next is the model is used to predict human plasmas total chlorotriazines, the four compounds, resulting from drinking water exposures in order to get margin of exposure for effects in the rat studies.

DR. DANIEL SCHLENK: Okay. Thank you, Dr. Clewell. Let's go ahead and address questions of the model right now because I think we're going to be getting into more of the risk-assessment component after this. So let's go ahead and do that; yeah, so, Dr. Greenwood?

DR. RICHARD GREENWOOD: If you look at slide 30 where you were looking at the area under the curve for the two, I really do not believe that the area under the gavage curve is the same as the area under the distributed dosing curve. Have you actually measured them or is it just --

DR. HARVEY CLEWELL: Oh, yes; we characterized those. I don't actually remember. Frankly, I don't pay much attention to the non-model-based comparisons.

DR. RICHARD GREENWOOD: I would say it is at least double. You have a look at that. I have looked at DIA, and if you take one of those peaks and then you lay that flat, two of those peaks, you're at least going to cover the blue lines. So if you are thinking in terms of the area under the curve, I suggest that that is something that really ought to be checked. Before you make a statement like that you really ought to measure them. I don't believe, just looking at those, that that's going to be the case, that they're similar.

DR. HARVEY CLEWELL: Okay.

DR. RICHARD GREENWOOD: So I think that really is something that needs checking. The other thing that hit me, because I was really impressed with the fit, on slide 35 I was

really impressed with the fits there for the dietary exposure. I think those are excellent, but at the end you are actually under-predicting quite markedly, not just for DACT; I could understand that because I think well maybe it is binding, as you said. I think that depends on turnover of albumin. But what about DIA and DEA, because they are similarly over-predicted at longer times by the model? The fit is wonderful up to there. Have you any explanation?

DR. HARVEY CLEWELL: Actually, I forgot to point out what that horizontal line in each of these plots is. That's the limit of quantification. So actually we are fighting with the question of is that a real measurement or not. We see this, and very often that we get data that is below or in the vicinity of the limited quantification and then we're really not sure what it's meaning is. So in the case of DACT, this would not be because of the binding to albumin because this is free DACT that is being measured, and so the covalently bound would not be included, so even in that case it's not clear what that reflects. Charles, did you want to say something?

DR. CHARLES BRECKENRIDGE: Yes. I think that explains a lot. It's very difficult to put a number below the level of quantification. Thank you.

DR. DANIEL SCHLENK: I have a quick question for you. On slide 26 and 27, the rat hepatocyte and human hepatocyte data in your statement there is that atrazine disappearance completely accounted for by production of the three phase 1 metabolites. If you are doing hepatocytes wouldn't you

expect to see the glutathione adduct as well being produced in that?

DR. HARVEY CLEWELL: I believe it's a matter of rate. The oxidative metabolism happens very fast.

DR. DANIEL SCHLENK: So would the GST though. I mean, you should get the primary metabolite off of atrazine. You should get that adduct immediately.

DR. HARVEY CLEWELL: You can see the slight decrease in DACT, later on, most likely reflects glutathione conjugation. The rate was rather slow.

DR. DANIEL SCHLENK: Well, again, if you go back to metabolic scheme, for example, if you go straight down from your atrazine to your first GST adduct, that doesn't require any oxidative metabolism and that should take place immediately. If I understand it, I am pretty sure that is the primary metabolite of atrazine metabolism, isn't it?

DR. HARVEY CLEWELL: No, I don't believe so, actually. When you have high affinity, oxidative metabolism like this, the concentrations of the compound in the liver stay very low, and the glutathione pathway generally is actually quite slow.

At very high concentrations, you would expect, as you saturate the oxidative metabolism, you would expect more production by the glutathione pathway. But we are trying to characterise the glutathione metabolites better now, as I mentioned. It does appear that most of it is DACT,

glutathione conjugates, which makes sense because DACT isn't subsequently oxidatively metabolized, so it circulates while it's being conjugated.

And we will be running cytosol experiments looking especially for rates or reaction with glutathione, both with and without GST. So we will be in a better position to address that. Unfortunately, there really has only been identification data and really not quantitative characterization of which are the key glutathione conjugate metabolites.

DR. DANIEL SCHLENK: Yes. It just seems a bit overblown that you say it completely accounted for production. That just doesn't jive with what you would expect to see in hepatocytes, I guess, that's my whole point.

DR. CHARLES BRECKENRIDGE: Dr. Schlenk, could I perhaps try to address that question? We are doing cytosolic fraction studies with hepatocytes so that we can remove oxidative metabolism from the picture altogether. Like Dr. Clewell mentioned, we can actually then evaluate the rates of formation there. So that's the first line of attack.

We also note that, as you cryofreeze hepatocytes for the purposes of processing, you actually deplete the GSH, and perhaps that is maybe complementing to the fact of not observing. The hepatocytes are obviously taken from whole animals and provided to the lab for assessment and they have to be provided in that manner. So it perhaps is a component of depletion of GSH that could be happening.

So we're trying to address the rate by actually looking at freshly harvested livers and looking at the cytosolic fractions.

DR. DANIEL SCHLENK: Great; thanks. Dr. Chambers?

DR. JANICE CHAMBERS: Two questions, Dr. Clewell. Do you consider any of the protein binding to be reversible or is it all covalent?

DR. HARVEY CLEWELL: That would probably be a better question for Mel Andersen, if we could get him to come up here.

DR. MEL ANDERSEN: Mel Anderson from the Hamner Institutes in North Carolina. The binding that's been observed in the red cells and in the plasma is covalent. That has been actually evaluated by direct evaluation of the adducts. We don't have any evidence. I have done kinetic models for things that bind strongly. I have no evidence with atrazine that any of this is strongly bound, but reversible.

DR. JANICE CHAMBERS: Thank you. And the second question is, do you have enough information about the partition coefficients to estimate how much is getting into the hypothalamus or the parts of the brain?

DR. MEL ANDERSEN: At this point, it appears that there is a fairly equal distribution of atrazine throughout the tissues, and it has a partition of about one. What you see in the blood is about what you see in the tissues. That's true of all the tissues that have been looked at.

DR. CHARLES BRECKENRIDGE: I'd like to add to that. Effectively in the study where we did the pharmacokinetic characterization of atrazine, those same animals provided us with tissue samples on the last blood collection, so we have the pituitary hypothalamus adrenal gland and we're in the process of analyzing for the amount of atrazine, DEA, DIA and DACT, in those tissues, and we already know what the plasma concentrations are. So that was what we meant by in vivo characterization of partitioning will verify the components that were derived from an in vitro modeling system.

DR. JAMES MCMANAMAN: Yes, two quick questions. One is that you show human -- and this is on slide 24 -- human and rats cells. What cells were those? I mean, they were hepatocyte, but presumably liver-derived somehow, or are they cell lines, or what exactly were they?

DR. CHARLES BRECKENRIDGE: These were human donor samples that we obtained there. I think there were three different donors, so they are real obtained, fresh and cryopreserved.

DR. JAMES MCMANAMAN: The second question is, do you see any other protein adducts, for instance, with these cells? Your primary ones may be albumin or some of the others, but are there protein adducts within the cell themselves?

DR. MEL ANDERSEN: In the original work that was done at Colorado State to pay for that was published in 2003, we looked at these terminal half-lives that you see in the

plasma, as well as we saw them existing in tissues. It appears that atrazine, at a slow rate, cannot react with a variety of protein cysteines so you get can some level of adduction.

So after the identification of persistence of binding in the red cells and in the plasma and in tissues, another student at Colorado State, Greg Dooley -- so Tammy McMullin did her PhD at Colorado State with me and Bob Handa, and Greg Dooley with John Tessari and others in the Department of Biochemistry. He actually evaluated directly the binding, both the hemoglobin to albumin. And then they looked at, I believe it was hypothalamic, the hypothalamic binding. They found a variety of binding sites in the hypothalamus all associated with cysteine adducts being formed in the tissue.

DR. JAMES MCMANAMAN: So they were associated with cysteine adducts, but did they localize where in the cell those cysteine adducts were? Were they in the endoplasmic reticulum? Were they in the mitochondria? Where? Do you know that information?

DR. MEL ANDERSEN: It wasn't done by isolation within the cell; it was done by identification of the proteins to which the adducts had formed. And they were not proteins that were expected to be found in any one particular sub-cellular compartment but more uniformly distributed.

DR. SUSAN AKANA: Short question. I am back on slide 30. These animals - were these the first four doses that they saw either by gavage or the four nights of feeding?

DR. CHARLES BRECKENRIDGE: Are you referring to the effects slide of that slide?

DR. SUSAN AKANA: Well, what I wanted to know is had they been maintained on the gavage and the diet for a number of days before the sampling started or were these the very first four doses that you are characterising?

DR. CHARLES BRECKENRIDGE: Again, on the right hand panel, they represent the progression of doses for the animal. So that animal went on study for blood collection, it was dosed everyday for four days. And if it was scheduled to have a blood collection in the latter part of the study, then that was after having had four days of dosing.

You cannot bleed the animals continuously through so you have to have sub-fractions of animals for the purposes of blood collection. The LH part are the animals that were treated for four successive days and look for LH surge suppression at that point in time.

DR. SUSAN AKANA: My question is, were they being given the atrazine when there is a known acute effect on food intake and body weight?

DR. CHARLES BRECKENRIDGE: So on the first day of administration, as indicated by the plasma data, that would be the first day they would have received the compound. They would have not received the compound prior to that, they're being gavaged. The other animals are being fed it. We actually were trying to achieve equal

doses by means of gavage versus feeding, and we had to estimate the likelihood that they would reduce their food intake and we increased the food concentration appropriately.

We slightly undershot -- and you can see that 40 mg per kg was an attempt to achieve a 50 mg per kg oral gavage dose, and that is attributed to the fact that we miss-guessed how much we needed to put in the feed, given the reduction in food consumption that was occurring as a result of the dosing. So it was our best attempt to achieve equivalence of dose. We were slightly under in that particular case.

DR. DANIEL SCHLENK: Okay. If there are quick questions we can go. My plan is that we would like to finish. We have one more presentation to go, and then if you guys are available tomorrow, which I am sure you are, that we could begin to tomorrow morning with questions as well because obviously we've had a lot to ponder. So if you can hold it to tomorrow that would be great. If you'd rather go it now we can go ahead. Okay. Go ahead, Dr. Greenwood.

DR. RICHARD GREENWOOD: Going back to slide 30, an explanation for the sort of behaviour if those areas really are similar, is that it's not total areas under the curve but area under the curve over some sort of critical threshold. Is there any evidence that there is a critical threshold for this action of atrazine?

DR. CHARLES BRECKENRIDGE: It's a very attractive hypothesis. We haven't yet figured out how to test that hypothesis. You move from correlation to causation, and that, as you

know, is always a difficulty and we haven't yet got there. Thank you.

DR. DANIEL SCHLENK: Okay. So let's go ahead. Again, we will have an opportunity tomorrow morning. You guys will be first off in the morning, if that's okay with you. And if anyone has questions over this evening to come back to the team, I'm sure that they would be happy to answer those.

DR. RICHARD GREENWOOD: Okay. And I'll move through this quite quickly and I'll try to setup a framework in which you can be able to understand the results that we are now going to be showing to you.

So we're using the model to -- and I'll go to the schematic on the following page -- we're using the model that we've generated, and which Dr. Clewell has describe, to provide a mechanism of calculating internal plasma concentrations following human exposure to atrazine and drinking water. So, the water consumption records that we see on the bottom effectively represent the details that Dr. Hendley spoke of. That is to say we have intermittent exposures to different concentrations as the DEA, DIA and DACT in atrazine and water.

We've taken those systems that were the most highly exposed, the 17 CWSs that were selected by the strategy that he described, and we coupled that schemograph intake profile with a survey of water intake record.

There were 885 individuals that were part of a 7-day water intake survey whereby they reported on an hourly basis

their water consumption records. And so, we took those as inputs into the PBPK model and derived from there, tracked the individual metabolites but calculated the total chlorotriazine concentration.

At the same time, we took a point of departure from the animal model, such that was described by Dr. Rodriguez, and converted into an internal plasma concentration for TCT. So now we have a reference point of no effect compared to exposure, and that magnitude difference is a margin of exposure, so it's a ratio of those two numbers. And we will be reporting throughout those data.

I won't go through this tiered strategy, but let me just say that we're actually using the 99.9th percentile of the MOE distributions. For each simulation we had roughly 10,000 iterations of the calculations of the MOE. We do that in the one hand by using a standard case with 28 subjects calculating a rolling 4-day average, sequentially, through the entire year. So there's 362 values, and from there you calculate distributions of MOEs, and we are going to report to you the 99th percentile of those distributions.

The standard case that we used was chosen as a point of reference so that other points of departure could be considered and we did a series of sensitive analysis. I'll try to move quickly through this. This is the 17 CWSs that we selected, the most highly exposed CWSs, and we are reporting now. In the top part you'll see the distributed dose NOAEL we picked from our feeding study that had no effect on the LH surge, 50 milligram per kg.

We used two metrics for characterization. One was a TCT peak and the other was TCT area under the curve. The second one you see is a bolus dose NOAEL from our Sprague-Dawley rat LH study. That was 10 mgs per kg. The next dose higher than that was 12 mgs per kg and we had an effect at that level.

We were pretty confident that we had isolated the point of break between effect and no effect on the LH surge. And the third endpoint was the EPA proposed point of departure, 2.56, based on the bolus dose experiment, Cooper in 2010.

So we selected those three different endpoints and compared or did the margin of exposure calculation. And here you see for the 17 CWSs, those margins of exposures; the mins, the maxes and the averages.

We then proceeded to investigate the sensitivity of those margins of exposure to the interpolation of peaks such as that was described by Dr. Hendley where between two measured peaks, seven days apart, and synthetic peak was inserted and we ran those chemographs through the model using -- on the left hand panel of the green bars, you'll see the linear model -- that’s the linear interpolation between measured values -- and then, on the right-hand side you'll see the synthetic peak and the impact of that on the margin of exposure calculation. You can see there is an obvious reduction. It's roughly a three-fold reduction in the margins because, in fact, it was roughly a three-fold increase or the inserted peaks.

In any case, that adjustment factor that he applied was approximately three-fold and we confirmed that. If you want to look at that on the right-hand side you'll see the MOEs are less in the other circumstance where we didn't have that interpolated peak.

In the next slide, we looked at the -- well, this is just the same information showing in a variety of different ways, and I'll slide through that.

In the next slide, we asked the question what would be the impact of calendar year, a year-to-year variation so that from the highest peak that was observed in a particular year to a year that had the lowest peak, so that's what this comparison is doing.

You should realize that when we do the simulation in a standard case, we pick that year which has a highest value, and in this particular instance we compared it to the year that had the lowest value for the TCT in drinking water.

And you can see that there's about on average a ten-fold difference between the variances from year to year and that's due to environmental factors that drive residue concentrations in water. The CWS-96 actually we know was an off-labeled use, so that one year that maximum difference that was observed is attributed to, in fact, that high value. But overall there are generally very large margins, and in some years those margins falls below 10,000.

The next slide, we asked the question -- and all of this is in the form of sensitivity analysis and you can see the standard case we're comparing to the 10 mg per kg NOAEL using TCT area under the curve.

We asked the question, what would happen if you modify the rolling average duration over which you're trying to integrate the internal dose; so one day of dose, two days of dose, three, four, seven, 14, 21, 28 and 90 days. You can see that it's virtually no impact of modifying the duration of the averaging period until you get past 28 days, and then there's a modest increase in the margins of exposure as a result of averaging over a longer period of time when you're simulating from the CWSs.

The next slide tests the question of; well what is the magnitude of the difference between the distributed dose versus the bolus does, so this is comparing the 50 milligram per kg dose translated into an internal dose metric for the rat; and comparing then the exposures of those individuals in the CWSs between those two metrics. You can see there's roughly a five-fold difference in terms of if you chose to use, for regulatory purposes, a bolus dose versus a distributed dose, and that implies a conservative judgement then that leads to a more conservative risk-assessment.

The next slides show the impact of choosing between a particular strain of animals versus another strain of animals. So the Sprague-Dawley rat, we did an extensive and clear characterization of the LH suppression following

four days of atrazine administration and the NOAEL was 10. Dr. Cooper's point of departure was 2.56 and you can see the magnitude of that choice of animal strain, relative to point of departure, is about a 3.6 fold difference in regard to the impact on the margins.

The next slide shows, well, what's the impact of actually using the peak versus the area under the curve, and this is kind of coming to the question of, well, what is the appropriate dose metric. If we don't know, at least we can measure what the consequences of choosing one versus the other. Intuitively, area under the curve makes some sense. Area under the curve above a critical concentration probably makes more sense, but we are just trying to determine the magnitude of that impact. It looks like, in fact, if you use area under the curve you are slightly more conservative by an order of 1.5 fold.

Then we assessed a number of other variables that actually did not make much difference. All of these standard models were done with females aged 13 to 19 using databases from CSFII, the Continuing Food Intake Survey for Individuals. The water consumption records, some body weight records, and so on, came from there. The frequency of water intake and the amount of water consumed came from a Bayer survey that had been embedded in those models for assessing the risk.

But as you can note there, at that age there is hardly any consequence between choosing 13- to 19-year-olds versus 20- to 49-year olds, so that variable didn't seem to make much impact.

We also evaluated the consequences of just including direct water where people reported having consumed directly water versus, in some of these other databases there is information about indirect water. That is to say if you cook food in water that has atrazine in it, that could get incorporated in your food and you'll get indirect water consumption through your food. By adding that variable, you can actually, again, decrease margins of exposure because you are effectively increasing exposure to the compound. So that was a sensitivity analysis around that aspect.

And finally, we did two types of simulation. The standard case was where we took 4-day rolling averages and we just moved that rolling average day-by-day throughout the entire year, so we generated 362 samples or margins for the individual we were simulating. Those are dependent MOEs, obviously, because what you see in the last on is not related to what you are going to get in the next one.

There was a certain desire by a staff at EPA to have that continuity of exposure so that that is what individuals will do in the real world. They will have a water supply and they will get residues that are coming through.

The other approach was to actually randomly draw from the CWS 4-day averages and just calculate the margins so it is a random draw, and that has an impact. It actually makes the analysis more conservative, as you can see, 4,000 versus the 1,700. So those were the forms of sensitivity analysis we have done, and we have done a lot of other

interesting things relative to predicting the kinetics in relationship to the dynamics.

We have not had a great insight out of all of that yet. I am kind of enamored by my modeling friends here, because what takes me six months to do an experiment, it takes them -- although Bob Silken (phonetic) will disagree with me -- it takes him half a day to do an experiment. So, in that sense we can do sensitivity analysis and actually find out things that might be useful for us to measure in experiments.

So to sum up, I would just say that all of the highly selected 17 CWSs, in terms of this MOE distribution analysis at the 99.9th percentile, we note in general that the MOEs are large and higher than a thousand. We note that there is roughly a three-fold impact of the synthetic chemographs on those MOE distributions. We note that the MOE distribution seem to be insensitive to the duration of the averaging period up to about 28 days, and then after that it makes a significant difference, or it at least increases those margins.

We believe that this modeling exercise can be used to inform the risk-assessment process. We are not making a risk-assessment here. We presented it in a neutral way by just discussing margins of exposure. And individuals and people who are responsible for those interpretations can judge the extent to which the factors that we have studied in our sensitivity make a difference or should be incorporated in such a regulatory decision.

We do note though, however, that as one makes progressively sequential judgements of conservative nature, you're effectively concatenating the margins or, shall I say, the protective factors. And in this way, we can actually quantify what you are doing by means of those judgements when you pick this animal strain versus that animal strain or this endpoint versus that endpoint. And really that was the purpose of that investigation was to assess in a quantitative way the impact of those judgements.

So I think, with that, I will stop and we could take whatever questions you want relative to this simulation part or anything else that you would wish to discuss.

DR. DANIEL SCHLENK: Thank you, Dr. Breckenridge. Just by a show of hands, how many actually have questions right now? Did I intimidate everybody? Okay. So what we'll do, if you don't mind, we'll start off with you in the morning and let the panel incubate a bit over the information that they've gotten. And then we'll hit you guys first thing in the morning with some questions if they're around, and then we'll move on from there.

DR. CHARLES BRECKENRIDGE: Thank you, Mr. Chairman, on behalf of Syngenta.

DR. DANIEL SCHLENK: Okay. Thanks. With that, let me turn it over to Joe. And the panel is going to meet for a few minutes in the coffee room afterwards.

JOSEPH BAILEY: Actually, I just want to thank everybody. I have no other closing comments. Just note that the meeting tomorrow morning starts at 8:30, so we'll be here then.

July 27, 2011 - 8:30 a.m. Day 2

JOSEPH BAILEY: Good morning everyone, just want to welcome you back to the second day of the FIFRA Scientific Advisory Panel. This is the Re-evaluation of the Human Health Effects of Atrazine: Review of Non-Cancer Effects, Drinking Water Monitoring Frequency and Cancer Epidemiology.

Just want to make a note about public comment; you are getting some handout this morning. Syngenta is going to have a few clarifying slide on questions that were raised yesterday. There was one other comment that was in the docket from the Physicians for Social Responsibility; you should have that now. There is one comment from Triazine Network; we may have additional handout for them, I am not sure at this point.

Finally, there is the Center for Regulatory Effectiveness's comments that you should also have. Just a reminder, please state your name into the microphone when you make any comment, and with that I will turn it over to Dr. Schlenk.

DR. DANIEL SCHLENK: Thank Joe. Let's go around the room one quick time, just have each panel member introduce themselves, where they are from and their area of expertise, for the general public. I will begin; my name

is Dan Schlenk. I am a Professor of Environmental Toxicology at the University of California Riverside. My expertise is in Fate and effects of Emerging contaminants and Pesticides and aquatic organisms.

DR. KENNETH PORTIER: Good morning, I am Ken Portier, Managing Director, Statistics and Evaluation at the American Cancer Society in Atlanta. I am a Bio-Statistician.

DR. JANICE CHAMBERS: I am Jan Chambers, with the College of Veterinary Medicine at Mississippi State University. I am a Pesticide Toxicologist and a member of the permanent panel.

DR. STEPHEN KLAINE: I am Steve Klaine, Clemson University. I am an Aquatic Ecotoxicologist and I am a member of the permanent panel.

DR. ELLEN GOLD: I am Ellen Gold. I am from U. C. Davis where I am a Professor of Epidemiology.

DR. FRANK BOVE: I am Frank Bove. I am with the Agency for Toxic Substance and Disease Registry. I am a Senior Epidemiology in the Division of Health Studies.

DR. HEATHER YOUNG: I am Heather Young, George Washington University, Department of Epidemiology, specializing in cancer reproductive outcomes.

DR. NELSON HORSEMAN: Nelson Horseman from the University of Cincinnati in the Department of Molecular and Cellular Physiology and I am an Endocrinologist.

DR. JAMES MCMANAMAN: Jim McManaman, University of Colorado, Department of Obstetrics and Gynecology.

DR. DANIEL GRIFFITH: I am Daniel Griffith, Ashbel Smith Professor of Geospatial Information Sciences, University of Texas at Dallas. I am a Spatial Statistician.

DR. HERBERT LEE: I am Herbie Lee, University of California, Santa Cruz where I am a Professor of Statistics and the Vice Provost for Academic Affairs and my research areas includes spatial statistics and deterministic Computer Modeling.

DR. ROBERT GILLIOM: Bob Gilliom, U. S. Geological Survey. I direct our pesticides studies for the National Water Quality Assessment Program and my expertise is in primarily the hydrology and water quality monitoring aspects of this problem.

DR. RICHARD COUPE: Richard Coupe with the U. S. Geological Survey out of Mississippi Water Science Center and I am a researcher on the fate and transport of agricultural chemicals.

DR. SUSAN AKANA: I am Susan Akana. I am currently at City College of San Francisco, which is my second career. I have retired from the University of California – San Francisco where I had a career as a research physiologist in stress and energy balance.

DR. KEVIN O'BYRNE: My name is Kevin O'Byrne. I am from King's College London. I am a Professor of Reproduction and Endocrinology and I am passionate about what controls luteinizing hormone secretions.

DR. KATHERINE ROBY: I am Kathy Roby from the University of Kansas Medical Center and my expertise is reproductive endocrinology.

DR. BARRY TIMMS: I am Barry Timms, Professor of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota with a specialty in reproductive biology and prostate biology.

DR. TRAVIS JERDE: I am Travis Jerde, Indiana University School of Medicine, Assistant Professor of Pharmacology, Toxicology and Urology and I specialize in prostate biology.

DR. PENELOPE FENNER-CRISP: I am Penny Fenner-Crisp, private consultant living in Charlottesville, Virginia and a member of the state's Pesticide Control Board. My area of expertise is toxicology and human health risk assessment.

DR. BETTE MEEK: I am Bette Meek. I am at the McLaughlin Center of the University of Ottawa and my background is in regulatory risk assessment and toxicology.

DR. RICHARD GREENWOOD: I am Richard Greenwood. I am an Emeritus Professor at the University of Portsmouth and my

expertise is in mode of action of pesticide and pharmacokinetics.

DR. WILLIAM HAYTON: I am William Hayton, Professor Emeritus, College of Pharmacy at Ohio State University, expertise in pharmacokinetics.

DR. DANIEL SCHLENK: Thank you everyone, I appreciate that. We are going to start out this morning; we have the Syngenta team up again this morning. They have provided some additional slides as Joe had mentioned. What I would like to do if it is okay, if we can maybe in five minutes go though a list of what those additional slides are and then open it up to the panel for additional questions. I think there were some follow up questions maybe that you may have and we will go from there. So Dr. McFarland if you can provide the list of the additional material that would be great.

DR. JANIS MCFARLAND: Thank you Dr. Schlenk and good morning everyone. Thanks again for all the time yesterday. We have three areas of clarification, based on questions we received yesterday afternoon, in the very brief handouts. The first area is some of the questions on food intake. The second area is on the pharmacokinetics – slide 30 questions with the area of under the curb. The third is areas of water from assessment of the upper centiles concentration in finished drinking water.

Attached to that are some of the reasons for a very few number of daily monitoring samples that were missing from the daily monitoring assessment that was provided to the

panel. So out of over 500 samples the reasons, for instance when the power went off at a particular site. I will turn it over to Dr. Breckenridge to quickly go through the slides on food intake.

DR. CHARLES BRECKENRIDGE: Good morning ladies and gentlemen, my name is Charles Breckenridge. I am a toxicologist with Syngenta. There were some questions yesterday afternoon relating to the impact of atrazine on food intake and body weight.

I selected one study to represent that impact; this was a multi-generation reproduction study in atrazine. It is a guideline study. The schematic of the study is laid out here where you see the animals beginning dosing early young adult, male and female F_0 generation. They are treated for several weeks and they are mated in driving a second generation.

So in those kinds of studies we track body weight and food intake on a weekly basis. This graph is a representation of food consumption in the male animals in F_0 and F_1 generation. You will note that there is an immediate and sustained impact on food intake in the 500 part per million (ppm) dose group at 40 mg/kg. The 50 ppm dose group has no affect on food intake in the male or F_0 and F_1. The same data is shown here for the female animals. Those asterisks below the data sets are indicating statistical significance, and again the 500 ppm group is having an effect whereas the 50 ppm is comparable to control.

There is a consequence of that relative to body weight progression over the course of that treatment interval, and you can note on the left-hand side there is a progressive slow separation of treated animals from the controls at the 500 ppm where there is significant reduction in body weight gain throughout the course of that continuous feeding regimen; for the males F_0 and F_1 generations and the same is observed in the female's. The indication where the elevation occurs, where the females are mated and they are developing a litter. So that body weight gain is associated with that event.

So in summary then, the continuous feeding-type studies suppressed body weight, suppressed food intake, and the same experience occurs with respect to gavage dose. The gavage dose is instantaneously on a day of treatment, impact food intake and body weight progression. Those effect and no effect level for those kind of studies are documented in probably the short-term experiments. The best one would be the CODAR study 0639081. If the panel is interested in seeing those data summarized, we could quickly do that and provide it to the panel as a handout later. But for now let's just say that there is clearly affects on body weight and food intake of atrazine and there is clear dosage that have no effect as well. I will stop there and pass the topic over to the kinetic question, unless there are any questions about that particular data set.

DR. DANIEL SCHLENK: Okay, any questions we have.

DR. SUSAN AKANA: I am interested in the group that had a lower body weight, lower food intake. It is likely their body composition is going to be different. Have you measures of Leptin for instance?

DR. CHARLES BRECKENRIDGE: No, we have not measured Leptin in any of those studies.

DR. DANIEL SCHLENK: Okay, let's go ahead and move on.

DR. HARVEY CLEWELL: Harvey Clewell, from The Hamner Institutes. I just was going to show a couple of slides about the question regarding the AUCs, because we did not have the quantitative information yesterday; I could not remember the details.

This is that same study that I described yesterday with the three gavage and three dietary dosages; and this is the slide that I showed. This is a slide I did not show and it shows the area under the curb versus dose with red being gavage, blue being diet. The DACT is on the right. The area under the curb is similar, slightly lower for the diet but then the mg/kg per day dose for diet was slight lower than the gavage. What you may not be able to see is the Y-axis on these. The DACT is about sixty times higher area under the curb compared to the DIA, and DEA and atrazine are even smaller. So the DACT really dominates the area under the curb exposure for these four compounds.

As I suggested yesterday, the area under the curb exposure in these dietary and gavage studies was pretty

similar and what the most striking difference is the pulsatile nature of the gavage exposure as opposed to more stable concentrations achieved with the dietary intake and the very striking difference in the LH suppression with the diet versus gavage is possibly, we think, associated with this more pulsatile nature and the higher concentration achieved.

DR. DANIEL SCHLENK: Okay, thanks. Any questions related to that?

DR. SUSAN AKANA: Yesterday there was some discussion of the fact that the atrazine and their compounds can bind to red blood cells and to albumin. Have you attempted to look at free circulating, unbound compounds in the experiment you just showed?

DR. HARVEY CLEWELL: That is what was reflected; there were free compound, not covalently bound compound, so that is what was measured, yes.

DR. SUSAN AKANA: And not bound to albumins, so totally free.

DR. HARVEY CLEWELL: That is correct. I mean the binding is covalent and so when you do the analysis, you don't get -- the coloring is gone, it doesn't come off.

DR. DANIEL SCHLENK: Okay, last but not least, we have Dr. Hendley.

DR. PAUL HENDLEY: Okay, thank you very much. Good morning, Paul Hendley, Syngenta. There are just a couple of quick

slides here, thankfully. The first one is just pulling one of the tables from Dr. Mosquin's report of April. This is really just pointing out the raw and finished data from the Safe Drinking Water Act and the frequent monitoring VMP and AMP. Just simply to give you some reference values of what those high centile concentration were in raw and finished from over 48,000 finished water samples, frequent monitoring. That 99.9 centile; it is 22.66 and the report will also show you what the bounds around that are.

The other thing we wanted to point out, one of the panel members had asked the question – very incredibly observantly – about some of the missing data. What we normally do when we submit data and we pull data together is, we have a spreadsheet of values and in this case, it is 500+ raw, and 500+ finished. We have another worksheet that says metadata, and the metadata includes this table. In the report, because it is only a preliminary data report because there are more samples being collected, we have not put the metadata statement in the appendix. Had it been the full year's report, you would have seen this table and I believe the questions were focus on June 29th, an auto-sampler failure. These auto-samplers are sitting in water treatment plants and sometimes folks either turn off the water streams to them or the electric sockets.

But just for the record that stretch in the middle for number 54 came from that dreadful period when the levees were being opened and that is why it was not just a minor

flood on the road; there was a foot of water shifting around.

So for the record, there was a good reason why those samples were not taken. Thank you.

DR. DANIEL SCHLENK: Okay, any questions for Dr. Hendley? Any final questions for the Syngenta team and the panel as a whole?

DR. RICHARD COUP: Can I ask a PRZM question from yesterday? It has to do with kind of how you scale it up to larger watershed size. Now, my understanding is this is an edge-of-field model. So you have a 500 square mile basis and you are treating that 500 square mile as an edge-of-field now. So everything is instantaneously transported in like one day to a point that you are using as your measurement. How would you ever account for transport through streams and hydrology or are you planning on scaling it up so you can go larger.

DR. PAUL HENDLEY: I think this is why I said this was the outstanding issue that maybe needs to be tackled, because it was originally a technology design for the ecological monitoring program where we were thinking of watersheds that were 10 to 50 square miles. We found it is fine working with some of the AMP watersheds, the smaller ones. But the challenge is working out exactly where some sort of routing comes in or weather.

I was trying to make the point yesterday, in fact, the moment you get to an area where averaging starts taking

into account a lot of what is going on in the environment, whether regression approach may be easier in terms of a simple way forward.

So we are still looking at how to account for the routing. You may have notice in one of the tables we did put time of concentration in for the various sizes of water bodies, which is exactly why we are looking at that problem. So work in progress and it is a good question and it needs to be address. But if you remember from many of the key watersheds the dominant, for example, 143 static watersheds are small, less than 50 square miles. And to be blunt, those are the ones that tend to have the highest residue so those are the ones that I think are of primary interest at the moment.

DR. JAMES MCMANAMAN: This is a question for Dr. Breckenridge. This is on the feeding question. So I notice that during pregnancy, there is really no effect of atrazine on food intake during pregnancy, but there is a difference in weight gain. Do you think that is significant? If you could comment on that please.

DR. CHARLES BRECKENRIDGE: We see an impact at higher doses on the weight gain in the mothers and it also finally reflects itself in the up weight at birth. There has always been a question in my mind about the food utilization efficiency that perhaps in periods of high demand that consequence on food intake becomes more important to the animal. So I cannot really say much more than that, but we do know that for the same amount of food in consumption, the relative body weight gain is

proportionally less. That is to say, food efficiency is less in atrazine treated animals.

DR. TRAVIS JERDE: This question is probably best addressed to Dr. Simpkin. You showed a slide yesterday where serum and therefore probably tissue concentrations of atrazine metabolite, particularly DACT, can reach levels of a micro-molar to ten micro-molar after a dose. But the doses you gave are fairly high, 3 mg/kg to 50 mg/kg and water exposure is about a hundred or less, but you only gave one dose. Are you planning studies to look at lower and repeated dosage and tissue concentration of what are metabolite gains? Because when you start to get to 1 -- 10 micro-molar concentration that is the concentration where pharmacologic and toxicological effects can happen. I am just wondering what your thoughts are on that.

DR. JAMES SIMPKIN: That is a Syngenta research planning question and I think it is best handled by Dr. Breckenridge.

DR. TRAVIS JERDE: Okay, that is fine.

DR. CHARLES BRECKENRIDGE: When we go to quantification and the presence of analytes in plasma or tissue, we run into limits of the quantification issue. So as we roll down to really lower dosages, we start to now not be able to measure. And I think that our intent, relative to the monkey study, is in fact to try a 20 part per million administered dose so we will achieve relatively low concentrations in plasma.

We think we will be able to measure DACT at that point, but we doubt we are actually going to detect the mono, the acolytes or the parent with that low level of an inputted dose. But we are trying to move down the region of relevance to actual possible human exposure and that is even a thousand-fold higher than the average of two part per billion.

So in some ways we start to run into analytic sensitivity questions as we are trying to quantitate a small dose entering a volume of distribution with rapid metabolism.

DR. RICHARD GREENWOOD: Yesterday you talked about two phases of elimination of DACT. One, which was rapid with a half-life which was in hours and the slower process, was tens of hours which you put down to the time to turn over the plasma protein. Those figures must have been derived from the *in vivo* data, I guess.

DR. CHARLES BRECKENRIDGE: I will take a first answer at this and then Dr. Clewell can comment more on the modeling part. But those observations came from two types of studies. One was the ^{14}C study in monkeys where we are looking for the urinary elimination rates and we note just that you can do a one compartment model, but in fact it seems like a two compartment model is better to characterize that. And that was also observed in the modeling of the human urinary elimination with the single .1 mg/kg dose as an input dose for those individuals.

So I believe that it is a matter of how to best characterize the rate of elimination. It seems like it fell into two different compartments.

DR. HARVEY CLEWELL: I just wanted to add that the work at Colorado State was some years ago while with Tammy McMullin is what identified the nature of the longer half-life being the plasma binding. Simulation was used in order to come up with the timeframe for that and it does coincide close to the turnover rate for plasma albumin but it has not been completely demonstrated I would say.

DR. RICHARD GREENWOOD: Just to follow up on that. So you have evidence from the monkey study but also the previous study in rats. And again you had sufficient data and details to get a good fix on that, I take it.

DR. DANIEL SCHLENK: Any other questions.

DR. SUSAN AKANA: A general point I hope you can clarify. With the atrazine, on one hand what I understand it is not that soluble in water so it is difficult to administer in fluids or to inject. On the other hand, when we talk about the metabolism of it, the patrician coefficient, if I recall correctly I think Dr. Rodriguez said it is a patrician coefficient of what. And that the one compartment model was appropriate; that there was not, for instance, a preferential uptake or storage of the atrazine compounds in fact.

Now, can you recount those two facts? One, that it is not very soluble in water; on the other hand you have a one compartment model?

DR. HARVEY CLEWELL: A problem with the one compartment model is that the half-life, the plasma kinetics is clearly not single compartment because of that long terminal half-life. And so there is a transition from the rapid clearance of the compounds themselves, which is reflected in the total radioactivity and then the longer half-life for clearance of the albumin adducts. So it is not actually a good candidate for single compartment modeling, if you are using the total radioactivity data.

You might be able to use a single compartment model for the total chlorotriazines. I have not really tried to do that. We started with the PBPK approach, but the problem with that is that does not consider the flow limited metabolism in the liver. So you would not correctly describe the presystemic clearance of the compounds in the liver before reaching the blood. So it is a good candidate for more physiological descriptions.

DR. WILLIAM HAYTON: That previous question triggers this thing that I wonder about, and that is the one compartment kinetic parameter value. Following the distribution numbers are, I think up around five or six liters per kilo. And I believe I heard you say, Dr. Clewell, that the distribution is fairly uniform across all of the tissues. I am wondering if there is any reconciliation of that.

DR. HARVEY CLEWELL: That is an artifact of the use of the terminal half-life, which is really based on one very small portion of the total radioactivity that does not really reflects the distribution of the vast majority of the compound. So that is the trouble with volumes of distribution of course, is they do not have a physiological meaning. So I think that that very high level is just because you have a very slow clearance and low blood levels and so then you have to impute a very high volume of distribution in order to put it into a one compartment description. So I think it is just artificial.

DR. DANIEL SCHLENK: Okay, thanks to the Syngenta team; appreciate that. Our next public commenter will be Wendelyn Jones from CropLife America. Dr. Fenner-Crisp?

DR. PENELOPE FENNER-CRISP: While they are changing folks around, I have a question Joe. Will the presentations that have been made by the agency and the commenters be available on regulations.gov during the course of this meeting?

JOSEPH BAILEY: They should be actually. I think they are on docket now; they are just waiting to be posted. The EPA presentations… yes.

DR. PENELOPE FENNER-CRISP: Since Harvey reminded me it is hard to see some of those numbers. My eyes are getting kind of old and I would like to blow them up.

JOSEPH BAILEY: I looked last night and the docket had not posted.

DR. PENELOPE FENNER-CRISP: They were not there last night.

JOSEPH BAILEY: Yes, but they are there, we just need to get to the docket and tell them to please put it up. So we will get a note out to them and they should be available very shortly.

DR. PENELOPE FENNER-CRISP: Thank you.

DR. DANIEL SCHLENK: Do you have a presentation?

WENDELYN JONES: You know, there are no slides.

DR. DANIEL SCHLENK: Amazing.

WENDELYN JONES: There are no reading materials.

DR. DANIEL SCHLENK: Okay, thanks.

WENDELYN JONES: Good morning, I am Wendelyn Jones and I am here today to represent CropLife America, and on behalf of our organization, we respectfully encourage the EPA/OPP and the SAP to remember their science background and ensure a science-centric path such that decisions are made on valid and reproducible science. CropLife America is a not-for-profit trade organization representing the nation's developers, manufactures, formulators, and distributors of plant science solutions for agriculture and pest management in the United States.

We are committed to the safe and responsible use of the industry's products in order to provide safe and abundant food as well as control for insect and plant disease vectors for the protection of human health and providing valuable benefits back to the consumer. Crop protection products require extensive data development for initial and continued registration. We respectfully note that this is the 11th SAP on atrazine since 2000. Atrazine was reregistered in 2006 based on a 12-year EPA review and the input from multiple SAPs.

This current SAP is part of a series scheduled by EPA beginning in 2009 to reevaluate atrazine. Both Syngenta and EPA have responded to this reevaluation of atrazine by providing many additional studies to characterize the toxicological characteristics and exposure potential. A robust scientific data base supports the continued use of this valuable product. A valid and reproducible sound science should always lead EPA's decision making.

The atrazine safety package represents one of the most advance sciences for the acquisition of hundreds of thousands of drinking water samples, detailed mode of action studies, and cutting-edge pharmacokinetic pharmaco-dynamic characterizations. Such a rich database should provide confidence in the safety of this product.

We encourage EPA/OPP to lead the way in utilizing advance scientific approaches such as evident by atrazine research being reviewed at this SAP. Such leadership would align with two recent NAS reports, Toxicology

Testing in the 21st Century and Science and Decisions: Calling For a Major Scientific Change in How Toxicology and Risk Assessments Are Done.

Among the cardinal principles of regulations of drinking water under SDWA, is providing the public with accurate and informative human health risk information and avoiding unnecessary public alarm by false allegations of threats to the safety of its drinking water. As respected scientists, we ask that the SAP exercise clear judgment in the report and recommendations that it provides in order to help assure that the work reflects the best scientific principles and avoids creating unwarranted public concerns.

Additionally, we encourage the EPA and the SAP to carefully consider the mode of action studies and the pharmacokinetic studies. EPA defines mode of action as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes and resulting in the adverse affect.

Previously the agency has noted in certain experimental rodent strains, that atrazine induces changes in luteinizing hormone secretion without any adverse consequences on reproduction. Within the white paper for this SAP, the agency is proposing to continue to use the change in LH secretions as the basis of the atrazine risk assessment.

We also note that the duration of exposure is an important parameter considered in evaluating the relationship between dose and attenuation of the LH surge. The ability to confidently compare dose response and exposure in rats to humans is extremely important. We are therefore very encouraged to see that the agency has paid particular attention to the elucidation of the pharmacokinetic behavior of atrazine. The integration of this understanding and other new studies is key to enable OPP's leadership and we encourage for the refinement of this approach.

Lastly, we would like to highlight a recent paper that was issued as part of the Agricultural Health Study. The AHS is a long-term research project that has been tracking the health of nearly 90,000 people, certified pesticide applicators and their spouses in Iowa and North Carolina since 1994.

The report recently published in environmental health perspective concluded that overall there was no consistent evidence of an association between atrazine use and any cancerous site. No one cares more about the safety of crop protection products including pesticides than the farmers who use them on their crops and soils where are own children play. Farmers have an important stake in keeping their land, rivers and ponds safe for their families, their neighbors and their communities.

If valid reproducible sound scientific research finds that any agricultural pesticide cannot be used safely, we will be the first to agree with increased regulations.

Sound science has found repeatedly that atrazine is safe when used responsibly and according to label recommendations. The comprehensive dataset on atrazine, over the years, including the more recent research, reaffirmed the safety of atrazine. Thus, COA supports this product as it helps us raise our crops affordablely and sustainablely. Thank you for your time and attention.

DR. DANIEL SCHLENK: Thanks. Any questions from the panel? Okay, thank you very much. Our next public commenter will be Scott Slaughter. I believe there is a handout associated with the comments.

SCOTT SLAUGHTER: I have been here before and it is always a pleasure to appear before the Science Advisory Committee. Hi, my name is Scott Slaughter and I am commenting today on behalf of the Center for Regulatory Effectiveness. I will be citing a number of documents. There are links to all the documents I refer to in the written materials you have. And I would like to first address the Cooper Study on LH surge. EPA has validated at least four tests for measuring the effects of atrazine on LH surge. Dr. Cooper's LH attenuation study differs significantly from the four LH tests that EPA has validated.

One difference is that the Cooper Study only tests Long-Evans rats. The four validated tests only use Sprague-Dawley rats. The Cooper Study on Long-Evans rats is also inconsistent with EPA's standard procedures for assessing potential endocrine effects of all pesticides including atrazine. In its Endocrine Disruptor Screening Program,

affectionately or unaffectionately known as EDSP, EPA used Sprague-Dawley rats to validate tests for potential pesticide endocrine effects on female pubertal development. The endpoints of these validations test in EDSP included vaginal openings and estrous cycling. Atrazine was utilized as a control in the validation of this EDSP assay.

EPA's report for this validation study adopts Sprague-Dawley rats as the strain to use in testing pesticide endocrine effects on development and reproduction. And I quote from EPA validation study in the EDSP as follows, "In summary, EPA is aware of the potential for differences between strains and therefore expresses a preference for standardization using Sprague-Dawley rat." If EPA still wants to rely on Dr. Cooper's study and its non-standard use of Long-Evans rats to regulate the endocrine effects of atrazine, then we ask that EPA first validate that study.

