U.S. ENVIRONMENTAL PROTECTION AGENCY

FIFRA SCIENTIFIC ADVISORY PANEL

OPEN MEETING TO CONSIDER AND REVIEW SCIENTIFIC ISSUES ASSOCIATED WITH THE AGENCY'S ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP) PROPOSED TIER-1 SCREENING BATTERY

EPA CONFERENCE CENTER

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DR. HEERINGA: Good morning, everyone. I'd like to welcome you to the first day of our multiple day session meeting of the FIFRA Science Advisory Panel on the topic of the Endocrine Disrupter Screening Program Proposed Tier 1 Screening Battery. I'm Steve Heeringa of the University of Michigan. I am the current chair of the FIFRA Science Advisory Panel and I'll be chairing this meeting. I'd like to go around the room here, around the table and have the panel members, our panel of experts, introduce themselves to you, give their name, affiliation, a little bit of their background and specific expertise. I'll begin with Dr. Portier.

DR. PORTIER: Good morning, I'm Ken Portier, director of statistics at the American Cancer Society National Office in Atlanta. I'm an applied statistician and a member of the permanent panel.

DR. CHAMBERS: I'm Jan Chambers, I direct the Center for Environmental Health Sciences at Mississippi State University. My area of expertise is pesticide toxicology with emphasis on the pattern of neurotoxicology and I'm a member of the permanent panel.

DR. ISOM: Good morning, I'm Gary Isom from Purdue University, professor of toxicology. My area of interest is chemical induced neuro-degeneration and I am a permanent member of the panel.

DR. BUCHER: I'm John Bucher, I'm the Associate Director of the National Toxicology Program. I'm a toxicologist by training, I have interest in carcinogenosis and general infusion toxicology and development of alternative methods.

DR. DELCLOS: Barry Delclos from the FDA's National Center of Toxicologic Research in Arkansas and I have research interest in endocrine disrupters and carcinogenosis.

DR. ELDRIDGE: Charles Eldridge, Wake Forest University, in North Carolina, Department of Physiology/Pharmacology. Interests are in neuro-endocrine steroid hormones, reproductive biology.

DR. DENVER: Good morning, I'm Bob Denver from the University of Michigan, Ann Arbor and

I'm in the Department of Molecular cellular developmental biology and also ecology and evolutionary biology and my interests are in developmental neuro endocrinology, thyroid stearic hormone interaction and also amphibian metamorphosis.

DR. VANDENBERGH: I'm John Vandenbergh, I'm a doctor of zoology at NC State University. My research interests for several years has been on behavioral endocrinology and its effects upon mostly female estrogen systems.

DR. LASLEY: I'm Bill Lasley from the University of California at Davis at the Center for Health and the Environment. My interest is in reproductive toxicology and development of methods used in population based studies.

DR. COOKE: Good morning, Gerry Cooke from Health Canada, in Ottawa, Ontario, Canada and I'm a reproductive toxicologist with expertise in steroid genesis, steroid metabolism and gene action.

DR. ZOELLER: Hi, I'm Tom Zoeller, I'm a professor of biology at the University of Massachusetts in Amherst, and my research interests are in thyroid hormone action, mainly on brain development, on prenatal brain development.

DR. BROWN: Terry Brown, from Johns

Hopkins University, and the department of biochemistry and molecular biology. My main areas of interest are in male reproduction specifically in androgen action and androgen receptor.

DR. BELCHER: Good morning. I'm Scott Belcher, I'm from the University of Cincinnati, the department of pharmacology, and my primary research interests are in estrogen receptor signaling and the role of endocrine disrupters.

DR. KULLMAN: Good morning. I'm Seth Kullman from North Carolina State University, Department of Environmental and Molecular Toxicology. I'm a molecular toxicologist with an interest in nuclear receptors and gene inhalation.

DR. HEERINGA: We have one additional panel member, David Furlow, who is on his way here from California we understand, due to a missed flight last evening, he should be arriving this morning so we'll have him introduce himself when he has arrived. At this point in time I'd like to introduce the designated Federal official for this meeting, Jim Downing.

MR. DOWNING: Good morning, I'd like to welcome everybody to this meeting of the FIFRA Scientific Advisory Panel. I'm Jim Downing, the designated Federal official for this particular SAP

meeting. As you know this is the first day of a planned four day meeting, on the endocrine disrupter screening program, EDSP, proposed Tier 1 screening battery. As the DFO for this meeting I serve as the liaison between the panel and the Agency and I'm responsible for insuring that all provisions of the Federal Advisory Committee Act are met.

I want to thank Dr. Heeringa for introducing the panel and acting as the chair for this meeting, and I want to thank the members of the panel and public for attending this meeting today, as well as all the people from EPA who will be giving presentations at the meeting. We mentioned briefly the function of the SAP and a little bit about the panel composition here today. By way of background, the FIFRA SAP is a Federal Advisory Committee under the FACA, F-A-C-A, provides for independent scientific peer review and advice at the Agency on pesticides, pesticide related issues regarding the impact of proposed regulatory actions on human health and the environment.

The FIFRA SAP only provides advice and recommendations to the EPA. All decision making and implementation upon remains with the Agency.

A brief word now about financial

conflicts of interest. As the designated Federal official for this meeting, a critical responsibility is to work with the appropriate agency officials to insure that all appropriate ethics regulations are satisfied. In that capacity, the panel members are briefed on provisions of the Federal Conflict of Interest laws. In addition each participant has filed a standard Government Financial Disclosure report. I along with the Deputy Ethics Officer of the Office of Prevention, Pesticides and Toxic Substances in consulting...in consultation rather with the Office of General Counsel, have reviewed these reports to insure all ethics requirements are met.

Public commenters - for members of the public requesting time to make public comment, please limit your comments to five minutes unless prior arrangements have been made. We do have a number of public commenters here today that have made arrangements and will take more than five minutes. For those that have not been preregistered for making comments today, please let me know or another member of the SAP staff know that you are interested in making public comments this afternoon.

Now a word about the public docket. There is a public docket for this meeting, all

background materials, questions posed to the panel by the Agency and other documents related to the SAP meeting are available in the public docket. Slides of today's presentations that will be available within a day or two, perhaps even by the end of the day, background documents are also available on the EPA website for the FIFRA SAP. The agenda prepared for this meeting lists contact information for the docket so you can refer to the top of your agenda to see the docket information.

FIFRA meeting minutes. After this meeting is conducted, the SAP will prepare a report consisting of the responses to questions posed by the Agency, considering all background materials, presentations and public comments. The report serves as the meeting minutes, and they will be completed within ninety days after the close of this meeting. They will also be, the final report will also be made public, both on our website, the FIFRA SAP website, as well as in the public docket for this meeting.

Again, I wish to thank the panel for their participation It's not always easy to take out a whole week out of one's schedule so I certainly appreciate everybody's efforts in participating in this meeting which I think will be very interesting and I

look forward to both a challenging and interesting discussion in the next two or three days.

DR. HEERINGA: Thank you very much, Jim. It's time I think we're set to begin and I'd like to open by introducing Dr. Linda Phillips who is Director of the Exposure Assessment Coordination Policy division of the Office of Science Coordination and Policy.

DR. PHILLIPS: Good morning. As the Chair said, I'm Linda Phillips with the Exposure Assessment Coordination and Policy division within EPA's Office of Science Coordination and Policy. Our acting director of the Office of Science Coordination and Policy had hoped to be here today to welcome the panel but unfortunately she is on travel, so she asked me to express her appreciation to the panel for participating in this important meeting.