Validation of the Cooper Study on LH attenuation should comport with first, EPA's guidance for validating endocrine disruptor tests; second, EPA's Information Quality Act Guidelines; and third, guidance produced by the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). Among other things, the validation process should determine whether Dr. Cooper's study results are reproducible by other independent laboratories.

As EPA noted earlier, Syngenta submitted studies that generated different results on LH attenuation than Dr. Cooper's study. The validation process should determine whether the NOAEL and LOAEL from Dr. Cooper's study are reproducible by other independent laboratories. The validation process should also determine whether the use of Long-Evans rats in tests for potential endocrine disrupting compounds is scientifically justified. If so, the EPA should reconsider its Endocrine Disruptor Screening Program.

I would like to briefly now address some of these specific charge questions. I am not going to repeat all of the comments in my written testimony, in the interest of saving time. Please I refer you all to those.

The next subject I would like to address is on page four of my written comments, which are Mode of Action and Adverse Outcome Questions, which I identified, perhaps incorrectly, as five, six, seven, eight and nine to the SAP.

To begin with EPA's Issue Paper, which was presented to you for this SAP states, and I quote EPA. "This Agency is using the 33% LH surge attenuation after a 4-day exposure as a precursor event to protect for other adverse outcomes including estrous cyclicity disruption, and delays in sexual maturation occurring at higher doses in laboratory animals."

Using the 33% LH surge attenuation after a 4-day exposure, like EPA proposes to do, is not based on any

adverse human health effect. In fact, the September 2010 SAP explained in its written minutes that 33% surge standard is not based on any adverse rat event. And I quote from the minutes of the 2010 SAP, "Greater than 80% attenuation of the LH surge, in any given 4-day estrous cycle, would be needed to observe deleterious effects in the reproductive systems in rats." And another quote from the 2010 SAP minutes, "Attenuation of the LH surge has no adverse effect on reproductive function and does not prevent ovulation until about 80% attenuation. Therefore, the proportion of animals and the latency to exhibition of delayed cycles might constitute a better endpoint or 'adverse response' for determining the effect of atrazine than is attenuation of the LH surge."

There are other quotes from the 2010 SAP minutes, which I will not repeat here but they are in my written documents. And it is quite clear that what EPA proposes to do is not directly connected to any adverse health effect that has been observed in a rat or a human.

I would like to close briefly by pointing out another quote from the September SAP. The introduction part is, Members of the September SAP "expressed the opinion that there doesn't seem to be strong evidence of adverse health effects from atrazine exposures at the levels found in surface waters; because of this, these Panel members believed it was unfair to ask the registrant to increase their sampling efforts." We agree. I will try to answer any questions you might have now.

DR. DANIEL SCHLENK: Any questions from the panel.

SCOTT SLAUGHTER: Thank you very much.

DR. DANIEL SCHLENK: Thank you Mr. Slaughter. The next public commenter will be Jere White from the Triazine Network and I believe there are several folks involved in that one. I think there are some slides.

JERE WHITE: Good morning Mr. Chairman, members of the panel, my name is Jere White. I am the executive director of the Kansas Corn Growers Association and the Kansas Grain Sorghum Producers Association. I serve as Chairman of the coalition that was formed in 1995 somewhat in response to the initiation of the special review of the atrazine. The goal of the Triazine Network, since its formation, was simply to see a scientifically based conclusion to the special review. We obviously are a coalition that represents the user community, if you will. And as such, we obviously have a keen interest if there are safety issues related to the use of the product. We represent over 30 commodities grounded in over 40 states and certainly commodities grounded in almost every state that has agricultural production, which is obviously most of the states in the country.

We believe the scientific weight of evidence continues to show that atrazine is both safe and effective and that is certainly the kind of tool that a farmer needs to have. We do look forward to a science based conclusion of the review of the use of atrazine on our farms. And it is not because of their uncertainty with the product but because of seemingly continuous review of the product has

literally surpassed the career of many at EPA; I assume probably some on the SAP and certainly several of our growers. In fact, I am wondering if it will surpass my career as well. This is also, as noted earlier; it is the 11th SAP since 2000. Network members have participated in every SAP since the special review began. Quite frankly at this point, Joe Bailey and I have observed a longer relationship than I have with my wife and I think she is starting to ask questions.

JOSEPH BAILEY: You did not need to state that publicly Jere.

JERE WHITE: As you know atrazine has been used for some 50 years by farmers; it has been used by more farmers in the US than any other herbicide. It is used on over half of the corn, 2/3 of the sorghum, 90% of the sugarcane. It is an important product and I think that has been well established, but it still bears repeating I guess 11 times since 2000. We use it because it is efficacious for wheat; it is cost effective; we believe it is safe. It is also a key tool that farmers use in conservation tillage and controlling soil erosion.

So we are here again today, and our sense is that much of the activist clamor that led to this re-review post 2009, has been properly vetted out by the agency and the SAP, and that we are moving on. However, we simply cannot disregard some of our specific concerns with certain Agency positions that are being discussed at this SAP. And that is why we have asked Dr. Lamb to join us again as he did in 2010. We certainly have shared concerns

with the SAP regarding some of these studies and Dr. Lamb will address those again.

We were pleased but not really surprised that the new version of the Agricultural Health Study adds further confidence to with the EPA has already established, that atrazine is not likely to cause cancer in humans and indeed other organizations and government agencies from around the world have concluded much the same.

In some of the previous discussions on, I guess what we commonly refer to as the Cooper Study, there were discussions about the appropriateness of the strain of rats, the appropriateness of the gavage technique. And I guess one of the things I took away from the earlier discussions was this whole issue of solubility and it seems pretty basic, it is the concept of saturation I guess is the same concept that we learn as elementary kids making rock candy. You super saturate the liquid and it forms the sugar crystals. It just seems like a basic concept that if you cannot get that much product into the water, then the exposure to humans or normally even to animals would be through the water and we are talking about regulatory discussion of water that is kind of a basic concept. Probably my understanding is limited to making rock candy, but hopefully Dr. Lamb can help communicate some of our concerns and I think others have addressed that.

Monitoring results clearly indicates that atrazine levels, even when they are detected in drinking water, are extremely low; do not exceed thresholds for human

health effects. Finished drinking water, in our opinion, should be the only water used in drinking water assessment, not raw water. This is the same requirement for all other potential contaminants including many with known health concerns at levels possible in the environment, again, not limited by solubility issues. There is no scientific justification to single out atrazine as being unique or different.

In addition, the use of Eco sites such as Missouri O-1 for risk assessment is just simply not appropriate. I believe in past SAPs there has been a lot of pictures shown of the Missouri O-1 site. One time it was a construction site and those of you that served for years on the SAP will remember the pictures of earthmovers in action.

There is simply no basis to assume that you could derive a minimum safe yield from these sites, it would be appropriate to site them for community water systems. There is no reason to believe that Missouri O-1 in and of itself would be appropriate for water supply and moreover, if you were to consider that you would build a reservoir. By doing so you would change the characteristics of what the exposure pattern would be from that water.

At this point, I would like to have Dr. Lamb share a review that he continued to do for us on behalf of the Triazine Network looking at the Cooper work and its appropriateness for this use.

DR. JAMES LAMB: Thank you Jere. Some of you, it is good to see you all again. I appreciate your taking the time to hear me, really I do. I am Jim Lamb, I am Director of Toxicology and mechanistic Biology and Exponent and I am here on behalf of the Triazine Network. I am going to comment specifically on the use of the Point of Departure that is proposed, which is the suppression of the LH Surge in the Long-Evans rats.

There are several major issues; this is going to be quite different actually, than the last time I spoke where I got into some of the nuts and bolts of the study. This is more about the science policy and the use of this endpoint and the use of the Long-Evans rat, the bolus dose, the LH surge for risks assessment purposes, which is really an important part of your charge. Should the reduction in LH be treated as an adverse effect? And I have got some bullets here but I am going to go into them more detailed further in the study, so I am not going to read them to you now.

I would contrast the study with the typical risk assessment study and the studies that already exist on atrazine, for which we all know there is a huge database. Specifically, this particular study, meaning the Cooper Study on the LH surge, it is an unusual selection of animals in this study design in order to measure very specifically effects on the LH surge. This was designed by Dr. Cooper for a specific mode of action purpose; it was not really designed to be a risk assessment study and you can see the way he designed and conducted the study

that that really was not the intention of this study. And for many reasons I do not believe it is appropriate.

It is a collection of three blocks of animals based on several requisitions of animals over a course of a year. It is a large complex study; it involved gavage administration for four days so that they could very precisely evaluate, in a subpopulation of animals, a change in the LH surge. And it required a group of very precise selection criteria. There is in addition, a very precise two-hour window in that surge, and you can see from his data how this is set up. It is not a conventional design; it is not validated; I do not expect as a study design there is any need that it ever would be validated. But I think some form of replication is critical if it is going to be used in a risk assessment. And for many reasons I do not believe there are serious issues with waiting to see that replicated, if indeed it is going to be used. And I will talk too about some of the questions about whether or not it even should be used for risk assessment.

I mentioned exclusion criteria… basically this study protocol called, at the beginning, for about 1000 animals; of which 359 were used. And the exclusion criteria, first and foremost was if the animals did not have a regular 4-day estrous cycle over the course of a couple of week, two or three weeks, they were not to be used in the study. So more than half of the animals or about half of the animals were eliminated from that 1000 at the very beginning of the study.

Then at the time of kill, after the 4-day dosing and the collection of tissues and samples and measurement of hormones, three additional criteria were applied. Did the animal have a proestrus smear; was there an increase in uterine weight, beyond a half of gram; was there elevated progesterone? All of these had to be satisfied to include the animals in his evaluation. So you go from 1000 to approximately 500 – I will have the numbers in just a second; and then from that 500 to 359 on that second set of criteria. Again, this is after four days of bolus dosing and he did not dose the animals that did not have a regular cycle. And then sample collection was every two hours you could not use an animal more than once because you were killing them every two hours.

I apologize for the size of the numbers on here, but this is a listing, by requisition, of the animals in the study. Go to the bottom line where it says total; there are 861 animals that we could identify in the information on the docket. The protocol call for 1000 but I never could figure out exactly how many animals started in that last group. And also could not tell how many were excluded after dosing. So there are some question marks here that you need to be aware of. So this numbers, especially the 861, is the total end is low. There are other animals because ultimately out of that group 31 were used, which you can see in the last group.

So you have total number you start with, followed by how many of those actually had the 4-day regular cycle and how many then were excluded after four days of gavage dosing and necropsy. So after the data is in hand, how

many did you then excluded and the total number included at the end, that we could calculate, was 359 animal.

It is an extreme design for a very specific purpose, and that is to answer questions particularly about Long-Evans rats. Dr. Cooper has done a tremendous of important work on atrazine and Long-Evans rats and his hypothesis, as I understand it, was to explore that mode of action. So this had a very particular design. But one thing that happened is, by removing animals after the data are collected, you are eliminating some of the variances in the study. The variance is still reasonably high in the included animals but if you, again, before you yell at me that this is unreadable, I am going to pull out part of this slide. What this is, is this is each of the seven groups, control and six treatment groups from the study and what are called excluded animals. These are excluded after dosing; this does not include the animals, which were eliminated for lack of an estrous cycle.

Then, included animals are on the last chart. I am going to just show you the control data from this and since you have the entire chart in the file, you can look at it at your leisure, which I am sure you will enjoy doing. You can see that the times for collection were – I guest Ralph calls it "rat time" – 1200, 1400, 1600, 1800 and 2000. And you see the variance in the animals excluded. Again, these are excluded after they have collected the data.

So he has LH numbers on these but they did not meet his criteria. Most of the animals were eliminated because

their uterine weight was below a half of gram. The other two criteria really came into play much less often. But you can see the variance in looking at the standard deviation versus the mean; in the included animals, it is much lower. If he had had to include these other animals, which he had removed for a purpose and through criteria, the variance would have been much higher. So you have a really well selected subpopulation of a 1000 animals. 40% of those animals -- less than 40% -- 35% ended up being used in this study. So this is not a study that represents even the entire population of Long-Evans rats, much less other rats or humans, for various reasons.

I do not believe this study is really designed to set a point of departure. I also think there are serious problems with the bolus exposure to these animals and the precisely timed sampling if you want to use this for risk assessment purposes. Other studies support the use of LH suppression as an endpoint, but probably at higher dose levels or longer exposure. There are various effects, vaginal opening, preputial separation, and other effects that indicate that some effect on LH may be a sentinel effect. I am not sure that the effect on LH surge in the Long-Evans rat though is a good sentinel effect for a human risk assessment.

This really repeats the point just made that you can link LH suppression, but probably the pulsatile LH suppression to adverse effects. The mechanism is really not that well sorted out at this point but on water exposure and solubility, an issue that has come up several times, this

chart shows a line and that is the limit of solubility, thirty parts per millions, of atrazine in water. And the reason you cannot do a drinking water study is because the top dose is actually right about where the current point of departure is, two and a half milligrams per kilogram per day. That does not even consider whether they are palatability problems or other reasons that they would not consume this water. So that is the highest you could go, theoretically it may be higher than you really could go in running the study. So we are sort of trapped back into either dietary or gavage studies.

As far as mode of action, the mode of action for the LH surge was described really well yesterday and it is dramatically different in the Long-Evans rats compared to humans. In rats atrazine does affect, in Long-Evans rats, the pre-ovulatory -- and probably Sprague-Dawleys as well -- the pre-ovulatory surge of LH, by blocking GnRH secretion in the hypothalamus. Ralph has shown this in his Long-Evans rat studies; I think this is very real; it is true for the Long Evans rat. That surge occurs over about two hours. But it does not alter the ability of the rat pituitary to respond or to produce LH; it really is a hypothalamic effect. And the *in vitro* study shows that atrazine does not seem to affect the response of the pituitary to GnRH.

Now, effects of atrazine then for the GnRH surge, appear to originate in the hypothalamus. In humans though, that is not really relevant. The pre-ovulatory surge, first of all, is two or three days not two or three hours. It is not triggered by GnRH surge. You have pulsatile GnRH

and estradiol positive feedback have been identified as resulting in that surge of LH. The atrazine exposure that inhibits the LH surge in certain strains of rats, whether it is Long-Evans, but not Fischer 344, basically this is not relevant to other strains rats and this mode of action is not relevant to humans.

Again, the timing in this study was very precise; it was gavage. You saw pictures of basically, even when you look at DACT, the saw-tooth pattern of daily gavage on the concentrations of DACT in these animals. It is not a steady state; it is not even really much of a pseudo-steady state. It is highly variable numbers based on daily gavage; they go up, they go down fairly quickly. Using the bound atrazine as noted by Harvey Clewell and the information he showed changes dramatically how steady you think this pseudo-steady state may be.

Also, human drinking water exposure is not like gavage. It is not modeled well by gavage; it includes many uses of tap water intake throughout a given day. It is not a single dose for most of us. Temporal considerations that I think are also important are – dosing is daily, but we are talking about through the 4-day cycle. It was not four days necessarily because of the cycle; it was again coming back to that pseudo-steady state as I understand it. But they did treat for the 4-day cycle and if it were relevant to humans, it should be compared to the 28-day human cycle.

When you look at an effect on a two-hour surge, well the LH surge is 48 hours, so if you are comparing the timing

of the effects you need to make some adjustments. Really, the 4-day study may relate better to a human 28-day exposure. But susceptibility is really dependant on the mode of action, and they are different between rats and humans. Humans are unlikely to be susceptible to changes in the GnRH surge. Typically, in a conventional risk assessment we are going to use a no observed adverse effect level or a lower confidence of a benchmark dose, or some equivalent number in a guideline study. But mechanistic studies do play a significant role in risk assessment. They typically do not involve the selection of a subpopulation, which this study does.

There are various endpoints relevant to adverse effects that have been studied over the years for atrazine. And no observed adverse effect levels have been established. In the end, the design of this study limits its usefulness in risk assessment; it was done for a very particular scientific purpose, and he answered his question. But such changes should not be treated as adverse effects relevant to humans. Other data are really already there that are more important for the atrazine risk assessment, but if EPA is going to regulate on such an unusual research study, it really needs to be independently replicated. That there is time to do this in that all the margins – we do know, you heard from Syngenta, you heard from EPA, the margins of exposure are sufficient. If you need to repeat or replicate this study, there is time and there are mechanisms by which EPA can demand that the study be done, and I have had great experience in the past working with Dr. Mendez and Dr. Cooper and others on unusual study designs for

regulatory purposes. There are ways to get these repeated if this is an endpoint that really is going to be used for risk assessment. With that, I will conclude, pass it back to Jere, or answer any questions. Thank you.

DR. DANIEL SCHLENK: Do you have any further comments Jere?

JERE WHITE: No.

DR. DANIEL SCHLENK: Any questions from the panel?

DR. PENELOPE FENNER-CRISP: Okay Jim, so if you were tasked to do the atrazine risk assessment, and you have available the current dataset that exists, what would you select as the appropriate dataset and study to derive a NOAEL and LOAEL or benchmark dose to use at the point of departure?

JIM LAMB: Good question, and I think I would rely on vaginal opening or preputial separation, which have no observed adverse effect levels point of departure at about six and a quarter milligrams per kilograms per day.

DR. DANIEL SCHLENK: Any other questions?

JIM LAMB: Thank you very much.

DR. DANIEL SCHLENK: Thank you. Our next public commenter will be Sarah Gallo from the National Corn Growers Association. We also have a handout for that.

SARAH GALLO: Good morning. My name is Sarah Gallo. I am the Director of Public Policy for the National Corn Growers Association, and I appreciate the opportunity to be here this morning. I am providing comments on behalf of the National Corn Growers Association, which represents more than 3600 members in 48 states, and 47 affiliated state organizations with more than 300,000 corn farmers who contribute to state check-off programs across the country.

Our members are proud to be a part of a sector that is one of the few bright spots in our country's balance of trade. USDA forecasts agricultural exports to reach a record $137 billion for this fiscal year – including a $44 billion trade surplus, which is the highest it has ever been. Our corn farmers represent an important part of these economic strengths. The United States is the world's largest producer and exporter of corn, and one of the key inputs that makes that possible is atrazine.

For more than 50 years, corn farmers have relied on atrazine to fight weeds effectively and affordably. It is applied on well over half of all corn acres in this country. By EPA's own estimate, atrazine saves corn farmers as much as $28 an acre and has reduced herbicide costs and increased yields.

Our confidence in this vital tool for corn farming has been bolstered by more than 6,000 studies and nine reviews conducted by the EPA. Just this past May, atrazine got another "all-clear" from a comprehensive study. A new report from the Ag Health Study - a massive,

government-sponsored epidemiological study of agricultural workers that has been on-going since 1994, found no association between atrazine worker exposure and any form of cancer.

This latest report studied more than 57,000 licensed pesticide applicators from 1994 to 2007. It is just the latest in a series of studies conducted by governments and international organizations that have found that atrazine is not a health risk. In 2007, the World Health Organization reviewed atrazine and concluded it is "not likely to pose a carcinogenic risk to humans." The World Health Organization is so confident of the safety of atrazine, in fact, that in 2010 it raised its acceptable drinking water recommendation from two parts per billion (ppb) to 100 ppb. That's far higher than the EPA limit of three ppb.

Over the past ten years, atrazine has been reviewed all over the world, in Britain in 2000-2003, Canada in 2004 and again in 2007, Australia in 2008, and the state of Minnesota again last year. In all of these cases, it has been favorably reviewed from a human health standpoint. Of course, the EPA itself re-registered atrazine in 2006, after a 12-year review.

The safety of atrazine, to people and the environment is clear. It has been verified by thousands of studies. The economic importance of atrazine is just as clear. It has been vouched for by corn farmers all over America. At a time when so much of the US economy is struggling, we cannot forget that agriculture is one of the few areas

that is competing better than ever; creating good American jobs right here at home in the heartland of America. Rather than do anything that would hurt our farmers' ability to compete, we should do all we can to ensure that America's farm exports remain strong in world markets. Atrazine helps us do that. Thank you.

DR. DANIEL SCHLENK: Any questions from the panel?

DR. KEVIN O'BYRNE: What is your major concern, anxiety?

SARAH GALLO: What is mine personally? We just want to make sure that this is a product that our producers are able to continue to use and just want to convey how important it is as an important tool for our growers.

DR. KEVIN O'BYRNE: So where is the stumbling block? What is causing the anxiety in your members?

SARAH GALLO: I think just the concern that there would be something that would prevent them from using the product.

DR. KEVIN O'BYRNE: Coming from what source?

SARAH GALLO: Nonscientific data or unwarranted concern.

DR. KEVIN O'BYRNE: Is that from government agencies or is that just…

SARAH GALLO: I am not entirely sure, I can…

DR. KEVIN O'BYRNE: Or is it from anxiety within the general population that have little or no understanding of what goes on in the fields.

SARAH GALLO: Well, sir, yes of course that is a concern of our, that people are misinformed.

DR. KEVIN O'BYRNE: So does your organization do anything to educate the general public?

SARAH GALLO: Yes, absolutely.

DR. KEVIN O'BYRNE: What is the nature of that?

SARAH GALLO: Both nationally and within all of our state organizations, Jere being one of them, we certainly have public outreach campaigns to education people about corn farming, about kind of the modern practices that our corn farmers have adopted to reduce herbicide use and transform their practices to be both economically and environmentally beneficial.

DR. KEVIN O'BYRNE: Thank you.

SARAH GALLO: I have no personal anxieties – I'm good.

DR. DANIEL SCHLENK: Thanks Ms. Gallo. Any other questions that relate to the panel charge questions? Let me ask that. Any questions related to the charge questions we have been given? Okay. Thank you very much. Our next public commenter is Tyler Wegmeyer from the American Farm

Bureau Federation. And we do have a handout as well for this.

TYLER WEGMEYER: Good morning everybody. My name is Tyler Wegmeyer and I am Director of Congressional Relations for the American Farm Bureau Federation. I am also a fourth generation farmer, growing mostly specialty crops in Western Loudoun County, Virginia.

The American Farm Bureau Federation is the country's largest general farm organization. Farm Bureau members grow, produce and raise the food and fiber and energy sources that feed, clothe and fuel the U. S. and the world. Our farms and ranches are found in all 50 states as well as Puerto Rico, and we represent producers of every size and scale of operation.

The American Farm Bureau Federation welcomes this opportunity to speak to the benefits of atrazine and what it means to the American farmer. Having access to important crop protection products is vital to the success of providing a safe and abundant food supply. I appreciate this opportunity to be able to express our views before this Scientific Advisory Panel. Atrazine has been in use for more than 50 years and has proved to be a safe, valuable and a cost-effective herbicide that farmers across the country use to manage the spread of weeds that rob crops of nutrients.

Today, US farmers safely and successfully use this herbicide on over 50% of corn, 90% of sugar cane and two-thirds of sorghum acreage. Corn is a base commodity for

inumerable food products, and corn and sorghum are key feedstocks. If these sectors are undermined, the repercussions would be felt throughout the US food industry, including weakening the economic health of the America's farmers and ranchers. No degree of economic dependence would matter if atrazine were a problem, but it's not. We believe sound science shows it to be safe for use.

Atrazine has been the subject of intense scrutiny since it has been on the market and has been the Subject of eleven SAPs since the year 2000 by the EPA. Recently, as you know, the Agricultural Health Study, a large government-sponsored study of agricultural workers, going on since 1994, found no association between atrazine worker exposure and any form of cancer.

In addition, the World Health Organization raised its acceptable drinking-water recommendation from two parts per billion (ppb) to 100 ppb, far higher than the EPA limit of three ppb. Atrazine has been examined by the international organizations and countries including the World Health Organization, the United Nations Food and Agriculture Organization, the governments of Great Britain, Canada and Australia, and the state of Minnesota, which have all deemed it safe for use.

The American Farm Bureau Federation has participated in every scientific advisory panel convened to examine atrazine's safety since the first special review in 1994. More than 6,000 studies on atrazine have been commissioned since its introduction to the market, and it

is one of the most complete scientific databases of any crop protection product. Our members look at EPA's recent actions with dismay and frustration. Farmers are deeply concerned that this process will result in an unjustified restriction or elimination of an important crop protection tool.

At a time of continuing high unemployment, and enormous trade deficits, agriculture is providing a much needed bright spot in our economy. And yet, we find ourselves fighting off ill-considered proposals, such as the one before this panel, that have the potential of making farming more difficult, less efficient and more expensive.

We hope that this atrazine review process is not being subjected to an unseemly rush to take unwarranted action and we urge that you ensure that the principles of sound science remain our way forward. Again, we appreciate this opportunity to submit our comments. Thank you.

DR. DANIEL SCHLENK: Again, any questions that relate to our charge questions.

DR. HEATHER YOUNG: I just want to comment since this is the second public commenter that has made the comment about the Ag Health Study; have you reviewed the 2011 Beane-Freeman study that show the four-fold increase risk with thyroid cancer? Because, you are stating here that they are showing no association with any forms of cancer and we have the 2011 study that shows us the four-fold

increase risk with thyroid cancer. I am wondering what your thoughts are on that?

TYLER WEGMEYER: Thanks for bringing it up. I have not looked at that specifically but I will.

DR. DANIEL SCHLENK: Any other questions? Thank you, Mr. Wegmeyer. The last public commenter that I have on my list is Stephanie Whalen from the Hawaii Agriculture Research Center. I believe she has a couple of slides.

STEPHANIE WHALEN: My name is Stephanie Whalen. I am the Executive Director of the Hawaii Agriculture Research Center, and some of you may be wondering, what is someone from Hawaii doing here. And so I thought I would put that a little in perspective.

HARC, our organization is over 100 years old. It is a private agricultural organization that supports agriculture in Hawaii. It began with a dominant agriculture product of sugarcane and then pineapple, along came coffee, macadamia nuts, papaya and now more vegetable production. Currently we have very little sugar left and our work is involved with the diversity, which includes those that I named, plus herbs, seed products, cacao tea, et cetera.

Our organization has been focus on scientific based information and technologies to transfer to our client, essentially the farmers in Hawaii. We have been delivering that type of information and it has been very

science-based. We are an organization that is very focus on science-based information.

My personal responsibility, throughout my almost 40 years with the organization, has been a background in pesticide residue work and I was around at the beginning of EPA when it was transferred over from HEW, and was involved in their original training operations. So that is where my background comes from, basically pesticide residue work, which then involved working with all the chemical manufactures throughout my last 40 years.

I also then was responsible, as EPA developed in more regulatory areas in air, water, clean water act, safe drinking water act, non-source pollution and then chemicals with pesticides, and manufacturing them, basically because the sugarcane industry was vertically integrated from the field to the table, so all those regulatory statues affected operations. That has been my history in terms of why I am here.

It is very obvious why I have been involved in the atrazine process; because of my history and my responsibility to the industry in Hawaii. And so I have been involved in all of the SAPs, I believe, except one. I provide the comments for -- I think it was the 1988 -- where the special review was first announced by EPA, and they asked for comments and got over 80,000 comments for that. I think that was the highest in their history up to that point.

A little bit about sugarcane in Hawaii, we were a 250,000 acres in its history; we are now down to 40,000 acres. Partly or mainly due to the stable price for the last three decades while the input cost increased over that time, and regulatory being just one of those cost and atrazine being a major concern.

In Hawaii, we estimate there is about 10% loss on 60% of our acres if we were to lose atrazine; that works out to be $130 per acre or about $2.3 million. Using Hawaii's numbers and then take that across the nation to Louisiana, Florida and Texas; their cost would be about $280 an acre and they are looking about a $90 million loss if we lost the use of atrazine. One reason of why there are more restrictions maybe the manufacture would say, Okay, we are going to just get rid of the sugarcane tolerance or the ability to use it for sugarcane because corn and sorghum maybe higher and therefore, we will give the agency sugarcane and that is why we have been at the table from the very beginning with the manufactures also, making sure they save our use as well as the more major uses.

And just to let you know that as new chemistry are found we, part of our role has always been testing all of the new chemistries for the industry in Hawaii, and so far we have always had to add and most of the new products have a little bit of atrazine still left in them to make them at least equivalent to the regular effective use of atrazine. There just has not been one that came along that could totally substitute for it.

I was going to talk a little bit about the Agriculture Health Study, but I think you have heard enough about that so I am not going to do that. So I will look at that first slide, because the National Corn Growers Association and the Farm Bureau talked about other countries and I thought it might be useful for you to have that in a table format, and so there it is. It is just put in a nice format that you can look at to see what the other countries have done about atrazine.

Then the bottom row there is for the water levels. Just to emphasis those; in Europe it is 14 parts per billions, that is a health-based standard; Australia 40 parts per billions. And institutional research center there is not applicable; they do not set that. US EPA has the lifetime MCL at three ppb. And I was very involved in an early period before that and it was 25 parts per million. It was a health advisory and we happen to have an area in our state that was close to three and so we voluntarily stopped the use of that compound in that particular area because there was this concern which was not fully fleshed out that three would be the new level set, which it eventually was. Though we did not hit the three; we were always at two and went down from there, but we are a very conservative industry when it comes to environmental issues.

Also under the US EPA they have the DWLOC of 12.5, which is regulated under now to 68 parts per billion. World Health Organization, which was pointed out already, went from two from prior to 2010 to now 100, based on the same data that is being reviewed here.

So my next slide, I just out of curiosity did a little calculation based on some of the data that has been presented in the last couple of days. Anyway, so I did a calculation for the 2.56 milligrams/kilograms/day which was the number that came out of the Cooper Study, and converted it to ppm in water for adult females, 60 kilograms instead of the 70 kilograms male and then the consumption rate of 2 liters per day.

If you put those numbers in -- and then for the uncertainty factor on the bottom there -- that is the ten times ten for the standard intra and interspecies safety factors. Then three has been talked about here for the FQPA number and then the two liters per day. And you will get a level of 256 parts per billion and it is my understanding is we have seen some of the monitoring data, which we did some of ours ourselves very early on, no numbers have even come near that. I think the highest in the community water system is something like in the high 60's to 70's.

The only other thing I wanted to say was that since I do really have a long history with EPA in terms of just paying a lot of attention to regulations and statues that are done by Congress, I wanted to express the agriculture community's appreciation of the deliberative and transparent and open process that now allows dialog and input from all of the stakeholders.

Now really almost previous to the atrazine thing, the growers just sat back and let the EPA, the Agency and the

registrant just do their thing and come out with the numbers and then we followed the labels. But, in the atrazine, it was really hitting many of the growers hard in that they had worked with this compound 50 years -- or back then it was probably only 40 years or 30 years, since we have been doing this for almost two decades -- and felt that, gee they are really the first target of any toxicity that comes about. So they were very concerned about this and wanted to follow the process a whole lot more than we had in the past.

So we are pleased to be able to be at the table as a stakeholder in this process and we are glad that the process has developed to that, because that is not where it was before. So we recognize the hard work and long hours everybody involved has put into the regulatory process, including yourself, and regardless of the topic, not just about pesticides but all the environmental statutes. And we appreciate that through the open process the Agency may often feel like a bull's eye, which everyone is taking shots at. And surely we have heard some of that this morning but all of you are scientists, you go to the conferences and workshops and that is where we have dialog and we are able to talk about experimental designs and the rest of it, and this is really the process that is set up for regulatory things, which does not allow that same kind of stuff that the scientists do. And so unfortunately this is the way that we can have scientific discussion and although it may be difficult sometimes, you feel that people are being critical unfairly, unfortunately, this is the

process we have and we are glad that we have some process.

So again, I want to express the growers' gratitude to you folks and to the Agency that opens up the process for input. In the end, we believe that we will get to a fair and reasonable policy and safe and effective pesticide use that is based on science. Thank you.

DR. DANIEL SCHLENK: Thank you. Any questions, clarification? Thanks. This concludes our public commenting period and I would like to thank each of the participants in that for coming forward with their comments. At this point in time what we would like to do before the break, if possible, if we could have the agencies come forward. We mentioned yesterday that we would have them come forward for those of you that have any questions that sort of came up over the evening from the plethora of presentations that took place yesterday. And, give you guys the opportunity to ask one final sort of batch of questions or clarification if you had any. So I thought it would be good before we got into the charge questions that we could ask any questions or clarification if anything came up through some of the oral presentations.

DR. HEATHER YOUNG: I think the epidemiology group would just like some clarification to make sure that we are on the same page. One of the charge questions that we are being ask is to look at the descriptor for the cancer risk assessment and so last night we looked online and we found that using the 2005 classification. And so I wanted to make sure that our choices would be inadequate,

not likely, suggestive; are those the categories that we are making recommendations as to?

DR. ELIZABETH MENDEZ: With regard to cancer classification within the Agency, that is indeed how we do it, suggestive, not likely, et cetera.

DR. HEATHER YOUNG: Okay. Thank you.

DR. DANIEL SCHLENK: Dr. Greenwood…

DR. RICHARD GREENWOOD: I wonder if you could give me a little bit of help on some of the pharmacokinetic data because I have not been able to get hold of some the original reports. It is just about the methodology, if you could just help me. I wonder can you tell me whether when they measured the total radiolabeling in the plasma, was that just taken at spinning down the red cells and then combusting the plasma or was it whole blood?

DR. CHESTER RODRIGUEZ: Yes, that is my understanding. So the cellular component of blood like it was removed, like it was spin down, like you said, to actually isolate a plasma component. I should also mention that the Simoneaux 1995 study that you requested it was sent to Joe Bailey so he should be able to provide that.

DR. RICHARD GREENWOOD: Thank you for that because I think interpreting the data, the methods that we used from the fraction that was actually counted I think you will realize there is a big difference.

DR. DANIEL GRIFFITH: For the monitoring group, why did you choose GEOEAS for your semi-variogram modeling?

DR. NELSON THURMAN: This is Nelson Thurman. I am going to let Jim Hetrick who used GEOEAS come up and explain why he chose GEOEAS.

DR. JAMES HETRICK: It's a simple answer actually. We used it because that is the software package we had available. How is that?

DR. DANIEL SCHLENK: Any other questions or clarification.

DR. KEVIN O'BYRNE: I just have one, yesterday you were talking about the pseudo plasma steady state levels; in the rat it is four days and it has a 4-day cycle and it takes four days of atrazine to reduce LH secretion. Then in the human, it takes 28 days and they just happen to have a 28-day cycle. It all seem terribly simplistic to me. Is that because women are bigger than rats? It is body weight that leads to that?

DR. CHESTER RODRIGUEZ: Basically, the 28 days comes from a range of values. For a human body weight of 60 kilograms, we came up with an estimate that ranges from 21 to 30 days based on allometric scaling or the rat elimination rate constant. So we decided to just use the value within that range and it just makes sense to use 28, which just happens to be the human menstrual cycle. So that was our thinking. But it was a range it was not 28 days exactly.

DR. KEVIN O'BYRNE: Yes, but in your slide you had 28. It's just terrible emotive. If we turn the clock back and think about the mammoths, assume they are like elephants; then what would you predict for them? 112 days?

DR. CHESTER RODRIGUEZ: The scaling that we have done is based on body weight, and that is the best information we have available. In the absence of specific human information, that is the best choice we have.

DR. TRAVIS JERDE: This question is for Dr. Cooper, regarding mechanism of action. It seem most of the research has been on luteinizing hormone and some on GnRH, and yet there is also affects on prolactin and the description has been that we have differing modes of action potentially. But one could also imagine that there would be a single or similar mode of action at the molecular level. I am wondering what kind of studies are being undertaken or proposed to look at signaling mechanisms or imprinting mechanisms, changes in DNA, things of that type that might help us assess more subtle effects particularly in the low dose range that seem to be overpassed in a lot of these studies.

DR. RALPH COOPER: There have been a number of them undertaken, not by us but by different laboratories, both in academia and elsewhere. And we still get bits and parts, but I can speculate a little bit on the suppression of prolactin by atrazine.

It is curious that in the ovariectomy estrogen-treated animal you can see a clear affect on prolactin

suppression. It is also clear that prolactin regulation during nursing, there is an effect. The reason that we even evaluated it, at the time I was reviewing a manuscript depicting the unique control of prolactin during lactation. However, when you go back into the cycling animal it is difficult for us to see -- under this condition that we have examined prolactin -- to see changes in the intact animal prolactin release.

Then the last part of that question is if there is a common mechanism. The one thing that we see consistently in the brain -- although I am not a big believer in the catecholamines driving any of this -- is under the acute experiments anyway, there is an increase in dopamine. That one possibility would be that it could influence GnRH neuronal activity, especially at the axonal level for the GnRH neurons, and then also of course dopamine being a prolactin inhibiting factor.

I am not aware of other studies looking into that. We are in our lab looking at other peptides in those things but we have limited resources.

DR. PENELOPE FENNER-CRISP: I guess this is for Dr. Rodriguez, since you were the one that raised the issue about the 60 kilogram person; what was the selection criterion for that?

DR. CHESTER RODRIGUEZ: None actually, it was just a typical body weight that we selected. But the good thing is that you can use any body weight that you think is appropriate for an adult human. It was just arbitrary.

DR. PENELOPE FENNER-CRISP: Once upon a time, it was estimated that the average female weighed about that, but if you look at the CBC data and the recent NHANES data, the average female in the US now weighs 74 kilogram. So the question becomes if you used the current average female as your sentinel for determining a number, it may not be 28 days anymore.

DR. DANIEL SCHLENK: All right, well thank you. We will go ahead and take a break now and begin the charge questions after the break; let's be back at 10:30.

DR. DANIEL SCHLENK: Everybody please take a seat. We are going to get started on our 14 questions. Before we get started, I think Dr. Fowle has a few comments.

DR. JACK FOWLE: Yes, I just wanted to just kind of review the biding for the purpose of the Scientific Advisory Panel. I admit I could be reading this wrong, but my sense of hearing some of the comments we heard this morning is basically that we are presenting with you a final risk assessment.

I just wanted to note that we are not coming to you today, to the Scientific Advisory Panel, and not sharing with the public, frankly, because we do not have it yet, this is not a final risk assessment. It is not even a preliminary risk assessment. We will not have a preliminary one until late 2012 or 2013.

What the purpose of this is to come to the Scientific Advisory Panel and share with you some of the conclusions we are coming to; some of the methods and models that we will be using and these will be inputs into the risk assessment that we will come up with. So we are coming to you to try to get your scientific advice and guidance as to our thinking; are we on the right track, or are there other things that we should be considering what you view as the tools; the strengths and weaknesses of tools and models and the types of endpoints we are thinking about right now.

Also, with respect to the epidemiology, we are really not asking for a judgment of the overall epidemiology risk. Basically what we are doing is saying, in terms of thinking about the considerations we have that go into our evaluation of the various studies, and think about how we might come to an overall judgment of the epidemiology data; also, more importantly, how we might integrate that with the toxicity information to come up with an overall weight of evidence approach.

We have tried to share with you, as best we could, what our thinking is, our line of reason, our logic and that kind of things. We would like your feedback on that. Also, to the extent that we are trying to move -- as some of you heard in May -- we are trying to move more towards implementing the toxicity testing in the 21st century, "Approach to Toxicity". We mentioned it would not happen fully for 15, 20, perhaps more years, but we try to do this incrementally as we went along.

We are using an adverse outcome pathway as a basis to try to lay out what we know, however much or however little in terms of toxicity, kind of use that as a framework. So we have given you, as best we could, what we know about atrazine in that context. So if you could give us feedback about that as well, that is the kind of thing that we are looking for in these charge questions, not a risk assessment, per se.

DR. DANIEL SCHLENK: Thank you very much. With that we will go ahead and start the reading in of the questions and as we discussed, if it is okay with you guys we are just going to read the letters of the questions rather than the whole question. Nelson, you are going to read the questions.

DR. NELSON THURMAN: Given the example dataset, we presented a matrix approach for deriving bias factors. So the questions we have related to that approach. 1. a) Given that the factors are likely to vary based on watershed size and water-body type, please comment on the level of detail we would need to develop for that. In other words, flowing water versus reservoir, and small versus medium versus larger watershed area. How many datasets would we need to analyze to provide a reasonable representation of a bias factor for each category? Then part b) Please comment on the advantages and disadvantages of deriving bias factors based on analyses of individual sites and years compared to taking percentiles of averages across sites and years.

DR. DANIEL SCHLENK: I guess you guys have the choice of doing this separately or together. Did you want to separate them into a and b or did you want to do them both together?

DR. ROBERT GILLIOM: I have it all together. I mean it is a… b, but it is sequential.

DR. DANIEL SCHLENK: Okay, so lead discussant on that is Bob Gilliom.

DR. ROBERT GILLIOM: So at risk of being a bit more boring because I read this, I would like to get it all down as I wrote it; so bear with me.

As a context for answering this question, the bias factor approach is probably best viewed as an early step in the type of systematic process that you show in Figure 22 of the issue paper, albeit with some different methods in the different steps.

Application of a bias factor to exposure statistics calculated from simple linear interpolation of sparse monitoring data is a potential simple and practical approach to evaluating data from a variety of monitoring frequencies to get either unbiased or conservatively high-biased preliminary estimates of exposure metrics, depending on how the factor is derived. The approach is primarily applicable to sites with moderate frequency monitoring data, such as weekly or biweekly, so that initial biased sample estimates are more or less in statistical control.

Quarterly data, for example, would be too sparse for use for short duration sample estimates. This said there is not a simple answer to the questions posed, because there are not enough data over an adequate range of sites and years to reliably organize the entire problem. The reality, as shown by the available calculations of site-year bias factors, is that each individual site has its own characteristics that govern the adequacy of different frequencies. And here are some observations about those.

Each site year has a different concentration distribution compared to other sites that same year and to other years for the same site. These site to site and year to year differences in the temporal distribution and magnitudes of concentrations also apply to the specific exposure statistic of interest, such as a particular maximum rolling average of 4, 7, 28-day levels.

Both the annual maximum of specific rolling averages and the temporal distribution of rolling averages, including total area under the curve for selected intervals, may turn out to be important. The implication of this is that focusing only on a bias factor for annual maximums may not fit all the needs for future risk assessment. Sparser sampling as compared to the actual population of interest, for example daily values, results in uncertainty in estimates and a tendency toward low bias for estimating high-end statistics.

Bias tends to be low because short-lived high-concentration events have a higher likelihood of being

missed with sparser sampling. Some broad differences among different types of sites are evident, such as according to basin size and reservoirs versus streams, but we really do not have adequate sample sizes across the gradients of all these conditions to quantify the relations with a sophisticated approach.

Potential Approaches that could be taken to address the problem include, and I will just name three. The first is evaluation of "relatively homogeneous" groups to develop a categorical system of bias factors. And this is the approach that is really referred to in the charge question. And if there really are useful groups, as opposed to a continuum of conditions, then – to give you a specific answer – perhaps on the order of 30 sites per group, each with several, 5-10 years, worth of data, might be the kind of adequacy for approaching on that level.

Reservoirs, however, which account for a large proportion of the community water supplies, will probably be difficult or impossible to categorize because of the highly variable characteristics, such as volume and residence time, which are not readily attainable.

An alternate approach to this categorical one that is mentioned would be to basically use a regression of bias versus explanatory variables, such as basin characteristics and water-body type, thus expressing bias as a continuum governed by specific characteristics. This approach could be promising for at least certain parts of the problem, such as watershed size for flowing

streams, but more data would be needed for multiple years at selected sites and at additional sites with intermediate basin sizes.

A third approach is a worst case group approach, such as small basins, to yield a conservatively high bias factor for protective screening that then would trigger monitoring. This could be a practical approach that can be used now, because we are relatively confident that flowing water sites with small basins, such as the AEMP sites and other small-basin sites, define the worst case bias factors, both for larger flowing streams and also probably for reservoirs, at least regarding short-term duration concentrations.

There are a significant number of community water supplies with the watershed size range within the range of the AEMP sites. A remaining weakness overall for these approaches is the lack of sufficient multi-year data. This is a problem for approaches other than the worst-case group approach above, because extremes do not happen every year. So that is the answer to part a). Should I stop here?

DR. DANIEL SCHLENK: Yes, let's stop here and we will split them up. Dr. Coupe…

DR. RICHARD COUPE: The only additional comment I have is just to reemphasize what Bob said was that I do not know that you could really develop categories of these community water systems. I think there are enough variables in

there that you would have a category of one for every water system.

DR. DANIEL SCHLENK: Okay. Dr. Lee…

DR. HERBERT LEE: I do not have too much to add to that, I largely agree fully. I did want to comment on one thing that Syngenta mentioned, they said "database sample number provide high confident on exposure". I just want to add in another piece of uncertainty that we mostly have been glossing over, which is measurement error. We do not have a good idea about what the magnitude of measurement error is. If you say go and take multiple samples at the same time, how similar did they turn out; or if you have multiple people taking samples at the same time, how similar did the turn out. And how similar is it if you have a person taking measurements versus an auto-sampler.

These are probably going to be relatively small compare to the big peaks, but if we are looking at extended durations of exposure, these sort of errors could add up.

DR. DANIEL SCHLENK: Any comments from the other panel members? Okay, you want to go ahead and do b)?

DR. ROBERT GILLIOM: So part b) to remind folks is, please comment on the advantages and disadvantages of deriving bias factors based on analyses of individual sites and years compared to taking percentiles of averages across sites and years.

My answer is the fundamental unit of exposure assessment is the site-year combination. And each community water system site has a unique watershed with corresponding hydrologic behavior, pesticide use, etc. A unique population of people served, and every year is different. Generally, analysis needs to focus on each individual community water supply as a unit. The condition of greatest concern is when the maximum of a selected rolling average duration exceeds a level of concern, yet to be defined, and this tends to be more likely in high use seasons during years when runoff after applications is high. Commonly, the most extreme conditions happen one or more times every few to several years, as exemplified by the Honey Creek and Maumee River multi-year results submitted by Syngenta.

Bias factors, to the extent they are used for screening-level analysis, should be developed with the objective of identifying sites that merit direct monitoring. In this application, they can be biased in the conservative direction and used to identify individual sites with an unacceptable likelihood, yet to be defined, of exceeding threshold, based on the available sparse monitoring data. These sites would then be monitored more intensively to more accurately assess the actual condition.

The bias factors may also be useful as a simple and transparent approach to estimating exposure for sparsely monitored sites for other purposes, such as for large-scale risk assessments or correlation with epidemiological results. In these applications, the starting point for analysis and the endpoint of interest

is the individual site, not groups of sites. However, there may be certain data analysis approaches that use data from groups of sites to make inferences for individual sites. This can be done as long as the uncertainties in predictions for individual sites are properly represented.

DR. DANIEL SCHLENK: Dr. Coupe…

DR. RICHARD COUPE: I do not have any additional comments.

DR. DANIEL SCHLENK: Dr. Lee…

DR. HERBERT LEE: I just want to clarify that – I agree with Bob – but want to say that it is important to look at each site by year when looking at the bias. So if we have comparison daily data and then we look at sub-sampling weekly or 14 or 28 days, compute by interpellation, compute the bias factor. You want to do that for each site for each year and then look at the distribution of bias factors, say across sites or across years. And you can gain information by pooling that way. But for computing the individual points in the comparison, you want to do it by site by year.

DR. DANIEL SCHLENK: Other panel members… All right, moving right along. Mr. Thurman, are you clear with what you have?

DR. NELSON THURMAN: I think we are very clear with what we have.

DR. DANIEL SCHLENK: Okay. Let's move on then to question two, and again you can feel free to read the sub-headings on that.

DR. NELSON THURMAN: Question number two; please comment on the Agency's method of estimating time series using conditional simulations of variograms for monitoring data sets such as the AMP community water system monitoring that have 7-day sampling frequencies. And part b) is; based on the US EPA's analysis using WARP with longer duration sampling intervals, what advantages does the SAP see of including WARP modeling in this approach, i.e., better estimation of the daily maximum value?

DR. DANIEL SCHLENK: Okay. Dr. Griffith, our lead discussant.

DR. DANIEL GRIFFITH: I have addressed these sequentially, so I will present part a) and then part b) separately. I wanted to prefix this with two comments. One is that the other discussants and I realize that probably some of what we will raise, the EPA scientists are fully aware of. Second, I do have tables in my report, which I will summarize.

So, this methodology acknowledges the serial correlation latent in time series data. Note that Table D1.1 NCWQR 1995 Maumee River Data Set contains substantial temporal autocorrelation. Conventional Box-Jenkins type ARIMA models require uniform spacing in time, but more effectively address seasonality. As an aside, the daily measures for 2011, the Syngenta report 2001301-03, imply that, for finished water, an ARIMA (1,1) model adequately

describes these data. And with those data that were released, most recently the ARIMA (1,1) model, the autoregressive term, was consistently above .9 suggesting that perhaps even differencing would be effective and the moving average term was roughly around -.4 across those six data sets.

Also, CWS-71 had a suspicious correlagram, but it could be a result of some of what we saw in the metadata this morning. Restricting attention to the days of interest appears to handle the stationarity issue in an effective way, but the Table D1.1 sample atrazine data implies that Julian days 101-200 may be the wrong time interval; the start time seems to be closer to Julian day 130 for that time series, and seems to go beyond Julian day 200.

The most recent Syngenta data support this contention for some of the other watersheds. And in fact, we saw a map presented yesterday and there was a similar map that was in one of the background material reports that showed the variation in latitude, which may well correlate with different start times and support this geographic variation consideration.

The complication here may well be that different CWSs will have different Julian day time periods; in other words, geographic variation in the windows across these CWSs.

Any methodology that focuses on mean responses, such as moving averages, the rolling averages, will tend to underestimate peak atrazine concentration. Expectation

maximization imputations are conditional expectations; in other words, they are means. The presence of autocorrelation implies that these conditional means are locally adjusted. Substituting conditional means into a time series for missing data values suppresses variance; they only represent a trend line. This is one reason for the underestimation of a 1-day maximum concentration, while obtaining reasonable estimates of rolling average concentrations.

This variance suppression also raises questions about assuming that standard time series developed by unadjusted kriging are representative of true daily time series. Virtually all software packages report standard errors for the case of random sampling. The assumption that they are the same for systematic or stratified random sampling, or for the observed non-probability sample of monitored days and I think diagnostics should be performed to evaluate the assumption of a pseudo-random unequal probability design, which appears to be at odds with their voluntary, truncated and mixed water gathering nature, may well seriously impact upon uncertainty assessment.

In addition, assuming that un-sampled days are missing at random seems questionable. In contrast, assuming missing years for any CWS are by design, and hence eliminating those years from the population of interest, seems reasonable. Perhaps assessments within the context of mixed modeling could furnish insights here. And I will come back to this in terms of pooling of time series.

Other principle issues of concern include the following: (1) impacts of misspecification on the semi-variogram model – a wide range of forms should be examined; (2) impacts of assuming that atrazine concentration data conform to a log-normal rather than another extreme value distribution, which can be dramatic in terms of the estimation of 95th percentiles; (3) impacts of ignoring spatial autocorrelation, which are less on mean patterns, and much more on 95th percentile estimates through effects on variance; (4) impacts of assuming a linear relationship between atrazine concentrations and covariates; again, potentially more dramatic on 95th percentile estimates, and add to uncertainty rather than improve estimatability; and, (5) impacts of not performing a sufficient number of simulations to really establish the uncertainty distributions; again, especially on 95th percentile estimates rather than on mean patterns.

Issue (1) here is of particular concern. Empirical semi-variograms estimated with sample data can be extremely variable and unstable. Subsequent SAS 9.22 PROC VARIOGRAM results include standard error estimates for semi-variogram model parameters. Many geo-statistical software packages fail to report these values, because, for example, some use visual curve fitting. Those reported here highlight this degree of variability.

One option is to exploit spatial autocorrelation by pooling data for similar watersheds when estimating semi-variograms; the subsequent discussion addresses this topic, too.

Finally, relatively large nugget effects tend to overly smooth rolling averages; in the absence of any autocorrelation, the E-M solution is the sample mean. Kriging produces the best linear unbiased predictors, and is one way to deal with irregularly spaced data through time; treating it like a linear geographic landscape, as well as a time series with a sizeable amount of missing data. For instance, the selected subset of Table D1.1 data has 43% of its values missing. It also is substantially better than the simple linear interpolation used in some of the preliminary research, and I have seen it in some of the more recent research since I have been here, although some substitutions, such as the one with PRZM model are conditional. But the implemented methodology appears to suffer from a number of weaknesses.

One drawback is considerably restricted candidate set of semi-variogram models available in GEOEAS, which no longer is a state of the art software package. It has a few exponential Gaussian, spherical; semi-variogram trend lines portrayed in Figures D-3 and D-27 appear unconvincing. A mis-specified model here is another source of the nugget effect.

In other words, you get a non-zero intercept value arising simply because of specification error. The autocorrelation in the selected subset sample time series is considerable, and appears to be much better described by a Bessel function, which more directly links to an ARIMA (1,1) model that is reflected in the most recent

data; if not a stable function, which is similar to a Gaussian function, but with an exponent other than two.

These models, as well as other valid semi-variogram models, can be estimated with ArcInfo's Geostatistical Analysis module, which I note the software supported the research for Report MRID 48470008. They also can be estimated with SAS 9.22 PROC VARIOGRAM. And they also can be estimated with modules from the R project, which are free and can be downloaded.

These two latter software packages furnish analytical rather than visual model estimation routines. SAS quantifies uncertainty associated with the semi-variogram model estimation, which is alluded to in the reports, but without specificity, and differs from kriging prediction error.

For the log-normal transformed atrazine example time series data; I did an estimation with SAS PROC variogram for the spherical Gaussian, which are two models that were reported, and the Bessell and the spherical clearly is not the best descriptor of the data. And depending upon criteria that you use, the Bessell and the Gaussian are competitive for that one-time series.

When I repeated this analysis, for the six most recent daily time series, in all six cases the Bessell function dramatically outperformed the spherical and the Gaussian. So those are two tables that I am just summarizing here. And it outperformed it on both criteria that are reported for goodness-of-fit.

Comparable results for other semi-variogram models can be obtained with SAS, PROC and NLIN – so you can actually program these for a couple dozen possible semi-variogram, but as I argued I think the most consistent one is the Bessell function – and it uses weighted nonlinear least squares.

A second weakness is the log-normal distribution assumption. Although the three-parameter logarithmic is the best Box-Cox power transformation enabling the selected subset of the Table D1.1 data to mimic a normal frequency distribution, the transformed data still are far from bell-shaped. The Shapiro-Wilk statistics, which are normalcy diagnostic statistics, improved from .9 to .93; so there is some improvement but there is still quite a bit of deviation entailed. This same description also applies to the six recent daily sampled CWSs. Of the six, only one achieves something that is indistinguishable from a bell shape curve with the three parameter log-normal distribution.

The log-normal conceptualization describes an outcome that may be viewed as the product of many positive-valued independent random variables. It has been used to analyze extreme values of, for example, rainfall quantities and river discharge volumes, and often is acknowledged as being a heavy or fat-tailed distribution.

One of the following extreme value distributions, which mean that their probability distributions have extreme deviations from their medians, may well be more

appropriate: the Weibull, the generalized extreme value, Gumbel, and the Frechet. The selected subset of Table D1.1 data better conforms to a Weibull distribution than a log-normal and slightly better conforms to an extreme value distribution, but the difference between the latter two is not very much.

Nevertheless, in all three cases, evidence exists suggesting that the empirical distribution still differs significantly from their counterpart's theoretical distributions. The largest extreme value goodness-of-fit appears to suffer from too many low values in the beginning of the selected subset time series. I think one of the reasons these goodness-of-fit are not coming out better is because there was a set day 101 that the time series started and if you inspect the time series, what you see is that that varies quite a bit. Which is what I was talking about earlier, a variation in the stationary part of the time series start and end date.

One stated ultimate goal of the methodology is to be able to predict values greater than those sampled. The Weibull, or perhaps another extreme value distribution, offers more potential for doing this than does the log-normal distribution. The most recent Syngenta report T001301-03 furnishes data for an additional six CWSs, and these data yields, almost across the board, support for the Weibull distribution over the log-normal distribution although there are some cases in which the log-normal distribution does slightly outperform the Weibull distribution.

These findings support the contention that atrazine may be better described by a Weibull distribution. They also suggest that such a characterization may be watershed specific. A third weakness is the overlooking of spatial autocorrelation. This is somewhat surprising because geo-statistics was developed to handle this data feature, and because of the extensive relevant discussions in Report MRID 48470008.

Many geographically distributed variables within a watershed exhibit spatial autocorrelation. Time series for different watersheds also may be correlated. Depending upon such parameters as planting timing and the occurrence of storm events, watersheds of similar size and similar characteristics may well generate similar but perhaps lagged time series of atrazine concentration.

If so, information can be borrowed from one time series to help complete another time series. And, information in comparable time series may be pooled to better estimate the semi-variogram models.

Planned research apparently seeks to address a forth weakness, namely the use of covariates, which are called soft data in the reports. Co-kriging allows inclusion of additional information. One concern here is the assumption of linear relationships between atrazine and selected covariates: scatterplots appearing in Figures D-23 and D-24 do not support this assumption. The furnished reports state a number of R square values without including scatterplots: a random scatter of n-1 points of approximately the same coordinate pair

accompanied by an extreme outlier can produce similar results.

Some linear regression analyses involve too few points. Linear regression with four, eight, or 15 observations I think tend to yield questionable results. Results have been obtained with analytical routines from Microsoft Excel; various analysts have shown many Microsoft Excel routines to be unreliable.

Conditional simulations are an efficient and effective way to produce confidence intervals for the atrazine time series. A fourth weakness, which is easily remedied, pertains to these simulations. Simulation experiments exploit the Law of Large Numbers and the Central Limit Theorem. Those based upon 10,000 replications should be sound. Those based upon 1,000 replications could be bolstered. Those based upon ten replications, for instance Figures D-28 and D-29, are unacceptable. Except in extenuating circumstances, the number of replications should be the same across all simulation experiments.

DR. DANIEL SCHLENK: Wow… Thank you Dr. Griffith. Dr. Lee, anything to add to that?

DR. HERBERT LEE: Not much, he has already incorporated pretty much what I had to say. I do want to just get on to the record a conversation I had with Nelson Thurman's group yesterday, after his presentation.

I think they are estimating an overall mean when they are doing what they are calling ordinary kriging, and that it

is skewing their results. The particular data structure here, when nothing is happening it returns to zero rather than to some overall mean level that is nonzero, which would be the normal case with spatial patterns. But here it returns to zero and so if there is a way to set the mean to zero, rather than estimating the mean, I think that will improve their results especially for the confidence bands.

For example, on their slide 16, looking at the Maumee 28-day results; those are sort of wondering around and they just have the wrong mean there I think is the main problem. On the 4-day average simulation on slide 19, for the Maumee and then the daily 4-day, 14-day averages for the Missouri -01, you see these weird bubbles that show up early on in the confidence bands and the confidence bands tends to be stretching higher than I think they really should be. And I think that is all because of having a mean that is nonzero; and that moving the mean to zero will help with a lot of the results.

DR. DANIEL SCHLENK: Thank you. Dr. Portier…

DR. KENNETH PORTIER: I should point out that we did communicate on Dr. Griffith's report before the meeting and so it really represents the three of us kind of thinking through this and we iterated it a couple of times.

I just have one additional issue that I want to bring up at this point, with using a 1D geospatial approach to model what is essentially a non-stationary time series.

And that is the assumption is made with the geospatial approach that observed data are known without error. And the impact of this assumption is most evident in figure 27-A, where the 95th and 5th percentile curves from the conditional simulations, coincide every seventh day at the known sample points. And if you look at the curve it is kind of weird; it goes up and than down… up and down.

We know in fact that these values are really estimates; for grab samples, they are simply snapshots of concentration at the time of sampling in the location that is actually being sampled. Looking at the actual data from, for example, NCWQR 1995 Maumee River dataset in Appendix D, section 1.1, we see that for some dates multiple samples were taken and that there is substantial variability evident in these estimates.

I think, although I am not certain of this – I did talk to my colleagues and I think they agree – that this variability is over and above the variability modeled by the nugget effect in the kriging model; this kind of needs to be confirmed and then incorporated into the model. Doing so will add additional variability to the simulations making 95th percentile curbs higher and 5th percentile curbs lower and having variability at the sample points.

DR. DANIEL SCHLENK: Any other panel members want to weigh in on this?

DR. ROBERT GILLIOM: I did not directly collaborate on the answer and I wanted to add that I think in this panelist

member's view I do not think kriging is the way to go to fill in this and a lot of the problems brought up maybe reinforce that.

I still feel that the better approach is to use a statistical time series model that links the temporal patterns of occurrence to some predictive factors like precipitation and stream flow and so forth, which is exemplified by the SEAWAVE model that was talked about in previous SAPs and recommended for this purpose. And I think, if I understood it right from the comments in the issue paper, it was kind of too much in the timeline to really get to that and try it. But my encouragement would be to still try that approach as a way to fill in data.

And the advantage it may have is an addition to being able to produce realistic time series that are unbiased for sampling frequencies like seven days and so forth. It can also be spread across wider sampling frequencies and more variable conditions with one tool. So you would have the advantage of having one singe tool be able to be used across a much wider range of circumstances where as with kriging you are going to have to have 7-day or better data probably to make it work. Thanks.

DR. DANIEL SCHLENK: Dr. Portier…

DR. KENNETH PORTIER: Bob, I was thinking kind of the same way. The only problem is the real non-stationarity of these time series, the fact that they have this jump up patterns and then decline for an event. And that is kind

of hard, unless you have a factor in the environment that mimics that kind of effect; say like stream flow, which we are going to talk about in a few minutes. It is going to be very hard to capture that with traditional time series modeling, and I do not know of any kind of approaches that would easily do it. But, in general, I kind of agree with you. The kriging is nice and it is taking into account all of this temporal autocorrelation, but like you I am kind of not convinced, and I think we will get to that in the subsequent questions.

DR. DANIEL SCHLENK: Anyone else in the panel on this? Okay, let's go ahead and go to b).

DR. DANIEL GRIFFITH: The WARP, which is the watershed regression on pesticides model, furnishes predictions of the distribution of atrazine concentrations in specific watersheds. Its input includes the following variables: atrazine use intensity, precipitation and rainfall intensity, a soil erodibility factor, percent runoff, and watershed size.

Competing models include: PRZM, which is the pesticide root zone model, EXAMS, which is the exposure analysis modeling system, and mass-balance. WARP model-generated output synthetic data for a 1-day temporal resolution would allow the use of co-kriging to secure missing atrazine concentration data imputations in a time series.

These supplemental data would need to be properly integrated with monitoring data. The reported experiment reveals that conditional simulations of merged WARP

model-generated and sampled monitoring data are highly dependent on the WARP-based imputations. If these data are equivalent to conditional expectations, then the associated imputations will have considerably less variability; in other words, the varian variation is suppressed, which given the large percentage of missing values, could overweight these portions of a time series.

In other words, WARP estimates do not really add the additional variability that would be expected in ambient measurements. Potential impacts include compromising the upper percentiles of observed concentrations, as well as reducing the likelihood of observing 4-day, or any x-day, rolling averages of concentration above some threshold value.

Perhaps one way to temper this effect is to add random noise to the deterministic values in such a way that they are indistinguishable from the observed monitoring data. One ultimate goal is to establish an upper percentile threshold that is not excessively conservative, in other words, orders of magnitude beyond the observed data.

The final percentile should furnish adequate protection, but not far more protection than is necessary, which could cost society benefits of atrazine while really not significantly improving the likelihood of avoiding adverse health effects. Within the context of this goal, error propagation merits evaluation to see whether or not compounding occurs, with this evaluation being balanced against returns on an investment of resources in such a study.

Conceptual arguments in terms of plausibility may be sufficient to dismiss some propagation possibilities. Sources of error meriting consideration range from merging spatially gridded field data that are 4x4 kilometers for rainfall with 85x74 kilometers for temperature, to raster images of rainfall totals from historical radar weather data – all of which may involve raster-to-polygon conversions - to the numerous assumptions employed by model-based imputation, for example, the 1:1 relationship between relative percentiles of flow and atrazine in the WARP model.

One concern expressed in the reports is the need for a priori knowledge about reasonable upper limits for peak concentration estimates. Although such figures furnish checks for synthetic results, percentages of these peaks are not being estimated. Furthermore, because imputations are conditional means, estimation of extremes is unlikely. Replacing a log-normal probability model with an extreme value probability model may help remediate this situation.

Research establishing a valid auto-Weibull type of model might be useful. For example, the autocorrelation trend in the specimen atrazine data suggests a Weibull distribution with a shape parameter of roughly 3.2, which implies that it approximately mimics a bell-shaped curve. This may be one of the reasons why, for that specimen dataset, the log-normal distribution performs well.

With an accompanying scale parameter of 2.3 and a suitable autocorrelation factor, which was estimated from the observed data, the resulting daily time series resembles the observed atrazine time series. Based on a simulation with 10,000 replications of 100 draws from the auto-Weibull distribution; the average almost perfectly replicates the base time series. And the approximate 95% confidence intervals based on 10,000 replications of 100 draws gives an upward bound of roughly 23.5 for the maximum observed concentration value of 14.058. This latter result supports the need for a priori knowledge of reasonable upper limits for peak concentration estimates, as well as indicates that imputations based upon a deterministic model, such as the WARP model, combined with an extreme value distribution, such as the Weibull, could allow prediction of values much greater than those sampled.

This example also illustrates that imputed values tend to be highly dependent upon the deterministic model predictions. In this case the synthetic temporal autocorrelation component employed accounted for roughly 87% of the variance in the observed time series, allowing much less variability for the stochastic component.

In closing, recognizing that EPA seeks reasonable estimates of exposure to atrazine from limited data, fine-tuning of the Agency's current approach may yield a number of benefits, whereas diminishing returns in additional accuracy of atrazine estimates almost certainly will set in as the complexity of its methodology increases. Furthermore, as methodological

complexity increases, chances of user error also increase. The final methodology needs to be implementable by various EPA scientists with a diverse set of expertise. In other words, EPA must establish acceptable trade-offs between the theory and the practice in these assessment too.

DR. DANIEL SCHLENK: Dr. Lee…

DR. HERBERT LEE: He has already incorporated all of my remarks. Thanks.

DR. DANIEL SCHLENK: Dr. Portier…

DR. KENNETH PORTIER: I just wanted to make one more point. Discussion of these fill-in models has centered on the potential of producing series with maximum closer to the single-day expected maximum. WARP PRZM combined models could help inform the estimate of the single-day maximum concentration in those sampling situations where the maximum has a low chance of being observed in the sample.

There was relatively little, if any, discussion in the white paper, on the ability of these simulated series to recreate the distribution of what I will call "durations of time exceeding a specified threshold"; in other words, estimating the likelihood that the concentration series produces a pattern with x-days in a row above some threshold concentration.

This to me seems to be a much more important statistic than the single-day daily maximum. Primarily because it

is more directly related to the regulatory decision, as we have had discussions about area under the curve and days of exposure.

Concentration time series with WARP PRZM infills, seem much more likely to more accurately estimate this distribution then would be simply using weekly samples or just modeling from the sample data rather than taking into account the basin and meteorology data that WARP and PRZM would do. It remains to be seen whether a running average time series properly scaled would produce a better estimate of this distribution than would a WARP PRZM infill series. And I think that remains an area of research.

DR. DANIEL SCHLENK: Thank you. Any other input from the panel?

DR. ROBERT GILLIOM: So I guess in further comment on the application of the WARP model is that I view it as its most valuable role as in applying to sites that you have no monitoring data for or data that is so sparse that you cannot fit a time series model or equivalent fill-in method. In that role what it is doing is simply giving you a prediction of the central tenancy of a chosen concentration statistic for all similar basin in a region.

So it give you an approximation of what to expect for that basin that could be used as has been mentioned to reconstruct a synthetic time series for sites with no data, basically. If you have actual data, just to stress

again, it should be used as opposed to trying to use the WARP regression model.

I guess the last thing I would say is that as the target statistic of interest get refined, such as a 4-day moving average or 14-day moving average or whatever, you can pretty readily refit a model like the WARP model to make that the dependent variable and just directly predict it. and directly predict the 4-day max based upon the watershed characteristics, and put confidence bounds on it and then that gives you a direct way to get right to the problem rather than having to reconstruct the whole time series. That is basically how we have applied it to date. So I will just leave it at that and we can follow up later if you like. Thank you.

DR. DANIEL SCHLENK: Any other input from the panel on this? Okay, we will go back to Nelson. Did you have everything you need on these? Do you need clarification at all?

DR. NELSON THURMAN: I think we have what we needed. Well…

DR. JAMES HETRICK: I guess I want a little bit of guidance here. Because I am hearing that if we are going to continue down this path of doing variogram analyses we have to upgrade our software, correct?

DR. DANIEL GRIFFITH: I think so. You can do it with R if you wish.

DR. JAMES HETRICK: Okay, that is fine. The other thing is I would like to just maybe in a little bit more plain

English here ask the question, are we on the right track as far as the conditional simulation approach?

DR. DANIEL GRIFFITH: I think you are. One of the concerns that I see is that if you do the imputations and use those – I saw several statements in the background materials that were completing a time series and now we have this time series of atrazine values. Well, all of those imputations are like the trend line and so you tremendously suppress the variance especially if in most of these cases you are estimating nearly half of the data.

And I realize that if you go into the spatial domain, that will estimate 90% of the data and there is controversy in the spatial domain about using that as well. So if you look at the missing data literature, people like Schafer, what they do is they then sample from – if it is a Weibull distribution, I would take this as my mean now that I have and I would draw a sample from it, with that mean. And then you might do that so many times to get some idea of the variability.

What I did in my example simulation, was I did 10,000 replications and so I had the basic imputed time series that was the trend lines and so I was sampling at each point from a Weibull distribution with that auto-correlated mean and then I get my upper and lower bounds. So it is a conditional simulation in that sense.

But I think that if you just impute and than use those imputations as though they are real values, you have

dramatically underrepresented the variation that you have in an actual time series. And you can even compare that with these daily time series that are available now; would give you some sort of benchmark to get an idea about that with.

DR. DANIEL SCHLENK: Dr. Portier and then Dr. Lee.

DR. KENNETH PORTIER: I think you are and as I was looking at what we were talking about, what you have not done here is really helped us split out uncertainty in variability. And it is something we keep coming to in front of the panel. But we are dealing with a time series and typically there we are talking about variability, right. We are trying to really explain that variability, the auto-correlation structure, making sure that we are not losing sight of the fact that yesterday's estimate has some information on what we expect today.

The other thing is the uncertainty. We are sampling from these systems. We are fitting models; the models themselves have uncertainty. Some of what Dr. Griffith talked about is uncertainty in estimating the semi-variogram, which has a big impact as it propagates through the model predictions. And probably in the next iteration of this, you really need to be kind of laying that out maybe a little bit more clearly. When are you addressing variability, which is a model component, and when are you addressing uncertainty, which is really a component of this simulation; to a certain extent, what you are capturing in the simulation.

DR. HERBERT LEE: As much as I really like kriging in general, in this particular case, as we have discussed, there is a lot of uncertainty. So I want to ask explicitly, are we getting any better results then just doing a linear interpolation and using a bias factor in terms of being able to predict accidence over a certain threshold of time.

It may be that in terms of the accuracy of our results, because of all of the uncertainty, we may be able to do just as well with the linear interpellation and the bias factor; that would be a lot easier and a lot simpler and probably cheaper to do in practice. And so I want to ask explicitly, is it worth the extra effort to do the kriging – as much as I like kriging in general.

DR. DANIEL SCHLENK: Okay, did you get your clarification?

DR. JAMES HETRICK: Yes, I think we are on the right track and I know where we need to go at least.

DR. DANIEL SCHLENK: Great. All right I think we have time for maybe one more question we can move in through here to get to lunch. Let's move on to charge question number three. And Nelson I will let you decide how much you want to read of that one. If you do not want to read the whole question, or just the subheading that is fine.

DR. NELSON THURMAN: This question is relating to some of the modeling approaches and methods we looked at applying to less frequent sampling intervals. And hopefully some of this is a spillover from what was discussed earlier.

Please comment on these additional modeling approaches, that we have presented both in the background paper and in our presentation, for interpreting sparse monitoring sets; in other words, sampling less frequently than weekly.

DR. DANIEL SCHLENK: Lead discussant on that, Dr. Lee.

DR. HERBERT LEE: So in some ways this is a continuation of the previous question but it has some different flavor to it. I do want to repeat when the data is sparse, you just cannot fit a variogram; you cannot get accurate results solely from kriging or from linear interpolation.

So various approaches have been explored; one of them is to use flow as a covariate. But the initial result has not been particularly promising. There are more complex relationships than just a simple linear relationship with flow; it depends on also the application timing and it is about transported materials rather than just the outright flow. So instead of thinking about flow itself, one direct improvement is to think about WARP, which is actually developed to model the situation; and so it makes more sense to use WARP rather than try and reinvent WARP.

Alternatively, we have looked at some other approaches. Syngenta's approach using PRZM appears promising. Looking at infilling points particularly around precipitation events; filling that in and using that to predict peak areas. You could set up a fairly conservative approach using PRZM. They also looked at

some methods using sort of the three times infilling, saying you expect a point, as a conservative approach, probably not more than three times what is observed nearby; and then you can infill using that. You can also look at building time series models or the SEAWAVE model, specifically for time series. So there are a number of different ways that this can go that I think are better than just looking at flow as a covariate.

I want to say sort of ideally, we want to set up a regime such that sites can move between different frequencies of monitoring. Right now we have some sites that are monitored weekly, during the application season, and others that are just monitored quarterly; and that is a really big gap. And it is unclear exactly how much information we are losing in there, but on the other hand if a site has been relatively clean, it does not necessarily need to be monitored as frequently.

So there needs to be a good way for moving up and down in terms of frequency, perhaps with something intermediate between weekly and quarterly like monthly. And using these sorts of different models at different levels would be a way to help guide when sites needs to move between levels. When you have weekly data you can do things like kriging or linear interpellation with a bias factor. When you have monthly or quarterly data we are going to need these models to help guide, do they need to be looked at more closely.

DR. DANIEL SCHLENK: Okay. Dr. Griffith, you are next.

DR. DANIEL GRIFFITH: The WARP, watershed regression on pesticides model, furnishes predictions of the distribution of atrazine concentrations in specific watersheds. Its input includes the following variables: atrazine use intensity, precipitation and rainfall intensity, a soil erodibility factor, percent runoff, and watershed size.

The PRZM predicts chemical movement in surface soil, yielding a daily time series of potential runoff event-based concentrations, and requires more input, for instance temperature, land use, and soil type than the WARP model. It uses spatially specific NEXRAD radar data, requiring additional data merging. The EXAMS model predicts the fate, transport and exposure concentration in surface water by combining chemical loadings, transport, and transformation into a set of differential equations using the law of conservation of mass as an accounting principle.

Its data inputs include fundamental chemical properties of atrazine, and up to 32 different segments for a given watershed, for each of which up to 28 different substances may be simulated. The EXAMS model also requires more input than the WARP model.

Finally, the mass-balance model, which describes variations in atrazine concentration as a series of storm-event associated peaks that taper off over time, produce atrazine discharge mass quantities that are often different by orders of magnitude in neither a positive or

a negative direction. Consequently, WARP appears to be a reasonable choice for obtaining supplemental data.

Output from that model yielding the best estimate of daily atrazine concentrations should be employed as the covariate in kriging. If output for no single model appears best, perhaps a weighted average of daily model output could be utilized. Reconsidering the semi-variogram models for the specimen atrazine data, including the synthetic spatial autocorrelation factor as a covariate for co-kriging produces considerable smoothing of the daily experimental variogram.

In addition, the resulting goodness-of-fit diagnostics improve for all candidate model specifications, and furnish additional evidence that the Bessel function may be the preferred model. These results corroborate that imputed values will tend to be highly dependent upon deterministic model predictions used as covariates. The lack of a stochastic component for the imputations will tend to suppress variability; deterministic model predictions are similar to conditional expectations.

Theoretically, if no relationship exists between the model-generated data and the observed monitoring data, then the deterministic values do not impact upon the kriged values. As the relationship between the deterministic model output and the monitoring data increases in strength, increasingly more information can be borrowed from the deterministic model output to complete each daily time series.

This procedure is far superior to linear interpolation. One principal weakness of using deterministic model output arises from the assumptions involved. Because values between observed monitoring points in time are unknown, they may not coincide with model output, even if the observed data perfectly align with the corresponding subset of model output.

This weakness furnishes a strong argument to employ a time-interval stratified random sampling design, rather than a systematic design with a random start weekday. Sensitivity analyses, especially with regard to error propagation, could shed light on the magnitude of impacts of certain assumptions.

Critical ones include the following: 1. in the PRZM model 60% of atrazine is applied at four uniformly distributed major pulses, with the remaining 40% being applied uniformly across all other days; 2. movement through a watershed is indexed to the longest shortest path between its outlets and its headwaters; 3. growing degree days are defined by – and they state a formula – difference between temperature extremes divided by 2-50 and that is in Fahrenheit. 4. all corn and sorghum crop areas are treated; 5. for PRZM all watershed farmers use atrazine in a similar way to those in the baseline CRC survey; 6. for PRZM the atrazine use rate is uniform across all soil types; 7. watersheds experience no conservation practices, and have good hydrologic conditions; and, 8. the non-random sample of monitoring data can be treated like a random sample.

Assumptions that most likely have little adverse affect on results include: 1. results for an irregularly space time series can be adjusted by weighting each value by 50% of the time distance between its preceding and its subsequent value; 2. multiple sample values for a day can be represented by their geometric mean; 3. values substituted for those quantities less than the detection limit; 4. designing analyses in such a way that concentration estimates tend to be conservative; and, 5. the half-life of atrazine is 61 days in a watershed although evidence exists suggesting that it has a much longer half-life in subsurface soils.

In the end, a meaningful model is only as good as the ability of its assumptions to mirror the real world. One notable trade-off is between the expenditure of resources to collect reliable sample data, in a design-based context, and the use of model-based techniques that require resources to assemble massive amounts of ancillary data properly and then convert them into sample data equivalences. Physical sample collection requires retrieval followed by storage of specimens, and is plagued by instrument malfunctions as well as human error. Model generated results suffer from data availability as well as human error. One cannot go back in time to correct the former; updates followed by model re-executions allow corrections to be made for the latter.

DR. DANIEL SCHLENK: Okay, next discussant, Dr. Portier.

DR. KENNETH PORTIER: Thank you. So I will start by saying I do not have a lot of experience with this kind of modeling, but that do not usually stop me from commenting. I should say that that experience is evening worst when I sit down with Dr. Gilliom and Dr. Coupe and start talking about what PRZM, EXAMS, SEAWAVE, and WARP, really produce in getting a better understanding of that. So some of this I may have to change as my understanding changes from the discussion. But I'll go on.

I can only assume that the WARP or PRZM, EXAMS, or the SEAWAVE models have the potential to produce a daily time series that could be used as a covariate time series, for example, in a co-kriging approach. That if sufficiently correlated with concentrations, would allow one to essentially fill in mean concentration pattern between sampling dates and the concentration time series. I am assuming that the estimated variogram, for the concentration time series, would possibly demonstrate better properties because it would not have to be accounting for as much of the total variability as it had to do without the covariate.

So I think if we get the right predictions from these deterministic models, it is quite possible we can get much better synthetic kymographs. So that is the first thing. But I wondered, as have some of the previous discussants as I read this section a number of times, if a simpler model might result in similar results and actually be easier to understand. For example, what happens if one were to simply regress the sample concentration time series on the PRZM or other model

predicted time series, using some kind of nonlinear function, a polynomial or something else or use some kind of robust smoothing approach such as low est. and properly lagging these results, whether we would get just as good a prediction. I do not know if we have really looked at that.

If one finds that the resulting R square was pretty high, close to one, I would suggest that the PRZM model or other model would be a good predictor of the concentration time series for the un-measurable time points. This approach might work quite well for what Syngenta referred to as the small AMP size classes, because the smaller areas allow for only one or a few fields to be impacting the water concentration and time lags would be reasonable on the order of a day or a couple of days.

So those small size classes where we have things that are really spiky, some of these model that might actually work because it is a close connection between what is going on in this small watershed and what is happening at that sampler in the community water system.

For larger CWS size class areas, incorporating many more fields and longer transit time, it is more likely that the concentration time series is some kind of weighted sum of variably lag WARP model prediction for all the fields impacted by significant rainfall event. So the larger the area the more kind of random of incidents occurring that you are trying to add up and integrate

through to get to a concentration at a community water system.

And all I could think of is for one thing this would be a very challenging model to fit; that is a statistician way of saying it is impossible. But then on the other hand I keep thinking this is some kind of sum of correlated series model. There must be some kind of limit theorem going on here that says when you get a number of these things happening and they are lagged, you would expect to see some kind of log-normal type pattern or something like this.

So for the medium to larger systems you are having to deal with less of the spikiness of the pattern and it is much more of a modulated pattern, which I would expect from this kind of sum of correlated series kind of thing in that. So you might be really looking at two different kinds of modeling scenarios.

This kind of regressed time series approach do not preclude the introduction of further autocorrelation structure in the residuals of the model fit. So the regression is fitting kind of the long-term pattern and then you have correlated noise around it as well and you could add that; some kind of again nonlinear ARIMA model or where the mean is not constant.

To some extend Syngenta is taking this approach by using the modified PRZM model to fill in the extreme events between sample concentration value. But if you are going to fill in the extreme events, why not go the whole way

and use WARP to fill in the rest of the sequence. This is not what Syngenta is doing because if you look at slide 20 of the Syngenta 'Occurrence in Drinking Water' presentation in the meeting docket, it clearly shows they are using linear interpellation between the WARP inspired maximum and the observed sample points. One other issue with the Syngenta approach is that they assume an atrazine runoff event occurs between every pair of sampling days.

So for a 7-day sampling this essentially assumes a runoff event every week and from some of the date we have seen in previous SAPs, and I will have to go back and look and see whether it was the February or April 2010 SAP, where we looked at a lot of these patterns, we know that for the most part it is one or two event a season that any of these things observes; so why would we even assume a priori a maximum every week. We really should be saying, "Oh, we will throw one somewhere in here" and that is a more reasonable model. And for small watersheds I might throw that maximum in closer at the beginning of the week and give myself time for that decay that would match the next sampling point, right; a very simple modification of their method.

Are there other time series that could be used as an explanatory model in this kind of regression approach? I think most hydrologist would agree that water flow would not be expected to be highly correlated with concentration except in possibly the very smallest basins; and even then both concentration and flow would be highly correlated with rainfall or irrigation patterns

and all of that is assuming atrazine has been recently applied in the field. There has to be some material there to flow off.

So if I have anything else I would say for small fields we really need to look at rainfall and irrigation patterns; that would be the only other time series that I could think of that might have any effect. And I will stop at this point.

DR. DANIEL SCHLENK: Any other panel member have anything to add.

DR. ROBERT GILLIOM: I guess one thing I would add is that personally I would encourage the continue development of the PRZM approach and especially the underlining data that is needed to drive it at a national level. Because the transfer value from that whole methodological approach from data to model will have a lot of transfer value to other chemicals and it is a good investment to make I think. But, on the other hand my other comment would be is, I would not get bogged down by holding off on all of the related decisions for a compound like atrazine while you work through the whole process, which might take a while of seeing how an edge-of-field model applies to different size systems and so on and so forth that we have not all got in to. So I guess I am in the camp of continuing to move ahead on development, but do not let it get in the way of progress.

DR. KENNETH PORTIER: This morning we had some conversation like this. So there is the atrazine issue and then there

is a longer term risk assessment paradigm for EPA and we see this kind of PRZM modeling time series stuff as part of a longer term paradigm that EPA is going to need nationally to be able to make these kinds of decisions. It just happens to be you are doing it with atrazine because it has this fantastic dataset and a lot of information, and you have a little bit of a push now to incorporate that. But I tend to agree with Dr. Gilliom in that we do not want that longer term goal to hold up your shorter term decision-making process.

DR. DANIEL SCHLENK: Any other panel input on question three. Okay we will go back to Nelson, do you have everything; do you need any clarification on this?

DR. JAMES HETRICK: In our attempt to try to infill that in that example that we have in the white paper in the appendix, we use flow; flow may not be the best covariate to use to try to take the WARP estimates and put them into a time series. Do you have any other suggestions of other covariates – I was thinking like nitrate or nutrients that might be something to look at – that you would suggest looking at as a covariate?

DR. ROBERT GILLIOM: I think certain aspects of flow will work if our time series modeling is an indication. So if you are within the seasonal window and you focus on anomalies of flow conditions from normal, for that season, then I think you will find them to be predictive; in the same vein I think daily precipitation will prove to be predictive as a explanatory variable and a time series

model. And then I guess those are the two main ones I think of and I can let you know if I think of others.

DR. DANIEL SCHLENK: All right, everybody good on number three?

DR. KENNETH PORTIER: When I looked at the flow data and you plot it out, it is very clear you have kind of a bi-modal thing going on. You have these periods of low concentration and high flow; so the stream is flowing but there is nothing in there. There is no chemical in there and that is because there probably was no chemical in the field or previous rainfall events washed out whatever was available to be washed out. But on the other hand there is a period where concentration is correlated to flow and so you kind of get this bi-modal process going on. And I do not know how to model that because the information on how you switch between this one and that one is essentially what PRZM is providing you.

PRZM integrates that field level fertilizer application and stuff like that. So just kind of naively using flow is not going to help you because I think you are adding a lot of variability because of what we looked at there.

When you look at other agrichemicals like fertilizers, clearly they are not put down at the same time as atrazine is, atrazine being a pre-plant. Sometimes they put fertilizer down, I guess, for some crops; some crops they make a separate trip at a different time through the field. I really do not know whether looking at those things are going to help you – especially nitrogen.

When you said that I had a flashback to some time series data I looked at of North Florida nitrogen flow in these clay areas, and the data was a nightmare because I could not predict when you would get these spikes. This was off of the chicken farms where they spread the fertilizer out in the pasture and then you are wondering when it is going to hit the stream. And it was not correlated with rainfall; we needed a PRZM model to begin to understand why it showed up today and it did not show up yesterday or a week ago.

DR. DANIEL SCHLENK: Okay, any other comments on that? You guys good with that?

DR. NELSON THURMAN: Yes, I think so.

DR. DANIEL SCHLENK: Okay, debating on whether to go on to four, but I think since we are so far ahead I think we will go ahead and just take a lunch break until 1:00. Let's go ahead and do that and we will reconvene at 1:00.

DR. DANIEL SCHLENK: Welcome back. Let's go ahead and move on to our next charge question which is charge question #4 which is, I think, the last in terms of our water sampling section and Nelson if you want to read that into the records that would be great.

DR. NELSON THURMAN: The preamble relates to our focus on trying to characterize year to year variability. Part A: Please comment on the sufficiency of existing atrazine/triazine monitoring data available to the Agency

– in particular the Atrazine Monitoring Program (AMP) coupled with the earlier Voluntary Monitoring Program (VMP), which conceivably span from 1993 to the present for some community water systems (CWS) – for use in characterizing the likely range in year-to-year variability in atrazine or total chlorotriazine (TCT) concentration.

Part B: Please comment on the Agency's suggestion for using a PRZM hybrid model, calibrated on the current years of monitoring, to provide estimates for a wider timeframe by modeling additional years using weather data that span a 30- to 50-year period.

And Part C: What other possible approaches can the SAP recommend for capturing year-to-year variability?

DR. DANIEL SCHLENK: Okay thanks. Our first lead discussant is Dr. Coupe.

DR. RICHARD COUPE: Thank you. This is Richard Coupe. Sadly I think everything I am going to say has already been said, at least once in our first three questions but I am going to say it anyway just because I have the floor.

Before I address the question directly, though, I want to make a couple points. They have been made by other people but I kind of want to put them together. One is that atrazine is an extremely important economic chemical. The other one is that atrazine is found in the source water from a number of community water systems. Material that was supplied by Syngenta indicated that

during the period 2001 to 2009, greater than 1.5 million of our fellow citizens were exposed to concentration of atrazine greater than 3.0 in their drinking water and that was just from quarterly samples.

I think these two facts are why we are here. In my mind it is right and appropriate that we discuss this. There have been some comments about how many SAPs there have been over the last 10 years or so but again, in my mind, it just shows how important the topic is.

I want to mention that when I was first asked to be on the SAP last year, I called Bob and asked him whether I should do it or not. He said sure, it is fun, come and watch government sausage being made, it will change your life.

So the question presupposes that you can never stop collecting observation data. By using historical data we can characterize the distribution of atrazine in drinking water in the future or that would mean for that to happen we would have to be able to say that what happened in the past is what is going to happen in the future.

So some of this data that we are looking at now is collected from as early as 1993 and that is getting close to 20 years old now and the question that comes up is, has anything changed over that time that would make you think that perhaps the delivery of atrazine to the water shed had changed? Of course there is a whole list of things that make that true and one is conservation tillage. We have a whole lot more conservation tillage

than we did before and the other one was the introduction of genetically modified crops, specifically for atrazine and some of the other crops; I mean for corn, for glyphosate, so this changed how atrazine is used.

So if you consider the drivers of what makes atrazine appear in your water, basically you have kind of three drivers. One is hydrology, one is flow path and the other one is use. All three of these work in combination to move atrazine into your service water. Then the question that comes up is, if you want to stop your sampling or if you are going to change your sampling pattern and rely on historical data is, will these change in the future? Well, hydrology kind of reflects rainfall and we do not have to look too far past this last May to see how much rainfall can change over time.

All you have to do is look at some of the WEB sites and show how many sites in the Midwest during the month of May had historic periods of record in their flow. It was just tremendous. So we had a record flood in May right there in our application time period. And you can ask these questions of flow path. When we talk about flow path, flow path is how water and atrazine moves over the landscape or under the landscape; how it moves into the stream and then into the drinking source. And, has that changed over time or can it change over time?

And then in reality it can change and it is going to change probably in the future. Conservation tillage is a big part of it. I mean, one of the big drivers for atrazine in service water is how it flows over the

surface and so if your conservation tillage leaves a lot more material on the surface it slows down the water so this has probably changed a little bit how the atrazine appears in surface water.