As you already know from the materials you've read, this is an important milestone for the endocrine disrupter screening program. We have worked for many years on the development and validation of a candidate assay for the battery, Tier1 battery and now we have proposed a Tier 1 Battery and we look forward to the comments that we receive from the SAP on the adequacy of the battery in covering the mode of action for endocrine.

The development and validation of the candidate assay has been a collaborative effort between our SCP scientists and ORD scientists and we have a number of them here today so I'd like them to get up and introduce themselves so you know who the scientists are that have worked on this process and then I'll turn it to over to Gary Timm who will give an introduction of the ESP and then Les Touart will talk about the proposed battery.

DR. HEERINGA: Thank you, Dr. Phillips. You can introduce your staff.

DR. RAY: I'm Earl Ray, EPA Research Triangle Park, research laboratory.

DR. WILSON: Good morning, I'm Vicki Wilson, reproductive tox division and Office of Research and Development.

DR. FOLKER: Tammy Folker, EPA, Research Triangle Park, reproductive toxicology division.

DR. COOPER: I'm Ralph Cooper, reproductive toxicology division, Research Triangle Park.

DR. LAWS: Susan Laws, reproductive toxicology division, Research Triangle Park.

DR. ANKLEY: I'm Gary Ankley, ecology division, Duluth, Minnesota.

DR. TEASY: Joe Teasy, ecology division, Duluth.

DR. FRANCIS: Anne Francis, I am the National Director for the Endocrine research program.

DR. HEERINGA: Thank you very much, any additional contributors please?

DR. MILLER: I'm Jessie Miller, I'm from OSCP.

DR. GROVE I'm Christiana Grove, Office of OSCP.

DR. BURTOFF: John Burtoff, OSCP.

DR. HALL: Dr. Hall, OSCP.

DR. CULYA: Jim Culya, OSCP.

DR. HEERINGA: Well, thank you very much, I think it's very useful to introduce the people, the staff, scientific staff who are working on this and I appreciate that. Gary Timm I think is going to do an overview for us.

DR. TIMM: Yes, thank you very much, good morning. As you'll hear two talks this morning, the one that I will give which is an overview of the endocrine disrupter screening program and I will start off by reminding people the statutory mandates and to fax recommendations and tell you about the development validation of the assays and the programs with clearly

an emphasis on Tier1 and then talk about some of the other mutation activities very, very briefly so that you get some idea of the other elements in the program and then last we will follow up with a presentation of the screening battery and illustrate how the screening battery works with a couple of model compounds or mode of action.

Try the computer into the microphone. EPA statutory authority dates back from 1996 with the passage of the Food Quality Protection act which amended the Federal Food Drug and Cosmetic Act and it actually mandated EPA to develop a screening program using validated assays to identify pesticides that may have an effect in humans similar to the effect produced by naturally occurring estrogen but that law also gave us some authority to extend that to other endocrine effects, made by the Administrator, and in language that I am sure was written by a lawyer, not a scientist, it could include non-pesticide chemicals that have an effect cumulative to that of a pesticide in which a substantial number of humans were exposed.

About the same time, I think it was actually about three weeks later, the Safe Drinking Water Act Amendment of 1996 was passed and they again gave EPA authority to require testing of chemical

substances found in the source of drinking water to whom a substantial population was exposed and this was really an add on, a build on to the basic authority in 408C, it would operate in the very same fashion.

Well, in anticipation of the passage of the Act back in May of 1996, EPA gathered a number of interested parties, stakeholders, together and asked if they would be interested in forming an advisory committee to guide us through this process to help us select screens and tasks for the screening program. That committee was chartered October 16, 1996, it had broad representation, there were members of the pesticide industry, the chemical industry, state government, federal government, health and environmental people so I think that the expertise and the breadth was quite considerable.

At their very first meeting in December of 1996 there was some debate about the scope of the program and there was a quick consensus reached that it should expand beyond the statutory minimum of estrogen. They said yes, although estrogen probably has gotten the most press, the most play, sometimes what you're seeing really is not a feminization of males but really a demasculinization so you need to look at androgens too because what you're sometimes seeing is anti-

androgens and clearly thyroid had a huge impact on development as well so they studied them.

Scientifically minimum program to be really credible needs to look at all three hormone systems and they said all the acts specified human health in the Environmental Protection Agency and the best evidence that we have for effects are really not on humans, but on wildlife. You clearly should look at ecological effects within the scope of this program and we know that chemicals other than pesticides are potential endocrine disrupters and so you need to look at the broader universe of chemicals to which people are exposed. If you're going to do all of this you really need to have a two tier approach to screening.

In Tier 1 which should be composed of in vitro and in vivo screens, you will detect the potential of chemicals interactively and endocrinally. Chemicals that are positive in Tier 1 then on the weight of the evidence basis is you have multiple assays in Tier 1 would then go on to Tier 2 which would be multi-generation studies comprised of a range of taxa and they would be designed to provide the kind of dose response information that you would need for a hazard assessment.

EDSTAC laid down for itself criteria for

the Tier 1 screens. It says it should, they should be able to detect all known modes of action for the endocrine end points of concern and they recognize that a battery was required because and a battery composed of both in vitro and in vivo systems because simple magnetic screens didn't exist for all modes of action, and so you needed to include the more complex multi model assays for Tier 1 and this is to some extent even a bit of a concern today that you will see a spectrum of opinion on and I think this afternoon you will hear some discussion about this formula. EDSTAC clearly wanted to minimize false negatives, EDSTAC clearly wanted to look at the full life cycle of the hormone from synthesis, to release in the blood stream, to finding its way to its target tissue, binding with a receptor, the downstream consequences from that binding and finally metabolism and elimination of the hormone, because they said at any of these various points there's a potential for interference with the system.

They felt that you should include a sufficient diversity among endpoints, to permit a weight of evidence conclusion, so, to that end there are multiple endpoints, the in vitro assays and the assays and the end points are complimentary.

EDSTAC, as I mentioned before, clearly

wanted to maximize sensitivity to minimize false negatives and that of course doesn't mean you can forget about false positives but that was the bias that was built into the system and they noted that the in vitro mechanistic screens are highly sensitive, but that as I mentioned before the in vivo apical screens were necessary to encompass all the known modes of action and to take metabolic activation into account and knowing that there are some differences between species, it should include a sufficient range of taxonomic groups to represent the differences in the endocrine system and metabolism and to that end fish are included because they are fish, they differ to some extent in hormones, they clearly differ in the way that they are exposed and they also differ to some extent in metabolism.

So the battery of assays that were recommended by EDSTAC were the estrogen receptor binding in rat uterine cytosol or the transcriptional activation system, the androgen receptor binding in using rat prostate cytosol or androgen transcriptional activation system. A steroidogenesis assay utilizing minced rat testes as the source of the enzymes and then the in vivo components, the uterotrophic, the Hershberger, a pubertal female, an amphibian

metamorphosis assay or a thyroid, and the fish gonadal recrudescence assay, fish are aquiescent and then they become up to reproductive capacity when the APG axis turns on In the springtime.

The, the EDSTAC also noted that these aren't the only assays that were available. They looked at a large number of candidates and they said the pubertal male might be a good substitute for the pubertal female and the adult male might be also a substitute for the female. But if you do use one of the male assays you would need as a complement, aromatase because the male is not a very good model to detect, not very sensitive to detect interferences with aromatase.

And they didn't recommend a specific protocol for in utero lactation but that was a goal. They said, you know, that would really be the best thing if we could get a screen that looked at the in utero phase and of course this panel looked at that issue and gave us a recommendation that there was nothing that was available, and EPA searched for a number of different protocols, nothing that really looked like a screen, it was a more complex, more expensive assay than the screening profiles.

For Tier 2 EDSTAC recommended the

mammalian 2-gen with some endocrine endpoints added to it, an avian reproduction test, amphibian growth reproduction fish life cycle and the invertebrate mice life cycle. EPA accepted the EDSTAC recommendations. We published that on December 28, 1998 in the Federal Register and proposed that as policy and the basis for the EDSP, stating that we thought that the recommendations that EDSTAC gave were scientifically rigorous, they represent the best science at the time and we felt that obtaining a consensus from such a wide group of stakeholders was quite a remarkable feat and was quite compelling.

EPA then, and I don't have a slide on this, but we went to the SAP in 1999 with that program and got some additional advice from the SAP, the SAP said that we should focus on about fifty to a hundred chemicals in the initial group to really try out the Tier 1 battery. The EPA went from that point on, we also looked at some items between what the EDSTAC recommended and we found that the existing off the shelf assays really were not suitable for use to detect chemicals. They were great for pharmaceuticals but the pharmaceutical industry had not optimized them to detect compounds of lower focus.

So we went forward and carried out three

activities to implement the program, priority setting or picking chemicals for that first tier of 50 to 100, jumping procedures that would be needed to implement the law, typically the Agency does that, the Agency has to develop detailed procedures to implement authorities that were granted by statute and then the biggest activity of all of course was the development and validation of the assays.

I'll mention the first two briefly just sort of to provide perspective. In terms of priority setting we, because the heightened chain did not work and we didn't feel like we had the time and resources to, to optimize it, we went ahead and proposed an approach and after comments adopted it, it was based strictly on exposure and we were looking at pesticide active ingredients, looking at food, water, residential and occupational exposure pathways and high production volume, inerts, pesticide active and HPB inerts looking at human and ecological effects biomonitoring data and also data that showed the presence of chemicals in water and air.

We then, using that approach, drafted a list of chemicals for initial screening and published that on June 18, 2007 for public comment. That list contained sixty-four pesticide actives and nine high

production volume pesticide inerts and again because these chemicals were chosen strictly on the basis of exposure, not at all habit information they are not a list of known or likely endocrine disrupters.

In terms of procedures in parallel with this other activity, we drafted a policy as to how to implement this authority, published for people to comment on the pest ordered templates and issued information collection requests which is required anytime an Agency requests information from a regulated entity and that was published on December 13 ,2007. In that notice EPA said it would direct test orders under 408P and also using its Authority under FIFRA 3(c)2(b) to the technical registrants for the active ingredients.

For the inerts it would send orders under the FFDCA408P authority for manufacturers and importers and now the big activity. The validation. The validation is required not only by FFDCA, the part of the law that I read to you in the very beginning, 408P, but also by the ICCVAM authorization act of 2000. It was recommended by EDSTAC and it was later endorsed by OECD, Organization for Economic Corporation and Development which now as a matter of policy says that new test guidelines need to have validated methods as

their basis. The validation has been defined as an assessment of the reliability, high relevance of a test method for a particular purpose, relevance being the extent to which test methods will correctly predict and measure biological effective interest and reliability to the extent to which a test can be performed reproducibly within laboratories and among laboratories and over the course of time.

A number of principles were set down by ORD at its conference back in 1996 by ICCVAM, by ECVAM, generally agreed upon that one must clearly articulate the scientific and the regulatory rationale for the method. One must describe the endpoints of the test method to a biological effect for the toxicity of interest, that there should be a formal detailed protocol available such that a competent laboratory who has not run the assay before can in fact follow that protocol and conduct the assay. There must be an assessment of variability again within labs, between labs, and over time. An assessment of the performance of the assay with known reference chemicals. It's like having the answer key to a quiz, giving the quiz. You must describe the limitations of the assay, pay attention to data quality issues, and typically conduct the validation in GLP laboratories, and then make the

data available for public inspection and send it to an independent scientific review.

Now even with the publication of these methods and they were really geared initially to assist the development of alternative assays; that is, methods, in vitro methods that would be replacing in vivo methods. The community developing these alternative methods was having difficulty and so as a matter of practice as they gained experience in the literature for validating alternative methods some of these concepts came out. The alternative test method should consist of two parts, the test system and prediction model. That the prediction model is an algorithm for converting the in vitro data into a prediction of in vivo toxicity and the validation is essentially in this approach, a test or measure of the performance of the prediction model and that the prediction model needs to be developed prior to validation because it has to be a prospective evaluation of the prediction model, not a retrospective one, and that the test set of chemicals used in validation should be different from the set used for model building so you have the idea again of a train set and a test set.

Well, there was a very, very different

set of approaches, a different discipline for the eco toxicity test methods and I would guess that that's been around for what probably twenty five, thirty years. They were conducting what they called ring tests where a new method would be tested across laboratory with a limited number of chemicals to test the reproducibility of a method. The relevance was assumed; they did not worry about relevance because they said we're testing him in an environmentally relevant species. They didn't have a prediction model because they were relying on direct observation of the toxicity of interest looking at critical life processes, and they based their standardization after the fact on the protocol assessment rather than on prevalidation. So recognizing that more guidance needed to be given in the area of test method validation OECD decided to develop what they called Guidance Document Number 34, to provide practical guidance on the validation test methods. And it was really to provide some not only guidance but flexibility in applying the criteria and guidance document 34 recognized that the amount and kind of information needed and the criteria that would be applied to a new test method would depend upon the type of test. Its purpose and use and what's known about

the test, how long it's been around, whether it's a mechanistic test, et cetera.

Guidance Document 34 I think sharpened the debate but I don't think it settled the issues. Guidance Document 34 came along in 2005, we had already been in business trying to validate things for some time and we looked at what we were doing in light of the guidance that we were receiving from our advisory committee as well as from OECD and said Tier 1 is for screening, it's really for the detection of potentially interactive endocrine system. And it's a battery of assays, it's not a single assay and the assays are already there to complement each other and the strength of one assay should offset the weaknesses in another. There...even though they've been around for thirty or forty years, in one sense they're new assays, they have not been validated before and they're not replacements to the existing streams so that means we have a limited number of reference chemicals, and frequently the best reference chemicals are not pesticides or chemicals we find in the environment but in fact pharmaceutical compounds and there are practical limitations regarding the numbers of tests that we run during validation and the numbers of chemicals that we test especially when you get into in vivo methods.

So we said it's important for us to challenge the assay with carefully selected benchmark chemicals. That number of chemicals will vary with the assay, maybe twenty, fifty for in vitro screens but it's going to be much more limited to the in vivo screens, five perhaps to fifteen, and much more limited than that for Tier 2.

The goal we set based upon advice we were given that somewhere around ten to twenty five percent of the chemicals would be negative and we didn't always meet that goal. I think that the probably the clearest example of not meeting that goal were the pubertal assays where we considered probably close to a hundred chemicals, picked one to run as the negative, and found that in fact it did not...it was not a negative. And that was frustrating, but in fact when you look at the assay and you look at the thyroid active compound assay and they don't interact with the estrogen and androgen system and vice versa and so there is still evidence of specificity in that assay even though we failed to find that clear negative that we wanted to find.