We are also now having -- in the Midwest especially -- we are having subsurface drainage which is being directly run to the surface of the soil. So we are having a flow path that is now changed. We are not running off into the stream now, we are running off into a low depression on the field. It is going into the service drain and is moving down through this drain and then into the stream. So that could actually elongate the distribution of atrazine in the stream.

We could have changes in flow path. All this is just to say that we could have changes in the future. And then use; could we look at use? Can use change in the near future? That is again true. If we just take a look at what happening with glyphosate now, we are having resistance appear for glyphosate for the chemical roundup and that could change how atrazine is used. The use of atrazine could increase quite a bit in the future because of resistance to glyphosate.

Also the biofuel's initiative, although it does not directly increase the amount of atrazine use, it increases the incentive for farmers to grow corn which does actually increase the amount of atrazine use. So we could be having big changes in all three of the major drivers for our flow path.

So then the question was is do we have enough data or when will we know we have enough data. Well, you are never going to have enough data is my conclusion on that. But do you have to sample everywhere all the time. Well no, of course you do not have to. And we have this atrazine monitoring program, which has a way into it and a way out of it which is really pretty good.

I do not think it is completely predictive of all the systems because to get into it you look at a quarterly sampling and have to exceed -- I forget what it is -- to exceed, 1.6. So we can probably use something like a WARP or a PRZM model to kind of look at places we do not have which are more vulnerable, but we do not have exceedances in their STWA samples.

In addition, you could use those to move them out of AWP or the monitoring program as you can use that in combination with your sampling to indicate that conditions have change, that you will not have the issues with the distribution that you had. I think that was all my comments on that part.

DR. DANIEL SCHLENK: Thanks. Bob Gilliom.

DR. ROBERT GILLIOM: Mine are just a few specific things to point out regarding adequacy of the historical multiyear data.

One is just along the lines of… and I am not trying to parse out which ones have which, but the total chlorotriazine were not measured in all of them so that

is just one thing to keep in mind. Also, in particular, simazine has been, as an example, some of the long term trends has been more up-trending in use and concentration. So those kinds of things always have to be watched out for as Richard alluded to for applying historical data to the future.

I did think that the VMP data, the voluntary monitoring thing that is weekly but since the early 90's, does present a valuable database to examine year to year trends as long as the bias and the short duration of statistics is either avoided or accounted for. It still does provide a really valuable database that has multiyear data for over 100 sites.

In terms of sufficiency of annual data, you know, sufficiency depends on the application; I guess is the glib way to say it. What we have now is sufficient for some purposes and not for others. If the objective is to estimate it conservatively, protective bias factor to apply to sparse monitoring data, and you can be comfortable with a conservatively high estimate from the data arranged, it is probably adequate. If you want to estimate actual within a narrow bound of uncertainly like plus or minus 20 or 50%, then it is not.

I think the reliability criteria just need to be stipulated and used to gauge whether accuracy is adequate or not. I think that is all I have.

DR. DANIEL SCHLENK: Thanks. Dr. Lee.

DR. HERBERT LEE: I just want to add one quick old detail in that there is a lot of year to year variability but particularly in, say, if you are looking at the magnitude. There is a lot of year to year variability. I would expect the shape to be more similar year to year. So things like the modality, is it one peak, it is multiple peaks, what is the correlation structure as far as estimating variograms? I would hope that that would be more consistent year to year but the overall magnitude of these peaks may be highly variable depending on these other factors that have already been discussed.

DR. DANIEL SCHLENK: Okay. Dr. Portier?

DR. KENNETH PORTIER: I guess I tend to agree with Syngenta that for atrazine we have a very rich database, one that should provide more than adequate information on year to year variability in atrazine and TCT concentration. The issue is more how to effectively utilize this database; how to extract the necessary statistics with the appropriate models to be able to produce, what I call, less bias estimates with within year and among year variability. Bottom line, I think we have a very rich database; we just have to figure out how to use it.

DR. **DR. DANIEL SCHLENK:** Okay, other panel comments? Yes, Dr. Griffith.

DR. DANIEL GRIFFITH: This links back to some of the other questions that we discussed before lunch as well. In part, when I think about… and this question, right at the beginning, characterizing overall uncertainty and

exposure… It seems to me that one of the critical questions -- and I commented briefly about it before and it was discussed in your presentation to us as well -- one of the critical issues is if we start looking at all the sources of variability or error, do they compound? How does error air propagate through all of the analyses and in part there has been focus on sampling error in terms of frequency in time and to some degree in space in terms of doing the sampling.

There is going to be, sort of, inherence stochastic variability in atrazine and that is going to be through time, that is going to be over space and that also is going to be how it gets into the water supply, which I think links back to the type of water shed, et cetera. We have talked about measurement error and I have not seen anything about the degree of measurement error in the water assay, but certainly imputations are going to introduce measurement error and type of sampling.

If it is the auto sampling which, at least what was described in the background material, is sort of averaging across the day because there is a little bit taken at different times of the day versus a one-time scoop. There is going to be, what really is measurement error there because it is a different type of sampling. And then I noted the specification error in terms of the semi-variogram model, but specification error also could enter through, is WARP the right model to use, should it be a different type of model and so there could be specification error there.

And these are some of the basic sources of error and I think there needs to be some type of a conceptualization as to how these interact, if they interact, and do they compound so that if in the end the 95… well I guess they are 90% confidence intervals if what you are really establishing, I would prefer 95%, but 90% confidence intervals, if they are based just on the sampling error, are they adequate.

If you assume that all of these actually compound, you could end up stretching those dramatically and unnecessarily. And so I think that there needs to be some consideration about what is going to compound and what is not in order to go back to this characterization of overall uncertainty. I think the answer to this question probably focuses a bit more on specification error in that whole topology.

DR. DANIEL SCHLENK: Thanks, Dr. Griffith. Any other comments on letter A, question 4? Okay let's go on to B. Dr. Coupe.

DR. RICHARD COUPE: Richard Coupe. Let's talk about the PRZM model. I just wanted to say that I really like what Syngenta has done with it. It is a very innovative approach. I particularly like the process-based work so you will incorporate whether atrazine use and the degradation characteristics for atrazine and the partitioning of atrazine and use soil characteristics to kind of predict how atrazine moves off. Their initial results certainly look promising.

On the other hand, PRZM really was not specifically developed to look at surface water runoff. It was developed as a root zone model and hydrology is really not model directly within the model so I am not sure how useful the model might be in the long run if you wanted to look at… say you wanted to look at what process was moving your atrazine along. If you do not have hydrology models specifically, you cannot really eliminate or look at it.

And then that kind of restricts your watershed size unless you figure out a way to handle that. Otherwise, I think it is a really good and worthwhile effort to move forward with and we need to have some sort of model, such like this process base, like the WARP does or PRZM, it looks really good for future results. That is all I have for that.

DR. DANIEL SCHLENK: Okay. Bob Gilliom.

DR. ROBERT GILLIOM: I think the only thing that I want to add is that the added benefit of continued development of PRZM is in relation to the database that supports it and that also may help support moving into also try to SWOT the STA model, which may have more applicability to the watershed scaling up in the long run. I think the overall effort is really good in that it is just going to enhance over time the ability to make the modeling approaches a more sophisticated addition to the tool box.

DR. DANIEL SCHLENK: Dr. Lee.

DR. HERBERT LEE: I concur and have nothing to add.

DR. DANIEL SCHLENK: Dr. Portier.

DR. KENNETH PORTIER: Sounds like a good idea if we can make it work.

DR. DANIEL SCHLENK: Any other panel members for B? Okay. Letter C then. Dr. Coupe.

DR. RICHARD COUPE: I really do not have much to add to this. We have been given an awful lot of ways to fill in data to kind of look at how to predict this. I do not want to overwhelm us too much.

DR. DANIEL SCHLENK: Okay. Bob.

DR. ROBERT GILLIOM: I would just return to the old topic of what kind of time series model to approach for fill in. I think if you use a more deterministically-based statistical model that has predictors in it like precip and flow anomalies and so forth, those could actually prove useful to reconstruct historical records from historical climate data and historical use data. That is one of the reason I favor that direction as oppose to a pure statistical fill in.

DR. DANIEL SCHLENK: Dr. Lee.

DR. HERBERT LEE: I just wanted to add in another comment that I was trying to figure out where to fit in and this is the best place I could figure to fit it. We have had

some discussion brought up by other presenters yesterday about quotes from previous SAPs. I want to note that previously, for example, in April 2010 we were asked to consider exposures as short as single day maximums and there was a lot of emphasis on trying to estimate a single day maximum from a weekly sample.

And then even in September of 2010 we were considering ranges. But at the time that we were discussing the hydrology and the water monitoring aspects we were still keeping single day maxima within the scope of what we might be trying to estimate. And at the conclusion of that panel we were moving towards area under the curve for a minimum of a 4-day moving average and that seems to be where we are moving now.

So going forward I think we are interested more in time periods of four or more days. So some of what we have suggested in previous SAPs may not be as applicable anymore now that the time period has shifted. We were trying to be flexible in the earlier SAPs depending upon what came out the biological side and it seems like there is some convergence now on the biological side that it is not really a daily maximum that is of interest to human health, maybe not conclusively, but that seems to be where we are headed. Some of what we said in earlier SAPs should be taken in the context that that has changed.

DR. DANIEL SCHLENK: Thank you. Dr. Portier.

DR. KENNETH PORTIER: So kind of building on that theme, you know, all of the approaches discussed up to this time have centered on the full period of interest, time series or concentration, right? And the focus has been on being able to simulate this time series with all of its correlated temporal value. Well when you actually stop to think about it, the periods of most interest are really event related; that is those day of high concentrations that we know are related in some way to the timing of chemical application and location and duration of significant rainfall events.

So what happens if you only look at these significant events? Forget the times of base flow where nothing interesting is happening. Let's look only at the significant events. I am thinking about this in terms of -- I am going to get to year to year variability but I think my bottom line is -- it's one thing to try to simulate 20 years of time series and it is another thing to think of 20 years of events, of significant flow events that are going to lead to some kind of exposure that we are going to be interested in.

So one is a time series… 20 sets of time series and another one might be 35 events in those 20 years that you are trying to understand. Can we identify how many and when significant rainfall events occur in the basin of interest. With the historical meteorology data, the answer to this is likely yes. Can we identify which of these events is likely to product a concentration increase event at the monitoring station or at the community water system?

If we can, then we are able to say something like this rainfall event in this area would produce this kind of schemograph at the community water center. This next rainfall event, coming say three days later, would add this additional bump to that schemograph. Those two rainfalls relate to one event that I am calling a concentration increase event.

When we can identify each of these concentration increase events, we can use them to develop distribution such as maximum daily concentration or duration of days above threshold concentration during the season or over a period of record. Can we relate site specific weather station time series data to these significant rainfall events? We are thinking initially spatially… spatial rainfall events relate to an event that happens at the community water system.

Now I am taking one step further back and saying, well, really the long-term data we have is rainfall gauge data. We have 50, 60, 70 years of daily rainfall gauge data. You kind of like to look at that in terms of year to year variability. Well that means you have to kind of relate this point specific rainfall gage data to some area rainfall so you can pass it through some kind of PRZM model to figure out an event at community water system, or, some kind of regression model.

To me that is going to be the hard thing to do, is to take that point data that we have, that rich rain gage time series data and try to relate that to something that

is really happening on a rainfall event on a basin-wide basis. I do not know anybody who has been successful in doing that. That is kind of where being able to tackle year to year variability falls down.

Now on the other hand, we had an SAP not too long ago where we looked at climate change impacts on risk assessment. I am sitting here thinking a lot of what we concluded there was that yesterday's rainfall is not necessarily tomorrow's rainfall in our current scenario. Maybe the 20 years of meteorological data that we have right now is a better tool for looking forward than 60 years of rain gauge station data. Maybe we should not be wasting our time trying to think that far back and just use the 20 years, and use that moving forward as our baseline because the last 20 years are probably a much better picture of the next 20 years than is the last 60 years a picture of the next 60 years.

And I think I will stop at that point. That is my thinking. The basic idea being an ultimate approach to year to year variability might be not to look at it from a time series but look at it as a set of events and break it up into how many events can happen in a year and of what magnitude and duration of those events.

DR. DANIEL SCHLENK: Okay. Other panel members? Okay. Let's go back to you Mr. Thurman, do you have any questions or clarification?

DR. NELSON THURMAN: Okay, it's Nelson Thurman. Actually I want to followup on a comment Dr. Portier had made about

using the existing database. I think one of the comments in terms of either taking the bias out of it or accounting for the bias and you said something along the lines of we need to figure out how to use it. Do you have any suggestions on how? Because we are wrestling with that, how do you use that?

DR. KENNETH PORTIER: You're talking the last 20 years where we have pretty good meteorological area temporal spatial data on rainfall from radar?

DR. NELSON THURMAN: I am actually talking about Part A. Your response to Part A which was on the atrazine monitoring database and you were…

DR. KENNETH PORTIER: Oh yeah, okay. Well I think that is what we have been talking about all along in question 3 and 4. It is going to depend on the size of the basin, right, and in the small basins we might be able to use PRZM to really feed some information to what these events look like and as the basins get larger somehow we are going to have to integrate that information and PRZM may not be an acceptable model for doing that kind of integration.

I keep thinking, and my understanding from talking to Coupe and Gilliom, is that they are really field-level based models and so you either have to run it on every field, which is something Syngenta showed us in a previous SAP that they could do with sufficient computing power and given enough time, they can run every field in the corn belt of the US and run it for the season and

accumulate all that information and all I could think of at the time is more power to them. I would love to see them do that, that maybe one approach.

And then you are god, with a small "g", you know everything. Then you can aggregate, you can compute, you can do everything that you want assuming the model is correct, right. Assuming the model works and you are doing all those kinds of things.

I think that is kind of what we have talked about today and what we have talked about in previous SAPs on this. We are still working on the same premise that we need these kinds of field-based models to do the integration for us. There is no kind of simple in between. And when you get the year to year variability that is just another level of uncertainty that we are having to factor in, and right now the only way to tackle that is year-to-year measurement of some type.

DR. DANIEL SCHLENK: Okay? You have everything you need Mr. Thurman?

DR. NELSON THURMAN: I think we have plenty. I do appreciate the thought and effort that the panel members have put into these questions. It has been helpful or us.

DR. DANIEL SCHLENK: Fabulous. Let's move on then to question 5 and change out the table there. Dr. Mendez were you going to read that? You do not have to read that whole paragraph. You can just read the little A if that suits you.

DR. ELIZABETH MENDEZ: Good afternoon. So we are going to have a little bit of a shift now in the types of questions you are going to be hearing. We have been talking a lot about the drinking monitoring program and now we are going to start talking a little bit about the hazard characterization and the neuroendocrine mode of action that we have been working with for the past few years.

Before I get started with that I wanted to reiterate something that Dr. Fowle said earlier this morning. We are at this point in time looking at these data. We are not really doing the risk assessment. What we are trying to do is come to you to seek your advice on how we are approaching, where the approach is that we are proposing, and how we are interpreting these data as we may eventually start applying them to our risk assessment process. So with that I am going to ask Dr. Cooper to join me at the table and then I will start asking the questions.

Charge question 5 has to do with the neuroendocrine mode of action and the disruptions to the HPG access that has been the basis of our risk assessment in the past. And the first question that we have is currently available data show that in the rat, a brief exposure, as brief as four days to low levels of atrazine can elicit decreases in LH. Please comment on the biological plausibility of these brief changes leading to an adverse outcome taking into account typical variability and how long and how much an LH surge reduction is needed to cause the

observed adverse effects; i.e., disruptions in cyclicity, delayed puberty and prostatitis.

DR. DANIEL SCHLENK: Our first lead discussant on that is Dr. O'Byrne?

DR. KEVIN O'BYRNE: First of all, I'd like to say that Dr. Timms and Jerde are not going to participate as associate discussants of this question because there is no obvious link between LH secretion and prostatitis. So they are going to convey their deliberations in charge question number 7.

So we can see here that four days exposure to low levels of atrazine declines LH secretion. But if we consider what we heard yesterday concerning a paper that's published in 2001, by Goldman et al. that showed that absent at 100 milligrams per kilo per day, which is certainly not a low dose by any stretch of the imagination to ovariectomized estrogen-primed Sprague-Dawley rats attenuated the LH surge by a mere 54 percent, and that was AUC. Actually the peak levels of LH were not significantly suppressed in that paradigm.

I'll remind you that two-day treatment with the same dosage had absolutely no effect on the LH surge. So the question is, might the surge reduction cause these disturbances to cyclicity and the onset of puberty. Well, we know from this historical data of laws from 2000 that 50 or more milligrams per kilograms per day, starting on postnatal day 22, which is when rats are

usually weaned, did delay puberty. However, 12.5 and 25 milligrams per kilogram per day had no effect on puberty.

And when you consider that they were being treated with 25 milligrams per kilogram per day for almost two weeks and it had no impact on puberty or cyclicity, then it's difficult to image that a 4-day treatment at 100 milligrams per kilograms for just four days would impact on those systems. That would be unlikely, in my view.

Now we know from the work way back in the early 70s by Everett that there is a huge variation in the levels of LH in the spontaneous surge, and I think this was touched on in the September meeting. And we are talking about a range here of 200 to 1000 nanograms per mil. And despite that huge range, the rate of ovulation -- in other words, the number of ova that were expelled from the ovary was not different.

So there is a huge redundancy within this system, and that's perhaps just as well because without reproduction, we wouldn't be sitting around this table discussing atrazine. Now if you think about a hypothalamic level, Fred Carr (phonetic) showed some years ago that you only need 10 percent of the GnRH surge to drive a full-blown LH surge in the U; so again, huge redundancy.

So perhaps this 50, 54, 55 percent reduction in LH that is seen with 4-day treatment with a hundred milligrams per kilograms per day is unlikely, again, to -- in my opinion -- to disturb cyclicity and other aspects of reproduction. And there is a sort of slight digression.

Allan Herbison published a paper in 2008 where he used a transgenetic model to selectively knock down GnRH neurons, and he showed quite convincingly that you only need 12 percent of GnRH neurons in a mouse brain to go through a normal puberty and have your first estrous cycle. So again, that's another example of a huge redundancy and robustness within this control system.

But perhaps, what's more important and troubles me is the consideration, which I mentioned 16 months ago, that the toxicological doses that we are discussing are so far removed from what one would be exposed to in the normal environment unless, of course, your little rat manage to nibble it's way into the subnet atrazine in the farmer's shed. I mean, I think this is something that we, on this side of the table anyway, are quite concerned about and I don't know how that's really going to be addressed.

I am delighted to hear that people are beginning to lower the dosage that they're exposing their animal models to, but there is a real need to think outside of the toxicological mindset. So the other point that I would like to make is that we saw some rather nice data from Syngenta yesterday that we simply cannot ignore. They showed that 50 milligrams per kilogram for four days attenuated the spontaneous LH surge by only 50 percent, again, in Sprague-Dawley rats.

So we've heard a lot of discussion about Long Evans versus Sprague Dawley. Well here there are two studies in Sprague-Dawleys from different sources and I think

it's speaking volumes to how much of a reduction is taking place in this surge-generating mechanism, which we know has a huge built-in margin of safety in terms of reproductive outcome in terms of ovulation.

They also showed that 10 milligrams per kilogram for four days had absolutely no effect on the surge at all. I'll just remind you that 10 milligrams per kilogram for four days is a whopping great dose of atrazine. So, I also feel that the spontaneous LH surge may be much more fragile and vulnerable to perturbations compared with the steroid-induced, because in the spontaneous circumstance you're relying on the ovary to release a certain amount of estrogen, which is driven by what one can only assume as a normal functioning pulse generator; in other words, pulsatile release of LH, and obviously FSH. We can't ignore FSH as well.

If you contrast that with pouring in buckets of estrogen or estrogen plus progesterone in your ovariectomized model, then I think we should focus in on those data that we saw yesterday in the gonadol intact animals.

My conclusion is that four days exposure at the doses that we are considering is unlikely to have effects on onset of puberty and the ovarian cyclicity. I am also quite pleased to see that there is a move to use other models, apart from the rat, and I think Syngenta need to be highly commended for putting some money into sort of primate research. I do wonder why that has taken so long to be put into effect, because I think we do need primate data. So I think that's all I have to say.

DR. DANIEL SCHLENK: Thanks. Dr. Akana?

DR. SUSAN AKANA: I'd like to add only two personal points of view. One is, I totally concur with Dr. O'Byrne.

What I do want to add is that it is highly unlikely that the doses we're using giving for four days in adult rat are going to have adverse reproductive outcomes. However, what we don't know is, if you go back in, say 10 days later, and give them a second exposure, if they'll have an additive or interactive effect.

So in my person view, it's important to recognize that when these animals have received such a dose and you see no apparent adverse outcome, that doesn't mean they're necessarily a normal animal from that point on. The second personal point of view I have is, I'm very mindful from the datasets and the docket and that we've seen yesterday that very frequently animals administered the atrazine have a drop in food-take immediately, and a drop in body weight.

In my world of physiology, this is not a normal behavior in an animal. It is something to be very mindful of how these animals are going into negative energy balance; their metabolism is shifting. And what's more important in this case of atrazine is they are choosing not to eat. And the results on their physiology can look similar to animals, a different cohort, where you artificially food-restrict them.

So we know from some studies in the literature and some nice studies done by Susan Moz (phonetic) here with food restriction that, yes, if you decrease food intake, you get a different metabolic shift, and they look similar to the anorexia that you might see with atrazine administration. But there are differences in the brain neurocircuitry and that's something to be mindful of as you pursue the atrazine studies.

DR. DANIEL SCHLENK: Okay. Dr. Jerde, do you have any comments? You're going to delay those to seven, right?

DR. TRAVIS JERDE: Yes.

DR. DANIEL SCHLENK: Do you have anything on the nonprostatitis endpoints? No? Yes?

DR. TRAVIS JERDE: Well, the only thing that I would add is, I've sort of alluded to it a little bit before. The mechanisms of action that we haven't really defined yet could turn out to be very important. I would encourage more research in this area looking at sibling mechanism, genetic imprinting and things like that that may occur. Because these hormonal changes, systemic changes, are oftentimes associated with those sorts of more subtle effects that probably ought to be addressed.

DR. DANIEL SCHLENK: Thanks. Dr. Roby, next?

DR. KATHERINE ROBY: I have no more to add.

DR. DANIEL SCHLENK: Okay. And Dr. Timms, do you have anything to add?

DR. BARRY TIMMS: At this point, no. We'll refer to our charge question.

DR. DANIEL SCHLENK: Okay. Other panel members, anything to add? Yes, Dr. Horseman?

DR. NELSON HORSEMAN: I want to go in the charge question here. It says that these perturbations are being considered as the basis for atrazine risk-assessment. So I hear a lot of skepticism, and this may be just stating something that Dr. Jerde just asked in a different way, but my question is does the use of this LH surge, as a sentinel effect, plausibly capture an apparent hypothalamic mode of action that is, as yet, poorly understood at a site or molecular level, but is important to understand.

In other words, it seems to me like we're being asked to consider whether this effect that is hard to understand its particular physiological meaning, is telling us something else. And if that's what we're being asked to consider, we need to see if we can give you better advice as to how to go about finding that something else, it seems to me. And maybe that's a poorly worded question but maybe not.

DR. DANIEL SCHLENK: Other comments? Dr. O'Byrne?

DR. KEVIN O'BYRNE: I mean, the surge generating mechanism in the rodent is a little bit odd. But nevertheless, it is a central mechanism and it's reasonably well understood. And if you do perturb it, then you are going to impact on the reproductive cycle because it's part of that control system. So if you don't have a surge then you're not going to have a normal estrous cycle, and that would be true in other species as well, so it is quite important.

The problem is the dose that is used to completely wipe this out is a hundred milligrams per kilo. So I appreciate what you're saying. It is important. And these guys have already shown the effects of atrazine on LH pulses, and we heard a lot yesterday about LH pulses are so important. Well of course they are, but they have to give a hundred milligrams per kilo and they still don't see any significant effect. I'm not quite sure when that was published and Ralph will remind us. So in the context of the pulse generator, pulsatile LH secretion, you need even higher doses.

DR. DANIEL SCHLENK: Dr. Roby?

DR. KATHERINE ROBY: I think your point is a good one though. I think the measure of LH is really just a measure of what's happening upstream. I think everyone agrees to that and there are a lot of studies, although they would be difficult studies to do to look at what's changing in the hypothalamus. But I think it's also interesting to note the studies where during the LH-dependent portion of gestation, resorption occurs, and I wonder if those point

to some alternative or additional points of input of atrazine in that maybe the regulation of LH at that point is different than the regulation occurring during the surge. But I think the point of understanding the molecular mechanism is important.

DR. DANIEL SCHLENK: Dr. Horseman?

DR. NELSON HORSEMAN: Okay. To come around to a different part of this, so we've talked a lot about roots of administration; and may mean gavage or in the food or in the water or whatever. But if the EPA is asking us to help them understand whether this apparent hypothalamic mode of action is relevant for the basis of atrazine risk-assessment, I wonder if we shouldn't see data where atrazine is applied to the hypothalamus.

I don't know if there is any literature out there. This is a fairly straight-forward type of ICV infusions, because -- I'm uncomfortable with the notion, well there's a mode of action that we are hanging a general physiological toxicological affect on, but then everybody knows for sure that that particular mode of action isn't directly coupled to an adverse outcome in terms of ovulation, and I keep saying that.

DR. KEVIN O'BYRNE: I think if you knock the LH surge sufficiently then you do impact on the other aspects of the reproductive cycle. So from that point of view, I mean, I think it is a good marker.

DR. DANIEL SCHLENK: Dr. Akana?

DR. SUSAN AKANA: I had a conversation with Dr. Handa yesterday and actually almost the same conversation in the April SAP.

In the April SAP he presented some c-fos information in the brain and I asked him specifically, "Did you look in the paraventricular nucleus," that being my favorite part of the hypothalamus. In conversation yesterday he said, "They looked extensively through the hypothalamus. They're looking at c-fos an hour after atrazine injection -- and I'm sorry I can't remember the dose -- but the hypothalamus was totally quite.

I did ask him in April, "Check the distributed CRF system; look at amygdala." And yesterday he told me, yes, they saw some c-fos in parts of the amygdala. So that's what I know of the hypothalamus c-fos studies. My comment about, for instance, ICV injection into the brain is remember the solubility of this compound is pretty poor. It almost has to be a crystalant implant.

DR. DANIEL SCHLENK: Okay. Any other input? Yes, Dr. Griffith?

DR. DANIEL GRIFFITH: A question that I think is relevant to answers 1 through 3 questions; what would be some good dose levels to explore then if you think 100 milligrams per kilogram is too high? What would be some good dose levels, lower dose levels to explore?

DR. KEVIN O'BYRNE: Well, we rely on you guys to tell us what make it in the water. And my understanding is its so low, and that's what we should be considering, in my opinion. We should be administering the doses that replicate the maximum levels that are found in the water and it should be given chronically or intermittently. It doesn't really matter. Those would be just part of the experimental strategies. This hopefully will be what will be done more carefully in the primates, because when you start working with primates you control and design your experiments exquisitely. The rat guys -- and I'm now a rat guy -- tend to be a little complacent.

DR. DANIEL SCHLENK: Yes, Dr. Timms?

DR. BARRY TIMMS: Actually, I think the answer to that question is more relevant if you consider the levels that may be predicted or found in humans because those are the levels at which we are exposed and those would be relevant to the dose exposure that Dr. O'Byrne was talking about.

DR. DANIEL SCHLENK: Yes, Dr. Roby?

DR. KATHERINE ROBY: Is also though seems to me that the experimental approach breaks down two different but important questions, and one is the biology and what happens with an exposure to atrazine, and secondly is what is the real risk factor? And one is assessing effects at realistic exposure levels, and one is doing more of the kind of pharmacologic type of studies.

DR. DANIEL SCHLENK: Dr. Horseman?

DR. NELSON HORSEMAN: To bring in a concept from a different SAP we had recently -- and maybe that's the source of this question I'm asking -- moving toward the notion of an adverse outcome pathway requires understanding the biological substrates of that adverse outcome. And from the diagrams we've seen here, you know, we give to the organism and something happens at these lower levels of organizations. We have no information about those other levels of organizations.

I think the question about these relevant doses and such, also can break down. If you consider the fact that we're going to understand this toxicology or ovarian physiology from studies of a small number of animals, relatively small number of animals, but millions of people are going to be expose to this.

So I don't think it's as simple as saying nothing happens as three parts per billion and only one and a half million people were exposed to more than three parts per billion. You know, that's you guy's problem there, essentially, but understanding the biology and from this toxicology in the 21st century notion of adverse outcome pathways and that sort of thing, I think you're a long ways away from that for this hypothalamic mechanism that seems to be proposed. That's my only comment.

DR. DANIEL SCHLENK: Okay. Dr. Chambers?

DR. JANICE CHAMBERS: I just want to pick up the same thought that Dr. Horseman was having. Again, the last SAP we were talking about the mechanisms involved at the adverse outcome pathways. When so little is known about the mechanism, then it's really hard to tell whether this is a biologically plausible phenomenon in the rat compared with the human or not anyway. So it just really seems like it's very important to try to identify some of the real mechanisms going on and find out whether those are relevant in humans.

DR. DANIEL SCHLENK: Yes, Dr. O'Byrne?

DR. KEVIN O'BYRNE: Well, I think the comments made by Tony Plant yesterday that there should be a focus on the pulse generator might be something that should be explored a little bit more. But I'll remind you that Ralph has already shown that LH pulses are not affected with 100 milligrams per kilo.

DR. DANIEL SCHLENK: No further comment from the panel at this point? Dr. McManaman?

DR. JAMES MCMANAMAN: It looks like it's 50 mgs per kg does reduce the LH surge. This is from Syngenta data. So I think that we're in the ball-park of -- and that corresponds to about 500 PPMs -- so we're not too far off. We're in the toxicological area, but we're not so far. We're getting close to the physiological area, I think.

DR. DANIEL SCHLENK: Okay, alright. So we'll throw it back to the EPA folks. Do you have comments requiring clarification?

DR. RALPH COOPER: I just have a point of clarification, I think, for dose selection and what seems to this panel to be a disparity between environmental levels and the doses that are used in laboratory studies.

I think it's a good thing that we're seeing this disconnect between what's needed in order to identify a potential adverse effect in the test species and what's in the environment and what's potentially exposure to the humans. And this didn't just happen by chance with a chemical that's been around for 50 years, there have been programs to maintain low concentrations.

I look at it a little bit differently. I think what we are looking for is, as we felt that, as we look at the mechanisms or the potential mechanisms involved in the test species, we want to make sure we're using the right set of tools, measuring the right parameters so that we can be sure we're making a good guess as to what's going on.

And what we're doing essentially is reinforcing the safety factors, if you will. I mean, look at what we're talking about. We're talking about very slow or very small differences in previous LOAELs, NOAELs, points of departure that have been used for the risk assessment. We may be tweaking the timing of those events and things

like that but we're pretty much still at the level we were back in 2000 when we did the six-month evaluation and you had the 1.6 NOAEL and 3 point something LOAEL.

What's changed maybe -- and I don't think it's earthshaking. It's not an order of magnitude -- is the acute exposure, if you will, for four days. Previously, it's my understanding it was 10. And we're identifying something in an intact animal, and I certainly agree with O'Byrne. He made a very good point when he said, "The less contrive that you have your experimental model perhaps the better information you'll get. Study an intact animal. Study that animal at the times when you anticipate the effect to be taking place that you're looking for. In the intact animal you'll see lower LOAELs and NOAELs.

When you give estradiol to an animal, you've taken its ovary out, you give estradiol to it; the rules change. You can still use it as a model and get information, but this dose response information falls apart. Secondly, when you give estradiol plus progesterone, which is what most of the registrant studies are, I've seen that totally blow out in effect that you may have seen elsewhere.

So these are not all comparable studies. When you talk about the ovexed animal, I mean, they're just as different as the monkey and the rat. They're different tools and they are different approaches that you use to get at things. But the two main points is that the LOAELs that we see in an intact animal -- I totally

concur with. It's really difficult to say that we see changes. I don't even recall the percent change in the amplitude of the surge of the area under the curve in our data but it certainly wasn't going to be one that John Everett or many of the people who later on looked at the threshold level of LH for ovulation.

The question that always remained -- and I worked with John Everett for years -- and the question was, well what is the rest of that LH doing then? Is it just there as a frill or is there some other important function that that may have? If you look at the literature on reproductive physiology, there are a lot of other things that we haven't explored.

DR. DANIEL SCHLENK: Yes, Dr. Fowle or Steve, did you want to say something?

DR. STEVEN BRADBURY: Yes. Along the lines of what Ralph was just saying, but also what Dr. Horseman was saying, I guess one of the things is we're at the point now is from a regulatory perspective we want our decision to be informed by the best available science. I mean our decisions must be informed by the best available science.

However, we don't have the luxury of waiting until all the T's and crossed and I's are dotted. So at various periods of time we have to pull together the science and take advantage of that best available science and make a decision at that particular point. And we have our re-registration view process that's periodically come back and we keep looking at these chemicals in repeated

fashion in the future so we can add additional science as we proceed.

So what Dr. Horseman is saying, at one point in time, we're kind of faced with a dilemma. It's not likely we're going to be able to have a lot of new data along these lines in the next couple of years, so to the extent that we don't have data that would get at these issues, what would really help us is what kind of guidance can you give us in terms of stitching together the best assessment we possibly can, given the science we have right now.

So one of the things we may be hearing, and I'm not exactly sure of the clarification, how, for instance, Dr. O'Byrne, you were saying that at the environmental levels that we would encounter with respect to atrazine, would not likely have an impact on reducing the LH surge.

We could interpret that a couple of ways. One would be that we would need to basically sort of eliminate that surge to be able to -- well, I might be saying this wrong. If we're not seeing the effect of the LH surge disease, environmental rapid exposures are those higher level doses where LH surge events intact might serve as a node, which would lead to a variety of adverse impact.

Is there any advice or guidance you'd give us for how we might use that data in the context for all the other data we have available to try to stitch together as best we can right now? What effect, if anything, atrazine might be causing?

DR. KEVIN O'BYRNE: I think the data we saw yesterday, for example, from Syngenta where they showed that 50 milligrams caused a reduction of about 50 percent of the surge is very clear and unambiguous. And with 10 milligrams there is no effect. I mean, that's hard evidence and I think you can work with that.

What I was trying to explain to you is that a 50 percent reduction in the LH surge, in the spontaneous LH surge, may have absolutely no impact on the ovulation and the cyclicity that is driven by that. So I think you've got some very hard data there. And so, I think that's important.

The other thing is, the discussion yesterday about the problems associated with the surge generating mechanism in the rat as opposed to other species, and particularly human, is something that you ideally would like to have that data. But if you don't have it then you have to go, as you say, with the best that you have.

So perhaps in the timeframe that you're talking about, you are not going to get any further data but you've got hard evidence now. And when some primate data does come out on terms of pulsatility, it would be just interesting to see how that fits because there's no doubt about it, what Tony Plant said yesterday in terms of the LH pulse generator, you don't have a surge unless you've got a normal functioning pulse generator. So you know you've got a normal functioning pulse generator at 10 milligrams per kilo. Otherwise, these rats would not be having

spontaneous normal surges. So I think you've got some good data.

DR. DANIEL SCHLENK: Any other comments? Okay. Back to the agency, is this the answer that you need?

DR. ELIZABETH MENDEZ: We've heard a lot to think about, so we appreciate the input. So if you'll allow, I'll keep going then to charge question number 6. And charge question number 6 has to do with some advice or some comments that we've heard from the panel back in September about the significance of 1-day versus 4-day exposure. And in response to those comments, our scientist went back to the lab and conducted some studies. And we've sort of started alluding to them in the previous question, but we are going to ask the question anyway. Please comment on the potential relevance of 1-day exposure to elicit an adverse outcome and the significance of an increase versus a decrease in LH.

DR. DANIEL SCHLENK: Before we move on, I just want to remind the lead discussants of each of these questions, it's your responsibility to summarize all of the panel's discussions for that. And I know that, based on the last question, it was somewhat widespread and unclear. So again, that's sort of the responsibility of the lead discussants to do that, so I just want to make sure you're aware of that as we go forward. So with that, Dr. Roby?

DR. KATHERINE ROBY: Okay. This question does, I think, segway from our discussion that we've been having. And I think to specifically address the absolute question on a 1-day exposure, we have to go to the new data that was presented with the ongoing study, as I understand it, where the model system of ovariectomy and estradiol replacement was used in looking at a single versus multiple-day exposure.

A single day exposure elicited an augmentation of the LH surge, so that's 1-day exposure. So what would be the significance or outcome of that, probably very little to nothing. An augmented LH surge, we've already talked about the excess in the LH surge in what we considered to be the redundancy in the mechanisms downstream of the LH surge.

So probably ovulation would have occurred just as it would have if the surge had been at those "normal levels." Now, the significance of a decrease; the new experiment shed some additional light and I think highlights the complexity of the system. And I think the model system was appropriate to address the question. The results, again, are interesting in the augmentation of the surge and it's relation to the progesterone that was probably secreted by the adrenal gland with the atrazine exposure.

Then, with the subsequent administrations and either the down regulation or desensitization of the continued exposure to progesterone each time, and then you see the

attenuation of the LH surge. I know this gets into multiple exposures, but if there were a single exposure that resulted in a decrease, to directly answer the question again, the overall outcome again would be probably pretty minimum. If the LH surge was inhibited enough, it would result in an anovulatory cycle. From a practical standpoint, there is no ovulation, and the cycle would probably be extended slightly in a rodent and a couple of days maybe in a woman. But subsequent cycles would then be normal. This is just single exposure.

So, I guess, bottom line is a single exposure would really have minimal effect downstream of the effect on the LH surge. I think something that the study presented that goes back to the pretext to the question is; 1) is the effect on the LH surge due to the peak exposure to the atrazine or is it due to this accumulation and this potential pseudo steady state? Or is it really just that multiple exposures eliciting those multiple increases in progesterone are -- you need to have so many exposures to have the down regulation in response to progesterone, which is maybe different than reaching a steady state or a pseudo steady state level. So multiple, short exposures may still be relevant to effect. And I think that this experiment leads to more questions, based on the result.

The other point that is in the discussion, but is not addressed at least in the data that's been presented recently -- and I think it's an important question -- is when during the cycle is the exposure relevant? So these

exposures were done at 1300, I believe, which is really basically the onset of the LH surge.

If exposure occurred at 0900 or the previous day, would there be any effect on the surge? I don't know. I guess that's to be shown. We certainly know of other compounds that, when administered, based on time of day, will either shut down the surge or have no effect on the surge. And atrazine could be functioning through similar mechanics. We don't know.

So, I think time of exposure is still a question that's a little bit open. I think when we want to translate that then to the human, obviously the amount of time within any menstrual cycle further away from the LH surge is significant relative to the time near to the LH surge.

I think the other question then related to pulsatile exposure versus what might be a pseudo steady state relates to the amount of time that the drug might need to be at a certain level to elicit a change in a woman compared to in a rodent, given the different dynamics of the LH surge in women versus rodents.

I think those are still some questions that are uncertain. Okay. So in summary, directly for the question, 1-day exposure, I think, really will have little effect on ultimate outcomes in fertility.

DR. DANIEL SCHLENK: Okay. Dr. Akana?

DR. SUSAN AKANA: I have nothing to add.

DR. DANIEL SCHLENK: Okay. Dr. O'Byrne?

DR. KEVIN O'BYRNE: I concur with what's been said. But actually, the 2-day exposure, I just realized, 2-day exposure had absolutely no effect, so that's quite relevant. It's giving us a little bit more information about that window that you are sort of asking about, so you've already got part of the answer.

DR. DANIEL SCHLENK: Dr. Roby?

DR. KATHERINE ROBY: If I could comment though; I think what we don't know is if that isn't due to now two exposures to progesterone and down-regulating that system and the sensitivity or adjusting the sensitivity, so the duration of exposure to progesterone in combination with exposure to the drug atrazine.

DR. KEVIN O'BYRNE: But I think one of the questions these guys were asking was if they started the treatment at a different phase of the cycle -- you've only got four days, so I think that is relevant to the question that was being posed.

DR. DANIEL SCHLENK: Sure.

DR. KATHERINE ROBY: Can I also add --

DR. DANIEL SCHLENK: Sure.

DR. KATHERINE ROBY: I did want to say that the rodent obviously is probably not the optimum model system to really look at time during the cycle. The estrous cycle is so short. It's not a true luteal phase, for example. Probably other model systems, obviously, like the nonhuman primate would be excellent to look at what effect exposure across different times of the cycle would actually have.

DR. DANIEL SCHLENK: Yes, Dr. Cooper?

DR. RALPH COOPER: I understand your comments and they are right on target with the question that was asked in the September SAP. We didn't have all that time to address them, but the one question that we didn't get to, and I think is still possible, is let's say for example you dosed on estrous only: there is a literature that says, and again goes back to Dr. Everett's lab where if you give one dose of progesterone, on the estrous early diestrous-1, I think it is, it would delay ovulation one day, so there is another example of timing, and timing is critical.

One other point that I'd like to make is that we've done studies with other chemicals that show that if we dose the animal between the hours of two and four in the afternoon, a vaginal pro-estrous, we can get a blockade total 100, which I guess you'd consider relevant, with 7 milligrams per kilogram of the chemical. But yet, if we drift back in time and give it earlier and earlier, so now the peak, the area under the curve, is earlier that

day -- so this just emphases the critical nature of the timing -- that there would be no effect at all.

I mean, you know, it's tox, you like to have effects. They are just so robust they last forever, but the real nature of this cyclical beast, the circadian rhythm, makes it such that timing is important. And that takes me to the question of why does one guy got 50 milligrams, another guy has got 10 and another guy has got 5 milligrams, that's a LOAEL? Well, if you go back and look at those studies you'll see, in those cases, timing wasn't always the same, and I think that could be contributing to the part of this. That cycle, there are certain things that are sensitive at different portions of the cycle.

DR. DANIEL SCHLENK: Okay. The rest of the panel? Yes, Dr. Lee?

DR. HERBERT LEE: I want to tie this back to the hydrology a little bit and ask a different question. So I hear you saying that one exposure may have a few short-term effects, but really no long-term effects. But if you're living in a small watershed community water system area you may get a high dose once a year because there is a one peak and then it comes back down. Is there any consideration as to what happens if you have a once annual 1-day exposure or, in a rat, maybe you want to translate this back on the length of the cycle, it'll be a lot faster than annual for a rat, but that sort of slow repeated exposure.

DR. DANIEL SCHLENK: Yes. I think we're going to kind of get to that in some of the later questions, actually. Yes. Feel free to answer, if you like.

DR. KATHERINE ROBY: Well, I think that even a once annual exposure that, say in worse-case scenario completely inhibit the LH surge, would still have very minimal effect on your overall reproductive capacity. You would have one anovulatory cycle. In reality, women very often have anovulatory cycles. It's not an uncommon event.

DR. DANIEL SCHLENK: Okay. Any other comments from the panel on this particular question? Okay. Let's go ahead and break for our afternoon break here. Let's try to be back at 2:35.

DR. DANIEL SCHLENK: Okay. Let's go ahead and get back at it if we can. Let me just remind the panel members if you do have discussions with agency folks you need to state that for the record kind of what you were talking about, in terms of your outcomes there. Yeah, only if it's relevant to the charge questions, of course. Don’t get too personal there. So let's go ahead and get started on number 7 and we're going to read that into the record.

DR. ELIZABETH MENDEZ: This one has to do with the prostatitis findings that we're seeing in the rat and that we're seeing after exposure from PND-1 to PND-4. And the question that we have is, given the biological processes involved in atrazine-mediated prostatitis in rats, please

comment on the human relevance of these findings in rats, for the overall hazard characterization of atrazine.

DR. DANIEL SCHLENK: Our lead discussant on that is Dr. Timms.

DR. BARRY TIMMS: This morning we were exposed to a very sophisticated, statistical model. This afternoon I'd like to expose you to some sophisticated anatomical models. If I could have the slide up I'll be referring to that during our presentation.

I'll present some background information and then address our responses to the agency, but I think it's important just briefly to summarize some of the information regarding prostatitis and it's relevance to the human condition. In men, younger than 50 years of age, it's a very common neurological diagnosis. In men over the age of 50, it's the third most common neurological diagnosis.

What that translates into is that, in this room, approximately half the men will experience prostatitis sometime during their lifetime, so it's not an insignificant health-related disease. The National Institutes of Health has re-categorized prostatitis into four types; the bacterial, acute and chronic, and the nonbacterial chronic pelvic pain syndrome and an asystematic histologically evident inflammation.

So there are different types of prostatitis that’s mostly divided into the bacterial and non-bacterial. When we use animal models such as the Lewis, Copenhagen and Wistar rats, they are animal models that develop a

spontaneous non-bacterial prostatitis with advancing age. And so they'd been used as animal models for investigation of the disease with the premise that if you increase or exacerbate that incidence or severity, it can be a reflection of a particular treatment.

I should point out that you can also increase the incidence and severity of prostatitis by treating male rats with estradiol. And a number of studies that have used these models have also shown that the spontaneous non-bacterial prostatitis observed in rats is very similar to the histological profile to that observed in humans. So it, again, is a good model.

Come back to estrogen, and estrogen-inducted prostatitis is partly related to the inhibition of dopamine secretion at the hypothalamus and that can result in the production and secretion of prolactin that eventually is associated with inflammation in the prostate that's been reported in 1993 by Tandin Lucal's (phonetic) group in which they showed E2-induced prostatitis was correlated with increase serum prolactin, elevated pituitary weight and that the administration of bromocriptine, which is a dopamine D2 agonist, was effective in suppressing the pituitary weight in hypoprolactinemia and it mitigated the prostate inflammation.

So the studies that we've been asked to review have implicated that exposure to atrazine during the late gestation period, that's days 15 to 19 in the rat, just prior to birth, or the early postnatal period, just after birth, days 1 through 4, in male rats can lead to

inflammation of the prostate. And in these studies they looked at ventral and lateral regions at later stages of postpubertal growth. It was considered that the inflammation was a result of elevated prolactin levels and these elevated levels were through to play a significant role.

The underlying mechanisms for that cause of prostatitis are not yet defined, but may be related to hormonal changes during what we call critical periods of development that may have subsequent adverse affect during agent. It's also important to note that all these studies have used short-term exposure with a range of doses and different rodent species.

So our response then with regard to these studies and the human relevance are the following: We believe it's unlikely that exposure in these animal model systems follow the same pattern of exposure that humans are exposed to in terms of atrazine and degradates which are more likely to occur over a lifetime and at much lower levels than those used in the animal studies. This refers to the comments that Dr. O'Byrne made earlier on about the relevance of the 100 milligrams per kilogram dose to actual human exposure levels.

In vitro and in vivo studies which have looked at cellular and molecular expression changes in response to human exposure levels should therefore be conducted. I'm going to refer to this diagram here. The prostate of the rodent consists of distinct lobes, which is not the case in the adult human gland. But the prostate of the rodent

has been the animal of choice for reproductive biology for many, many years, the mouse and the rat. One of the values of this model is that we can examine the effects of treatments, such as endocrine disruptors and observe the region or regions which have a specific sensitivity to the compound under investigation.

So based upon such studies, it became clear that the region of the rodent prostate exhibiting the most sensitive response to the effects of estrogen and endocrine disruptors, for example, was the dorsal lateral lobe. What I show here on the left-hand side is a schematic diagram of a rodent prostate showing the early developmental stages. On the left, it is showing the individual budding outgrowth and in the middle is a reconstruction of a late gestation day of birth approximately rodent prostate where all the regions of the prostate have been color-coded.

So you can see green, yellow and bluish grey indicating respectively the dorsal lobe, the lateral lobe and the ventral lobe of the rodent prostate. These are the lobes that we typically see described in these studies.

On the right-hand side is a 13-week human fetal prostate showing -- and we have shown and others have shown -- that the green area and the yellow area are homologous to the dorsal lateral lobe in the rodent model. And this region in the human is the one which is important because, if you notice, in the human, the human does not have the equivalent ventral lobe, and I want to make that point very significantly. The human prostate in that

region has particularly stromal component. So it's important for us to understand these homologies and their relationship to any studies that are done in the animal model. I'll come back to that.

While this wasn't the primary objective of this atrazine study in the issue paper, there is some evidence that the atrazine effects may be mediated through alterations of steroid agenesis, including estrogens. It's important to emphasis that the dorsal lateral prostate, which I said is homologous to the equivalent peripheral zone in the human, which is the zone which develops a preponderance for cancer, is an important correlation.

Most of the studies that we reviewed have actually looked at prostatitis in the lateral lobe, that’s the yellow region up there. The dorsal lobe, or combined dorsal/lateral lobe have typically not been examined. Of interest in an earlier study was, as I said, that this region, the dorsal/lateral region is actually homologous to what we call the peripheral zone in the human prostate where cancer develops.

An endpoint in the atrazine rodent studies is the use of tissue weights to determine growth and/or adverse effects. In a recent national toxicology program scientific review of an endocrine disruptor, a specific concern was raised regarding the reliability and usefulness of this parameter as a measurement of physiological effects. As stated in that report, and I quote, "Perhaps the most important confounding factor in all of the prostate studies is that prostatic wet weight

is an extremely poor measure of prostatic growth, which substantially diminishes the strength of data advanced both for and against an effect of whatever substance on prostatic growth.

So in the light of that statement, you'll notice that many of these studies report on ventral prostate weight and, as I pointed out, ventral prostate really doesn't have relevance to the human, and that prostatic wet weight is a poor measure of growth parameter.

So in the light of this, the inflammatory response should be characterized in this model, we feel, using contemporary approaches such as BrdU-labeling, immuncytochemistry to determine proliferation rates as a consequence of treatment, and more importantly, characterization and histological quantification of the hyperplastic or inflammatory response.

Furthermore, there are very significant modern contemporary approaches such as tissue microarray analysis that can be performed using laser-capture micro-dissection, and these might better define the cellular mechanisms responsible for the region-specific inflammatory responses.

Based on several animal models, these inflammatory mediator induction responses would likely occur prior to and at much lower doses than histologically-evidenced cellular inflammation. What that means is that, when you see the inflammation, what precedes that initiation might

have much more consequence in terms of an effective mechanism of the action of atrazine.

Cytokines induction, for example, can induce a number of effects in the tissue, micro environment, which can result in hyperplasia, dysmaplasia and dysplasia. In addition, cytokines are known to induce developmental growth regulators, including IGF, TGF and FGF, which are very important growth regulators during development and growth of the gland.

We feel that levels of these factors should be evaluated as part of this process of understanding the mechanisms behind the initiation of prostatitis. And finally, inflammation is associated not only with that, but also with DNA damage and loss of imprinting of certain genes and that should also be evaluated.

One interesting aspect of the gland in the rodent, and also in the human, not discussed in any of the relevant publications is the fact that the prostate, and especially the lateral lobe of the prostate in the rodent model, has very high levels of endogenous zinc. For example, tissue zinc levels are reported to be actually lower in prostate pathology, benign prostatic hyperplasia and cancer. While we don't know and fully understand the mechanisms and the reasons for the high levels of zinc, we know it's important in fertility. It might be relevant to an understanding of why inflammation specifically develops in that region.

So a question might be, are the dorsal lateral levels of zinc reduced in earlier prostate development by atrazine exposure and does this play a later role in the development of prostatitis? And we feel that the relevance of this to the incidence may be important with regard to mediators of inflammation. Zinc concentrations and their correlations with inflammatory mediator expression and cellular inflammation should be evaluated.

To date, there's been no causal link between atrazine exposure and prostate cancer from the studies that we've heard about. However, several important questions remain unanswered and unresolved from the present literature, such as the effects of repeated life-long exposure to low doses on both prostate cancer incidence in grade and any effects on progression to advance disease. The causes of prostate cancer are unclear but several studies have indicated that chronic prostatic inflammation may proceed benign prostatic hyperplasia and/or cancer in humans depending, again, on the region and the zone. In fact, inflammation is the most tightly correlated histological anomaly to prostate cancer development.

If the link between prostatic inflammation and atrazine exposure is confirmed, this ascends to added importance and implores us to consider the related consequences. An additional component of these studies is that treatment of Wistar dams with daily doses of atrazine on postnatal days one to four resulted in suppression of what's called the suckling induced prolactin release in offspring.

Taking the same animals and looking at them at 120 days of age, the male offspring showed increased incidence and severity of prostate inflammation in the ventral and lateral lobes. There was significant increase in ventral prostate tissue weight at the low does 6.25 mgs per kilogram, but that was calculated as non-significant when body weight was taken into consideration. No other weight changes were observed.

The lateral lobes were examined using mylar peroxidase assay for looking at inflammation responses and also histology. The type of inflammation was characterized as a focal neutrophill infiltrate, and in the lumen of the glands and focal mononuclear cells in the stroma. Though in these studies, the reaction was described as a chronic inflammatory response, the authors did not make a comparison of the inflammation with a classification of human prostatitis types that have been described by NIH.

A major point in considering the relevance in this type of study to the human health hazard is with regard to the modus operandi for the exposure. So in the rat model, offspring suckled from atrazine-treated dams, so we have breastfeed pups and at PND, postnatal day 120, the lateral prostate, the right lobe, was taken for histological examination; the left lobe was taken for mylar peroxidase.

What I find interesting is that a recent report from the CDC indicates that only three out of four mothers initiate a breastfeeding regimen, and by three to six

months, this rate is not maintained. So the study that was reported by Stoker et al. in 1999 suggests that early lactational exposure to prolactin is important for the normal development of the tuberoinfundibular neurons. However, according to the CDC data, approximately 25 percent of US babies are not exposed to breast milk prolactin. This may pose the question as to whether these individuals are at the same risk for later development of prostatistic if they're not exposed to the mother's breast milk prolactin.

And a question again from us is do we know or do we have an estimate for the measured levels of atrazine and its metabolites in human breast milk.

One other consideration is that, if you belong to the baby-boomer generation, you're the ones that are going to be the most likely to be affected and subsequent generations by any health hazards from atrazine exposure, given that the chemical has only been in use since the 1950s.

Let me summarize our responses. We believe there are several unresolved questions which limit the conclusions that can be drawn regarding the human relevance of atrazine's affect on the prostate. The results in rodent models are of limited applicability due to inconsistencies in atrazine exposure levels and methodology.

Secondly, the inflammation atrazine causes in rodents has not been sufficiently characterized regarding molecular

and cellular events that may indicate critical changes to the tissue microenvironment leaving open the possibility that lower doses of atrazine could produce subtle but very biologically significant events.

The cellular signing mechanisms involved have not been elucidated and molecular events such as DNA damage and imprinting changes may be possible at low levels, and this has been shown in other studies of low level exposure to environmental endocrine disruptors. Such changes may accumulate during the aging process of men.

And finally, it is unclear what effects atrazine may have on the truly relevant measures of prostate cancer effects, including grade, progression and aggressiveness. We believe further research is needed to confirm or refute a possible role for atrazine in human prostate disease.

DR. DANIEL SCHLENK: Thank you, Dr. Timms. Dr. Jerde?

DR. TRAVIS JERDE: Thank you. I agree with the sentiments that Dr. Timms has just presented. I would like to reiterate that the data so far in both the epidemiological association of prostate cancer to atrazine exposure, as well as the animal studies, do remain inconclusive with regard to any role hat atrazine may play in disease.

A little bit about prostate cancer; to my view, prostate cancer is not a disease. It's probably five or six or ten different diseases. And when you look at

epidemiological evidence of a disease that's going to affect half the population, a one or two percent change in incidence that may or may not occur is really not going to show up. But if there is an increase in the clinically significant, highly aggressive forms that some men get -- and those would be the very small percentage of the men that have the disease that will actually end up dying from this disease -- that would be a more applicable measure.

So when Dr. Timms talks in his report here about the more relevant measures, what we could be looking at are those cancers that are a Gleason 4 plus 3 or higher, those cancers that occur earlier in men, those cancers that are faster or achieve in androgen-independent state. Okay, those are the prostate cancers that kill people.

So that's something that I hope that the data can be extracted from in what's been published so far. And I agree with the dosing regimens, repeated dosing throughout one's life, because that is what one sees with prostate cancer, it's a disease of aging.

Now if it is true that this compound could cause prostatic inflammation, particularly as a life-long exposure, the results of this on human health actually could be quite profound. As Dr. Timms pointed out, inflammation is the most associated histological feature associated with prostate cancer. And we have another very common disease in the prostate, the second most common neurological condition diagnosed, and that's

called benign prostatic hyperplasia or LUTS, lower urinary tract symptoms in men.

I chaired a session as the SBUR this spring on new advances in BPH research and, among other things, one of the themes that came out of this meeting was that inflammation is the most tightly correlated histological feature to symptoms of BPH, moreso than is prostate size, in fact. And so, one thing we haven't addressed so far is the presence of BPH LUTS symptoms in exposed individuals.

We have no idea what causes prostatic inflammation in humans. It is not likely to be bacterial infection. Those prostates have been cultured. We've looked for 16S ribosomal RNA; inconclusive results in those studies. And so this gives rise to all sorts of different hypotheses. And there are hormonal changes, the presence of the metabolic syndrome, type 2 diabetes seems to be correlated a little bit, lifestyle, diet, and environmental exposures are one of those things, including some of the endocrine disruptors that Dr. Timms is well-known for investigating.

But I'll just leave you with a little bit of epidemiology that I think is quite striking and is why we care about these issues in our field. In the United States, 50 percent of men will get prostate cancer. That’s well established. In Asia it's less than 10 percent. In China it's about one and a half percent. If you look at full-blooded Chinese-Americans who have lived in the United States for greater than two generations, their

lifetime incidence is 40 percent, suggesting that a lifestyle change of whatever is a very important progression of this disease. That is also associated with an increase in the clinically significant deadly forms of prostate cancer. So these are the reasons why we care about this and why -- there's a lot of recommendations for you guys to go back to your lab and say, now do these studies. But over the course of the next few years, these things continue to -- it's like a continually evaluation process, but those are some of the important questions that need to be addressed from our end as prostate biologists.

DR. DANIEL SCHLENK: Okay. Any other comments from panel members? Okay. Let me go back to EPA. Do you guys have what you need for that particular question?

DR. ELIZABETH MENDEZ: Yes. We have a lot to think about, but we wanted to also circle back, if we may, to a question that we have as we were sitting here during break thinking about what we've heard this afternoon.

One of the things that, if you remember when I first started my first presentation yesterday, the introduction, and I was going through the adverse outcome pathway. There was a bifurcation in the road of when do we see something that is just a mere perturbation, that it's not going to lead us to a biological adverse effect, and when do we trip or go over that line where perturbation does become clinically significant.

The BND that we're working on right now that was brought to the table, it's based on a one standard deviation. It's not a BNDL10. It's not a BNDL15. When we do the BND at one standard deviation, we're talking about a 33 percent decrease in LH surge or attenuation of LH surge. And I guess, what I would like to get a little bit of clarity is, during the September meeting, we heard some comments about 80 percent being necessary for the ovarian cyclosity disruption, but there was also some comments about, well there might be something else happening at lower levels of LH attenuation and you may just not have the data and we can't negate that.

I would like to get a little bit of feedback from the panel in terms of do I hear that you're a little bit closer to giving us a range at least between 33 or 80, somewhere in there, that you feel is where we trip that censor that we go from a mere perturbation to something that leads to an adverse effect, given that we have at about 12 and a half megs or 25 megs, we start seeing things in terms of apical endpoints. So I guess that's sort of a question that I would like to get a little bit of clarity on.

DR. DANIEL SCHLENK: This would probably be a follow-up to question five, right? Yes.

DR. ELIZABETH MENDEZ: Correct.

DR. KATHERINE ROBY: I'll try to get the discussion started. I think, from last September to now, we have no greater

insight in the significance of depression of the LH surge to subsequent outcomes downstream. I think, still, 80 plus percent needs to be reduced before you see a shift in ovulation, for example.

I think what was mentioned last September is what is an unknown is if there were a 30 or 50 or 60 percent decrease over time, it's completely unknown -- I know of no literature that has addressed what might be the overall effect in your lifetime fertility. So I think we still don't have a great idea of where you go from a biological modifier that you're measuring LH and a concrete downstream negative effect or negative outcome in regard to fertility in the female. I'm not sure if that was very clear.

DR. DANIEL SCHLENK: Any other comments from the panel? Yes, Dr. McManaman?

DR. JAMES MCMANAMAN: Yes. I think that you're asking for a tertiary affect when we don't even know what the primary affect is. And so I guess I would echo the sentiments that have been express by Dr. Horseman and others that we really begin to look at what's going on at the level of the hypothalamus because that's the most likely affect, because you're affecting not only LH but you're affecting prolactin. And unless somebody can explain to me how LH is causing a decrease in prolactin then I think that it looks like, I'd say, probably a global affect or a potentially global affect on the hypothalamus and not specifically on an LH affect.

DR. DANIEL SCHLENK: Yes, Dr. Mendez?

DR. ELIZABETH MENDEZ: Just a little bit of a follow-up then just for my own personal understanding. The way that we've been thinking about the LH surge is sort of a node from whereas some other effects may make happen that LH diminished decrease. So I understand the 80 percent for ovarian cyclicity disruption but I'd like to hear a little bit more discussion about other endpoints like the puberty onset or something along those lines as well.

DR. DANIEL SCHLENK: Dr. Horseman?

DR. NELSON HORSEMAN: This gets to the point that we were discussing earlier. And I think the problem and the reason Dr. McManaman is suggesting other hypothalamic effects is there is no coherent way that any of us, I thin, can figure out that this suppression of the LH surge and these other reproductive physiological affects ranging from delayed vaginal opening to estrous cyclicity to prostatitis in the male to whatever, and then add on top of that affects on appetite that are presumably hypothalamic.

As far as we can tell, or at least me, there is no way to consolidate those under one umbrella at this point. That doesn't mean that there isn't a final common pathway that explains all these things, but it's just that I don't hear any of us saying, "Oh, yeah, we can consolidate those things under one mode of action." Thank you.

DR. DANIEL SCHLENK: Yes, Dr. Mendez?

DR. ELIZABETH MENDEZ: One last follow-up. I just want to make sure that I'm hearing the panel correctly. So as I'm sitting here, am I hearing you say that you're not entirely certain that this is a node from whence everything else comes down? Is that what I'm hearing panel say?

DR. DANIEL SCHLENK: Anybody want to respond to that?

DR. KATHERINE ROBY: I will.

DR. DANIEL SCHLENK: Dr. Roby?

DR. KATHERINE ROBY: It might be a node, but I'm not a 100 percent convinced it's only the surge that is a node. And you asked a question about other downstream effects. We measure vaginal opening as an indication of onset of puberty in a rodent. And that occurs because of changes in LH, IA, GnRH, pulse secretion around the time of puberty stimulating the ovary, increasing estradiol production, which ultimately affects the vaginal tissues and allows for vaginal opening through some mechanisms that are well-known now.

So that ties back to LH. It doesn't tie back to a change in what we call the LH surge, the ovulatory surge, but probably a change in the pulse or the increase in pulse and amplitude that occurs around the onset of puberty.

So in the sense, that LH is still that node, but again, the mechanisms are not explored.

DR. DANIEL SCHLENK: Yes, Dr. McManaman?

DR. JAMES MCMANAMAN: Yes. So to follow-up on what Dr. Roby said, it potentially could be a node, but if it is, it's not the only node. Because the atrazine causes a decrease in LH, that was suggest a decrease in GnRH. Atrazine also causes a decrease in prolactin, so prolactin is regulated in a different way, so if it was affecting the hypothalamus to affect dopamine release, decreased dopamine release would actually increase prolactin and not decrease prolactin.

So I suggest that there's something more complicated going on at the level of the hypothalamus and not at the level of -- I mean, LH is the secondary effect to the GnRH -- it's just hard to measure GnRH, that's why everybody measures LH -- but it suggests that it's at the level of the hypothalamus and not at the level of the LH, per se.

DR. DANIEL SCHLENK: Yes, Dr. Akana?

DR. SUSAN AKANA: Yes. I think LH is a node in the net, so it's just one of many, and it's the one that's probably most manipulable and measurable for you. But on a different tact, when I study male stressed rats, the first general rule of thumb we look for an unhappy rat is a drop in body weight. Like, a 10 percent drop in body weight is an automatic flag, regardless of whatever the

provocation is. Now you're working with a lot of female rats, which have a much slower growth rat. So I think it would be even tighter, maybe a five percent drop in body weight.

DR. DANIEL SCHLENK: Okay. Does that clarify your question there, Dr. Mendez?

DR. ELIZABETH MENDEZ: I think I'm starting to get an idea that we're talking about, as Dr. Akana said, a net. We may have a part of the net. We don't have the whole net. And I guess, at this point in time, as Dr. Fowle mentioned earlier today, we have to, from a regulatory standpoint, go with what we have in front of us but remain vigilant to what may come further down the line.

So I guess that's the reality of the situation were in the regulatory arena, but we'll certainly keep our eyes open. Thank you.

DR. DANIEL SCHLENK: Okay. At this point, I believe -- no? She answered it; okay. I guess we've got that one done; awesome. All right. Well there's no further comments on 5, 6, 7; let's go to question number 8 then.

DR. ELIZABETH MENDEZ: Number 8 question, the genesis of that was we had some conflicting data about rat mammary gland development that was presented during the September SAP. And some other feedback that we got from the panel was, well, we have these two studies; one uses subjective measures for mammary gland development, the other one is using objective measures, namely morphometrics, but we've

never seen the two methodologies compared side-by-side. Based on the feedback that we heard during the September SAP, our colleagues in the Office of Research and Development tried to address that question.

The question is, please comment on the agency's findings in addressing the issues raised by the SAP during the September 2010 meeting. Please comment on whether this study, along with the negative studies by CODAR adds to the weight of evidence that it is unlikely that atrazine impacts mammary gland development.

DR. DANIEL SCHLENK: Thanks. Our lead discussant on that is Dr. Horseman.

DR. NELSON HORSEMAN: So a simplifying answer to this question would be a simple yes. But as you might expect from the panel, you're not allowed to give just that. Let me read from my answer that Dr. McManaman and I have discussed somewhat.

The new data presented from Dr. Cooper's study address the concern that the studies from Fenton's group and those from Hovy, contracted with Syngenta, used quite different approaches for capturing mammary gland morphology. In the former, a ranking system was used; in the latter, a set of measured morphometric variables was applied.

While the ranking system has been referred to a subjective and qualitative, it is in fact neither. The method, when done in a blinded fashion with trained

application and morphological criteria is clearly objective and no less so than a morphometric approach. It is also quantitative because the established morphological criteria converted to quantities, that is ranks that can then be compared with standard statistical methods. So it is, in effect, objective and quantitative, ultimately. Morphometric measurements may be objective, but the only objective is if the measured variables are not chosen subjectively. So it's nothing magic about these two approaches, I think. And the fact that the data come out the same then isn't too surprising.

There's a 2009 workshop that's been referenced in the white paper that did a good job of summarizing best practices for using morphological variables to characterize rodent mammary glands. So based on using both approaches, the ranking approach and morphometry in a careful manner with Sprague-Dawley rats -- and again, rats trained is an issue that runs through a lot of these questions -- the Cooper study presented in the white paper demonstrates, number one, that both approaches produced similar conclusions, and number two, that any effects of prenatal atrazine exposure on mammary gland development early in life -- and I think the measurements were done at day 45, which is just after puberty is finished -- are very subtle and are not measurable by any of these techniques.

So while Long Evans rats might be a different case, good arguments have been made that the Sprague-Dawley model is

appropriate and adequate, and this study is definitive in that regard.

So to the larger questions; first the use of "mammary gland development" as an index of adverse environmental chemical effects, has been advocated based on a number of features of mammary gland growth, morphology, pharmacology and physiology. There are several papers from the number of groups advocating this as a model system.

These features that are used in this advocacy include the exquisite hormone responsiveness of the mammary glands and a distinct developmental sequences of events, most of which occur after birth and are therefore accessible in ways that some other developmental events might not be. These are compelling notions, but I would say, thus far, implementing this practically has been difficult and not finally proven to be that helpful.

Given the centrality of lactation, though, in the life history of mammals, continued concern about the affects of environmental chemicals on mammary gland biology is extremely important. Therefore, inadequacies in the literature relating to atrazine effects on mammary gland development should not deter future studies.

So the direct answer to the charge question is that the new evidence does not provide any support for an effective atrazine on mammary gland morphology. In the new studies, SD rats were treated in utero with a wide range of doses. Tissues were taken for analysis on

postnatal day 45. Mammary gland morphology was measured by both the arbitrary ranking system and by morphometric quantification using image analysis. And because certain morphological characters occur in a predictable manner, measurement of this presented here are taken to signify development. It's always important to remember though that development refers to processes that underlie these morphological changes, not the morphology, per se.

So while the charge question is focused on resolving differences of experimental design, data gathering interpretation between studies primarily from the Fenton lab and those from Cooper's lab and the Davis 2011 paper and the Hovy lab, also published this year, these ambiguous findings need to be considered in large context. So stepping back, earlier concerns about breast carcinogenesis are mammary gland cancer in atrazine-treated rats were resolved satisfactorily by discovering that mammary tumors came about by a process of disorder postnatal development.

It's driven by accelerated reproductive senescence and appropriate secretion of gonadotropin steroids and prolactin and that was reviewed by Cooper et al. most recently as 2007.

So these well-accepted conclusions lead to the simple deduction that atrazine does have effects on mammary gland development, even if those effects did not appear unambiguously in the results from the early life studies from Fentons or the other labs cited here.

So the more relevant question seems to be whether development in the rodent mammary glands early in life provides any adequately robust model in which to observe subtle adverse effects of potential environmental toxicants such as atrazine. For a variety of reasons, it seems unlikely that mammary gland morphology, standing as a surrogate for underlying developmental processes, is adequately sensitive to fulfill this role.

One limitation of rodent mammary gland morphology is it is subject to wide variations among rodent strains, depending on differences in hormone secretion patterns, the presence of endogenous retrovirus, particularly, MMTV, and on their nutrition. In addition, there are internal differences between morphological characteristics of the glands within an individual and even within regions of a particular gland. So robustness is difficult to come by there.

The second limitation is one's ability to define differences in morphology or development as being adverse. Given that the function of the glands is to produce adequate milk for the offspring, for any change in morphology to be defined as adverse it would need to be connected in some objective way to a deficiency in milk supply. And given that the glands are controlled by a host of intrinsic and extrinsic homeostatic mechanisms that are focused on ultimately regulating milk production, it's not surprising that subtle effects of environmental chemicals on morphology may not, by

themselves, perturb function sufficiently to be definitely adverse.

Concerns remain, however, as to whether an environmental toxicant such as atrazine which affects reproductive hormones or other mammary-related physiological variables might interact in important ways with other environmental factors that predispose individuals to poor mammary gland function and ultimately to inadequate lactation and poor breastfeeding outcomes.

In particular, obesity is a known risk factor for poor mammary gland function in humans as well as in rodent models, and is the number one contributor to failure of breastfeeding and failure of women to implement their breastfeeding goals. It's certainly conceivable, maybe likely, that subtle affects of an environmental chemical will have important consequences in overweight individuals.

In conclusions, it is true that the current data "adds" to the weight of evidence that it's unlikely that atrazine impacts mammary gland development, which is the statement in the question. However, the evidence off effects in some studies, combined with the known affects of atrazine on reproductive hormones provides an important basis for continued concern in efforts to design better studies that would determine whether these hormonal effects could contribute to poor lactation, a clearly adverse outcome, in susceptible individuals.

DR. DANIEL SCHLENK: Okay. Dr. McManaman, anything to add on that?

DR. JAMES MCMANAMAN: As Dr. Horseman said, I concur.

DR. DANIEL SCHLENK: Great. Thanks for working together on that. Any other comments from the panel? Okay. I think that's pretty clear; yes?

DR. ELIZABETH MENDEZ: Yes. Thank you.

DR. DANIEL SCHLENK: Okay. All right. So we're moving right along. Let's go ahead and read in charge question 9 and hopefully call it a day after that, maybe.

DR. ELIZABETH MENDEZ: All right. Charge question 9, it speaks to the sensitivity between the adults and infants and children in the analyses that has been conducted by the agency regarding the studies that we have in front of us and the evidence or lack thereof of an enhanced sensitivity of the young.

So the question is please comment on the weight of evidence analyses conducted by the agency and the extent to which the uncertainties related to the potential for differential sensitivity of the young are addressed with the additional data. Let me clarify that by additional data, what we mean is, all of the life-stage data that we have that is typically not available to us.

DR. DANIEL SCHLENK: Okay. Our lead discussant is Dr. Fenner-Crisp.

DR. PENELOPE FENNER-CRISP: Okay. We've crafted three questions that I think re-characterized your request for comment, and they are as follows.

Does the existing body of data exploring the potential for adverse consequences following exposure of either direct or indirect at relevant life stages, in fact, encompass all of the life-stages of interest prenatal, perinatal, pre- and peripubertal and adult.

Secondly, do the study design employed in this existing body of data allow for an adequate assessment of the potential for differential sensitivities in light of the fact that these studies do not necessarily include measurement of the same endpoint or phenomenon that currently serves as the basis for the quantitative characterization of hazard, in other words, the suppression of the LH surge.

In reading the issue paper, we've concluded that chapter 5 is supposed to serve as the weight of evidence and the uncertainty analysis. So the question becomes, does chapter 5 adequately present the weight of evidence and the uncertainty analyses on the question of potential age-related differences in sensitivity? Let's answer this question first.

Chapter 5 is not a weight of evidence analysis of the datasets and it does not present the uncertainties of the database as it currently exists. The truly robust weight of evidence discussion would include a recapitulation of

the previously submitted and reviewed studies, along with the newer ones, so that one can understand where and how each contribute to the determination of whether or not sufficient information exist to answer the question of whether or not we know enough about differential sensitivities.

Secondly, there seems to be little or no discussion of the extent to which the uncertainties related to the potential for differential sensitivity of the young are addressed.

With regard to the first question, it would've been helpful or enlightening to have had available either a table or a figure which summarizes all of the relevant studies bearing on this issue. Dr. Mendez included several tables in her third presentation yesterday, which could've served as a starting point for a composite table of such studies. And we had, among the slides presented by Syngenta, a figure that could've been useful for assembling the dataset and had visual display of the available data to help answer the question.

Nonetheless, the panel believes that there is sufficient information available to reach the conclusion that the issue of differential sensitivity has been adequately studied if one accepts the premise that the data on the LH surge is the appropriate one for making the comparisons.

The panel continues to agree with the agency's conclusion that exposure during the earlier life-stages does not

lead to greater sensitivity, again, when comparing with that, the BMDL-based dataset.

Speaking to the second question, which was the issue of whether or not the parameters evaluated in the studies put forth to assess the potential for differential sensitivity are adequate or appropriate for that purpose. There are a wide variety of studies and a wife variety of endpoints that have been evaluated in these other studies, and I guess, in general, we feel that they do provide a nice variety of things against which to compare.

I'm going to follow with what I have characterized the subgroup as mission creek. In other words, we're going to answer a question that you haven't asked. That has to do with the FQPA safety factor. Selection of it is a combination of what one does or doesn't know about the science and what one applies in terms of policies. So we're sticking the toe on the water on the hook of the science side.

As summarized in the agency's policy guidance entitled Determination of the Appropriate FQPA Safety Factors and Tolerance Assessment, Section 408(b)(2)(c) that Liz mentioned yesterday instructs the agency in making its reasonable certainty of non-harm finding that it apply an additional 10-fold margin of safety. I won't read the rest of that. The section for the administrator may use a different margin of safety if she wishes.

It should be noted that the law does not impose any directional constraints on the choice for a different margin of safety. The different margin of safety could be greater than 10x or less than 10x. While this flexibility exists in the law, there is little precedent explicitly or implicitly for application of an FQPA safety factor greater than 10. I'm not going to tell you what the example is. There is substantial precedent for the reduction of the FQPA safety factor to either 3x or 1x, but there is no precedent for application of an FQPA safety factor less than 1. Should note, cases where the FQPA safety factor has been removed equates to a safety factor of 1x.

As articulated in the 2003 IRED, EPA retained the FQPA safety factor of 10 for atrazine and its metabolites to protect the safety of infants and children in assessing risk from dietary, that is in food and drinking water exposure, and they offer the rationale as to why. I'm not going to repeat that here. And for residential exposures, they applied an FQPA safety factor of 3x, was reduced by roughly half.

In summary, the 10x factor was applied in the dietary risk assessment reflecting concerns both with regard to the neuroendocrine MoA and the uncertainties regarding exposures in drinking water. And the 3x factor for residential exposure reflects concerns only with regard to the neuroendocrine MoA.

The July version of the issue paper, we have available for this meeting, summarizes the results of a series of studies, some predating and some postdating last September's SAP meeting and concludes, although additional experimental toxicology studies are still ongoing to better characterize the potential adverse health outcomes resulting from atrazine exposure, including the duration of exposure that may lead to an adverse health outcome, available data do not indicate that pre- and/or postnatal exposure leads to increased sensitivity in the young relative to the attenuation of the LH surge and serves as a basis for the atrazine risk-assessment. The panel did agree with that last fall.

If the panel continues to agree with the agency's conclusion with regard to the lack of early age-related sensitivity to the neuroendocrine effects that are driving the hazard assessment, at least two options with regard to the appropriate magnitude of a revised FQPA safety factor could be considered. In the current issue paper, the agency proposes to replace the old NOAEL from the Morseth study with the new BMDL to .56 to serve as a point of departure. Going on to note three additional studies evaluating the effect of atrazine exposure across life-stages have become available within the last few months.

These studies reinforce the conclusions reached during the September 2010 meetings since all of the affects observed in the young in these set of studies occurred at

doses roughly 25 times higher than the dose EPA is proposing to use as the point of departure.

So we see two options that one might elect to use for reevaluating the safety factor in the new upcoming risk-assessment. The 10x safety factor currently applied in the dietary risk-assessment could be reduced to 3x. Removing the 3x or reducing to 1, that portion of the safety factor addressing concerns regarding the hazard potential. The other half, which is currently applied, because of the exposure issues, would be revisited when they are resolved. The 3x safety factor currently applied in the residential risk-assessment also could be removed or reduced to one. That's the more conservative approach.

The second option is, given that one could conclude, from the agency's statement above about the 25-fold difference thing, is that not only is there no differential increase in sensitivity in the young as a consequence of pre- and/or early-postnatal exposure; there is, in fact, the decreased sensitivity when compared with the adult female. On the basis of this finding, one might argue that the 3x FQPA safety factor currently applied to account for the uncertainties are concerned around the neuroendocrine effects could be reduced to less than one.

For example, on page 29 of the issue paper, the agency states that all of the effects and sexual maturation and altered androgen status reported after about 30 days of exposure occur at dose levels 5 or more fold higher than those leading to the LH surge. Further, they say the

dose level eliciting the increase in the incident of prostatitis in the offspring is greater than 10-fold higher than the dose leading to the LH surge attenuation used as the basis for the risk-assessment. This argues then, that the FQPA safety factor component addressing the hazard potential could be reduced not just to 1x, but further by at least 5-fold.

DR. DANIEL SCHLENK: Okay. Thank you, Dr. Fenner-Crisp. Dr. Akana?

DR. SUSAN AKANA: I have nothing more to add. Thank you.

DR. DANIEL SCHLENK: How about subtract? Dr. Chambers?

DR. JANICE CHAMBERS: Penny is a hard act to follow. I don't have much to add either. I do agree that there is nothing in the evidence you provided to us that indicates that the young are more sensitive. I also agree with Penny that there wasn't a good compilation of the studies for us to try to sort that out very easily, but the issue paper does indicate that additional studies are ongoing, so I assume that when those data are derived, they'll be looked at.

Some of the studies that you did quote in there did show functional endpoints, in terms of looking at vaginal opening and behavior and that sort of thing, and I tend to think that those sorts of functional endpoints are more important to look at than something that may not be a true adverse effect.

DR. DANIEL SCHLENK: Dr. Meek?

DR. BETTE MEEK: Right. Well, I agree with most of what's been said and maybe all of it if I understood all of it, so just a couple of additional points.

I really didn't see a weight of evidence analysis, so that was really to underscore the point. I think a weight of evidence analysis of being kind of a simulation of the data looking at the consistency, the dose response concordance, et cetera. So I didn't really see that in the issue paper. Of course, consideration of FQPA really should be based on transparent and systematic consideration of the most important qualitative and quantitative uncertainties associated with both exposure and the effect, and we've restricted this discussion principally to hazard; our relevant to susceptible life-stages, and again, I really didn't see that discussion in the paper.

A couple of points, though I probably stated them in a slightly different manner but underscore some of the previous points. I think that we need to take into account the interplay between uncertainty and safety factors, and the point of departure. And we need to recognize that the point of departure in this case is likely very protected, having been based on the lower 95 percent confidence interval for the benchmark response for an early precursor event rather than an adverse effect.

So I agree that, based on the data that we have been presented, there appears to be no basis for application on the factor on the basis of hazard. So in relation to exposure, I'm just going to leave one point with you, although we haven't discussed that at this point. I wondered if any thought had been given to estimating the internal dose metric for younger age groups of the human populations. Human population for consideration of the context of FQPA recognizing that the chemical specific adjustment factor for interspecies or animal to kinetic differences are likely to be less than default. So that is just something to keep in mind for future.

One other point, given the interplay between the point of departure and applied uncertainty or safety factor, I think that the question gives me -- I'm taking license anyways to raise a recommendation that I made at the September SAP meeting. In the interest of transparency, I think it would be helpful to consider an array of points of departure for various endpoints and their biological significance with a view to bounding potentially the degree of conservatism associated with the ultimate choice.

This would include but not be limited to the benchmark dose for the impact on the LH surge, but including also those for more traditional endpoints generally considered to be adverse. This seems rather critical as a basis to interpret the derived benchmark dose in the context not only of its biology significance, but also its degree of conservatism in the risk-assessment construct which we

use traditionally to address more severe endpoints. So again, that really builds on some of the earlier conversations that we've heard today as well, and I think I'll leave it at that.

DR. DANIEL SCHLENK: Thank you, Dr. Meeks. Any other input from the panel? Dr. Portier?

DR. KENNETH PORTIER: I got a question of Dr. Meek. If I understood what you were saying, when we talk about the LH surge we're talking about something really, really, really early in a process to an adverse event. When you think of FQPA factors, there's like a 10-fold multiplier from the normal population that's susceptible.

Is that something that's on the table when you're talking about now setting an endpoint that's very early in some kind of process? Is that one way of addressing that susceptible individual in the population or are you thinking about something else? It just kind of came up in my mind as you were saying this, and I was thinking, "Is that what she thinks?"

DR. BETTE MEEK: To me, you can't separate the discussion of the point of departure, its degree of conservatism and how health protective it is from the discussion of the uncertainty factors. Ultimately, those uncertainty factors have to be informed by the degree of adversity of the critical endpoint, the extent to which you think sensitive populations are protected, the interspecies differences in kinetics and dynamics.

So I find it difficult to discuss a single factor outside of all of those considerations for which there is interplay. I also am not particularly fond of collapsing an entire database to one point of departure, which is why I think it's important as well to consider several points of departure in terms of the increasing degree of adversity of the effects for which you're trying to protect and bound those points of departure with considerations related to their adversity.

DR. DANIEL SCHLENK: Okay. Any other panel input? Okay. Back to the agency. Are you content with that answer, I guess?

DR. ELIZABETH MENDEZ: Well, I have to say an FQPA factor of less than one is -- it's one of those wow moments, but I certainly recognize what I am hearing from the panel and we'll do a better job of compiling the data for the weight of evidence. We were trying in the interest because we had presented a lot of that data in September, we were trying not to repeat everything again, but it appears that it would've been helpful at this point in time to do so, so we'll certainly take that under consideration.

Other than that, no; I think you've answer our question. I am going to, for question 10, pass it on to Dr. Christensen because I think I'm starting to lose my voice a little bit here.

DR. DANIEL SCHLENK: We're not going to go to question 10.

DR. ELIZABETH MENDEZ: You're not going to go on to--

DR. DANIEL SCHLENK: No.

DR. ELIZABETH MENDEZ: You're going to call it a day?

DR. DANIEL SCHLENK: We're going to call it a day; yes.

DR. ELIZABETH MENDEZ: All right. In that case, any other questions?

DR. DANIEL SCHLENK: Yes. Dr. Fenner-Crisp, you have one more comment?

DR. PENELOPE FENNER-CRISP: One of your last points, Liz, about a document; I see the issue papers at any one time as a living document there, and how many of them -- at least three that gets bigger and bigger and bigger. But this one should have built on the last one and should have acknowledged the work that was done in the earlier ones, particularly on this point.

So it should've been brought forward into that chapter, because ultimately you're going to have a last one and it's going to become the background document for the risk-assessment and you're going to want to have all of that there. So grow it with time.

DR. ELIZABETH MENDEZ: Yes. We'll certainly do that.

DR. DANIEL SCHLENK: Okay. Have a question, Dr. Griffith?

DR. DANIEL GRIFFITH: Listening to the responses to questions 5 through 9 and linking that back to the water monitoring, my impression is, there is some need to also look for the repeatability, the cyclability that's in the water monitoring data from year-to-year. Most of what I've seen has been concentrated on what's going on in a given year. But if there is something such as atrazine damage accumulates through time, then it would be useful to see how those time series are replicating themselves from one summer to the next. And I didn't see any of that in the background material, but it seems to me that that might be something that need to be inspected.

DR. ELIZABETH MENDEZ: I don't know if our water-monitoring colleagues are still in the room.

DR. DANIEL SCHLENK: Yes. That may be something we'd want to do on the last question, I believe; yes. Okay? With that, I'll turn it over t Joe Bailey and he can close this out.

JOSEPH BAILEY: Thanks to everyone. I don't have any closing comments except thanks for your input today.

July 28, 2011 - 8:30 a.m. Day 3

JOSEPH BAILEY: Let's get started here. My name is Joe Bailey, and I'm with the FIFRA Scientific Advisory Panel staff, serving as designated Federal Official. This FIFRA for Scientific Advisory Panel meeting, reevaluation of the human health effects of atrazine, review of non-

cancer effects, drinking water and monitoring frequency, and cancer epidemiology.

I don't have any announcements to make this morning with regard to the docket or anything. The EPA presentations are there, available, and the public comments are not there yet, but they will be, hopefully by the end of the week. And that's it. I'll turn it over now to Dr. Schlenk, our Chair.

DR. DANIEL SCHLENK: Thanks, Joe. Good morning, everyone. I think we'll skip our normal round-robin introductions this morning since I think everybody kind of knows everyone at this point. So we're going to begin with Question 10. And, Dr. Christensen, you're going to read that into the record? That would be great.

DR. CAROL CHRISTENSEN: Thank you. Good morning; Carol Christensen with EPA. So we are moving into cancer epidemiology portion of the meeting. So Questions 10 and 11 relate to Chapter 3 and Appendix B of the draft Issue Paper from EPA. So at this time we're looking for feedback on our evaluation of the individual studies, our synthesis across the epidemiology database, as well as the integration with the experimental toxicology database. As was mentioned yesterday, we're sort of stopping short from making a request as to what the cancer classification should be, per se, but more looking for feedback on the process at which we came to our preliminary conclusions and the extent to what the data support those conclusions at this time.

So having said that, Question 10, Part A reads: "Please comment on the sufficiency of the Agency's cancer epidemiology reviews with respect to identifying the major strengths and limitations of each study in the overall synthesis of results by cancer type."

DR. DANIEL SCHLENK: Thank. And our lead discussion on that is Dr. Bove.

DR. FRANK BOVE: Good morning, everyone. Having sat on the 2000 and 2003 science advisory panel meetings for atrazine and registered my frustration in each of these meetings that the EPA had not done a systematic and comprehensive evaluation of all the cancer at the work that had been done up to those times, I'm pleased that EPA has finally done so. And I think that they've done a pretty good job. Although we have some differences with EPA on some of the evaluations, for the most cancer sites we're in agreement with EPA's assessment. We think it's pretty comprehensive.

Focusing first on the methodology of EPA's literature search, we find that the methods were sufficient, thorough and transparent. EPA's method of evaluating studies was, in general, sufficient and, as I said, comprehensive. Important aspects of the studies were considering, including accurately measuring the cancers and exposures, issues of bias, sample size and statistical power.

Major strengths and weaknesses were identified, and in particular, whether exposures were assessed

quantitatively; the ranges of exposure, whether critical windows, time windows of exposure were evaluated. And they used the usual etiological criteria, such as temporality, magnitude of the measure of association, which would be the relative risk or the odds ratio, for example, exposure-response trends, consistency of findings across studies and biological plausibility. However, there are some issues with EPA's methods of assessment, at least I have.

First, the focus of the assessment should be on the individual level studies. Ecological studies should be evaluated only if there are compelling reasons to do so. For example, if there is no other individual level studies to evaluate.

Secondly, the focus should not be on whether a finding is statistically significant, especially given the low statistical power of most of these studies. Emphasizing statistical significance when power is low will likely result in Type II errors. I did notice a couple of times in the text the notion of borderline significance was mentioned. There really is no such thing; a finding is either statistically significant or it isn't. And there was also a statement in the text that the findings that are borderline are more likely to be chance findings and that's not accurate either. We can get into that if we want, but that's --

A third issue is concerning biases. It's important to provide some evidence, not just charge that the bias might be there, but actually provide some evidence as to

why a particular bias is likely to be present in this study; the likely magnitude of the bias and the likely direction of the bias. In particular, the likely impacts of non-differential exposure misclassification be taken seriously. That is, bias towards the null for dichotomous exposure variables and distortion of exposure-response relationships, in particular, attenuation at the high end so that monotonic trends are not observed.

This is also the case with the healthy worker survivor affect biases and since we're studying occupational cohorts most of the time here, that's also an issue. Again, these kinds of biases tend to distort exposure-response relationship so they're not monotonic.