So in validating the assay, EPA asked the question is the variability satisfactory for the purpose and with the results that we wanted to obtain

with the benchmark chemicals. How do we proceed, what is the process, this is the process that was found in the ICCVAM 1997 document. First of all I'm start talking test method development. You go into the library because you can save a lot of time in the library versus going to the lab so we had scientific literature review prepared to look at the relevant kinds of assays and recommend to us what protocols we've got were the best to proceed with. Took that protocol, tried to demonstrate how feasible that protocol worked, demonstrate its relevance to the end points, and then work on optimizing the conditions of the protocol and when we were satisfied that we could do that, then we went into an inter-laboratory study with three to five laboratories, and then sent, we collated all that information, put it into an integrated summary report and all that information in the back up studies went to the peer review panel and they gave us their report, we developed a response to comments to the peer review panel and then we moved to the fifth stage which is regulatory assessments which is really where we are now, the proposal to the Tier 1 battery, your review of that battery, advice to us and then adoption of a Tier 1 battery.

There was a big challenge of how to get

everything done and we worked with OECD on the guidelines of international interests. The methods there would be developed and validated through OECD and interestingly enough the DUX was a leading country on most of those test guidelines. The test methods that were not of interest to other member countries in OECD were developed and validated by EPA with advice from our advisory committee, the Endocrine Disruption Methods Validation Advisory Committee and this is kind of a scoreboard of where we now stand. As you can see the uterotrophic assay was completed and peer reviewed through OECD, the Hershberger went to a similar process except EPA serves as the lead country on that assay, leading laboratory, the estrogen receptor transcriptional activation assay was validated by Japan, through OECD and you can see they have the adult male, pubertal female, pubertal male, AR binding, aromatase assays were done by EPA. Amphibian metamorphosis assay in fish, again, EPA leads validated and peer review in conjunction with OECD. Steroidogenesis assay I'll tell you a bit more about, but it is to be validated next month and the ER binding assay will probably be validated I should say peer reviewed, both of those peer reviewed, next month for steroidogenesis and peer review for ER binding in June

of this year.

This slide compares the recommendations of EDSTAC with those proposed by EPA. As you can see I've highlighted in the right column those differences, the first being the androgen receptor transcriptional activation assay, we have not yet validated that assay. Steroidogenesis, they recommended the minced rat testes assay, we went through a prevalidation with that assay, we found that variability was very high, but the real coup de grace for that assay was the fact the lighted cell is only about one to two percent of the match of the testes and your typical site of toxicity assays could not differentiate between the rest of the cells and the lighting cells so it was felt that we really could not tell when we had a lighting specific toxigen versus a general toxigen and we didn't see any good way to solve that problem. So faced with two difficulties, and knowing that our lab down at RTP and some of the literature had suggested that the H295R assay looked promising in that it was probably the only cancer cell line that we were aware of that had all of these enzyme, the steroidogenisis pathway. We shifted our resources to that assay and the validation of the core chemicals, testing the core chemicals, as I noted validation report is in preparation and that will all

go to peer review this next month.

The other assay, the next one down that is different, it's the fish gonadal recrudescence assay, I have described that briefly to you and we switched to the fish short term reproduction assay because we found that the variability in the recrudescence assay was just much too huge and so it was not a sensitive assay.

Placental aromatase assay, we actually validated the placental aromatase and human recombinant aromatase assay, we felt that given the ease of the recombinant assay compared with the placental aromatase assay that that's the one we would require. Obviously if somebody is bound and determined to go with the other assay, they could petition us to use that, but we suspect that, we opted for the recombinant aromatase assay.

The adult male assay went through a validation program. It was originally anticipated that this would be a very strong specific mode of action assay using hormone measurements as kind of a fingerprint for mode of action. Unfortunately when it got to the validation it seemed as though that really no longer was the case. There was too much variability in the hormone measurements, they were measuring out

only the thyroid hormones and the steroid hormones but LH and FSH and the idea was that you could get, you could really get an understanding of where in the HPG axis you had a problem by looking at the different hormone measurements, but that was not practical and so you were left really with the histopathology of the main endpoint. EPA felt that that really put the adult male at a big disadvantage relative to the pubertal assays.

The last one as I mentioned before was the Utero-lactation assay which you gave us advice on about a year ago and we abandoned work on that as a Tier 1 assay.

Just the next few slides do summarize things. Everything is supposed to converge and I know that physicists will say a pre-body collision is a very rare event. We're going to try to have that happen, having assay validation, priority setting, and implementation procedures all come together in August of 2008. We have been told by our appropriations committee that this is your deadline and I don't know what happens if you break it, whether the appropriations committee says, sorry, you're out of money or what but this we're taking this very, very seriously so we will issue in August 2008 a Federal

Register Notice of our final battery, issue the final list of chemicals and publish the final policies and issue test orders.

So what happens after August 2008? Well, there's still work to be done, clearly. We need to complete the validation of the Tier 2 assays so that people have a place to go with their options on Tier 1 and so the mammalian 2-gen after the end points were added in the 1998 guidelines would work, they would be acceptable. However, we think we can improve upon them, we're...our efforts are now directed toward a guilty, hefty modified protocol on an extended one generation test in which more animals are carried forward. We have greater sensitivity for some of the androgen endpoints and, plus we're picking up neuro tox, developmental neuro tox and developmental immuno tox, so we think that that's an improvement, it will be probably an improvement over the expanded two, and we're working with OECD to insure that that becomes an OECD guideline if that is an acceptable satisfactory procedure.

We're working on protocols for the avian 2-gen, for the amphibian growth reproduction study, for a fish 2-gen, for a Mysid 2-gen and of course we're looking at having all of that completed by the end of

2010 which is about the time we expect to have data in from Tier 1 and have the Agency review those data and be in a position to make some decisions on what chemicals need to go in Tier 1 and what do not. So in summary it's a two tier program, chemical assays for Tier 1 screening battery includes both in vitro and in vivo, mammalian and nonmammalian assays that have gone through validation process and peer review, EPA considers them to be validated and ready for use. We will implement the screening programs on the first group of chemicals, seventy three chemicals in August 2008, with orders and protocols for the assays in the Tier 1 battery. We'll continue to plug away on Tier 2 with a target of 2010 and that brings us back to again the purpose of this meeting to review our battery, Tier 1 battery and to give us advice with respect to the battery's ability to meet its intended purpose which as EDSTAC articulated, it's to distinguish chemical substances that interact with the endocrine system, that is the EAP system, from those that do not and it should then provide a reasonable assurance both to EPA and to all stake holders that upon completion of Tier 1 screening the chemical will either have low or no potential for endocrine, that is EAT activity, or if it in fact such has such a potential. Thank you very

much.

DR. HEERINGA: Thank you very much. At this point I'd like to turn to the panel to see if you have any questions and clarifications for Dr. Gary Timm. Dr. Denver?

DR. DENVER: Bob Denver, University of Michigan, I'm curious about the ability to refine or add to the Tier 1 screenings and if there is an ability to do that but that has not been mentioned.

DR. TIMM: There clearly is the intention in the future of doing that, I mean one of the purposes for the SAP's earlier recommendation to test a limited number of chemicals to stop and evaluate what you have, look at your current Tier 1 battery, the other component of that is to look at how the science has changed and if there's something better there to put it in. In terms of whether we would permit substitutions now or not, I don't think EPA has really reached a decision on that, I tend to think not just because that's a difficult thing to do and one has to go through validation but we will see what things look like, we're open but clearly we've got a job to do and not much time in which to do it.