Also, the issue of confounding: It's important to keep in mind it's not just that a factor is correlated with, for example, pesticides correlated with another pesticide. There really needs to be a strong risk factor involved. An example, with asbestos and lung cancer and smoking as a possible confounder; even though the work forces that are studied have a high prevalence of smoking, the confounding effects of smoking in these studies are usually no more than 20 to 30 percent. And smoking is an extremely strong risk factor for lung cancer and is also very highly correlated with asbestos exposure and yet that's about as much as the confounding that exists. So when we're talking about confounding by other risk factors that are weak risk factors -- even though they may be correlated with pesticide exposure --

you do not see much confounding. I'll talk about that a little bit later and I'll give you an example.

Fourth, it's problematic to make a general statement about all cancers. That's true whether you've studied all cancers combined, as sometimes the Agriculture Health Study does, or whether you make a blanket statement -- as in page 71 of the text -- that atrazine is not likely to be carcinogenic in humans.

The evidence across cancer sites is considerably mixed, with some sites having evidence of no association and other sites having at least suggestive evidence. So lumping, making these kind of blanket statements -- and also lumping all cancers together to analyze them -- is not very helpful.

Fifth, the appendix: The text, actually, was very good in describing each cancer site and the evidence for that in the epi literature. The appendix, on the other hand, had studies of different sites all mixed in together. It would be helpful if the appendix was better organized, like the text was. And that also includes the tables that are in the text. And actually, Dr. Gold has put together a table that we'll put in our report that is an example of what might be done.

Okay. The EPA discussed the strengths and weaknesses of the Agricultural Health Study and we thought it was comprehensive. One strength that the Agricultural Health Study has is that it is a longitudinal follow-up and exposures and risk factor are updated every five years.

Information is updated every five years; however, this information has not been used -- at least analyzed yet -- in these studies we evaluated. The information is still from Phase I.

Another issue with the Agricultural Health Study cohort is that it is predominately white and may therefore not include more susceptible populations; for example, prostate cancer if more prevalent among African-Americans. And another limitation is there is a small number of female applicators making it difficult to study cancers in females occupationally exposed.

One other point that EPA makes in the text is they state that most of the agricultural health studies were hypothesis generating. And actually, all of them were hypothesis generating and that's true of most scientific research, but they were also all hypothesis-testing studies. They all had an interest in particular cancers as well as other cancers, but there were particular cancers of interest and hypotheses were tested.

Okay. Now let’s get to the particular cancer sites. EPA pointed out correctly that most of the studies focused on cancers of the lympohematopoetic system, the reproductive and the endocrine system, and that's what we’ll be focusing on. First, prostate cancer; it was evaluated by the 2003 Science Advisory Panel. Back then, the Science Advisory Panel's position was that the database was insufficient to support a conclusion regarding the potential of atrazine to cause prostate cancer.

This conclusion remains valid today even though the agricultural health study cohort provides evidence against an association. And the reason it's still valid is that there are still lingering questions about the Saint Gabriel triazine manufacturing studies. These studies were initiated because of an excess of prostate cancer -- five observed and two expected -- that occurred prior to the start of the prostate screening program at the plant. Now, the screening program can explain most of the excess cases at the plant and we said that back in 2003 -- but not the excess represented by these five cases. The exposure experience of these five cases has never been presented. The follow-up case control study could've compared the exposure experience of these five cases with controls that were employed prior to the screening program, but that wasn't done.

At the previous 2003 SAP panel, it was concluded that "Lack of association among farmers does not preclude the existence of a positive association with triazine manufacturing plant workers," and this is because the exposure experience is different. Farmers had more intermittent exposures; workers would have more chronic exposures.

Moving onto breast cancer, a study of the agricultural health cohort observed relative risk hovering around 1.0. So this is negative evidence. On the other hand, there is a drinking water study in Wisconsin where the mean atrazine level in the high use area, high pesticide use area, was very low: less than .5 parts per billion. And only a handful of cases were exposed to well water with

equal to or greater than three parts per billion atrazine. But the odds ratios range from 1.2 to 1.4, again, based on small numbers of cases.

The evidence for association is extremely weak in this study, it shouldn't be dismissed. EPA correctly identified the limitations in these studies, including the agricultural health survey and that was small numbers, low power exposure, misclassification, inability to evaluate critical time windows of exposure and inability to evaluate exposure-response relationships; however, EPA states in the text that there is a lack of evidence, and that's not correct either. There is some evidence. It's very weak. So it may be better to characterize breast cancer as having inadequate information to assess whether atrazine can cause breast cancer.

Ovarian cancer: EPA focused on four studies, three of which were individual level studies; a study in Italy, a study in Central Valley, California, and the Agricultural Health cohort studies. Both the Italian case control Study and the Agricultural Health study observed positive associations and the Italian study observed higher odds ratios with longer duration of exposure to triazines. It did not look at atrazines specifically. And when they more precisely defined the exposed group, they also found higher odds ratios. Although it didn't evaluate atrazine specifically, the Italian study reported that the sales of atrazine in the area where these pesticides were used, the sales of atrazine were 10 times higher than the sales

of other triazines. So it looks like atrazine was predominately used in that area.

The Central Valley, California case control study used pesticide usage reporting data and questionnaire information to construct the job exposure matrix to assess occupational exposure to atrazine. They also evaluated residential proximity to areas where the pesticide was applied. Based on two exposed cases, the odds ratio forever occupationally exposed to atrazine was .76, and for residential proximity it was .88, based on eight exposed cases. However, the study excluded cases that died, they were too ill to participate, which EPA pointed out may have introduced a selection bias. Given the positive findings in two relatively well-conducted studies, EPA should consider the evidence as suggestive for an association between atrazine and ovarian cancer.

Moving on to lympohematopoetic cancers, the evidence is negative for leukemias, except for hairy cell leukemia -- I'll talk about that in a second -- and multiple myeloma. There are two studies, both of which are hospital-based case control studies -- I think both are French studies -- that evaluate hairy cell leukemia. Both were positive for triazines. Atrazine wasn't looked at separately. Both of the Italian studies used similar methods for control selection. EPA had some problems with their control selection, but in reviewing these studies, I don't find any problem whatsoever. They also had similar exposure assessment methods.

In the earlier Italian study, an odds ratio of 2.4 was observed for definitely exposed to triazines, based on 20 cases. The analysis was restricted to cases and controls unexposed to organophosphates, the odds ratios were reduced to between 1.5 and 2, depending on what variables were included in the models. No exposure-response relationship was reported, but they did not present any data in this study.

A more recent Italian study observed an odds ratio of 5.1 for hairy cell leukemia, based on four triazine-exposed cases. This study evaluated several exposure lag periods and didn't find any differences when they did that, but they did not report any of their exposure-response analysis. However, given that there are two positive studies here, there is, I would think, suggestive evidence for an association between triazines and hairy cell leukemia that should be followed up.

As for non-Hodgkin's lymphoma -- and this was discussed briefly in the 2000 Science Advisory Panel, but again, not in a comprehensive fashion -- there were several positive individual level studies of atrazine and non-Hodgkin's lymphoma, including a pooled analysis of Midwestern studies that used hierarchical methods to take into account a large number of pesticides simultaneously. I think it was greater than 40 pesticides.

Interestingly, the study concluded: "Adjustment for multiple pesticides suggested that there were few instances of substantial confounding of pesticide effects by other pesticides. Again, confounding, it's very

important to remember, it has to be strong risk factor as well as being associated with the other exposure of interest. This study of hierarchical pooled analysis observed an odds ratio of 1.5 for atrazine and non-Hodgkin's lymphoma.

Another study found an association between atrazine and a specific sub-type of non-Hodgkin's lymphoma, the chromosomal translocation T-1418, and the odds ratio was 1.7, based on 15 exposed cases. On the other hand, for the negative T-1418, the odds ratio was 1. There were some issues here; they could not get tissue samples for most of the cases. They had to do a missing value algorithm to impute values. So there are some issues there. But in any case, they did see a positive association for that specific sub-type.

A French study that examined hairy cell leukemia also observed associations between triazine and the non-Hodgkin's lymphoma subgroups diffuse large cell, odds ratio 2.1 based on eight cases and follicular lymphoma odds ratio 2.3, based on four cases. On the other hand, the Agricultural Health Study recent update was negative for atrazine and non-Hodgkin's lymphoma, including the subgroups. But given the positive studies, there is suggestive evidence of an association between atrazine and non-Hodgkin's lymphoma and I think that should continue to be evaluated.

Thyroid cancer: Here we have just the recent update of the Agricultural Health Study which observed elevated risks within the highest three quartiles of exposure

lifetime days. But the trend was not monotonic, again, probably due to exposure misclassification bias. The categorization was, as I think it was pointed out a day or two ago, was kind of funny. The categorization used in the analysis was based on all cancer cases and was not really appropriate for this cancer. Unfortunately, researchers could've used some smoothing methods to evaluate how the curve looked and maybe done the categorization based on that but they did not. In any case, this study provides suggestive evidence and should be followed up.

A number of other cancers that have been studies, usually there's only one study for them, except for gliomas, and they've been negative. And those include lung, pancreas, melanoma, colorectal, and as I said, gliomas. In the recent Agricultural Health Study update, non-monotonic exposure-response trends were found for liver and esophageal cancers. So you may want to say the evidence is inadequate and requires follow-up for those two.

Finally, two studies evaluate childhood cancers. The Agricultural Health Study evaluated paternal use of atrazine in all childhood cancers combined and observed 1.27. They only had small numbers of particular cancers, so they had to lump them all together. I think that is problematic. The second study in California evaluated residential proximity to areas where triazines were applied and acute lymphocytic leukemia and observed a non-monotonic exposure-response trend. When lifetime exposure -- lifetime of a child -- was evaluated, but didn't observe any elevations whatsoever. They just

looked at the first year of life proximity. They reported limitations in both, which EPA pointed out in its evaluation. So this again, inadequate evidence of an association here as well and requires follow-up.

So in summary, the cancers for which one can consider the suggestive evidence of carcinogenic potential -- I'm using these categories that EPA uses -- would include ovarian cancer, non-Hodgkin's lymphoma, hairy cell leukemia and thyroid cancer. Cancers for which there is inadequate evidence would include prostate, breast, childhood cancers, liver cancer and esophageal cancer. Both categories I would say the cancer sites require follow-up studies. And then cancers not likely to be caused by atrazine include oral, lung, colorectal, pancreas, bladder; leukemia, except for hairy cell, multiple myeloma, melanoma, kidney, larynx, brain, gliomas. That's it.

DR. DANIEL SCHLENK: Thank you, Dr. Bove. Dr. Gold?

DR. ELLEN GOLD: Thank you. Good morning. I also want to commend the staff on a really much more thorough review and a very thoughtful one. We thought it was very good. We've consulted with each other, so I don't have a whole lot to add, but I did want to underscore just a couple of things. So the point about adjusting for the multiple other pesticides, I think is really important because the statement is made in multiple places that adjustment was made if these pesticides were used together a lot, if they were highly correlated. But if they're not related to the outcome, then I think what you're seeing,

potentially, you're over-adjusting and potentially seeing an attenuation, an adjustment toward the null in general. So I think that needs to be considered when interpreting the results.

Also, this is a relatively minor point, but I also saw in several places that the comment was made that the agricultural health study had minimal selection bias because they had only two percent lost to follow up. Well, that's not the only source of selection bias. So they enrolled 82 percent, which is a really good enrollment, but the other 18 percent could be completely different and so could introduce selection bias that way. So the lost of follow-up is not the only source of bias.

The third point, which Dr. Bove touched on and I just want to highlight a little bit more, is this is a pretty typical occupational prospective study in that exposure assessment was made at the beginning and then long intervals passed before it was ever assessed again, and then people were followed up for outcomes. The reason I asked the question when I did the first day about the data collection is because while that's typical of some occupational studies, it's not typical of well-done cohort studies that at regular intervals reassess the exposure and the covariates. So this could potentially relate in either under-ascertainment or over-assessment of exposure, number one, and inadequate assessment of covariates. So that's why I think that's a limitation that could be added to the design features.

And then finally, the issue of the non-representativeness of the AHS cohort and the small numbers of women. So it's impossible to know if the most highly susceptible group, namely black males, have a different -- if there's an effect modification by race in terms of the effect, say, on prostate because they have a much higher risk of prostate cancer.

And in terms of women, we know, as it was said about prostate cancer yesterday that there are probably 10 different types, the same is true with breast cancer. So it was not possible to look at whether there were particular subtypes that -- in other words, whether the exposure might predispose to certain subtypes; you simply couldn't do that. So that's a limitation as well. That's all I have.

DR. DANIEL SCHLENK: Thank you. Dr. Young.

DR. HEATHER YOUNG: I mostly concur with what's already been said because we've consulted on the answer. I just want to make one other point, is that when you're doing a literature review, I also think it's important to note the gaps in the literature. And for the most part all of these studies are looking at occupationally exposed populations and there's a real dearth in the literature of drinking water exposure and residential exposures. And that is what the primary concern is when we're thinking about the population as a whole. So I think it's worth noting and acknowledging in the literature review that there's a real gap in the literature, looking at exposures other than occupational exposures.

DR. DANIEL SCHLENK: Thank you. Yes, Dr. Gold?

DR. ELLEN GOLD: Because Dr. Bove mentioned this table that I drafted yesterday, I'm going to as my colleagues to double-check it because it was done kind of quickly. But also, all I did was take the papers since 2003, and that were individually based. So we excluded the ecologic analysis because we felt they should be down weighted. If -- and we would kind of encourage EPA to consider this if they use such a table -- the idea is to use the weight of the evidence, then the papers that came before 2003 that are individually based, case control or cohort, should be included and I have not done that.

DR. DANIEL SCHLENK: Any other panel comments? Dr. Portier.

DR. KENNETH PORTIER: One of the reasons I kind of asked about thyroid cancer the other day was of all the cancers they looked at, that's the one that seems to be not going down and possibly going up. So, you know, a lot of the other cancers, it's kind of hard to get excited because they're all kind of declining and you would expect it would at least be staying level if atrazine and the long duration of cancer was occurring. But with thyroid cancer, we really do suspect something is going on. And with the odds ratio of 4 in two of those four categories, our flags went up and said this is one we should be looking at.

DR. ELLEN GOLD: Can I just point out that thyroid cancer is another good example of where the high-risk group is

really not looked at and yet you still see an elevation; it's much more frequent in women, and they are under-represented here.

DR. DANIEL SCHLENK: Any other comments from the panel? Question 10? Okay. Let me go to the EPA. Do you have any questions or clarification at all?

DR. CAROL CHRISTENSEN: No, not at this time. Thank you very much for the time and effort you put into that question.

DR. DANIEL SCHLENK: Okay. Would you want to read in Question 11 into the record?

DR. CAROL CHRISTENSEN: Yes. Question 11, subparts A and B: "Please comment on the extent to which the scientific information supports the integrative analysis contained in Section 3.3 of EPA's draft Issue Paper with respect to the similarities, differences of the experimental toxicology and epidemiology findings. Please comment on any significant uncertainties in the epidemiologic findings."

And Part B: "Please comment on whether the epidemiology literature published since the last SAP review, including the findings from the Agricultural Health Study is sufficient to justify changing the Agency's conclusion that atrazine is not likely to be carcinogenic to humans."

DR. DANIEL SCHLENK: Thank you. Our lead discussion on that is Dr. Gold.

DR. ELLEN GOLD: Thank you. So we've consulted on this as well and we felt that there was a lot of overlap between this question and the prior one, especially Part A of this one. So if it's okay, I mean, we do go cancer-by-cancer, but Dr. Bove did a really good job of that so I'm not going to repeat that for Part A if that's okay with you, unless you want something specific.

DR. CAROL CHRISTENSEN: Yeah. Certainly no need to repeat concerning the epidemiology finding. On Part A we were looking for sort of cancer-by-cancer, considering both the epi and the experimental database; is that correct?

DR. ELLEN GOLD: I don't have anything to add to what Dr. Bove said. I can ask my colleagues if they do.

DR. DANIEL SCHLENK: Well, is that consistent with Dr. Bove and Dr. Young? You have no further comments on A?

DR. HEATHER YOUNG: No. No further comments on A.

DR. DANIEL SCHLENK: Okay. Let me go back to the EPA just to make sure. Do you have some questions, clarification you'd like to ask about A?

DR. CAROL CHRISTENSEN: Right. Yeah. So in Part A, we're specifically thinking about that Section 3.3 again of the draft Issue Paper, pulling together what's known from the experimental toxicology, the animal bioassays, the in vitro studies for each specific cancer site. You know, for example, thyroid cancer that was just mentioned, you

know, the animal model is particularly sensitive to thyroid tumors, kind weighing that, providing some feedback and opinion, kind of weighing the fact that we have a very sensitive animal species in which we're not seeing.

In fact, we have a high quality epi study in which we are seeing something, but only one, you know, that kind of thing - pulling that kind of thing together. So any feedback that any member has on that kind of thing by anatomical cancer site, that was ideally what we were getting at, and if we were not clear, hopefully that clarifies a little bit.

DR. DANIEL SCHLENK: Yes, Dr. Young?

DR. HEATHER YOUNG: I think I'm correct in stating for all of us that the recommendations that Dr. Bove went through for each of those cancers individually, we really did it considering both pieces of the evidence. For Question 10, we weren't really just looking at the epi studies in a vacuum, but we looked at them looking at also, what were the experimental toxicology findings, mode of action, those types of things. And so the recommendations we made in Question 10 would really be consistent with what we would we say for Part 11(a). We didn't look at the epi studies in a vacuum when we were doing that. Am I speaking for everyone correctly?

DR. ELLEN GOLD: And also I will say that some of this will come up in response to Part B.

DR. DANIEL SCHLENK: Let's go ahead with B. Yes, Dr. Fowle.

DR. JACK FOWLE: I don't know. Perhaps not sufficient amount is known, but I was just wondering is enough known about the cancer of mechanisms leading to thyroid cancer in experimental animals versus humans to say that they're some different mechanisms that would make the humans more sensitive? Any information that could shed light on possibly evaluating that because rats tend to be exquisitely sensitive to thyroid cancer and we didn't see it at all. So we were really contemplating that fairly deeply.

DR. DANIEL SCHLENK: Yes, Dr. Mendez.

DR. ELIZABETH MENDEZ: Also, another little bit of information that I got this morning from our colleagues in ORD is that there are some data in frogs who are also very sensitive to thyroid hormone perturbation and that does not seem to be affected either. So we're struggling as to what appears to be a little bit of a disconnect there.

DR. DANIEL SCHLENK: So if I understand you correctly, you're asking the panel to comment on other modes of action related to thyroid hormone impacts? Is that kind of the question?

DR. ELIZABETH MENDEZ: I guess we're trying to understand what the etiology might be that would lead us to a different path in a human.

DR. DANIEL SCHLENK: Okay. Understand the susceptibility issues between species then for those particular effects. Does anyone have any input with regard to that? Yes, Dr. Meek?

DR. BETTE MEEK: I just wanted to make the point that when we first discussed the framework for integration of epidemiological and toxicology data at one of the earlier meeting that in fact it seemed relevant to walk through the weight of evidence for causality for the epidemiological data, initially, and then to integrate with the toxicological data, including weight of evidence for mode of action. So I think this would explicitly call out, addressing for each of those cancers, biological plausibility. So again, I'm not sure the extent that you did that, and certainly from the thyroid cancer perspective, it's a bit difficult to understand the human findings based on what we know about modes of induction of thyroid cancer in animals.

DR. FRANK BOVE: I think what we're saying is this, that first of all, let's evaluate the epi evidence separately. And when we do that, we see that for most of the sites there's no evidence. For some of the sites, there's some suggestive evidence in the epi literature, which need to be followed up. I don't think we're ready to talk about mechanism at all. When we say suggestive evidence, we mean that yeah, there are a few studies out there that seem to be positive, but, you know, we're not ready yet to merge the tox with the epi. If you do that, what you end up doing most of the time is ignoring the epi findings and letting the tox findings trump it. So what

I would prefer we do is to take the epi evidence seriously, for a change, and see where the gaps are, where the research needs to be done and do it. And then when we get to a point where we feel that the epi evidence is pretty good, then start merging it with the tox; otherwise, you'll ignore the epi evidence.

DR. ELLEN GOLD: I think also we have to be a little bit careful. I mean, I understand, looking at the weight of the evidence and looking at mechanisms and so forth, but in the early days of cancer epidemiology, we didn't know mechanisms. We still don't. For many, I would contend, maybe for thyroid cancer we don't. We know relatively few risk factors, probably three that I can think of. And so sometimes the epidemiology will spur people on to do the mechanistic studies. And so I think in the case of -- well, let me back up.

For many of the cancers, the approach was well there are some neuroendocrine mechanism and that justified looking a prostate over and breast, for example, maybe thyroid, I don't know. You know, if it's having an effect on pituitary, maybe. But I think for some of these others -- and I think we do have it in our comments -- that we perhaps, don't know enough or the animal literature might inconsistent, but I think as epidemiologists, we worry about extrapolating farm animals in the fact that the mechanisms may not be the same. So I would put some cautionary notes like that. And I think we did try and integrate in what we wrote, sort of consideration of that.

DR. KATHERINE ROBY: I just want to comment with respect -- I'm not an expert on thyroid cancer, but I know ovarian cancer and I think it was well added into the position paper that actually, there's a real dichotomy there because what we do know about ovarian cancer is that an inhibition of LH should actually be protective. So there is really a separation between what I think, based on the epidemiology you say is suggestive, but the evidence that we know, mechanistically, would indicate really the opposite should be the case, which may point to some other mechanism if the suggested comes to be correct. So it could be pointing to some different mechanism.

The other point that I wanted to make is that, again, just specifically with regard to ovarian cancer, there really are no animal model systems for the initiation or causal factors of ovarian cancer. So whatever is in the literature that might indicate there's a model looking at this compound or this toxicant causes ovarian cancer, there are no model systems to address that issue.

DR. ELLEN GOLD: I would just point out that it was also mentioned in the issue paper that that mechanism that was originally based on the animal studies for breast cancer doesn't apply in humans. And so I think we have to be really careful on two scores; one is can you extrapolate from one species to the other in terms of mechanisms? And secondly, the fact that you don't have an animal model doesn't mean that you shouldn't pay attention to the epidemiology.

DR. DANIEL SCHLENK: Any other comments? So back to Dr. Christensen and Dr. Mendez, is this something you find useful, I guess, that you need?

DR. CAROL CHRISTENSEN: Yeah. Maybe we can hear the responses to B and we'll kind of try to sum up what we've heard at the end.

DR. DANIEL SCHLENK: Yeah. I think that'll probably be a good strategy. So let's go ahead and move onto B. Dr. Gold?

DR. ELLEN GOLD: Okay. By the way, in the introduction to Question 11, it seemed like you were asking whether there was a basis, sort of cancer-by-cancer, to change your opinion from the 2003 SAP decision. So that's sort of the orientation of the comments for B. And I have sort a bullet point for each sort of category, if you will, and mostly grouped by cancers.

So the epidemiologic evidence compiled since the last SAP review in 2003 regarding the carcinogenicity of atrazine does not justify changing the Agency's conclusions regarding prostate cancer, breast cancer, adult gliomas, oral, esophageal, pancreatic, melanoma, renal, laryngeal, lung, bladder, colorectal and liver cancer, or leukemia -- with the exception of the hairy cell leukemia perhaps -- chronic lymphocytic leukemia or multiple myeloma.

The epidemiologic evidence regarding a potential association of atrazine exposure with ovarian cancer is suggestive of an association, but still inconclusive and requires more rigorous investigation with larger sample

sizes, which is difficult for this rare cancer that is likely to have a long latent period, and which also greatly complicates the exposure assessment.

For thyroid cancer we only have one study, the recent AHS cohort analysis, but it suggests a strong relationship -- fourfold increased odds ratio -- that is unlikely to be due to residual confounding. We've already mentioned some of the concerns about how the cut-offs were made for exposure. So those might be reconsidered, but that might explain why there's a non-significant exposure-response relationship, you know, like cutting it off at the median might have worked better.

So this is very suggestive finding from a single study and is not sufficient to be certain of a causal relation between atrazine and thyroid cancer and thus requires replication in a larger study and more experimental investigation with regard to potential biologic mechanisms.

The epidemiologic findings regarding an association of NHL and hairy cell leukemia with triazine use after adjusting for other pesticide exposures, although having small numbers of exposed cases in most of these studies, suggest about a one and a half to two-fold increase. Some of these estimates were statistically significant, and some findings had non-significant exposure-to-response relationships, although the numbers of cases for each of these malignancies were fairly small.

However, these findings were not duplicated in the most recent cohort analyses from the AHS, which had twice as many cancer cases. Thus, while early studies suggested possible relationships of atrazine use with NHL and HCL, the more recent better designed and controlled studies with larger sample sizes did not replicate these findings, indicating, as mentioned in the Issue Paper, that sufficient evidence for associations of atrazine with NHL and HCL is lacking in humans or animal experimental studies. Although, the limitations of the AHS that we noted in response to Question 10 should not be ignored in considering these results.

And then studies of pediatric cancers -- by the way, I would just agree with the comment about not lumping all cancers together, and so with the pediatric ones, this is a little bit problematic in some that have teased out acute lymphocytic leukemia and found an increased risk, although a monotonic exposure-response relationship was not observed. This is also an extremely rare cancer, but the most frequent one in children.

So the Issue Paper correctly concludes that the evidence is currently insufficient to determine if atrazine exposure increases the risk of pediatric cancers, particularly leukemia. That is all I have.

DR. DANIEL SCHLENK: Dr. Bove, anything to add?

DR. FRANK BOVE: No. I have nothing to add.

DR. DANIEL SCHLENK: Dr. Young, anything to add?

DR. HEATHER YOUNG: No. Nothing to add.

DR. DANIEL SCHLENK: Okay. So we'll go back to the -- oh, let me open it up. Any other comments from further panel members? Yes, Dr. Akana?

DR. SUSAN AKANA: Just a mild observation. I might've missed it, but we know in the rodent data that the atrazines can activate the adrenal's downstream in certain situations. So notice here that adrenal cancers are not on the list.

DR. DANIEL SCHLENK: Okay. Any other comments from the panel? Okay. Dr. Christensen, any questions or clarification for you?

DR. CAROL CHRISTENSEN: Not specifically. Again, thank you very much for the time and attention to addressing this part of the question. In an attempt to sort of recap, maybe let me do so -- and you can correct me if I'm in error -- but what I heard you say, you know, in your evaluation of the cancer of the epidemiological evidence and the cancer-specific sites, you sort of automatically and inherently considered both the observational and experimental data within those evaluations and your comments regarding insufficient evidence or suggestive evidence or considering that information implicitly. I also heard a caution concerning moving, perhaps, too quickly to integrate the tox and the epi when there are some suggestive findings out there with some limitations and uncertainties as to how to interpret how far you can take that inference within the observational data at this

time. But still, your conclusions regarding specific cancer sites, again, inadequate or sufficient, in your opinion is informed by both toxicology and epidemiology.

DR. DANIEL SCHLENK: Anyone want to take that one on? Dr. Gold?

DR. ELLEN GOLD: I think that's a fair statement because we were also impressed by the fact that the Bradford Hill criteria were used, and one of those is biologic plausibility. And that sort of implies that you consider information about mechanism or evidence from toxicologic experiments to see if it's consistent.

So I think that it's fair to say that the emphasis of our comments was on the epidemiologic studies, but I think it was also in consideration of the animal experiments and toxicologic data as well, when it existed. You know, I think it's really important to remember that a lot of public health policy is based on sort of imperfect science and sometimes you don't have the animal experiments, but you still can take preventive action and influence the incidence of disease. I think smoking and lung cancer is a great example. We didn't understand the path of physiology, but it didn't prevent public health from acting.

DR. HEATHER YOUNG: I just want to emphasize again that just because we aren't sure about the biological plausibility, doesn't mean that it's implausible. And so because you don't know about the mode of action, it doesn't mean that there is no mode of action. So I think that's what we're

trying to say is that although it may not be known what the mode of action for some of these effects that we're seeing in humans, it doesn't mean that one doesn't exist or that may not appear in experimental evidence with animals at some point, or maybe it's not going to show up in experimental animals because we're not using the right models. And so I think you need to not throw away epidemiologic evidence that has really strong risks appearing just because we're unsure about mode of action.

DR. DANIEL SCHLENK: Okay. So do you guys have any other further questions? Oh, Dr. Bradbury, do you want to make a statement?

DR. STEPHEN BRADBURY: Question 10 and 11 really get to the crux of our February 2010 SAP where we're trying to bring together this framework concept of how to integrate experimental toxicology information with epidemiology information. And our goal is to more fully and completely and hopefully adequately bring epidemiological information into our risk assessment and evaluations. So to the extent you were thinking or the panel's thinking were not wanting to use epidemiology data on it, I want to make sure that's very clear, quite the opposite.

What we're trying to work through is when you get to a very specific case, like let's say the thyroid information, getting back to what Bette Meek was saying is with a very specific set of information before us and trying to exercise that framework is thinking through. So how do we try to reconcile and understand the uncertainties and try to articulate even qualitatively?

Here's what we know about these experimental models. How they react to what we do know in say, the rat or the mouse in terms of how cancers of the thyroid play out -- blah, blah, blah -- and here's this epidemiology information which is suggestive.

And given that at a certain point in time you have to make a decision about what's the likelihood of risk associated with exposures to different amounts of atrazine. How do you try to pull this together, qualitatively, you know in many cases? And so that's where you are hearing some of us trying to probe a little bit, not so much in the generic, but when you have a very specific example before us. For example, the thyroid cancer, any advice you have on sort of how to integrate the information. It's advice, you know, how do you pull this information together and try to reconcile some things.

DR. HEATHER YOUNG: So as a public health professional, I would say you proceed with caution because you don't have a lot of evidence, until you do have more evidence, to make a decision either way. I think that's why we are sort of in the suggestive camps. We're not saying that there really is an association. We have a population-based study that's highly suggestive. We're not sure about mode of action. So we're not saying there's a causal association, but we're also not prepared to say that it's unlikely. And so I think what we're saying is proceed with caution until we have more evidence.

DR. ELLEN GOLD: I would just reiterate once again that biologic plausibility is only one component of the Bradford Hill. And so I would agree with what Dr. Young said, but I think, you know, what you're doing is building a case and all the pieces may not fit perfectly, but if the case is strong for the other components, the fact that you don't know the mechanism, or that the mechanism appears to be different in a different species, would add a note of caution, but it doesn't down weight the rest of the strength of the evidence.

DR. STEPHEN BRADBURY: The word "caution" is an interesting word. In different context it means different things. So could I ask if you could discuss how to articulate this kind of information in the context of uncertainty, as opposed to caution? If I can indulge the panel to think about ways to talk about uncertainties as you try to bring different information together.

DR. DANIEL SCHLENK: Anybody want to take that on? Bette?

DR. BETTE MEEK: I'm going to push the agenda a bit more on this to pose the question, given the uncertainty that we have about the observed association, is there any way that we can use the information, quantitatively, in any context, to give us a comfort level, perhaps, or not, about the focus of any kind of dose response relationship modeling that we do? Because that would at least enable us to take the information into account. This really goes back to an issue that came up, I think, early on when we were talking about a framework to integrate, and in terms of the epidemiological data, was really to do a

problem formulation to consider where will the epi data play out in this risk assessment and how can we most meaningfully us it. Because I'm sensitive to the issue faced by the Agency to say well, we should follow this up, but on the other hand, they have to make decisions now. So how could we use that information, even in some kind of semi-quantitative sense to give us at least a comfort level or not? I think that's kind of the issue that I see.

DR. ELLEN GOLD: I think it's hard to do this generically. You kind of need to do it cancer-by-cancer as we tried to do it. So let's take the example of thyroid cancer. They had four categories of exposure, and in a couple of them the risk ratio is four-fold or more. But when I looked at the numbers, they had, I think in the four categories, 3, 12, 3, and 11. Something like that. I think we would agree that there's such variability around those estimates in the categories where you only have three people that trying to figure out -- you could have a lot of misclassification, a lot of variability. And the fact that you don't have a monotonic dose trend doesn't really say much. So I made the comment if it were my data, I think I would've looked at the distribution and maybe tried to make sure that I have enough numbers in maybe two categories, like above and below the median, for example, which is not as satisfying as having four categories, but if reflects the reality of the situation.

So that said, I mean, as the Agency has pointed out, you have the temporality of the association. You have a four-fold association -- even if you divided it on a

median, my guess is that would go down a little bit in each category, but let's say it's three-fold, which I think it would be because the two larger categories were the ones where it was more closer to four so that's going to heavily weight your estimate. So it might come out 3, 3.5. That's a risk estimate that's unlikely to go away with adequate control for confounding and stuff like that.

So my point being, temporality, strength of the association - if you looked at it like that, you might actually have a dose response. We know very little about the etiology of thyroid cancer. And we're sort of missing, maybe biologic plausibility. We also don't have other studies, so we don't have consistency. That's why we use language like suggestive, as opposed to unlikely. So when talk about caution, I think it's reflecting those kinds of words, "suggestive" rather than "unlikely."

DR. FRANK BOVE: Yes. And also "inadequate" as opposed to "not likely." Not likely is a very statement. Basically, you would have to ignore the epi evidence for many of these cancers to say not likely. So that's one of the things we're trying to caution you against doing.

As for risk assessment, it would be nice if there was animal data and human data. For example, for tricoethylene, there is some animal data for kidney cancer and there is some human data. You can check and see if you extrapolate from epi studies and animal studies, you can get some kind of bounding that is great. That's not what we have here.

On the other hand, suppose we have a situation where there's a birth defect cluster that's happening in a skyrocketing number of cases and there's no animal model, or the animal model is negative like flutamide for example, what do you do then? You obviously work on the epi data. So I'm just saying, you know, for risk assessment purposes, you have information in front of you. You have some evidence from tox; you have some evidence from epi. You may have less evidence in one or the other. You're just going to have to make some judgments. Okay. If there is evidence in both, that's makes it easy, but in most of these cases, that's not going to happen, especially in this case I don't see it happening.

So what do you do? The tendency has been -- and as I said, I've been on these panels since 2000 -- the tendency has been to really give sure shift to the epidemiological evidence. And this is a plea to not do that, even if it doesn't jive with the tox information because the tox information may be wrong. The animal models may be wrong. We may learn something down the pike. The epi information could also be wrong that's why we're asking for follow-up work, especially on thyroid because there's only one study. But even on non-Hodgkin's lymphoma where there are several studies and you can pool those studies, and that's been done, and get some kind of overall odds ratio somewhere in the range of 1.5 and 2 and you can use that if you want, but there's no animal information to bound that with. So what do you do then?

DR. DANIEL SCHLENK: Any comments that we have? Yeah, Dr. Meek?

DR. BETTE MEEK: Just a point of clarification, I wasn't think that we necessarily have to have the animal evidence to bound, but rather whatever approach is taken in the ultimate dose response characterization that we could say something, semi-quantitatively, at least, about the risk that we've seen or we suspect in the epi studies. So it would be bounding it for another end point.

DR. DANIEL GRIFFITH: You might think of it in terms of given the small numbers, your probability is somewhere between zero and one, and it might've only shrunk to somewhere between .1 and .9 because the numbers are just so small.

DR. DANIEL SCHLENK: Any other comments. Again, let me remind the panel, please send your comments to Dr. Gold as she has to basically put this together in a manner that reflects the panel's input there. So just be sure to send your comments there.

Okay. Are we ready to move on? All right. Question 12. I think you're going to switch out to readers here; is that right?

DR. ELIZABETH MENDEZ: I'm actually going to be reading. Dr. Rodriguez is going to come up to address any questions the panel may have. So good morning; Elizabeth Mendez, EPA. So we're shifting again and now we're going from epidemiology data to the pharmacokinetic information that we've been evaluating and considering in this

process. And I want to preface this by saying that one of the questions that you will see within Question 12 is about the PBPK model that we have no fully reviewed at this time. We felt that since we had you all in the room, it would be wise of us to avail ourselves of your expertise as we move forward towards the reg review process in 2013. So with that in mind, I'm just going to go forward and read the questions. Do you want me to read all four parts or --

DR. DANIEL SCHLENK: I think just A through D would probably be appropriate, yeah.

DR. ELIZABETH MENDEZ: Question 12, subpart A: "Please comment on the strengths and limitations associated with a simplified pharmacokinetic modeling approach for human extrapolation." And that is in regard to the one we've been proposing.

Subpart B: "Compare and contrast the strengths and weaknesses of using total radioactivity for pharmacokinetic analyses, as presented in Agency's Issue Paper, as opposed to using available pharmacokinetic data for the parent and the chloro-s-triazine metabolites that have similar toxicological properties to the parent."

Subsection C: "As pointed out in the Agency Issue Paper, we are still reviewing a PBPK model submitted by Syngenta. As we complete our review of the Syngenta model, please comment on key aspects that EPA should be considering, concerning a PBPK model, including model credibility and a structure parameter values and

documentation, model reliability. How well does the model simulate the dose metric relevant to the mode of action, and model applicability? Does the model have essential features for intended application?"

Finally, Subpart D: "Please comment on the extent to which the one-compartment linear model of total plasma radioactivity derived from ^{14}C labeled atrazine, may account for interspecies differences in pharmacokinetics."

DR. DANIEL SCHLENK: Okay. Our lead discussion on that is Dr. Greenwood. Let's go through them, A, and then break and then B and then break.

DR. RICHARD GREENWOOD: Okay. There is some overlap in some of these, but we'll be able to refer back to where we've covered it in earlier sections. We spent a little time discussing between ourselves and the people who were in discussions on this, some of the data and approach. What I'm going to say is sort of a compilation of inputs from other people, but then will also have some other comments to make. So I'll make a start with an overview.

I think the approach taken by the Agency assumes the area under the plasma concentration curve reflects the opportunity for exposure of the site of action to atrazine. That's one of the assumptions. And it also assumes that the toxicities of the metabolites are very similar. And on the evidence presented by the Agency, it's a reasonable assumption, particularly when you

consider that one metabolite, the deoxycholated atrazine dominates the profile.

However, when we look at some of the data presented by Syngenta, which compare dosing by oral gavage and the dietary route, this suggests that actually, it might be worth revisiting some of the assumptions that underlie the approach using total radiolabel, and I'll explain why. If you're wanting to use area under the curve as an appropriate measure of exposure of whatever the site of action or sites of actions are involved in the suppression of the LH surge.

Now, the Syngenta data show that when atrazine was administered by oral gavage, the area under the curve is larger than that found by dietary dosing, particularly for the parent compound and the mono-deoxycholated metabolite. But the major difference is, really, were a much smoother plasma concentration curve found with only a few fluctuations when it's given with the feed. When you look at what happens with gavage, you get these huge transient peaks. And this is something to bear in mind when you go back to looking at the radiolabel data.

Now, if we look at the relatively modest differences in the overall area under the curve for DACT, then it's surprising that the suppression of the LH surge was produced by gavage administration, but not by dietary administration. So there's still a reasonable area under the concentration curve when it’s dietary.

Now, several explanations offer themselves -- I've put a little thought into this with colleagues that might explain this -- it could be that for atrazine, total area under the curve might not be the appropriate measure for exposure. It could be -- there's only one explanation, there's no evidence -- it may be the area under the curve above a critical threshold concentration. So this is just a hypothesis.

Another hypothesis that might explain this is that a sustained constant low concentration may not be sufficient to cause the effect. So the gentle pressure, it may require pulses, intermittent pulses of high concentrations that you get with oral gavage. Again, no evidence at the moment, it is just potential correlations.

The other difference between dietary and gavage is that the dietary route takes about 24 hours to reach the high plateau of concentration but then is maintained. And because feeding goes on longer -- after the oral gavage finishes the last dose, they're still feeding -- then, of course, the peak is maintained for longer in dietary. So a lot depends, I think, on where that might fall within that critical four-day period because the effect on the LH surge.

It does open up some questions, and I think because of the approach taken by the Agency sort of depends on these assumptions being made, these really need to be checked out and looked at very careful. Given the importance of identifying an appropriate dose metric, I think it's

important that some effort is put in to just looking at that. I think a few suggestions for the Agency of how this might be tackled - you don't have to be hung up on giving this stuff by gavage or in the diet, there are lots of methods available now for giving, achieving constant plasma levels by subdermal implantation of slow-release formulations. And you can get very high pulses in a very short time just by intravenous injection. It's an old trick, but it's been used lots of times.

So some of these things could be tested with those sorts of experiments. But in the absence of information to where the interpretation of the Syngenta data of this link between pharmacokinetic behavior and pharmacodynamic activity -- this is for the LH surge suppression -- which is just one of the secondary lesions, resulting from some unidentified primary lesions. This is where we're stuck all the time. We don't know what the primary lesion is. But it's reasonable to examine all the available pharmacokinetic data in the way that the Agency has proposed. You've proposed to look at all the pharmacokinetic data, and that's the only way you can go forward. It's sensible.

The area under the curve is the dose metric that represents the exposure of all tissue, target and non-target, to the toxicant. You can't get away from that, so that area under the curve approach does give you a measure of the potential exposure of all tissues, not just target tissues, non-target tissues. Everything that gets a blood supply gets exposed. So it really is a sensible way forward, from the pharmacokinetic point of

view, to consider area under the curve as a reasonable dose metric. The problems come when you then try to relate to the pharmacodynamic activity. But I think the advantage of the use of the total radiolabel is that you can get quite a lot of reassurance from a mass balance check. It gives you some confidence that all the administered dose is accounted for. And I'll refer back to this when we look later at the physiologically based pharmacokinetic data because that is something that the registrant is going to look at, I think in trying to get a mass balance. It needs to be done. It's easy with radiolabel.

It's still unique to apply some caution when you're using the old ^{14}C label atrazine studies because I've now got access to these and I've looked at them. It's quite reasonable because it's the usual thing to assume first order kinetics, which is what you've done for the overall elimination process. But there's good evidence when you look at the data you've presented, the Agency presented and Syngenta presented, that that's what it is.

But the other thing that seems to hit me when I look at all of this is that there's evidence that there are two first order elimination processes going on simultaneously. And that in fact, if you had enough data, you'd get a pretty good fit to a double exponential model. I'm pretty certain if you fitted a double exponential model you'd get it because there seem to be two fractions of material in the plasma that have been operated on by a fast-rate constant, one by a slow-rate constant. The first one we will probably be

representing, elimination of that, in fact, the free material. And the slow elimination compartment, it would be probably that bound to proteins where the turnover is very much -- the proteins are slower.

So there is some evidence that that's from the non-human primate data for double exponential nature of the elimination process, but there's also evidence in the rodent radiolabel data which you use to estimate the fraction elimination rate constant. Because if you look at those lower concentrations linear time plots and you look at the pattern of residuals from the fitted straight lines, given that you've only got four points for these things, it's always difficult. You've got three degrees of freedom, so it's tough to try and get anything, but if you look at the pattern of residuals, it's actually consistent: high, low - low, high. And it's consistent across the different doses, across the different studies. And in fact, I've looked at the mouse study of Ross and co-workers, 2009, and you get a similar picture there. So there is quite a lot of evidence that there are two compartments.

Well, fortunately, I think the fraction which is operated on by the slow process is probably very small, negligible, compared with the fast process, the free process. So although there is some bias introduced into the estimates for the first order rate constant, it's not going to be -- when you look at the variability in the whole system, it's not going to be really too important, I don't think. Though you went on to look at quite a few studies and you get very similar values for the first

order elimination rate constant across the study, which is, again, given the small number of points in each, it's heartening. I always get a bit more confident when I see it's repeatable rather than just statistically significant.

So I think the approach is sound. And the only deviations that I find from this consistency, which you pointed out, are the high elimination constants observed for the 50 and 100 milligrams per kilogram doses in the feed data. If you remember, there were factor of three probably out -- well, in the grand scheme of things, that's again, not exactly a problem. But you do need to be careful about the interpretation of the radiolabel studies because all of them use a similar experimental design. That is, they use either single-dose or equally spaced constant doses. And they take samples infrequently, usually every 24 hours.

So this gives a really lousy definition of the pharmacokinetic profile. And you can't get anything else but a smooth plateau out of it because when you join two lines together it's a straight line. And if they're taken at the same point each day, they're going to be roughly the same height. So you end up with what appears to be an nice plateau, but actually, if you look at the Syngenta data or where some of the others were, they've got a better definition, you see these huge spike superimposed on the top of it. If it's by gavage and you still see wobble about it in the dietary dosing.
So again, that needs to be bore in mind in an interpretation. But despite all of this, the studies can

still be useful because the area under this apparent plateau is still consistent proportion of the total area under the curve. So if you're trying to use that and correlate it with pharmacodynamic activity, you're still in with a fighting chance. But the other thing that you need to be very careful about is this concept of the pseudo steady state being achieved after four days dosing. And there's no evidence of this in the Syngenta data or any of the others where they look at the individual components rather than the total radiolabel.

In fact, if you look those, you get a pretty steady state in DACT, even with gavage, after Day 1. So after 24 hours, it's up there and it's pretty well maintained. So there is very limited evidence for this. And the feed study is very difficult to explain. Now, there's one possible explanation -- well, there are several explanations, again, and I give them for what their worth. It's possible that some bindings occur in over four days and once that's all saturated, then you do get this pseudo steady state. I don't see what some of those binding sites would be, but you may have other ideas. But if the pseudo steady state did involve binding, then the area under the curve would not be the freely available, and that is the pharmacodynamically relevant fraction, but it would be material which is bound, which is not available for interaction with the site of action, wherever it is. So again, you've got to be careful about how you interpret it.