DR. BROWN: Terry Brown, what is the status of the energy receptor transcriptional

activation assay at this point? I know there have been problems in the past with the patent restrictions and you indicate that it's still in development or is that not the case?

DR. TIMM: The androgen receptor binding assay we actually have one that's been developed in our laboratory down in Research Triangle Park, we just have not had the resources to carry it through and validate it. It's been used in probably a dozen or more laboratories by now and the developer may want to add to what I'm saying but it's something that possibly could be validated using a paper exercise if enough laboratories have tested enough chemicals. That's something we want to look into. In terms of the other activities, estrogen receptor transcriptional activation assay has been validated by Derry of Japan. You will also hear this afternoon about a commercial system, the little cell system which does that. Our intention is to develop, I think there's a lot of interest in OECD and some of it really worked at OECD to develop test guidelines for activation, transcriptional activation assays.

DR. HEERINGA: I'd like to thank you very much, Dr. Timm. At this point I think we're set for a presentation from Dr. Les Touart, and I think

since we started at nine I'm willing to go a little while longer before our break and I think, Dr. Touart, I think your presentation is possible before we break. Panel members, it looks like copies of the slides are being circulated.

DR. TOUART: Thank you, Chair, and my name again is Les Touart and I'll be providing a presentation on the Tier 1 screening battery basically the rationale for the battery and provide some examples of some compounds and how they play out in some of the assays. The first point I'd like to make is what it is, the Tier 1 screening, and the goal of the Tier 1 screening as described by the staff is to detect chemical substances or mixtures capable of interacting with estrogen, androgen or thyroid hormone systems. What it is not...the objective of the Tier 1 screening is not to determine dose response relationships, confirm the mechanism of action or determine the adversity of the chemical's effect on reproduction and/or development. I think these are elements that the EDSTAC believed were more appropriate in the Tier 2 dealing with more definitive tasks. You know, the Tier 1 was designed to be more qualitative for screening, to provide suggestive evidence that a potential for interaction was possible.

Just to kind of go back and preview a little bit in the context of the endocrine system and these are the realities that it's an integrated, fairly complex, you know, system, it's designed to maintain homeostasis so with that there's a built in key feature which is the negative feedback. You know, hormones as they reach certain titers will feed back into the system so that they can be controlled and this is important in again maintaining the homeostasis. The estrogens, androgen, and the thyroid systems, you know, we understood are subparts of part of the endocrine hexis, and in investigating the potential for interaction or disruption of these systems one has to include the broad axis themselves. The system can be perturbed at multiple sites and by multiple mechanisms.

The next few slides are just to give a little bit more diagrammatic, you know, context, you know, for this, which I'm sure you all are familiar with context of the HPG axis as an example, you know, where you have hormones coming from hypothalamus to pituitary which then, you know, move down, stimulate production of the steroids in terms of the androgens, estrogens and feedback, you know, mechanisms and the axis is designed to generate a variety of and control a lot of processes in terms of food production and the

like.

Another example, just to quickly go through these is HPT and thyroid, you know, axis. Again the context, you know, here it's a complex system, there are multiple chemical signals that go through a lot of coordination that's needed for these examples hereto of sites where things could actually disrupt and interfere with the normal function of the axis.

The next slide is very designed to show a broader integration. Can't we look at the HPG axis, you know, saying, well, you can't look at the HPG axis, you're left saying, you know, these do interact, interact with other endocrine, you know, systems and mechanisms so they go very broadly in the context in controlling many processes in living organisms. Another context is to evaluate effects on a particular hormone, you really need an intact, you know, system, intact axis to be able to evaluate the full potential of effects that these will occur. To generally sum up the considerations here, it's, you know, given the complex interactive nature of the endocrine system it's clear that chemicals should be screened for their apical activity, the ability to alter things like growth, development, reproductive processes, rather

than just for their sex steroid activity in in vitro assays. If the objective is indeed such as effectively detect their potential to disrupt these endocrine regulated processes.

In developing the screening battery and the proposal to go forward, I've been following the EDSTAC recommendations, it's designed to insure detection of the estrogens, androgens, and thyroid hormonal systems. I think the, you know, key context, it would be naive to think that one could look at say estrogen, you know, alone without really considering the other, you know, hormones and the interactions that could take place because interferences, you know, even along those lines would have complications that would be manifest. The battery fulfills the EDSTAC recommendation for including a range of taxonomic roots. It includes sufficient diversity of endpoints to maximize sensitivity and minimize false negatives and I think the other context here as I've said is really designed to help minimize false positives too by having multiple assays and multiple endpoints, it kind of works both ways to use the weight of the evidence in determining what you have.

It emphasizes apical assays to provide a more comprehensive assessment, again, the concept of

needing those intact, you know, axis and looking at the summary outcomes of various processes involved in the development of reproduction.

It meets the overall objective of detecting the potential and the mediated effects regardless of mode of action. The assays, and Gary kind of mentioned these, but the ones that we are proposing in this battery include the steroidogenesis, it's an in vitro, you know, assay, the estrogen receptor binding assay, the estrogen transcriptional activation assay, the androgen receptor binding assay, an aromatase assay, not an in vitro, and then from in vivos we have a uterotrophic assay, a Hershberger assay, pubertal female assay and pubertal male assay. These are all our own assays.

Then there's amphibian metamorphosis and the short term reproduction. This slide is to display the various assays within the battery and to compare them across various modalities that we are trying to obtain information on in terms of the battery's ability to indicate the potential for some interaction. In the first column dealing with the estrogen agonists and the assays that are designed to work with that include the ER binding, the ER transcriptional activation, uterotrophic pubertal female and a history production

screen. Context here with the binding assay, that identifies whether you actually bind with the receptor. Transcriptional activation would actually give you information on function and be able to indicate that there's agonism that would be occurring. The uterotrophic is sensitive for, you know, the estrogen receptor mediated, you know, processes and so it provides a sensitive indicator of that fact. But the pubertal female and the fish referral, you know, these are intact organism, you know, tests that would include the entire HPG, you know, axis and cover the taxonomic range and again, the EDSTAC concept was to try to cover the bookends of the perfect plan. In terms of the, they've got them at locked processes, the other element that this brought in is roots of exposure in terms of the pubertal female or the rodent study would be more of a dietary exposure. For the fish in this case it would be more of a, I guess it depends on dermal inhalation since it's exposure from the water coming across the gills so there's some differences in that context. For the estrogen antagonist we have ER binding because the binding assay doesn't differentiate between the agonist or the antagonist.

The ER transcriptional activation has potential for actually identifying antagonism but it

hasn't been validated for that component yet so that's another reason that we've included the ER binding, the transcriptional activation, if it's not positive as an agonist, we could kind of secondarily interpret that it has a very, a potential for being an antagonist, but again that hasn't been fully validated in its context at least in all cases but the other thing is that the, we're limited then in terms of in vivo assays and the intact, you know, HPG axis context with the pubertal female and the fish repro, and that's a context in terms of the, you know, being able to have, you know, intact organisms and the taxonomic diversity.

When we get to the androgens for both the androgen agonists and the androgen antagonism. We have AR binding similar to ER binding. It, it will detect the ability of the receptor to be bound. Hershberger assay, which is a castrated rodent version that we use and within this context it provides a sensitive indicator for the agonism and antagonism of androgens. The pubertal male has an intact HPG as does the fish repro. And again the intact HPG with a taxonomic spread is covered with that one.

For steroid synthesis modulation we thought, you know, for both the synthesis in terms of the androgen as well as the estrogen. The

steroidogenesis assay which is a recombinant assay covers both and aromatase is also utilized but it's for very, for the next final step in the process which would be the conversion of the testosterone to the estrogen finally.

And then again for the, with both pubertals and the fish, we got the full HPG axis covered so any interference in steroid metabolism that would occur there or through any other process within the system that adrenal system in terms of adrenal corticoids. You know, those would be picked up in that context. For the HPG and we have the pubertals and then the fish repro. For the thyroid we really have no in vitro, you know, screens that have been able to conform to a point that would be useful that we have available to assist in identifying a particular mechanism, but with the intact HPG, with pubertal male, pubertal female we do have endpoints related to the, you know, hormones and the gland histology and then with amphibian metamorphosis we provide, you know, a developed male life stage and multiple end points for looking at final and I think the context here with the variety of life stages that we have in terms of pubertal or maturing individuals in the pubertal assay. In the fish assay we're dealing with reproductively

active adults and then with the amphibian assay we're dealing with larval stages which have relationships to, you know, to other like fetal developments and things and analogous frames so the context with multiple life stages which including intact HPG or HPT we've covered a life raft of potential interactions with the estrogen.

Okay, and just to briefly kind of go back over, you know, the modalities as far as which assays, you know, fit within the particular modalities, HPG is covered with the male pubertal and female pubertal and fish reproduction. Subsets of the HPG which include the estrogen androgens and then the steroidogenesis include some of the in vitro assays. On the estrogen side, you know, we have the binding transcriptional activations and then the female pubertal and fish reproduction. The androgen sides, the AR binding, Hershberger, the male pubertal and fish reproduction. The context for having both the female and the male pubertals was that the male pubertal couldn't, you know, cover the, in an intact HPG some of these estrogen endpoints that would be of use and again we wanted to make sure that we had the taxonomy spread out with both pubertals included that provides full coverage there but also allows interpretation in terms

of the other axes in terms of the thyroid axes. For thyroid it's just the male female pubertals and then the amphibian metamorphosis.

What I want to do now is go into at least in going back to the modalities and then look at some of the examples that we have for the assays related to those modalities. For the estrogen pathway, ER binding again is there to detect chemicals that bind with the receptor. The transcriptional activation is there to detect the estrogen receptor interaction and function and can differentiate the ER agonists. The Uterotrophic which, the preferred method that we are putting forward is the subcutaneous. At peak exposure it is an in vivo, it detects chemicals that act in vivo but also incorporates metabolism. The pubertal female and the fish brain are designed to detect compounds that act on the full estrogen system as intact HPGs. The first example of estrogen compound, methoxychlor, it's an organic chlorine insecticide, methoxychlor has been shown to be weakly active in in vitro ER binding assays, methoxychlor and its metabolites are much more active in a compound, and one of these is an ER alpha agonist but an ER beta antagonist and an androgen receptor antagonist as well.

In looking at the assay responses I'm

not surprised the ER binding and the transcriptional activation invoked weak responses in terms of the parent methoxyclor. In the uterotrophic assay when metabolism's incorporated, we get a definite positive. In the pubertal female there's a positive which substantiates the effect in terms of accelerated vaginal opening and age at first menstruous so multiple endpoints which are influenced by an estrogen hormone itself. The fish screen also positive, inducing a male tautologen which is a key endpoint within that assay but also reducing egg production and just to go through at least briefly some of the ASI's in this context.

The uterotrophic assay, you see the definite increase of the effect in terms and this is average data across three labs that were used in the OECD validation program and again we've heard the obex animals the subcutaneous exposure but the pubertal female and I think, I think the, what's indicated here, again, at both concentrations we have significant effects. The significance is identified by the colors of the cells to indicate that at both test, you know, concentrations there were significant effects, seeing the age at the vaginal opening, age at first address and also on the cytoxicity of the organisms.

This slide is just to indicate that the

pubertal female includes some thyroid end points but for this estrogen active compound, you know, the end points utilized for detecting thyroid activity, these were all negative in context, so that the context here is end points that are related to a particular modality, you know, will respond to a modality but for other modalities, you know, they will not so I'll just say that you can't get multiple mechanisms in action, but I think we'll have some examples of that later.

In terms of the, an estrogenic effect in the male, we see again on the male side a significant increase in vitellogen occurring which is a sensitive endpoint of the assay. There was also some significant findings in terms of the testosterone in reduction there from the, from the compound.

And this may be related to some of the androgen receptor antagonisms that is also associated with methoxychlor in terms of multiple pathways that might be involved. One of the apical endpoints, I'll vet it and then I'll look for this particular compound, but we can see and again, this slide is looking at cumulative number of eggs spawned and there's, in this case pre-exposure time the number of eggs identified and then the post exposure from time zero you can see that once exposure is initiated there is a effect on

the number of eggs spawned and the significant difference, you know, identified in the high exposure group.

The next example compound that was investigated through the estrogen assays is just an IA, a controversial compound. Its main use is in the synthesis of polycarbonate plastics. It's been demonstrated previous to be estrogenic in vitro and mixed results in vivo.

And these were played out again with the assays in our battery in terms of the ER binding and the transcriptional activation both showing, you know, positives, you know, indicating that, that it's an agonist. The uterotrophic assays subcutaneous also positive to indicate that at least in the in vivo system, you know, there is a positive response. The pubertal female, you know, was negative in this case. The fish screen was positive indicating also like we have seen for other estrogens induction of the male vitellogen and then decreasing in female egg production. The suggestion here is that perhaps there is some type of metabolism, you know, that would be going on that may be say detoxifying the compound in pubertal females since this is a dietary route. The subcutaneous with the uterotrophic and then in the fish

brain where you have transfer across the gills, you know, they tend to corroborate each other in a context so that would raise the question as to whether dietary route would be effective in terms of the, of the disphenol, you know, A type of mechanism involved, so there's other data again like you saw with methoxychlor, there is a dose related response in terms of the action and the increase in terms of either size. For the pubertal female the only significant findings in the study were on weight gain but the significance on the weight would tend to indicate that you might be, you know, adding, you know, the facts regarding those are really both. This is just another example of for thyroid endpoints also negative in the case.

For the fathead minnow you see again, you know, reduction in the reproduction which is shown but also the vitellogen response was a significant induction of vitellogen in males, all treatments, and in females at the high treatment also.

Switching over to the androgen pathway and looking at the assays associated which are AR binding, Hershberger, pubertal male and fish brain, they are binding again the test compounds applying to the androgen receptor. Hershberger is designed to detect chemicals that act through the air but to distinguish

the agonist and the antagonist and also incorporates the metabolic processes. Pubertal male and the fish detect the full androgen system, you know, having intact HPG axes.

Our first compound which wore the belt testosterone ordinarily a potent androgen in terms of what would be expected in terms of responses. A strong binder in binding, strong positive in the Hershberger indicate agonism. A positive in the pubertal male hitting on several of the key endpoints, the reclusive separation and tissue weights of various tissues and reduced things like testes and epididymis waste.

The fish brain was also positive for several of the endpoints and we'll go through these with some of the data from some example studies and at this point go on with this every day before that in the OECD Validation Program and again, important things like the ventral prostate, the seminal vesicles, the pelvic vc muscle complex, advanced penis toppers, all these were significantly affected at the higher doses.