But if the rise in plasma concentration is -- if we say that the plasma is in equilibrium with the tissues, then

the implication is that there is a compartment within one or more tissues which needs to be saturated before you can get the final rising plasma concentration. And in that tissue or tissues -- it could be any tissue -- it could be that there is a slower distribution process taking place over four days so that you get this rise that feed observed in the total radiolabel in the plasma. And it would be interesting to see whether there was also a slow elimination process from this compartment.

Well, if you look at some of the various studies, if you look at the poll dataset, there is some indication that following the single-dose by oral gavage, there is a very slow elimination for liver and kidney, but an even slower elimination from red blood cells, and we know that that's due to binding, covalent binding, to the red blood cells, but also in muscle. The rate of elimination from muscle, if you look at the poll data, is very similar to that from the erythrocytes. That's following the cessation of dosing and they did seven-day daily dosing in that study.

So I think that the whole topic has become rather confused in people's mind because the time to the pseudo steady state happens to be four days, which just by coincidence happens to be the critical exposure of which you got to hit in the rat estrous cycle. But the two are independent of each other; one is pharmacokinetic and the other is pharmacodynamic. So it wouldn’t matter if you had a study state when it is not at the critical period, it has no effect whatsoever.

Actually, if you say that you need a steady state in order to suppress the LH surge, then logically, that isn't achieved until after four days dosing. So you would never suppress the LH surge if you started dosing on the first day because it takes four days to achieve steady state. You'd always have to start dosing, if you apply that logic, four days before the start of the critical period in the estrous cycle. Because it's only then that you'd get this pseudo steady state. So I think it's actually been a bit of a red herring, this idea that you need a pseudo steady state and it's come out of the fact that there are limitations in some of the radiolabeled studies. I'm afraid I've spilled over into some of the others, but I think this is probably the place where I felt my comments would best fit.

So really, as far as I can see, there really aren't any grounds -- or there are grounds, certainly, for examining, reexamining this idea of what's required, in term of the nature of the exposure and the level of exposure to suppress the LH surge over that critical period. Whether you need spikes, whether a constant pressure ain't going to do, or whether you just need to get the concentration high enough that some critical period over those four days. I'll leave it there and hand over to colleagues.

DR. DANIEL SCHLENK: Okay. Just so I'm clear, Dr. Greenwood, it sounds like you kind of hit all A through D in some of your comments.

DR. RICHARD GREENWOOD: I've got some other comments which are specific to the others, but I will relay it back to this. It has been sort of a long explanation, but I thought it was the easiest way of doing it.

DR. DANIEL SCHLENK: Sure. No worries. I was just wondering how it was going to split up with everybody else. Okay. Thank you. Dr. Hayton?

DR. WILLIAM HAYTON: Well, I agree with my colleague's comments and I would add a few thoughts, in terms of Part A question to comment on strengths and limitations of the simplified pharmacokinetic modeling, using the one compartment model. I think one strength we could mention is that it is simple, in the sense that it has a minimal numbers of parameters to estimate. And because of that you need a fairly limited number of data points. So a lot of the radioactivity concentration time profiles really wouldn't support more complicated modeling. So I found that a strength that could be mentioned.

I also found another strength is that the one compartment approach does have utility in that the basic idea here is to estimate exposure to total triazines and because the data seemed to conform to the model that the purpose for which we want to use the model is satisfied. And then finally, we have data available from three species: from rat, monkey and human. I thought that was a strength.

In terms of limitations, you know, I think the point that Dr. Greenwood brought up that total radioactivity seems to include some fraction that is albumin adduct or plasma

protein adduct, which doesn't have toxicologic activity. So it would be nice to be able to get rid of that. From the data that I saw, particularly the CODAR 2011, study. It seemed that if you look at atrazine and its three chloro-s-triazine metabolites, the DEA, DIA, and DACT, the total radioactivity, if we could rid of the bound radioactivity, covalently bound, that that would track the sum of those four metabolites fairly closely. I think that's one weakness or limitation of using just total radioactivity and because it's data from the literature -- old data -- there's probably no way to subtract that out.

Another limitation, I thought of the one compartment system, its simplicity is a virtue, but also, some of the finer points of the pharmacokinetics tend to be obscured. So we don't know much, using that model, about saturable binding, transport of metabolism that could give some kind of a non-linear relationship between the administered dose and the exposure of the site of action.

I found some comfort from the fact the half-life of total radioactivity is dose independent over a broad range of doses. I thought that give some comfort that there are non-linearities that could confound the analysis. Thanks.

DR. DANIEL SCHLENK: Okay. Dr. Meek?

DR. BETTE MEEK: Yeah. I have very little of substance to add. I'm really encouraged that the Agency is moving along to estimate the internal dose metric. And I think

this is as step along the way. I think that you've also indicated that the ideal approach would be a physiologically based pharmacokinetic model for all of the reasons kind of mentioned by previous commenters, in terms on the limitations of the approach.

DR. DANIEL SCHLENK: Okay. Any other panel member input on the PK stuff here? Okay. Are you guys going to address B separately then? Okay. We have a question from Dr. Rodriguez - question/clarification.

DR. CHESTER RODRIGUEZ: Just a comment, actually. One of the issues that we found, especially with the human study that were presented is that it was a significant mass balance issue. When atrazine DEA, DIA, and DACT were monitored, those four species only accounted for 14.5 percent of the dose. So 85 percent of the dose, they don't know where it went. So in terms of us using caution, we feel at this point that radiolabel studies may actually safeguard against that. But until we have a better understanding of mass balance, I think this represents a reasonable approach at this time.

DR. DANIEL SCHLENK: So I'm assuming that's a question that you're asking. Is that what you're asking the panel to correspond on that?

DR. CHESTER RODRIGUEZ: No. It's just a comment.

DR. DANIEL SCHLENK: Oh, okay. I think we got that. Do you guys have anything to say about that?

DR. RICHARD GREENWOOD: I agree that you need to have mass balance, and it's one of the things I think that is going to be addressed if we look at the contribution by Syngenta in their paper they presented. It's one of the things they need to look at. It's true, it does need looking at, but the problem is it's not just mass balance with the total radiolabel. You know where it is, but you don't know how it's divided within compartments within a tissue. You just combust it and you get the total.

You often need to go on for a long time, the study, to make sure you can see what the real turnover rate of it is - get enough points to be able to do the proper analysis of the elimination to see whether it's single, double, or triple exponential, for instance, whether it's saturable and so on.

DR. DANIEL SCHLENK: Sure. The comment -- it's just my own personal comment; it seems you would also need to know what those glutathione adducts are as well because it seems that a fairly large chunk of the metabolism seems to be glutathione conjugated. So consequently, maybe that 76 percent that's there, a large amount of that could be some of these unknown conjugates that are present. So that needs to be characterized as well because that would not necessarily be toxic, per se, it would be a nontoxic metabolite at that point.

Any other comments on A? Okay. Let's go ahead and go through B and then we'll take a break after B. Dr. Greenwood, again, do you want to lead off?

DR. RICHARD GREENWOOD: Yeah. Thank you. Well, I think the question, it's the sort of thing that you might set for the undergraduates actually, compare and contrast is the wording. But it's a problem. This is not the straightforward business, choosing what sort of model to use because all of these methods have their own strengths and weaknesses, and I'll try to look at some of these in light of what we've seen with atrazine.

I think all people doing studies in pharmacokinetics have to ask themselves to start with, what do I want to use the data for because there are two extreme approaches; one you use a single compartment model, use total radiolabel, and it's got advantages, it's very simple to carry out. You've got a very, very good sensitivity and the modeling is easy. On the other extreme, you've got a big physiologically-based pharmacokinetic study, where you have lots of compartments, and when you do the modeling, lots of boxes connected by differential equations and you've got to parameterize all of those, you can end up with more than 40 parameters.

If you look at the Syngenta model, I think there are 40-odd parameters that I looked at. And it’s a lot of hard work to get sufficient data to estimate all of those, some available in the literature. So often people opt for a sort of middle path, which is a compromise between the two, in terms of the amount of work and what you get out of it. Because what you get out of a radiolabeled study is difficult to interpret. And the big advantage of the physiologically based pharmacokinetic models is that they are very easy to interpret, in terms of the

physiology and the lesions that happen, a disruption of physiology when you put in toxicant. And you can actually look to see whether the toxicant is affecting the pharmacokinetics, which can happen. In some of the studies I've done, it certainly does happen. So you can either go for the complex and the arguably more realistic physiologically based model, but you need to get the information to parameterize them.

These days, life has been made a little bit easier because you can use mass spec, and so the limits of quantification really have gone down. The problem is, in order to get it to the mass spec, the LC mass spec, you've actually got to do sample preparation, which all needs validation, involves dissection of individual body component, quantitative extraction, preliminary clean up, and often -- well, usually you have to, in order to get reliable results, you need labeled unalikes to correct for matrix effects in the mass spec analysis. And it is very difficult, as been pointed out, to achieve a mass balance. Even though mass spectrometry detectors can get down to lower levels of quantification, they still cannot achieve the lower levels of quantification that you can get with radiolabeled compounds with combustion and scintillation counting.

I guess the important thing is, though, that what the methods used for extraction of tissues do is make sure that you are actually extracting the free. Normally you do not extract if it's bound -- if it's covalently bound, you don't extract it -- but you extract the free

material, which is the material that's physiologically, toxicologically relevant.

Assuming a sort of mammillary model with the circularly system providing rapid mass transport around to every tissue. Then when steady state is achieved the levels in all the tissue will change at matching -- not necessarily at the same rate -- but matching rates and you get a sort of steady state achieved. That's with one dose and then elimination. The area under the curve does represent the overall opportunity for exposure of the site of action and it doesn't matter whether it's one or more tissues. It does not matter where it is located.

So these physiologically based pharmacokinetic models do provide information which is readily interpretable and it supports interpretation of modes of action. It doesn't assume that the parent compound and metabolites are equi-toxent, you don't have to make that decision.

But it also can help to identify where you get deviations from the expected behavior -- which do happen -- where you change the physiology by the poison in the animal. For instance, if you modify cardiac output or you modify hepatic function. Then you are going to change distribution and you are going to change elimination rates. That will happen in time, you get time-dependent parameters. I'm sure it makes life very interesting for the modelers.

Now, simple models which are based on far fewer samples to be analyzed, a lot less preparation required and

total radiolabel, it's easy to measure and it's sensitive and it could be automated so you can get through a lot of samples. As I said before, the mass balance is readily checked and that is important. However, I think as Dr. Hayton said, the problem is if you've got bound material mixed in that total fraction, then that's not available for distribution to the site of action.

So the modeling is simpler, but the interpretation, in terms of toxicology and mode of action is far more difficult and it could be misleading, particularly if there were big differences between the toxicities of parent compound and metabolites. If there is significant binding, then depending on the method of preparation, of course, the area under the curve might not provide a good measure of exposure of the site of action.

I mean, one way of getting around this is if you think that there is significant binding, you could always do a radiolabel study, ultra filtrate the plasma, count the filter and count the filtrate and you'll then see what proportion of it is actually bound and you can get a handle on it, but it is one more step. So without doing that, you can actually overestimate, if you like, the exposure of the site of action. So I'll leave it there.

DR. DANIEL SCHLENK: Dr. Hayton?

DR. WILLIAM HAYTON: Yeah. Let me just quickly summarize what I thought strengths of using radioactivity were, compared with specific assays for the chloro-s-triazines. I think with total radioactivity, you get some comfort that you

haven't missed any toxicologically active metabolites and you get good sensitivity. Of course that depends on specific activity, but that can usually be made very high. I thought that's the strength of the total radioactivity approach.

A weakness is that the label is distributed among multiple chemical species and each one of those species has its own pharmacokinetic behavior, so total radioactivity tends to hide much of the underlying kinetic behaviors. From what we know of the chloro-s-triazines, they seem to be equally equipotent, toxicologically. So to the extent that they represent total radioactivity, you know, that's going to work out okay. I guess the uncertainty that's already been mentioned several times is that there seems to be quite a bit of radioactivity in plasma that may not be the chloro-s-triazines and some of it has quite a bit longer half-life. So what's going on there introduces some uncertainly into the overall consideration?

DR. DANIEL SCHLENK: Okay. Dr. Meek?

DR. BETTE MEEK: Yeah. I have nothing much to add.

DR. DANIEL SCHLENK: Any other panel comments for that? Dr. McManaman?

DR. JAMES MCMANAMAN: You know, I think these were good comments from the group that explored this issue, but I want to add a couple of cautionary notes. If you look at your Slide 22 from the Agency, there is a difference in

the elimination rates for the various compounds of DIA, it is much faster than the DACT. If you look at the data presented by Syngenta, they have DACT as being the primary compound under which protein adducts can occur or glutathionylation.

So depending on differences -- and there be may be differences between species and the rates of these adduction processes or the elimination processes, so by using the radioactivity, I think you don't get at that. And using the single compartment model, I don't think you get at that. So I would be a little careful since we really don't know what's going on with the human as much as we do with the rat, I don't think you can extrapolate because the rates, you know, if the rate of adduction is different from humans to rats, then you may have a different toxicity and that has to be considered. So that's just a cautionary note.

DR. DANIEL SCHLENK: Thank you. Any other comments before we break? We'll just wait until we finish the complete question before we come back to you guys to finish up, if that's okay. Let's go ahead and move on to Question 12(c). Dr. Greenwood?

DR. RICHARD GREENWOOD: I'm not going to say a great deal on this, but one of my colleagues will, I think. I think we've already gone over a lot of this, but one of the big advantages, I think, of this model of mice worth pursuing and validating and so on is it does hold out the prospect of really reliable, scientifically based extrapolation between species. It's one of the advantages, I think.

So I think it is worth pursuing this because physiological models for humans there have been because of the pharmaceutical industry.

There are some problems with this model, as submitted by Syngenta, and one of the weaknesses is that there was some in vivo parameterization from in vitro metabolic studies. So that's always a problem. But the curves are well defined by frequent measurements in time. The predictions, though, if the tissue concentration depend heavily or will depend heavily on selected tissue plasma partition coefficient. These are really critical and you get this with lots of methods, both in environmental analysis and in pharmacokinetic analysis. Those values are critical and can introduce real bias. And I'm really glad to see that Syngenta intend to verify these in vivo. I think that's essential and it's one of the things that they said they would intend doing.

Amongst other things, it may actually help to identify binding within tissues in multiple compartments within tissues and might provide a check on what appears to be a slow distribution compartment as we mentioned earlier. I think the limitation of this, and again the Agency needs to think about this, is that because of the limits of quantification, it's going to be very difficult to use this to validate for low, probably human-relevant doses. I see that as something that needs looking at carefully by the Agency -- well, would need to be looked at before they can go ahead and adopt it. Thank you.

DR. DANIEL SCHLENK: Dr. Hayton?

DR. WILLIAM HAYTON: I'd like to pass to Dr. Meek because I think she is going to enumerate all of the information requested in the question. And if she doesn't I do have a laundry list, but I think she's got the better one.

DR. DANIEL SCHLENK: Okay. Dr. Meek, you've been tapped.

DR. BETTE MEEK: Well, the question is fairly broad, as you'll note. So we're trying to determine how best to divide this up. I wanted to say that I I'm suitably impressed with, first of all, the considerable progress on the development of the PBPK model and its review for all the reasons and the value of the model for all the reasons that we've heard here. It avoids a number of generalizations and the value of the sensitivity analysis associated with the model for testing hypothesis is considerable as well. So I would strongly encourage the Agency to work with the proponent to ensure that the model is sufficiently robust to meet their needs. I mean, given its considerable potential to more accurately predict interspecies and intraspecies differences in kinetics and to test a wide range of hypothesis regarding critical determinates.

So in relation to key aspects that should be considered in review of the PBPK model submitted by Syngenta by the Agency, I referenced the recently released WHO guidance on the Characterization and Application of Physiologically Based Pharmacokinetic Models and Risk Assessment. It was referenced by one of folks presenting to the meeting of the other day.

Development of the guidance drew broadly on expertise internationally in both PBPK modeling and risk assessment and involved protracted input from a drafting group in a series of related workshops. This group developed a comprehensive list of questions for consideration relevant to evaluation of the biological bases, model simulations, reliability and applicability of specific PBPK models for application in risk assessment.

I'd note also that a subset of these questions is equally applicable to other types of pharmacokinetic models and it would be helpful to step through them, then, in relation to the modeling approach currently proposed by the Agency.

I'm always concerned when we hold, often, more data informed approaches such as PBPK modeling to higher standards of verification than approaches which are based on less inference. So it's important to consider stepping through these questions for all modeling based approaches.

The document also makes recommendations concerning process for consideration of PBPK models in regulatory risk assessment. This includes early and iterative involvement of regulatory risk assessors in model development, access to both internal and independent expertise, documentation by model developers in standard format risk assessment applications and independent review.

So I'm not sure whether I want to go through the list of considerations for considering PBPK models in risk assessment. I'll briefly try to summarize what's here and submit, for the record, the more detailed listing. But for the biological basis:

- Are the major sites and processes of absorption, storage, transformation and clearance included in the model?
- Are the mathematical equations of ADME based on a sound theoretical biological basis?
- Are the input parameters related to the characteristics of the host, chemical or environment?
- Is the sum total of the tissue blood flow rates equal to the cardiac output?
- Is the ventilation perfusion ratio specified in the model within physiological limits?
- Are the volumes of compartments within known physiological limits?
- Is the approach used to establish partition coefficients within the domain of valid application?
- Is the method used for estimating biochemical parameters adequate?
- Is the allometric scaling of parameters, if applicable, done appropriately?
- Is the integration algorithm proven for solving differential equations in similar models?
- And has the computer model code been verified for syntax errors and the accuracy of units?

And then the model simulation of data:

- Has the model been evaluated for its ability to predict kinetics under various conditions, consistent with its intended application?
- Does the model consistently reproduce the general trend of the data, the peaks, bumps and valleys, saturation of metabolism, or only portions of one or more data sets?
- Are the model predictions within an acceptable level of correspondence with the experimental data that was considered to be within a factor of 2?

And the reliability for model testing, uncertainty and sensitivity.

- Is the model capable of providing predictions of the concentration time course of the candidate dose metrics in the target organ or a suitable surrogate compartment?
- Has the uncertainty in model predictions of dose metric been assessed for the relevant exposure conditions?
- What is the reliability of the data used for calibrating and/or evaluating the PBPK model?
- And is the sensitivity of the dose metric to change in numerical values of input parameters characterized for relevant exposures?

And then, of course, Applicability:

- Has the model been developed and evaluated in the species and life stage of relevance to the risk assessment?
- Do the exposure routes in the model correspond to those of anticipated human exposures, as well as

those of the critical studies chosen for the assessment?

- Has the model been tested for the exposure doses and durations of relevance to the intended extrapolations?
- And does the model contain point estimates of parameters, consistent with the purpose of application?

So one of the difficulties that we came up against is really this kind of transparent presentation of the model content for risk assessment and I was encourage to hear that, certainly, Syngenta was aware of the requirements for documentation and are presenting it in that context. I was also pleased to hear that they had the model evaluated by external reviewers as well. And I'll leave it at that.

DR. DANIEL SCHLENK: Anything to add, Dr. Hayton?

DR. WILLIAM HAYTON: No.

DR. DANIEL SCHLENK: Okay. All right. Open for any other panel members. Comments? Yes, Dr. Horseman?

DR. NELSON HORSEMAN: This is almost certainly a completely naïve question, but this last point here, model applicability, I wonder if I might hear some comments on how these models might be applicable, depending upon whether one is concerned about LH surge suppression versus thyroid tumorigenesis. It seems to me that model applicability implies a relationship to the

toxicological, physiological endpoints. That would be interesting for me to hear.

DR. DANIEL SCHLENK: Who wants to take that one? Dr. Greenwood?

DR. RICHARD GREENWOOD: I think that it's difficult. All the pharmacokinetic data will tell you is, what is the likely exposure of all tissues. And this is one of the problems we face here with atrazine. We don't know for sure where the site of people has suspicions. We don't know where the primary lesion occurs and we don't know how much actual exposure, if you like, of the primary site of action or sites of action to produce a sufficient lesion to cause the secondary lesions that we observed as the symptoms, including LH suppression and so on.

So at the moment, we're really in the dark. And what we're trying to do with all of this sort of approach, what people try to do, is to just get a measure of the opportunity for a particular compound or a group of compounds to interact with a site of action. If you know what that is, and with the pyrethroids, with OP's, then it's really easy, relatively. But when you don't know where it is and what it is, this is the best that you can do in order to try and decess overall exposure of the site of action because the area under curve, the other plasma, really, because all tissues get exposed to that. It really is the best measure of overall opportunity for a compound to interact with any particular site of action.

One of the problems is that you don't know that when you're looking at individual tissue distributions, for instance. You don't know whether that's just a sync which keeps it away from the site of action or whether it's a good thing, if you like, from the atrazine, if it's getting there to the site of action. So it is difficult, but I think at the moment, this is probably the best that we can do and you can use these such models, even the simple models to try and get an estimate of exposure over various time scales. And, of course, some things can sometimes take longer exposure, maybe, than for some of these things like interaction of an OP with an enzyme, where you get an instant effect.

DR. BETTE MEEK: Really, we're trying to get a little closer to the internal does even though we don't necessarily know what the target is to consider much more accurately, interspecies differences and human variability. So ultimately, you're trying to move from the external dose to at least a closer surrogate for the internal dose to be able to replace the kind of default uncertainty factors that we use for that purpose, but it relates solely to exposure when you don't necessarily understand the adverse outcome pathway.

DR. PENELOPE FENNER-CRISP: One of the areas that iterated in the WHO guidance, with respect to applicability, had to do with developing models appropriate to the subpopulation of interest that you were focusing on. And of course, with respect to atrazine, there's a whole lot of data and interest in defining the toxicological consequences of exposures at various life stages.

There's a lot data generated on that point, but as far as I can tell, from this point in time, the simple PK model only models for an adult; an adult which isn't even an average adult.

And to this point in time, seems not to be modeling for any of the younger life stages of concern that's really the focus of this risk assessment. So it's just a point to reemphasize in conducting and developing these models, whether it continues to be the simple one or the most sophisticated PBPK model, one has to consider having variations in the application of the model that are consistent with the life stages of concern.

DR. DANIEL SCHLENK: Any other comments on Letter C? Yes, Dr. Portier?

DR. KENNETH PORTIER: You know, in that long laundry list that you did, is there something in there that says implementing this on a platform that's transparent for others to look at?

You know, I was sitting there thinking okay, five years from now we'll be sitting and you will have implemented it in something that we can't run on our computers. So I'd like add that, way at the bottom of the list, that I think it's consistent with other things we've heard like with the dietary programs, you implemented in SAS, I don't have SAS, so why didn't you implement it in something I can run it in.

DR. DANIEL SCHLENK: Dr. Meek?

DR. BETTE MEEK: Just to underscore the point, there was a lot of discussion in this project on exactly that issue and the need for transparent modeling platforms that are available to all. And some of that is evolving. First of all, for the purposes of increasing understanding and uptake.

DR. DANIEL SCHLENK: Okay. Any other comments on Letter C? Okay. Let's go on to D. Dr. Greenwood, do you want to start off on that?

DR. RICHARD GREENWOOD: Yeah. It's asking us to comment on the extent to which this one compartment model can account for intraspecies differences. You can do it, but there's a lot of uncertainty associated with it because it's difficult to introduce into one compartment model differences in metabolic capabilities, for instance, binding properties of various tissues. But you can extrapolate using empirical allometric factors. You can do that, empirically. But what you can't do is carry out the extrapolation on the basis of any sort of good scientific physiological and metabolic information. So again, they'll be a large uncertainty associated with any extrapolation between species, using the single compartment model.

Another problem with it, really, it's difficult to compare the exposure time needed for infecting rats with that in humans. But a lot of the reason is the information we've got in rats is based on the same dose being given on each day. It's simply because people are

looking at the suppression of the LH surge. So all of the experiments are being carried out in the same way because that's what they're interested in. So it may be more difficult to try and extrapolate this sort of information across to human exposure because that's not the usual mode of exposure.

One worry for me is that the rat did not scale to monkey when they used the standard allometric scaling factors. In fact, he went the wrong way. Part of the problem, I think, is in different species, you need to consider binding sites as well as metabolism because, -- as I've said earlier, without going on about it any longer -- it's actually the concentration of free material that counts. And one area where I think these physiologically based pharmacokinetic models are going to have the advantage in terms of extrapolation over these simple models is because you can get the physiological parameters. You can actually parameterize a lot of these from the literature.

Well, it's got that advantage. It's based on something you can measure as well as cardiac output and so on, blood flow to various organs, organ weights. And there have been a couple of studies lately, one by Boudoir (ph) and one by Buoy last year, where they've actually taken human physiologically based pharmacokinetic models and then played games. They've used stochastic modeling. And what they've done is to say, okay, if you look at young children, you look at old people, then things like cardiac output, renal function, hepatic function all change, and we've got measures of how they change.

So you can also say, okay, what happens if you get somebody who weighs 120 kilograms instead of 60 kilograms? And you can play the games by altering the parameters of the model and then doing a stochastic approach and trying to generate a population based on the sort of population variability that you've got. Okay, it's in early stages, but what it's trying to do is to try to look at individual variability using the model. You can't use those sorts of games. You can't play those sorts of games with a single compartment model.

So again, going back to what Dr. Meek said, actually, you've got to decide what do you want to use this model for and if you want to extrapolate between life stages and if you want to extrapolate between species, probably it's not the best way of doing it. You may get more joy out of the more complex model, given all the drawbacks to those that we've already outlined.

DR. DANIEL SCHLENK: Dr. Hayton?

DR. WILLIAM HAYTON: I agree with my colleague and I guess I'll just emphasize, based on total radioactivity -- I don't know what to make of it, but I don't know whether it rises to the level of being disconcerting, but why the half-life in monkey actually came out to be shorter than rat when you'd expect it to go the other way. We have only have three species here. I mean, you know, rat, monkey, and a very limited amount of data in human for the scaling part. But other than that anomaly, it seemed

like the volume of distribution for total radioactivity is relatively constant across the species.

Certainly, the rat elimination rate constant seemed to scale to the human value, even though that's based on limited observation, it seemed to scale according to expected allometric scaling relationships, you know, three-quarter our body weight relationship. I'd say overall, from what we have now, it's seems to work satisfactorily. Well, let me hasten that it will be interesting to see what's going on with the monkey and it seems like those studies are in the works, right, that Syngenta is doing that?

DR. DANIEL SCHLENK: Dr. Meek?

DR. BETTE MEEK: Yeah. I have nothing to add. Thanks.

DR. DANIEL SCHLENK: Any other panel input on Letter D, Question 12? Okay. Go back to the Agency and determine whether or not you guys have what you need. Any questions or clarification? Okay. Let's go ahead and move onto 13. And before we do, I think Agency has some further questions or clarification for some of the previous questions we were given.

DR. ELIZABETH MENDEZ: Yeah. Before we proceed to Question 13, Dr. Dellarco would like to ask a question for clarification.

DR. VICKI DELLARCO: I'm Dr. Vicki Dellarco. I'm in the Office of Pesticide Programs. I'm the science advisor in

the Office of the Director. I want to come back to the question of how we integrate different lines of evidence that we're seeking your advice on. There's been logic in how we've proceeded with all these SAP reviews.

In the first review that we had in February, introduced a framework that we would use to pull together information to inform an opinion about what the compound might do in humans. And so, really this question that we're asking you about how to integrate the experimental, both mechanistic, empirical, and the epidemiology comes back to that framework.

What we're seeking advice from you on is this: if you remember that framework, there were certain attributes of it. It was a hypothesis-based framework, taking all the evidence and trying to understand what the compound does, kinetically, dynamically along a pathway. It was an evidence-based framework. And it was a framework that integrates different data streams and being able to characterize a conclusion and the confidence in that conclusion and the uncertainty around that conclusion.

So if we go back to the thyroid example -- because it was discussed a lot -- to kind of use it as an example of the guidance that we're seeking on. As we look at the different tumor sites that are suggested in the epidemiology and how we bring all information to bear on interpreting that is -- it's hypothesis-based, so you would ask, okay, what do we understand about thyroid cancer in humans because we're interested in humans?

Rather than saying we don't know. There could be any mechanism to try to lay down some reasonable hypothesis, based on the experimental, medical, epidemiologic literature about what could be some key events involved in that. So as an example, we know radiation is a factor, so one would want to look at urogenesys. One would want to go to thyroid human disease models and see what the association is in those models with thyroid cancer, and perhaps, perturbation of that thyroid axis and elevation of THS could be a factor.

But again, laying down that hypothesis, drawing on all knowledge. And then to start looking at the experimental evidence to see what it tells us about how it may evoke those key events. You know, is the compound a mutagen? Do you have a sufficient basis to draw that conclusion? Do we have studies that have looked at perturbation of the hypokalemic pituitary thyroid axis? What do we understand about that? What is the epidemiology telling us?

So where we could use your help is how can we structure that analysis in a scientifically rigorous way? How can we structure it in a way that is transparent in how we reach conclusions so that it's understood how we're weighing different line of evidence? In some cases, the epidemiology may be given weight. In other cases, experimental and epidemiology may be given equal weight. It depends on the tumor site that we're looking at and the information that we have. So this is basically what we're asking you to do so that when we go into the experiment, what are the things that we should think

about in characterizing the strength of that evidence and the limitations in that evidence.

When we look at the epidemiology, you've given us some very good advice there on how we should look at the epidemiology. Now we need your advice in how we bring this all together, again, in a structured, rigorous, transparent way so that in the end, when we reach conclusions, it's very transparent to everybody what the uncertainties were; how much weight you put on those uncertainties or how much confidence in the conclusion. And so it's a multi-discipline process. So this is probably a question that requires a multi-disciplined input. It would really be helpful if the panel could come together; the epidemiologist, the biologist, and the risk assessor to give us guidance on this.

DR. DANIEL SCHLENK: Does anybody want to sort of tackle that? We have Dr. Portier.

DR. KENNETH PORTIER: It's really good. I know what you're trying to get at. And I was sitting here looking back at the notes from the February meeting. You broke it out into the exposure modeling, the PBPK modeling, the PD modeling, and then you're trying to bring it all together. And nowhere in there did we really link the epidemiology quantitatively. It's kind of qualitatively. And that the framework starts with the exposure modeling. So we've been doing a lot of discussion about exposure modeling. And I think in this case, we have that compartment pretty well discussed. I see that going on.

The issue I was going to bring with the PK modeling is that in your framework, you kind of start with the typical adult, but I didn't see that PK model here. What I saw was an atrazine-specific model that's much simpler than your typical adult model that you kind of started with in your framework. And I felt like, in this case, you kind of did what I'd call "top down" modeling rather than a "conceptual up" modeling, which I thought what the framework was all about. Dealing with here's a typical adult, here's some typical processes; atrazine is going to impact these processes, these pathways and produce this kind of signal in the body that's going to result in this kind of -- and I'm not sure the PK model we're looking at here is quite -- and I'm looking at Dr. Greenwood because he was sitting there as well. I hope he knows what I'm talking about. I don't think the PK modeling in the atrazine cases is as good as what you would conceptually add in the framework. I have nothing to say on the PD modeling.

I've been sitting here thinking, though, about how we get the epidemiology end, and I understand their point. When we start looking at the PK and the PD modeling and there's no information to inform the typical adult model, then we have to fall back on the epidemiology and say, what are the associations? What's the strength of the association? Are these associations helping me to better understand whether something is really happening in the body that I should look at?

I don't think we really discussed that very well, even in the last meeting. We make the qualitative link, but then

we go looking -- and I think that's the epidemiologist point -- we go looking for excuses to throw the epidemiology out rather than think of excuses for why the epidemiology should force us back into the lab to look more carefully at mechanisms. I don't know how -- you're asking how we put those together. And I think I'll throw that back to the panel and say do we have any paradigms, any structures to help us think that way? And I haven't seen any.

I know what you're asking for, but I don't know how to take that loose epidemiology information and kind of link it in a qualitative way or as a driver for mechanism research, even if it's to push the mechanism research out, or push more epidemiology out.

Unfortunately, pushing the epidemiology out is typically too expensive, in terms of time and effort and timeframe - for EPA's timeframe. You know, they have to make a decision in a year and a half; they're not going to do a full-blown repeat epidemiology study in a year and a half. So I'll turn it over to Dr. Bove. Maybe that's kind of got them thinking, trying to translate some of this.

DR. FRANK BOVE: I don't know if this is going to help at all, but in the February meeting and in this meeting, again, it's important, first of all, to get the evaluation of the epi evidence right. There were problems with the 2010 Issue Paper. There are problems with this one. We see an advance, but we still see weaknesses in just evaluating the epi data. So that's the first thing.

Before we talk about integration, let's actually evaluate the epi data in the best way we can and maybe -- I mean, sure, you can't do an epi study right off the bat, but you can pool data. For example, for the prostate cancer situation with the Saint Gabriel plant, a simple thing could be done/should've been done to answer that question. What about these five cases? What were their exposure experiences? What happens when you compare those five cases with the controls that also were there before the plant started the screening program?

This is nothing to do. And you can actually then answer the question: Is there something there at that plant or not? If there isn't, then the case is closed on prostate cancer, for example.

So these are things that can be done if you evaluate the epi evidence properly and appropriately. Again, go over the years, there's a history to this, in 2000, there was hardly any. We called it a brevity and superficial evaluation of the epi evidence. In 2003, it was a little bit better, but there was a lot of evidence that wasn't discussed and it was focused on prostate cancer and there were problems with that.

In 2010, we had ecologic studies, mostly to evaluate, and the evaluations were problematic. That's the best I can say. This is better, but you still have a ways to go. So my feeling is let's get the evaluation of the epi data right. There are things that could answer some of these questions. Thyroid cancer - there's no way to answer that question without further study. Most of the cancers

where we talked about suggestive evidence or inadequate evidence, they require further study. There's no way around it. You could do some pooled analysis, though, in the meantime, but you have an ongoing agricultural health study. You could encourage states -- we were just talking about this -- you can encourage states to use their municipal drinking water data and their cancer and birth defect registry to start answering some of these questions too.

So that's on the epi side. First things first; before you talk about integration, let's see what evidence we have, evaluate it appropriately, and see what things we can do in the short term to enhance that information. Then when we've got that together, then we can start integrating. It's done all the time. If you look at the risk assessments for trichloroethylene PCE, so on, that are just recently being done, they're integrating to tox and epi information. In the case of trichloroethylene, it's the human data that's taking precedence. In PCE, it's not. And they're categorizing it for carcinogenicity based differently because of that. I think that those are examples that EPA can look at its own risk assessment process to see how these things are getting integrated.

DR. DANIEL SCHLENK: Dr. Meek?

DR. BETTE MEEK: Thanks. I think sometimes what complicates true integration of available data is that we really are reacting to chemical-specific data. So we're not drawing more broadly on, for example, what we know about human disease. And I also think that the way the questions are

posed here, looking at pieces of the -- we haven't been asked to look at the totality of the data. We've been asked to look at the epidemiological data or what's the critical effect. That kind of thing.

I think that there needs to be some broader thought about how we bring what we know about diseases models into account in interpreting both the epidemiological and toxicological data. When I read through the section here, which related to integration, I kind of walked away saying I don't feel there's been an integration yet. Again, I don't think we're drawing broadly enough on the available information.

I think the other issue that complicates this is we don't really have a hypothesized adverse outcome pathway yet. So it's as a basis for trying to integrate available information that's complicating the issue as well.

DR. DANIEL SCHLENK: Dr. Chambers and then Dr. Horseman.

DR. JANICE CHAMBERS: Well, I appreciate that you're going to have to deal with atrazine in 2013, and so it's on the table right now. It seems like for answering this broad question, which is a very good question, you need a case that has a little bit more solid information here and there. The epidemiology evidence seems to be suggestive, at best, at this point. The mechanisms we're looking at for the point of departure and everything is an entirely different type of thing. The mechanism isn't known there. The exposure data from environmental and to a certain extent, from the occupational are pretty fuzzy.

So you really don't have good solid information and enough mechanistic information and enough pharmacokinetic information right now to do that.

If you're going to look at integration, I think you need a more solid case where you have a mechanism solid epidemiology data and more information that all can be integrated as the first case to try to do that, and probably atrazine doesn't have all of those elements. I know it's on the table and all, but I don't think that's really feasible right now.

DR. DANIEL SCHLENK: Dr. Horseman?

DR. NELSON HORSEMAN: My point is very similar to Dr. Chambers' but maybe worded in a different way. I don't know whether you can call it pharmacodynamic, toxicologic, physiologic - the fact that we've been asked to consider as a point of departure, which is the LH surge suppression, I had a sense that that was related to epidemiological findings, if you will, related to menstrual cycle irregularities and reproductive senescence, and some other animal things -- so on and so forth -- which we heard nothing about in this meeting.

The epidemiology that we heard about is cancer epidemiology. We haven't heard anything about cancer pharmacodynamics or toxicology or anything else in this meeting or in the white paper. I get the sense that we're being asked to integrate two things that have nothing to do with one another, except atrazine - to go back to Dr. Chambers' point. Maybe that's a wrong

interpretation. If it is, I'd like to hear somebody correct me.

DR. DANIEL SCHLENK: Dr. Greenwood?

DR. RICHARD GREENWOOD: Again, I was at the earlier meeting where we started to look at epidemiology, and I think there, the message that came across to me was that one of the main weaknesses in many of the studies was poor exposure data. I think the Agency is trying to pool together the exposure data, but they're not there yet, but they're getting close now. I think once that's together, then it may be possible to do some stronger studies.

DR. KENNETH PORTIER: I wanted to follow-up on something Dr. Greenwood was talking about at the end of his discussion on Part D, which was that they're starting to use these PKPD models in a play mode, right, a "what if" mode, a hypothesis generation mode. So again, when I thought about the framework, I was thinking, oh, great, they'll have kind of a generic model and then they can look at the epi and say well, thyroid. What would have to be happening in this mode for us to see something happening with thyroid?

So in a sense, you're generating that hypothesis. You're looking at the epi and saying okay, the epi people are saying it's kind of possible. Can we do something in our generic model to produce -- you know, we know this is an endocrine disrupter. We know from the ecotox that it has affects on certain animals. Amphibian effects maybe are

not directly translatable to humans, but we know something about what's going on.

Can we just play with this model and see if something happens? That was, to me, one way of generating a hypothesis. It's not testing it, but it kind of feasibility. Does the model say it's feasible? If you can't tweak the model enough to get that effect, then you're at least in a position where you're saying, well, you know, we've looked at our current big knowledge, our current understanding of PKPD processes, and we can't make it happen. Then you can turn back to the community and say if you still think there's really something happening here, propose something different, but we've kind of exhausted the obvious avenues. To me, I thought that was part of the framework. What I'm calling "that" is kind bottom up from basic concepts and trying to make something happen in this conceptual model.

DR. DANIEL SCHLENK: Dr. Meek?

DR. BETTE MEEK: Just a brief response to that, Ken. I think what the modeling is telling us currently is that we handle the chemical very similarly to rats. In fact, that the chemical is rather evenly distributed. So it doesn't address the PD component because we don't have a hypothesized AOP here. So the PBPK model doesn't really help us in that context.

DR. DANIEL SCHLENK: Dr. O'Byrne and then Dr. Jerde.

DR. KEVIN O'BYRNE: I share Nelson's concerns about the fragmentation. One of the things that frustrates me is the lack of epi data on those reproductive impact of atrazine. Apparently in the September meeting there were two papers brought forward. I mean I looked at those. One wasn't worth looking at and the other one was just so weak. And I just wonder, why has that been neglected, in terms of -- I don't mean neglected from your perspective, but in terms of the epidemiologists tackling this issue. Is there a reason why it's being left, given that the gonadatropines seem to be so central to the mode of action, et cetera, with atrazine?

So that's one point that I'd like to make. The other is -- I think Richard Greenwood made some very good comments earlier on about the diet versus the gavage dynamics of atrazine and its metabolites in the plasma. And he's absolutely right. Those sorts of peaks following gavage versus their absence in diet could have major affects on how the brain is perceiving and processing those signals.

I think very recently, Stavert Lightman, in Bristol in the UK, has been looking at the brain's response to pulsatile release of corticotropins, up to the glucocorticoids. And it's astonishing, the sensitivity of the brain to the pulsatile reception of these hormones and how you get translocation and pulsatile transcription. It's absolutely fantastic what he's demonstrated the sensitivity of the brain to pulse modes.

When they gavage these animals and you get these huge peaks, goodness knows what's going on inside the brain.

I think the move towards the -- in my point of view -- as a sort of simple physiologist, moving towards feeding animals, if you can't get it into the water, is much more relevant to looking at the mechanisms of action of atrazine on the reproductive system, and I suspect other systems as well. I don't know if that's helpful at all.

DR. DANIEL SCHLENK: Dr. Jerde?

DR. TRAVIS JERDE: I wanted to second what Dr. Horseman said and offer this: so you read through a lot of this and what's available to the public and what's available to the scientific community we've heard from industry representatives, something along the lines of there's no compelling evidence available that atrazine may be carcinogenic to humans. And that sounds an awful lot like atrazine is not carcinogenic to humans, to a lot of people.

I guess I'd like to support the epidemiologic side of this because it seems like the conclusions that they're making, based on a very limited literature in most cases -- separate types of cancers, separate diseases, likely separate mechanisms of actions -- the conclusions they're drawing are, wait a minute, step on the brakes. We can't really say something so definitive that sounds an awful lot like this isn't carcinogenic.

Furthermore, a lot of these studies are on high exposures in the plant. As a public health issue, we could even deal with that personal protective equipment, help the workers and that sort of thing. And yet the water, to a

larger prospective, the exposure in water might be the bigger problem. From what I understand, from what you guys have been saying, there's almost nothing on it. And so this gets back to Dr. Horseman's point, integrating a model - we're losing what I think is a fairly strong statement from them, which is we need to understand this a lot more than what we currently do. That's becoming obscured in trying to get the right model and we really don't know what the right model is because we don't have any clue, from limited epidemiologic studies what that might be.

And once we have better data from say, the thyroid, and now we may look at the thyroid and say okay, in these patients that are exposed, if there is an increase in thyroid, we looked at the thyroids, this changes. Now we can go back to the model system and say, okay, we're going to change this and see what happens and now we've got our model.

So I'm just concerned as a scientist who looks somewhat translational, but I do consider myself a molecular biologist who uses models. I'm just concerned that some of those epidemiologic findings might be getting obscured by so much talk about modeling and integration.

DR. DANIEL SCHLENK: Okay. Dr. Young?

DR. HEATHER YOUNG: I think I want to come back to the point that I made earlier, too, is the huge gap in the literature is that it's looking almost exclusively at occupational exposures, which is why we have very few

studies looking at reproductive outcomes because the occupation that we're looking at is predominately male-dominated. And there are a few studies looking at female gynecologic cancers, but for the most part, again, we're focusing on occupational exposures, not on community exposures where you would expect to see, if there are any, the reproductive effects. Because then we would have a much broader population that we're looking at, a much better representation of female. So again, I think it's hard to ask us to integrate when we don't have all the pieces of the information.

DR. DANIEL SCHLENK: Dr. Roby?

DR. KATHERINE ROBY: And to take that to the next step, also, we need to continue to explore the mechanism of action. As we learn more about the mechanisms of cancer, and we learn about the mechanism of atrazine action, along with epidemiology, we can begin to make the ties, but there's not only gap in the epi data, but there's gap in understanding mechanism of action of atrazine and mechanism of cancer development. So all of these data need to grow together, but there needs to be continued exploration of atrazine's mechanism of action.

DR. DANIEL SCHLENK: Dr. Gold?

DR. ELLEN GOLD: I wanted to make a couple of points. First, I want to agree -- I think Mr. Horseman made the point -- in the Issue Paper, the mode of action that's emphasized is largely the neuroendocrine LH surge one. And it is true that that was discussed pretty thoroughly in the

September meeting to look at reproductive effects, but I'm not sure that it applies, necessarily. It applies, perhaps, to some of the cancers we're looking at, but not all of them. So point Number one is there was a little bit of a disconnect there, but it probably reflects what we know at the moment.

Secondly, the question was raised why we're not doing a better job of looking at reproductive, and part of it is because we're not looking at the community. But part of it is because the epidemiologic study that we're depending on the most was a cancer institute study that was focused on cancer outcomes. So when they made an attempt to look at reproductive outcomes, I think it was done in a less than optimal way, even in that cohort.

The other point I want to make is it's occurred to me, sitting and listening to this and being part of the SAP that talked about the framework for using epidemiologic evidence that I think the Agency is undergoing a little bit of a culture change in trying to figure out how to incorporate epidemiologic data; that it comes from a place of using mostly toxicologic data. And this is like, you know, a new piece. How do we deal with this? And culture change takes time.

So I think we've been trying to inform you about our feelings about if you have epidemiologic data but you're lacking toxicologic or mechanistic data, you shouldn't ignore the epidemiologic data. And finally, that leads to having the epidemiologic data drive -- or provide the impetus for doing future mechanistic -- but it shouldn't

-- just because you don't know, it doesn't mean you should ignore it and say there's unlikely cancer risk.