In pubertal male at single dose accelerated reclusive separation, increased ventral prostate, seminal vesicles, and then a decrease in the air testes and air epididymis. In looking at the fish and one of the end points within the fish assay is one that

looks at secondary sex characteristics. In this case androgens will cause the emasculization or male secondary sex characteristics to be manifested in the female. The muscular tubules which are present in breeding male fathead minnows is a pronounced element of the organism. It doesn't occur in the females, but with the, it's controlled by the androgen. In the presence of methyl testosterone the female fish, you know, demonstrates, will display these secondary sex characteristics. The tubules will be manifest.

In this case also vitellogen was, was, was increased, you know, and testosterone's an androgen but it's an aromatizable androgen, and this slide shows that the aromatized methyl estradiol and estradiol, you know, binding curves are, are very close to each other, so that would give an indication that the androgen manifested its effect in terms of the androgenicity on the organism but also as it's converted, you know, will affect some of our estrogen end points at the same time.

And this is an example of the pronounced effect that this compound has on the reproduction in terms of the, you know, pre-exposure time and production of controls times zero, but at times zero, it, it looked of the treatment concentrations we had

virtually cessation of production of the eggs, so again an indication that these endocrine active material, you know, will affect the summary endpoint. It's not surprising, 'cause this is actually reproductive hormones entered as well to affect reproduction.

For one of our classic campaigns, the closest one has been the Roth study. It's a fungicide used on fruits, vegetables, variety of pesticide uses. It's a classic anti- androgen, inhibits the air dependent gene receptor expression in vivo. There are a variety of in vivo effects that have been studied in longer term studies, you know, as well, so we know or at least have a fair understanding of the compound in terms of how it's likely to affect us.

As far as the assays in the battery and how they respond, AR binding assays are somewhat equivocal in terms of a response and we can in some cases or at least it may be possible in others. The Hershberger was, you know, clear positive. The pubertal male positive again for multiple hits in terms of pop your goose off in separation and testes weigh, fish brain also key endpoints in terms of egg production, increase in some of the histological findings of the gonad and the ovary and reductions of male secondary sex characteristics.

The context here is in closing is also a metabolizable material and it's likely that within the AR binding especially for parent effects, the reasons that the parent is not as potent as some of the metabolites so when you get into the in vivo, you know, assay we're seeing much more upgrafts response. In the Hershberger, again, you know, the key point here is that you would be getting multiple hits across the various endpoints, you know, so that they corroborate and meet each other in terms of the action. In other assays like the pubertal we're also getting, you know, multiple hits, again corroborating the response as being, you know, anti-androgenic. You know, the fish again corroborating what we're seeing in terms of effects, summary effects in terms of fecundity, the outside atresia, increases in GSI, for vitellogen there was a increase in the females, tubules of slow results are reduced in, in, in males. And I think another context here in terms of how things are interpreted, you know, and part of the reason for having a taxonomic representation in terms of effects seen in the mammal, effects seen in the, in the fish would give good indication that this effect is likely going to be transferred throughout the kingdom and referred in universal in that context. If it was just within one

group versus another then that might raise the question as to whether it's going to be active in all particular morphology type levels.

The next examples that I deal with as fallacies as pesticides is in the area of commercial products causes a variety of male developmental effects characteristic of an anti-androgen, again in a variety of studies. In the assays we tested in terms of the battery for the air binding, this compound was a non-binder. From the Hershberger assay was negative, you know, in this in vivo system but in the pubertal male, again it was positive for responses to give an indication that the effect was likely referred more of an intact HPG, you know, for the, for the action to sort of be manifest, that the Hershberger is a little too narrow to pick that up, but for the pubertal male they were providing a variety of end points to boost the separation which is a key end point of that particular assay. Incidentally on the effects on reduction waste testes and the epididymis, which would also be indicative of anti-androgen type responses.

Moving to steroidogenesis the assays that are associated are at least informed from the compounds that have been in effect in this particular pathway, given the recombinant aromatase and then the H295R

which recombinant steroidogenesis. You know, these assays won't detect inhibition of aromatase and the steroidogenesis chemical that induced recombinant enzymes throughout this steroid syndrome.

The pubertal female takes chemicals that act on the estrogen system's adrenals. You know, pubertal male will affect chemicals that act on the Mayan androgen system and they know that as fish bait, similarly affected compounds that act on the estrogen egg systems. It's the main thing that interfered with steroid metabolism will likely manifest in one of those steroid controlled processes.

One compound that's been fairly well studied is Ki-comosol, swedisol and a foamal compound, its action is to inhibit steroid synthesis in fungi, but inhibits a variety of the cyclin p450 enzymes. It's also reported to induce progesterone production, and elements of it has inhibited testosterone and estrogen production.

In the assays and battery for aromatase, ketacosol was negative. For the recombinant steroidogenesis assay it was positive, recording a 90 percent reduction of estrogen and testosterone in the in vitro system. In pubertal female it was positive with the effects seen on ovarian histopathology and on

fixed adrenal but no effect on vaginal. In the pubertal male was positive for delaying bruises and abrasion and for tissue weights and it reduced the testes growth.

In the fish brain, histopathology was the only positive with consistent positives in terms of providing cell proliferation. Just to go with the pubertal female, for vaginal opening it was negative. We had a significant, you know, effect on body weight gain which would give indication that we were right at maximum dose. In the other end points I could bring away, there was an increase in that to give an indication that it was a positive response. For the pubertal male more endpoints affected in terms of summary end points of reclusive separation, the seminal vesicles were all reduced and the testes and epididymis -- well, testes were reduced.

This is an example of the interstitial cell hyperplasia seen in the fish. I'm not sure that the pictures are that good, at least in terms of what the slides go, but the context if you can make it out is that the interstitial lighted cells are really small aggregates that are in between the seminal vesicles, but in the treatment these cell aggregates become much, much larger, you know, they do expand in the

interstitial spaces and you know, would be an indication that there is increased production of live cells which seek to compensate for production of additional stimuli.

Switching now to thyroid pathway, the three assays that are associated with this are the pubertal female, the pubertal male and amphibian metamorphosis, all with intact HPT.

One of the thyroid pathway compounds that we used across the, what is the chlore, it's a natural end result for chloric acid. It's used in solid rocket fuels, you know, it's also for treating thyroid disorders, it's also a constituent in some macro fertilizers which has led to a lot of contamination off site, but it's, the compound's a large one, it inhibits the thyroid gland's ability to absorb iodine. In the assays pubertal female was positive, increased flicker, cell height and color in the gland and decreased T 4 and increased the TSH. A pubertal male, same story, the glandular pathology and then the decreased T4 and increased TSH. In amphibian metamorphosis we don't have the hormones as endpoints in assay but for the ones that we do include the pathology was similar to what was seen in the pubertals in terms of increased follicular cell height, reduced color area and then

for further endpoints that we do have in amphibian metamorphosis, there were developmental stage delays and morphology changes, this slide's to highlight that. In the amphibian metamorphosis again gland histology was the most sensitive of the end points, you know, where we, follicular cell height, color area, you know, increase in cell height, decrease in color area seen. The hymen length was reduced, the weight binding were increased and in terms of the, in terms of compound and developmental stage was significantly delayed but the root was seen only at the highest concentrations.

There's an example of the plant pathology. Again the control you have large colored areas and standard oswego cells, but within the treatment the follicular cell height increases and lose the color area and more pronounced at the increased dose and also causing somewhat of a plan I believe.