DR. DANIEL SCHLENK: Okay. Nice discussion. I'm not sure where it's going to go in the report. Perhaps, yeah. All right. Yes, Vicki?

DR. VICKI DELLARCO: So I think we've gotten some nuggets of advice. If it could be pulled together in a logical way, it would be very helpful. And if I can summarize what I've heard, you guys don't have to integrate, we have to integrate. So we want your advice in things that we should be thinking about. And what I've heard is some advice.

1) Before you can integrate, you have to ensure that you've done a thorough analysis of the individual lines of evidence. We've been given some advice on the epidemiology and things that we should go back and look at more closely, in terms the analysis. And that includes the experimental data too.

2) Secondly, I heard that if there is an empirical finding, whether it comes from an animal study or it comes from epidemiology, you start to look to see do you have an understanding around that. So simply, you don't have the mode of action and the animal model, as Dr. Meek pointed out, start to look in the middle literature to see if you can begin to look at plausibility and lay some testable hypothesis down.

3) I also heard that we do have an understanding of some perturbations occurring that could lead to some outcomes. And as you look at that understanding, you

should look in the epidemiology to see how well the population of interest has been characterized.

So again, that helps you as you integrate, be able to begin characterizing strength, limitations and uncertainties.

I don't know if I've pulled together everything that you've said, but what's useful is the things that we should be thinking of, where we should be focusing as we bring these different lines of evidence together. Because we are going to have to do that at some point before more research or more epidemiology is done. With any pesticide, we continue to look at it because we have reevaluation schedules. Thank you.

DR. DANIEL SCHLENK: Thanks. I think what we're going to do -- Dr. Portier is taking furious notes here -- I think what we'll do is maybe put this on the end of Question 11. If you can do that, Dr. Gold, that would be great. So we'll tack it on to Question 11.

We have some travel issues that the panel needs to deal with, so think what we're going to do is take an early lunch and if the panel would meet in the coffee room, we need to very rapidly decide a few things because 12:00 is check out time. So we need to make a decision very quickly. So we're going to take an hour and a half lunch. We'll be back at 1:00 and finish the last two questions.

DR. DANIEL SCHLENK: Good afternoon, everyone. Let's go ahead and get started on Question 13. Dr. Mendez, are you going to read that?

DR. ELIZABETH MENDEZ: Good afternoon. Charge Question Number 13 has to do with the temporal relationships between exposure and tox endpoint.

Question A: "Please comment on the rationale used by the Agency for selecting these exposure duration options" that I mentioned in the preamble of the question. "Please discuss the rationale for other alternative durations of concern, if any."

Question B: "Please comment on which exposure duration in humans most closely corresponds to the exposure duration found to cause adverse effects in rats."

Question C: "Please comment on the approach used by the Agency, *i.e.* the one compartment linear model to relate atrazine levels from the water chemographs to predict corresponding human plasma triazine levels for the proposed durations of concern. In particular, please comment on the Agency's proposed approach to use water AUC estimates to calculate a time-weighted daily average of atrazine exposure for a given duration of concern. Please suggest alternative approaches as appropriate."

DR. DANIEL SCHLENK: Our lead discussant on this question is Dr. Bill Hayton.

DR. WILLIAM HAYTON: Our group did meet to discuss this and tried to come to some meeting of our minds on the response. So I'll read my response, and if the other members want to add to that, that is what we'll do.

So my response to Part A is a time to reach steady state and time to effect are not necessarily closely related. It could simply be a coincidence that they both take about the same amount of time. The time to accumulate radioactivity to steady state and the route with the oral gavage dosing takes about four days, and it also takes four days of exposure before you start to see LH surge suppression.

So we didn't really see any evidence for a cause-effect relationship there at all. It seemed like if one looked at the CODAR, 2011 study -- I better get back to my text or I'm going to get all balled up here. Where he measured atrazine and then the three DEA, DIA, and DACT metabolites, the exposure produced by the pseudo steady state level for those for compounds, in four days, was about the same as produced during the first day. In other words, the accumulation in the steady state for those substances seem to happen fairly quickly.

The CODAR study of four daily doses of atrazine by oral gavage, followed by a four-day washout period with plasma concentrations measured intensively during both the treatment and washout periods, showed very similar Cmax, Cmin and AUC for treatment days two through four, for atrazine and the toxicologically active metabolic. Again, DEA, DIA, and DACT.

Treatment Day 1 exposures were only slightly smaller than those observed for Days 2-4. So in other words, there was little accumulation of the chloro-s-triazines with daily multiple dosing regimen, which is very consistent with the relatively short half-lives of the triazines, compared with the 24-hour dosing interval.

The one dose I looked at fairly intensively was the 50 milligram per kilogram per day oral gavage treatment. The longest half-life of the four triazines was that for DACT, which was about seven hours. It should be noted it was a much longer half-life, starting around 36 hours after the fourth dose, and that half-life was about 17 hours. But that half-life controls just an insignificant fraction of the overall accumulation of the systemic exposure to DACT.

So the seven-hour half-life and 24-hour dosing interval indicate that accumulation would be negligible, and therefore, that exposure after the first dose is pretty much the same as you would see at steady state on Day 4.

So since the accumulation of nacreous triazines is negligible when atrazine is dosed daily by oral gavage, the time to effect is apparently not controlled by the time required for the systemic concentrations to reach a minimum critical level associated with the onset of effect, as the triazine exposure, after the first daily dose, is similar to that after the fourth dose.

So the logic here is we're not looking at a pharmacokinetically-controlled accumulation to some threshold level because you must hit that threshold level right after the first dose, so it doesn't take four days of dosing to get there.

So we concluded that it is therefore more probable that the time to onset of effect is controlled by the pharmacodynamics; in other words, the kinetics of events downstream from the chemical initiating event are in control of the onset of effect. And the kinetics of downstream adverse outcome pathway events for LH attenuation in human versus rat are not well characterized and it is therefore, not apparent what the appropriate duration of human exposure is to use in conjunction with setting maximum level of exposure to prevent LH attenuation in humans. Without the relative rat versus human effect kinetics, the conservative approach would appear to be to use the four-day duration identified in the studies with rats.

And so that's based on the individual chloro-s-triazines. For total radioactivity, plasma concentration, the elimination half-life is longer and the expected accumulation profile has a considerably longer time to steady state. In this case, the daily exposure would increase day-by-day, with three to four days of exposure, required to achieve 90 percent of the steady state plasma concentration.

An accumulation to a threshold concentration could define the time to onset of the LH surge suppression. As long

as the long half-life of total radioactivity likely reflects the half-life of albumin adducts, which are not active in LH surge suppression, this explanation of the four-day exposure being defined by the time to reach steady state is unlikely.

DR. DANIEL SCHLENK: Thank you. Dr. Chambers?

DR. JANICE CHAMBERS: I was actually not part of that earlier discussion group. I don't know what that means, but the only thought that I had that I think is worth reiterating is something you mentioned also, that it's probably just a coincidence that the four-day and the 28-day extrapolation are just a coincidence, nothing to do with a common mechanism and an amount leading to an effect.

DR. DANIEL SCHLENK: Okay. Dr. Greenwood?

DR. RICHARD GREENWOOD: I think I've already said pretty much what I needed to say earlier. I think I just, again, I support what Dr. Chambers' said. It's just really very difficult in the absence of any scientific knowledge to be able to extrapolate to human exposure that's equivalent to this exposure that's necessary to suppress the LH surge in rats.

DR. DANIEL SCHLENK: Okay. Dr. Fenner-Crisp?

DR. PENELOPE FENNER-CRISP: I don't have anything to add. I had my input yesterday.

DR. DANIEL SCHLENK: Okay. Dr. Meek?

DR. BETTE MEEK: I don't have very much to add. I think it's important to recognize that if you use the four-day kind of period, that's really a science policy choice to be conservative because essentially what we were saying is that there's this really limited information on which to base that period.

Just one other point I'd like to make is the allometric scaling that was done for the 21 to 30 days in humans, you normally wouldn't use allometric scaling where you're expecting the effect to be mediated by metabolites. So it's probably something to think about.

DR. DANIEL SCHLENK: Other panel members? No? Okay. Let's move onto B. Dr. Hayton?

DR. WILLIAM HAYTON: For this question we did consult, Dr. Greenwood and I, briefly with Dr. Rodriguez about what they were really looking for there. We had difficulty really responding to their question because the molecular initiating event and the adverse outcome pathway are not well enough understood at this point, we felt, to fully address this question. It seems possible that the kinetics of events downstream from the chemical initiating event control the time to onset of LH attenuation.

Another factor to consider is the minimum duration of LH attenuation that must occur before adverse toxicological effects ensue. Is a brief transient suppression for LH to be avoided or suppression of longer duration? How

large a suppression of the LH surge must be avoided? And we felt there just isn't the quantitative information available to answer those questions. So without answers, you know, I guess this would be science policy kind of consideration, but a conservative approach would be to avoid even a brief transient suppression of LH, but really no evidence to conclude that.

DR. DANIEL SCHLENK: Dr. Chambers?

DR. JANICE CHAMBERS: The only thing I wanted to add here is a couple of things that have already been said; 1) is I don't think that it is really understood whether a steady state concentration is what's causing the effect or whether it's a high-dose pulse. So it's hard to interpret this question in not knowing that.

And the other thing that I think that is worth reiterating is that it's been mentioned several times that the suppression in the LH surge may not really be a truly adverse effect at this point. So we don't know.

DR. DANIEL SCHLENK: Dr. Greenwood.

DR. RICHARD GREENWOOD: I don't think I have anything to add.

DR. DANIEL SCHLENK: Dr. Fenner-Crisp?

DR. PENELOPE FENNER-CRISP: I'd probably ask a different question here. I'd probably ask a question about what exposure duration in humans not most clearly corresponds to the exposure duration found to cause adverse effects

in rats, but rather, what exposure duration in humans would be needed to induce, in effect, representative or a correlate to those observed in the rat studies.

We've talked about the menstrual cycle correlations in terms of time. We haven't talked about expected durations of exposure for any of the plethora of other adverse effects seen in the animal studies that may well have human correlates like the delaying in puberty and all those kinds of things. I think that would be the appropriate question to ask. It may well be something other than either the 4 or the 14 of the 28, depending upon which adverse correlate you're talking about.

DR. DANIEL SCHLENK: Okay. Dr. Meek?

DR. BETTE MEEK: I think I would echo. I would certainly like to see the discussion broaden to consider other than simply the effect on LH attenuation. So again, it would be drawing on more of the data to consider what the appropriate kind of timeframe might be.

DR. DANIEL SCHLENK: Other panel members? Dr. O'Byrne?

DR. KEVIN O'BYRNE: I suppose the frustration here is the huge difference between primates, whether it's rhesus monkeys or humans and the rat model. The whole panel is mindful of this because it was discussed apparently in September when Tony Plant was here and gave a lengthy presentation. He touched on this just the other day on certain salient aspects of the difference. And that's just the surge generating mechanism. The different time

scale, you know, days rather than hours of the surge - the estrogen dependence.

I mean, you got to have 30, 36 hours of continuous estrogen stimulation to get a surge in a monkey or a woman, or a man if you remove his testicles. But in terms of moving to puberty, I mean, the time scales are even greater between primates and rats. So that's an even greater mind field, as far as I can see.

DR. DANIEL SCHLENK: Dr. Roby?

DR. KATHERINE ROBY: I think the other complicating factor is if you're talking about a one-time event where you were causing a one-time transient inhibition of LH, and if we're talking about onset of puberty. A one-time event really is not going to have a significant effect on the onset of puberty. If you're talking about eliminating LH for an extended amount of time, then you'll have a negative effect downstream. But again, the one-time very transient inhibition in itself is going to have minimal effect, even on something like the onset of puberty.

DR. PENELOPE FENNER-CRISP: My comment is, in the four-day window of exposure would be of no use in answering that question.

DR. KATHERINE ROBY: Correct.

DR. DANIEL SCHLENK: Any other comments from the panel on B? Okay. Let's move on to Letter C. Dr. Hayton?

DR. WILLIAM HAYTON: Yeah. Just to briefly review, I guess it's on the screen, isn't it? That they're asking for comment -- the Agency is asking for comment on using water; the area under the curve estimates that calculated time-weighted daily average of atrazine exposure for a given duration of concern.

And the response is that the approach is theoretically sound. The integral of the water chemograph, divided by the time span of the chemograph provides an estimate of the time-weighted average concentration of triazines in the water during the time span. And multiplication of the average concentration by the daily water ingestion rate quantifies the daily triazines dose. And the daily dose divided by the human triazine clearance, which is estimated allometrically, provides an estimate of the steady state average plasma concentration of radioactivity. And when that is multiplied by 24 hours, then the AUC for the human over a 24-hour period is obtained. I think we all agreed that if you make the assumption that it's behaving as a one compartment system that will work.

And then I had one little additional piece here. The use of water AUC to calculate a time-weighted daily average is theoretically sound. An alternative to the water AUC is simply to average the measured water concentrations, but this would be inferior. You wouldn't get a time-weighted average; it would just give you a simple arithmetic average. So we didn't see a better way forward there.

DR. DANIEL SCHLENK: Okay. Dr. Chambers?

DR. JANICE CHAMBERS: Nothing to add.

DR. DANIEL SCHLENK: Dr. Greenwood?

DR. RICHARD GREENWOOD: I think that the approach is sound. I agree with that, in terms of theoretically sound. I think it just is necessary to check some of the underlying assumptions about that pharmacokinetic curve.

DR. DANIEL SCHLENK: Dr. Fenner-Crisp?

DR. PENELOPE FENNER-CRISP: I don't have anything to add.

DR. DANIEL SCHLENK: And Dr. Meek?

DR. BETTE MEEK: Nothing to add.

DR. DANIEL SCHLENK: Other panel members? Okay. That completes 13. Let me go back to the EPA and ask if they have any questions or clarification.

DR. ELIZABETH MENDEZ: I'm just looking at the team, and I don't see anybody that is jumping out with a question. So I guess we could move onto Question 14.

DR. DANIEL SCHLENK: Okay. Sounds good. Hold on. Dr. Portier has a question.

DR. KENNETH PORTIER: And it's to Dr. Roby. You know, I was listening to what you were saying and I thought to myself

well, under a scenario, suppose that just slightly above background atrazine were enough to completely suppress LH in the human female. In a typical year it would be maybe three months, right? So what do you think the impact would be if three months out of every year you had that suppressed? And I was sitting there thinking, I don't know if we know the answer to that, although women take birth control pills. I guess if you're really forgetful, you could be doing it three times out of a year, but do you have any -- I'm thinking worst-case scenario here. I'm just trying to think --

DR. KATHERINE ROBY: Let's put that scenario in a mature reproductive woman. In that case, probably little because we have the example of oral contraceptives that are doing the same thing. And now there are oral contraceptives that basically inhibit the cycle for months at a time, in a row. So the effect, again, probably very little because we know that those oral contraceptives are safe, and when you stop using them, you reinitiate your cyclicity, which is the important endpoint.

Now, if that were to happen at maybe a different life stage, maybe the impact could be a little bit more, but if we take the mature situation where we assume the cyclicity in the brain, it's reinitiated when you stop the inhibitory effect. It would, again, just push puberty a little bit further and it might hasten senescence or menopause, or the transition through menopause. So whether those are significant adverse events, I guess remains to be decided.

DR. KENNETH PORTIER: This is Dr. Portier. I think that thinking would be good for Section B if you kind of added that in because you guys were focusing on the very short-term exposure and I was sitting there saying well, but the worst-case scenario is we use it during the season and it really impacts the woman. And what I'm hearing you say is well, even if it did that, you know, our current knowledge might seem to indicate, at least in a normal adult woman, if it were operating like an oral contraceptive, the long-term impacts could be mild.

Now, what we don't know is whether atrazine maybe is different than direct estrogen and progesterone in the sense of what it does to the brain and whether it short-circuit something else, and I think I got that implication.

DR. DANIEL SCHLENK: Just to clarify, what question do you think that should be added to?

DR. DANIEL SCHLENK: Oh, B -- on 13(b)

DR. KENNETH PORTIER: B, talking about exposure duration in humans.

DR. DANIEL SCHLENK: Oh, okay. Dr. O'Byrne?

DR. KEVIN O'BYRNE: I think there's a slight difference because when you're on oral contraceptive pill, then you're not hypoestrogenic. It's quite possible that if atrazine is switching off your pulse generator and that

may be common, maybe similar to functional hypothalamic amenorrhea, where you would be hypoestrogenic. So there you could end up with potential for osteoporosis, et cetera, et cetera.

But I'm just mindful of all the seasonal animals that switch off for weeks or months or even half-a-year, and there's no adverse effect there. I mean, they just come back and breed and switch off again, year in/year out. I still feel that even a brief loss of reproductive function would not have any great impact.

DR. DANIEL SCHLENK: Okay. Any other comment? Dr. Horseman?

DR. NELSON HORSEMAN: I think I'd like to speak for the males in the Midwest. Well, this discussion about integrating this into the female reproductive cycle and we haven't talked at all about suppressing LH pulse generation in males, which I don't mind too much seasonal suppression of reproductive function in ground squirrels. I don't think in men that would be such a great thing. There has been no consideration of how you might view this LH surge suppression in males.

DR. DANIEL SCHLENK: Okay. Dr. Roby first.

DR. KATHERINE ROBY: So I guess my comments are assuming that the only effect is at the level of the ovulatory surge, I think that if there's a level of effect at the pulse throughout the rest of the cycle, then it's a different story. Absolutely.

DR. DANIEL SCHLENK: And Dr. O'Byrne?

DR. KEVIN O'BYRNE: I can see a male contraceptive coming on line here, but I think the thing to appreciate here is that a reduction of post generator frequency in the female is serious business because the whole menstrual cycle is exquisitely sensitive to changes in post generator frequency. Us men, our pulse generator can be knocked down quite considerably, and we still produce testosterone to protect our bones and maintain our libido, et cetera, et cetera, and our spermatogenesis. So we're much more robust than women, in that respect.

DR. DANIEL SCHLENK: Nice addition there. Way to pull the foot out of the mouth on that one. Very good. Very, very diplomatic there. Any other comments, with that? Okay. Let's go ahead then and read in Question 14.

DR. ELIZABETH MENDEZ: Question 14 relates to the case study that was at the end of the Issue Paper and the Agency's use of the 95th and 5th percentile of conditional simulations of daily concentration. "Please comment on the use of a 95th percentile of the conditional simulations for providing an upper bound on rolling average concentrations in the case study."

DR. DANIEL SCHLENK: Our lead discussion for that is Dr. Portier.

DR. KENNETH PORTIER: Thank you. This is Ken Portier. While they're bringing up my slides, I just have some slides for illustration. I want to read this into the record.

EPA asked this panel to discuss its epidemiology and PK findings in light of the framework discussed before the SAP in February 2010, the consultation with the panel. I want to say for the record that as I write up this discussion that we had right before lunch, I'll be including in this report a copy of Figure 1 from the February 2010 SAP report, and that Figure 1 is of the framework diagram. So I can refer back to it and kind of put the discussion in context.

In addition, it's very likely that a second figure may be included in the report to allow better illustration of the issues and discussion on this topic. And the second figure is likely to be a slight enhancement or modification to the framework diagram. Of course, I don't have it at this point. So I can't show it to you, for the record. So just watch my hands, and we're going to do this. But I just wanted it for the record.

The panel likes to make sure that nothing shows up in our report that we haven't talked about in the room. And so I wanted to make sure that you were warned that when you see these diagrams, you're not surprised because I was trying to figure out how to write up the discussion without the diagram. I don't think that I could do it.

A lot of what we're going to be talking about here really follows on Questions 1 through 4. If we don't get what we talked about in one to four correct, we're not going to be able to do this correctly as well.

So the AUC water value is a time series that's computed from an input water concentration time series by successfully integrating, numerically, using this trapezoin method, over the period of concern, say four days, and then it uses this as input into computing the AUC plasma value.

The time step for the underlying concentration time series is daily, and the resulting time step for the AUC time series is also daily. The goal is the estimation of human average daily concentration of atrazine over the period of concern. For the four-day duration of concern, concentrations from four sequential days -- in this case, using five actual time points -- are input into the trapezoid method to produce an average AUC value.

For the 14-day duration concern, we used 15 data points. And for the 28, we're going to use 29 data points. An example of this, you can see Figure 21 in the white paper. Again, this is just trying to make sure everybody understands what's going on here because the write-up in the white paper was pretty tight. It wasn't a lot of illustration there.

The first period of concern, for example, uses concentrations from Days 1 to 5, and the second period using Days 2 to 6. So it's really a rolling average kind of thing that's going on. So we have a total of T days of data. We're going to have T-minus four data points in the subsequent AUC time series.

So consider first the daily sampling concentration time series. In Figure 29(a), it shows the actual daily time series curve in a simulated 95th percentile curve and a simulated 5-percentile curve. Passing the actual daily sampling time series through the numerical integration function produces the actual AUC water times.

So what I've done here is I tried to recreate what EPA did in that Figure 29(a), but I don't have all the kriging tools and everything else. So what I did is I took the data from the 1995 Maui River data and I kind of smoothed it out to produce a daily time series. I apologize for those of you way in the back, but right in the middle of all that green is a solid black line and that represents the actual smoothed time series for a concentration. And the green around that is the 1,000 simulated time series with uncertainty or noise.

Just as we did with the geospatial model where you added some variability for those points between the sample points, you get kind of noise. You get 1,000 realizations of what this time series might have really been. And the black line becomes the average. And what EPA did -- and again, I apologize for those of you in the back -- I've just drawn a dotted line across the top of all those greens and a dotted line along the bottom of the greens that represents the 95th -- or the maximum, if you like -- upper bound and the minimum lower bound. I could've done it on the 95th percentile. I just wasn't thinking at 11:30 last night as I was doing it. But that top curve represents -- you can think of the top curve as

representing the 95th percentile of all those simulations and the bottom one representing the bottom percentile.

So you have these kind of three curves. You have the top curve, the 95 max, and the bottom curve, the 95 min in the black line, and then this is past -- so here are these curves and we pass the concentration curve through this trapezoidal integrator, which computes and average, and you end up with these three time series. Kind of one in the middle which represents the mean or the median; a 95th percentile upper and a 95th percentile lower. So all I did was take that dotted line from the previous graph, pass it though the AUC formula and here's what I get back again. This is actually AUC times 24 hours. I didn't divide it by 24; it just would change the scale.

If I did that same AUC process and did it for every one of the thousand simulations, I get something that looks like this. And the point I was trying to make is that you can't take the percentile function from the concentration and just kind of directly pass it through the integrator. You have to integrate every one of the realizations and then compute the upper 95th percentile and the lower 95th percentile because that would be the top of the green line. The top of the green area is a better estimate of that.

So you can see what EPA did. My understanding from reading the document and then talking personally with Nelson, what they did was take the formula and pass it through and I think that produces a very conservative upper concentration AUC estimate. And a better way to do

it would be to pass your simulations through and then compute your statistics on the simulated numbers. I think that's the whole point of what I wanted to make in my comments, and these graphs will all be in the document.

So what I was saying is that the way EPA did it is not the way a statistician would have done it. We would have gone back to the original, individual simulations, run those through the AUC average and then compute the percentiles and use those as our estimates of upper and lower bounds. And I think they'd be more realistic, assuming everything that we discussed in Questions 1 through 4, we get the model fitting right. We get the infill correct and all this other stuff.

I want to make two other points that kind of came to me last night. So part of the discussion we have is where we're doing just averaging. You're saying well, what if I have the daily time series? What if I average and look at four-day averages or seven-day averages or 14-day averages or 28-day averages? Again, these don't show up really well in the back, but the point we were trying to make the other day is that as you average the variability and the process gets decreased, and that as you actually expand the average amount, you could lose some of the patterning. The point I was making, you lose some of the duration of exposure information, so that you can see for four and seven-day averaging it's not bad, but once you get to say, 28-day averaging, you've lost two of the major peaks. They've been averaged into one peak. And that one peak is much broader than the individual two

peaks that we average then. And so you get the wrong picture. So you don't see a lot of interest on our part when you're dealing with kind of an average time series. We'd rather you deal with the daily time series and infill.

And then the second point is that when simple averaging -- all I did here was take four days and averaged. You know, make a window of four days, and get an average. Move it one, get another average. That's what that red graph is. But for the AUC, when we use that trapezoid function, we're doing exactly the same thing, it's just a slightly different averaging. So on the previous slide I took four points and averaged it. On this slide you're really taking five points and you're giving half the weight to the first point and the fifth point, and full weight to the middle three. It's a slightly different average. It's going to be slightly smoother, but I think if I had taken the red line here and the red line on the previous graph, they would've been almost identical.

So what you're really doing when you're computing the AUC is very similar to what you're doing when you're averaging these series. I mean, they're the same thing, except that the AUC is scaled to an hourly value, at least using your equation. So to me, the bottom line here is I wouldn't want to be computing AUCs on five or four-day averages because I basically would be averaging averages. And you're going to end up with the equivalent of a 16-day average instead of what you think you're doing is four-day averaging. It's kind of hard to see that. I think that was the statistical way of looking at

this issue of the 95th percentile and providing an upper bound on rolling average concentration. So with that, I'll rest to the panel.

DR. DANIEL SCHLENK: Thanks, Dr. Portier. Dr. Fenner-Crisp is our first associate on that.

DR. PENELOPE FENNER-CRISP: I couldn't possibly add anything to that.

DR. DANIEL SCHLENK: Dr. Greenwood.

DR. RICHARD GREENWOOD: Me too.

DR. DANIEL SCHLENK: Ditto, I guess, huh? Dr. Griffith.

DR. DANIEL GRIFFITH: Sorry. I have something to add.

DR. DANIEL SCHLENK: I know. Fabulous.

DR. DANIEL GRIFFITH: I agree. It reflects back on some of the discussion from Questions 1 through 4. And again, I'll read this. I made a comment yesterday, which I think links to Questions 1 through 4 as well about sources of error and some of that will come out in here. I do have one table which I will describe.

Identified sources of error noted in the reports include sample size, to which sampling error links, spatial and temporal proximity of samples which alludes to coverage and hence, quality of samples. But I note that spatial proximity does not appear to be used, and the nature of a

given phenomenon, in other words, its inherent variability. Model misspecification should be added to this list, as should measurement error. This latter source of error could be linked to the substitution of kriged or deterministic model-generated imputations into a daily time series, as well as the handling of below detection limit values.

Although it furnishes a tool to ascertain uncertainty and risk, conditional simulation, which utilizes Monte Carlo techniques, does not embrace all of these sources of error. One weakness is that conditional simulation is sensitive to the data upon which conditioning is made. A simulation replicates its conditioning values on average. Frequently, the normal or log-normal distribution is the probability model of choice that is attached to the conditioning values. In other words, the conditioning values may be the means of a collection of normal distributions, one for each day in a time series.

For the specimen atrazine data, a log-normal distribution assumption fails to adequately track the serial correlation in the data and furnishes a poorer statistical description from the monitoring data than selected alternatives statistical distributions.

When considering a 90 percent confidence interval, which is what the 5th to 95th percentiles are referring to, a balance should be maintained between claiming an excessive atrazine concentration when one does not exist, and failing to detect an excessive atrazine concentration when one does exist.

The latter is the riskier of these two situations. For the purpose of atrazine impact analysis, this confidence interval focuses attention on the 95th percentile for conditional simulation. Because rolling average concentration are means, by definition they result from smoothing data so that peaks disappear. Consequently, actual peak concentrations are underestimated. These averages are easier to predict, specifically because they are means.

Figures 27 and 29 illustrate that 14 and 28-day rolling averages may be of little value for decision-making and monitoring purposes, even though they have relatively tight confidence intervals. Although four-day rolling averages are better, the crucial peak missed by them is a substantial peak. This point may be of less importance if the rolling averages represent duration of exposure.

The situation may well improve what the change in the variogram model as well as the change in the probability model employed for the stochastic simulation. A variogram model that better captures autocorrelation effects will better differentiate between the conditional and stochastic components. A probability model that allows more variability has potential to better capture the peaks. And those go back to some of the comments I made earlier.

A standard conditional simulation fails to capture all sources of variation. It assumes that the conditioning, the kriged values, are fixed and true. We saw a

reference to this before. What happens then is that the confidence band shrink to -- and in the classical case -- they shrink to zero at the observe data as though those data values are true values. And then simulate sampling error about these values. It fails to incorporate parameter estimation error. The log-transformed atrazine example time series data reveals the following additional sources of error, and this is the table.

So we see the spherical models being used quite extensively in the paper, and the spherical model has a relatively poor fit compared to other models. It's weighted some of the squared error, is 28, whereas, for the Gaussian, it's 13 and the Bessel function is 12.9. It gives a good nugget estimate because it can't estimate the nugget effect, so it defaults to zero. The Gaussian gives a slightly higher nugget effect. The Bessel function gives something very close to zero, but it's not statistically significant from zero. And the advantage of those two -- which is why they probably capture the pattern better -- is that the Gaussian and the Bessel have a cusp near the origin, and that cusp means that your autocorrelations structure actually goes out a bit stronger than what the spherical is capturing.

The scale parameters between the spherical and the Bessel are about identical. The Gaussian is slightly lower. And then when you look at range, well the spherical has a rang, by construction, the Gaussian and Bessel are asymptotic functions, so you can only get an effective range. The spherical suggests that there is a range of about 22 days. The Gaussian about 14 days, which is a

substantial difference, and then the Bessel actually suggests something more like 37 days. And if you look at the time series, just like the ones we saw, 37 actually seems more reasonable.

The conditional simulation fails to incorporate measurement error. The substitution of selected quantifies for below detection limit values, should have minimal impact upon these results. I saw in the reports where .05 were used in some cases, in the Syngenta six supplemental water system monitoring data, it looked to me like they used .03. I don't think that that's going to make much difference.

The variability in an assay to detect and quantify atrazine in water samples -- and my understanding from what I've reads on EPA websites is that the RaPID Assay Kit, analytical precision standards, apply and they're plus or minus 30 percent. So all of these measures can be off by as much as 30 percent above or below the reported values, which is substantial. And none of that measurement error is actually being captured in what has been done.

The cumulative effect of these errors, at least some of them may compound, which propagate through an analysis, may well invalidate the claim of a 95th percentile. Without tracing the cumulative effects of these different sources of error, perhaps a more representative approach would be to use a 97.5 percentile; in other words, switch to a 95 percent confidence interval to try and adjust for these, but as I said before, I think it would be much

better to see what is compounding, in terms of the error and how it's propagating through the system.

DR. DANIEL SCHLENK: Thank you, Dr. Griffith. Dr. Hayton?

DR. WILLIAM HAYTON: I can't add to that.

DR. DANIEL SCHLENK: Dr. Meek?

DR. BETTE MEEK: I have nothing to add.

DR. DANIEL SCHLENK: Okay. General panel comments? Yes, Dr. Gilliom?

DR. ROBERT GILLIOM: I guess I just want to add the perspective that I think all the statistical issues with the exposure estimates are manageable. And the exposure part of the equation is maybe two or three orders of magnitude easier to come to resolution on than the duration of exposure in organism that's of concern.

It's kind of perspective on the answer rather than adding to the statistics, but I think all the exposure statistical issues -- and there were a lot of great comments made to consider and work in -- but it's relatively manageable.

DR. DANIEL SCHLENK: Anyone else? Okay. Dr. Mendez, do you have any questions of clarification? Or Mr. Thurman?

DR. NELSON THURMAN: I just want to say I agree with Bob Gilliom that as I was listening to this, I was thinking those are things that we can account for, we can do it.

DR. DANIEL SCHLENK: Sounds promising. Okay. At this point, what I think we'll do is we'll just go around the table once. This is your chance to provide your final comments, anything that you would like to add to the record. Then we'll go back to the Agency and have some closing comments from them as well. Dr. Hayton, do you want to start us off?

DR. WILLIAM HAYTON: Nothing too profound. I guess the frustration is after so many years of looking at atrazine, we still don't really understand its chemical initiating event and all of that toxicodynamics. And if we knew that story, I think we would be quite a bit further down the road to solving a number of the questions.

DR. DANIEL SCHLENK: Dr. Greenwood?

DR. RICHARD GREENWOOD: I think we've seen a lot of progress over the last couple of years on the pharmacokinetic side. That's for sure. I feel quite confident with the way that's going forward, that we're going to have tools that are going to be able to help us with the internal exposure, just as we're getting the external exposure better defined. But I just echo what Dr. Hayton said, I really feel that until we can get hold of this primary lesion, at least one of them and maybe more, then we're still in the dark.

DR. DANIEL SCHLENK: Dr. Meek?

DR. BETTE MEEK: Yeah. I'm really encouraged by the progress on, for example, development of the physiologically based pharmacokinetic model. I think integration of information at the moment is a challenge, again, for the reasons that others have mentioned, but perhaps not insurmountable, given how much progress was made on the PBPK side in a very short period of time.

DR. DANIEL SCHLENK: Dr. Fenner-Crisp?

DR. PENELOPE FENNER-CRISP: I've been mulling over again the issue of determination of whether or not there is life state differences in sensitivities. And thinking again about the methodology that was used to determine whether or not that exists. And it’s coming out in my mind to be an apples and oranges kind of thing.

On the one hand, one's using an apparently not adverse effect precursor event to an apical event in the adult, the four-day LH surge suppression, comparing it against NOAEL's and LOAEL's for apical effects, generated in data for other life stages than the adult. And I'm wondering if it might not be appropriate to also do an analysis where you select an apical event in an adult that's a consequence of the LH surge suppression and compare it with the set of NOAEL's and LOAEL's that were used in the first instance; and then ask the question again, "Do you see a differential sensitivity", and compare it with the original analysis. It may well turn out to be you would

conclude the same thing. Obviously, in that amount of time, I haven't done that analysis, but in might be an interesting exercise to see if you could, in fact, come to the same conclusion.

DR. DANIEL SCHLENK: Dr. Jerde?

DR. TRAVIS JERDE: Yeah, I agree. It's great to see progress on pharmacokinetics because I think what those of us on the cellular and molecular side need is to be able to conduct mechanistic studies on how atrazine and its metabolites may affect cells, tissues, and organisms as a whole; using concentrations that are reasonable, in terms of exposure and ground water exposure to effected populations. And that's kind of what I think this side of the field has been waiting for. And I also think maybe that's what epidemiology has been waiting for as well because they need to -- it's clear that we're not ready to make a conclusion, definitely yet, particularly in terms of cancer or non-cancer effects until we know what the exposure are and we can effectively study it, epidemiologically in the human and molecularly in models.

DR. DANIEL SCHLENK: Dr. Timms?

DR. BARRY TIMMS: Yes. I concur with Dr. Jerde that I think it's important to look at molecular mechanisms of action and with regard to that, it's also going to be important to determine the actual level of human exposure so we can be working in a framework of exposure that we can mimic in model systems.

DR. DANIEL SCHLENK: Dr. Roby?

DR. KATHERINE ROBY: I really just agree with everything that's just been said down the table, understanding the mechanism, the actual exposure and the kinetics of the relevance to those two endpoints. That's all I have. Thank you.

DR. DANIEL SCHLENK: Dr. O'Byrne?

DR. KEVIN O'BYRNE: Well, I remain optimistic because the levels of atrazine that are needed to effect elements of the reproductive system are just extremely high. And in addition, my thoughts that the reproductive system has got such a huge margin of robustness that I have very little disquiet about atrazine.

DR. DANIEL SCHLENK: Dr. Akana?

DR. SUSAN AKANA: Much to my amazement, I concur with Dr. O'Byrne. When I first was invited to the panels and started reading the literature, every alarm in my head was going off, in terms of stress and energy imbalance. And through the long process, I am really persuaded that the margins of safety -- or at least the effective doses that are affecting animals are not a major concern for my favorite endocrine axis.

DR. DANIEL SCHLENK: I'm glad you're relaxed. Bob Gilliom.

DR. ROBERT GILLIOM: I guess one of the main gaps in our knowledge that’s frustrating to me -- and this has come

in a few side discussions -- is that there's so much more we could do with available data to look at the relationships between the population served by specific drinking water supplies and what's actually in the supplies. So I don't know enough about whether I'm calling it right, but to me, it's a category of an epidemiological study that can now be done fairly comprehensively and it's one of the main missing pieces, from what I hear, of linking all these theories and possibilities to increased incidence of adverse outcomes in people.

So the real life experiment is out there. We haven't sampled it or analyzed it. I would say we probably have sampled it, but we haven't organized and analyzed it yet. So whoever is to do it, I think there's a lot that could be done by mining the data and evaluating the relationships between these patterns of outcomes and the actual exposure that's happened over the last couple of decades. So that's my main point.

The secondary point, I would add that has just kind of come up in thinking about the future is that we're using past records of observations to reconstruct a lot of assumptions and understandings about future exposure and how to monitor it and so forth. And I think what we've seen with other chemicals -- and atrazine kind of stands out as an exception -- is that usually these things are going through changes over time that span over several to a few years and systematic downtrends or uptrends.

So I think I just want to put in the marker that we have to be aware that when we design the monitoring approaches, we have to anticipate that there are likely going to be long-term changes and shifts in regionality, perhaps, that just happen because of changing crop patterns - maybe genetically-modified crop come in and market forces, things like that, replacement compounds. So that we just have to have that built into the thinking of how we track future exposure and not assume it's a steady state. Thank you.

DR. DANIEL SCHLENK: Dr. Griffith?

DR. DANIEL GRIFFITH: Well, I guess I'd like to echo two things that were raised yesterday, and one has to do with when we look at the impact on drinking water, what are the actual dosages for exposure and will they really have a consequence when humans are exposed to them?

And a second point that was raised that I think needs to be thought about is sort of void in the analysis so far, of the water data, is the whole notion of repetition. If you have repeated exposure, even at small levels, but it has cumulative damage, then you need to be looking at long-time series of water levels - atrazine levels in water, not just one year, and then the confounding factor that's going to impact on that is people move all the time and so what happens as people move in and out of these water systems.

DR. DANIEL SCHLENK: Okay. Dr. McManaman?

DR. JAMES MCMANAMAN: I was struck last time and continue to be struck this time by the difference between what the epidemiology data is telling us and what the toxicology data is telling us. And I'm concerned that we really don't have a way of integrating those two and we may not be using the correct animal models by focusing a lot on the rat. It's a convenient system. We understand its biology pretty well, but I think we might be getting mixed signals about mechanisms. So it makes it really difficult for those of us who are interested in mechanisms to say very much about it.

DR. DANIEL SCHLENK: Dr. Horseman?

DR. NELSON HORSEMAN: The only thing I would add and I would echo the thoughts of some of the other panel members that it seems obvious that the EPA, and for that matter, the participation of the registrants and everyone else is doing a good job of regulating this in a safe way.

But I do think that one of the issues I don't think we have come to grips with is that the mode of action and maybe in both the cancer side and this other reproductive toxicology side, but maybe not, there's an implication that this has to do with effects that are happening in the brain, particularly in the hypothalamus. But we really don't have any studies, at least good studies, focused on that organ. And I would think that if that's the mode of action everyone feels like is relevant, then direct studies on the brain as an organ -- and the hypothalamus as an organ -- need really to be prioritized.

DR. DANIEL SCHLENK: Dr. Young?

DR. HEATHER YOUNG: I just want to say I was encouraged by the fact that the Agency undertook a comprehensive review of the cancer epidemiology literature. I think it was a major step in the right direction. So I hope that they continue to do that. Also to echo Robert Gilliom's comment in that there are big gaps in the literature with regard to community exposures and the data there, the water monitoring data is there. There are cancer registries, there are birth defect registries. And so it's a matter of really combining those and getting some information on what some of the reproductive effects may be, and also looking at the community effects for cancer.

DR. DANIEL SCHLENK: Dr. Bove?

DR. FRANK BOVE: Ditto. I don't think it would take that much effort either, to do these types of studies. It could be done rather quickly. The states have the data. Simple data linkage would be important. Moving away from the studies we evaluated last time, the ecologic study and doing individual level studies using the cancer registries and birth defect registries.

DR. DANIEL SCHLENK: Dr. Gold?

DR. ELLEN GOLD: Well, being toward the end here, I'm not sure I have a lot to add. I concur with just about everything that's been said. I think the importance of looking at community water supply exposure is important because it's

chronic low-dose, as opposed to the high-dose that you see either in the applicators or the manufacturing workers or in the animals for that matter. So it might be more relevant.

I just wanted to make one comment about the potential effect on menopause, age at menopause, which seems like it's not all that important, but there actually is a huge literature on age at menopause being an indicator for lots of long-term disease risk and life expectancy.

But I would also note that oral contraceptive use in high parity usually delay with the age of menopause, which is usually a good indicator of long-term survival, life expectancy, early age at menopause associated with smoking is usually a bad thing.

DR. DANIEL SCHLENK: Dr. Klaine?

DR. STEPHEN KLAINE: I wanted to comment on challenges with analyzing episodic exposure data and make the comment that, or suggest that in addition to looking at durations of exposure, you might also want to look at the duration of the periods between exposures. And they might be just as important.

I think that as you get to the point of understanding the atrazine receptor better and the reversibility of that particular process, it'll probably better drive how you analyze your exposure data. So it's just something to keep in mind.

DR. DANIEL SCHLENK: Dr. Chambers?

DR. JANICE CHAMBERS: I'll just kind of go back to the initial comments that were made at the first part of the table here; encouraged by the pharmacokinetics progress; kind of discouraged about not knowing what the mechanism is and do hope that a little bit more about that is known so that a good human relevant risk assessment can be done when you get to that in a couple of years.

DR. DANIEL SCHLENK: Dr. Portier?

DR. KENNETH PORTIER: I just wanted to point out, this is probably the first time in about 60 years that every member on the panel has had closing remarks to make. I think it speaks to the fact that everybody is engaged with EPA on this. The panel that has been here a number of times, those of us on the permanent panel, really want to kind of see this work. If we haven't answered all of your questions, it's probably because those questions can't be answered at this point in time and that there's a lot of research, epi and mechanism research, that needs to be done that we just kind of keep pointing at.

Unfortunately, we don’t know who's going to do it and who is going to pay for it, but if you're going to answer the questions, someone is going to have to do it and someone is going to have to pay for it.

DR. DANIEL SCHLENK: Yeah, I'll do it. Just closing comments: I also applaud the Agency's framework for this approach as we've had several panels, recently even, that have

pursued the adverse outcome pathway approach. I think it's the way to go.

I would like to encourage the Agency, even though things may not be going as quickly as possible, but in my opinion, I think you're on the right track. It's just a matter of time to get those linkages that are out there that we've seen with other compounds, such as some of the cholinesterase inhibitors and thing of that nature, where there's been some success in that capacity. I think it's just a matter of time, I think, before the top down and bottom up actually meet in the middle, perhaps, in terms of the effects that are present. So I'm encouraged.

As usual, when we come to these things, the amount of information we learn is amazing. So I'd like to applaud the Agency for the presentations and the questions that you are asking. I think that's very encouraging. With that, I'll turn it over to Dr. Mendez, if you have any closing comments for yourself and the Agency.

DR. ELIZABETH MENDEZ: On behalf of the team and the Agency, I want to express our deepest gratitude to the panel for their thoughtful considerations and deliberations during this process.

As Dr. Portier alluded to, there are a lot of questions. There are some questions that you couldn't answer because the data is just not available. I'm personally a little bit encouraged by the fact that I think we're wrestling with the right issues. That, to me, is encouraging that we are looking where we need to be looking at.

As you may have noticed, during the past few days I have been furiously taking notes. You have given us a lot to think about, and as we move forward with this process, we will take your advice under consideration.

As always, we remain committed to keep vigilant to any new developments, and as those become available, we will try to integrate it into our evaluations and we appreciate all the hard work. I mean, we understand that 600 pages plus of documentation to go through is a lot and the fact that you've gone through it with such care and such precision, we truly appreciate that.

DR. DANIEL SCHLENK: Thanks. I'd also like to express my appreciation to the panel for working together as well as you have. You know, on the permanent panel, we see a lot of these things and often times there's not the congruity or collaboration that takes place. And it's obvious you guys have gone the extra mile in working together to get us through a fairly lengthy process about a day ahead of schedule, and I really appreciate that and I appreciate you working together for that. That doesn't always happen and my thanks, at least personally, for that.

I'd also like to appreciate or give thanks to the EPA, again, for their staff for the presentations, the PIs for the research that they are doing, and the FIFRA staff, Laura Bailey and the staff for making it as easy as possible to get here and to get away and making our stay as comfortable as possible. They've done a great job for that. With that, I'll turn it over to Joe Bailey.

JOSEPH BAILEY: Dan certainly covered all of the thanks that I wanted to make, but first and foremost, I do want to thank the panel for their commitment to come here and take the time out of their schedules and prepare as well as they did for the meeting; the time at the meeting as well as the follow-up work we'll be working on to get the report finished. Thanks to EPA, OPP, and ORD for working with us to coordinate all of this information. And the public commenters for bringing their thoughts forward. The final report, we will have it done within 90 days after the meeting. So thank you all very much.

DR. DANIEL SCHLENK: Meeting is adjourned.

(Whereupon, at 2:15 p.m., the meeting was adjourned.)

* * * * *