In the pubertal it's a similar story, here we have the bar graphs of the follicular cell increased with both the female and the male and the colored area decreased and then examples also of the gland histology which is, you know, a similar story that we saw with the amphibian gland.

Hormones let's go over, the T 4 is decreased in both male and female and the serum TSH increased in

both the male and female although the female seemed maybe a little bit more interested in a sense.

The next compound I want to talk about because it highlights one of the elements of an amphibian metamorphosis and this is hyponoic acid. This is a pharmaceutical that's used in radiologic imaging, inhibits the aromatase activity. We tested it in the amphibian assay and it inhibits the deonase which caused a dysynchronous development, a development stage that's determined by valuating specific developmental landmarks, which are spatially and temporally coordinated. The effects were as listed here. Retarded development, accelerated craniofacial tail development, decreased whole body length, decreased weight and effects on histological changes in the man where, you know, it varies, like some we've noted.

This is just from a, we were allowed to participate in an elaborate study of findings of asynchronous development, meaning different parts of the amphibian going through development, you know, were affected and this slide tries to demonstrate this in terms of typical control development and these stages that you can favor, you can favor are outlined stage specific classification when you process for the

various stages that that form of development you both grew and in stages, you know, 59, 60, 61 you can see kind of a progression where you, you know, the craniofacial development, you know, changes, and actual head size reduces, but you see a lot more development as you move through progressions, the tail would be reserved and the limbs become more developed but in the typical IOP treatment situation the head fall is in a confused state in terms of development, in terms of a tail at the very advanced stage 63, the limbs are in kind of a retarded, stage 59, the head in somewhat of an advanced 62 so the context is that the peripheral tissue response wherever the deonase occurs is causing a developmental modification. In this case the amphibian assay is the only one assay that exists with an end point that would relate to the peripheral tissue responses or at least the deonase in peripheral tissue. So the context of that assay is one that would need to be, you know, present to be able to pick up those kinds of activities.

I'm just going to go through some kind of summaries now in terms of the strength of the battery and its ability to detect. And this slide's a little different from the original package that we have and I think that everybody has, it should have been

distributed, a replacement slide, for slide 60 where what we've done is just added which assays, the previous slide just has several assays, you know, given an activity but here we're trying to spell out the specific assays. For estrogenic activity, again, the idea of binding, we have transcriptional activation, which will cover the, you know, binding and inform whether we got agonists, for 3000 uterotrophic female pubertal and the fish covering the end points and taxonomy separation and also the metabolism in the anti estrogenic activity we've got neo binding but in the in vivo just the female pubertal and the picture will cover that activity and the taxonomic, you know, range. For androgenic activity we have in vitro and Hershberger and the intact HPGs, the male pubertal in fish and the same for the anti-androgens. And the population that's steroidogenesis, we have the H295R, male female pubertals and fish. I mean, what basis is the story the tail end of the steroidogenesis?

Altered effects on the hypothallic pituitary function and this would include HPG and HPT where appropriate and male/female pupils come from cobalt HPG to fish's HPG and then amphibian metamorphosis is the HP as it relates to the HPT.

For antithyroid activity we have both the male female pubertal and the amphibian metamorphosis.

For thyrominetic activities, you know, it has always been demonstrated as amphibian metamorphosis and as we mentioned the perfect example of that he for those peripheral tissues and responses and giving them rough effects.

In interpreting the battery, as was mentioned by Gary in his presentation, I mean, using the weight of evidence includes professional judgment, you know, some end points more diagnostic specific than others and, and really it's the weight of various effects seen in multiple endpoints and across multiple assays that carry the most weight. We're looking for that confirmation of corroboration across the assays and two possible interpretational outcomes, either the potential for the activity on estrogen action hormones, that would require some further analysis to the Tier 2 family of the patient or we can interpret that there's low and no potential for EAT activity so that the compound can be, you know, pushed aside instead of somewhat harping on it.

In summary, your multiple assays are required to comprehensively screen estrogens and androgens, the thyroid hormone systems. The in vivo assays are good

for well understood mechanisms like simplifying in vivo assays, in vivo assays with intact HBG, HPT axis are good for efficiently screening complex processes, multiple taxa and multiple modes of action end points provide a range of metabolism and corroboration that would be needed in interpreting, you know, that the effects are likely manifested through endocrine systems. The complete battery is needed to support a weight of the evidence finding something lower, low potential for EHE activities.

The next two slides pretty just repeat the third question which I think will be the subject for the next day, but the first charge question really gets at, you know, how effective is the battery at covering the extra damage in the thyroid system and then the second charge question where it gets at, you know, how well the battery works as integrated in a system in terms of what combinations are likely to be considered for that and so with that I thank the panel for their attention and try to answer any qualification questions you have at this time.

DR. HEERINGA: I thank Dr Touart, but first of all I'd like to commend both you and Gary Timm for very efficient presentations and I think made very good use of our time, well organized, thank you very

much. What I'd like to do is, I'd like to take a break and then return for questions from the panel so that we have a little bit of a chance to for people to get up and have a chance to stretch and we'll return and then we'll take questions on your presentations from the panel.

For the audience at large, this is a floating agenda and I think we're moving right along, I think we will go into the period of public comment so those of you who are prepared for public comment I'm not sure whether we'll start before the noon hour but we'll certainly start first thing after the lunch hour for public comment but when we return from the fifteen minute break, I have 10:36 so let's come back here at why don't we say five minutes of eleven and we'll return to some questions on the scientific presentations. Thank you very much.

(**WHEREUPON**, a brief recess was taken.)

DR. HEERINGA: Okay, welcome back, everyone. I'd like to again welcome you back to the second half of our first morning session, the meeting of the FIFRA Science Advisory Panel and the topic of the Endocrine Disrupter Screening Program Proposed Tier 1 Screening Battery. At this point we have heard and seen presentations from Gary Timm and Dr. Les Touart

and we have reached a point where I'd like to open it up to the panel members for questions of clarification for Dr. Touart or Gary Timm with regard to their presentations or any of the materials that were in the technical report. Dr. Kullman.

DR. KULLMAN: Seth Kullman. Dr Touart, you had mentioned the weight of the evidence several times and it appears that several compounds have slight differences from their modalities and was curious if you could expand a little bit more on the weight of the evidence process.

DR. TOUART: I'll try to expand a little bit. I think the context of the weight of the evidence again in terms of multiple end points within some of the apical assays, I think some of the in vitros or Uterotrophic and Hershberger have, have maybe fewer, you know, end points to consider but with the regular assays there are a variety of end points that touch on, you know, the range within say an HPG where you may have estrogenic, androgenic or even thyroid endpoints that could be affected at the same time. The context of the battery isn't to identify a specific mode of action but to be able to detect, you know, an activity if that mode of action exists, you know, so that if we see effects in multiple endpoints and in multiple

assays especially across various taxa in terms of the rodent and, and fish or frog, the indication would be that this is a potential, you know, for disrupting those types of systems whether it's the, you know, HPG, HPT or, you know, for some compounds maybe both in terms and by having multiple endpoints and multiple facts I think that the weight of the evidence would give an indication that that's real as opposed to if only, you know, one end point and one assay were to like I say you might get a receptor binding in the ER binding assay but everything else the transcription activation's negative, the, say, uterotrophic is negative, the pubertal, you know, female's negative and the fish repro is negative. The indications would be that while it may have some binding affinity, but there's not a potential to disrupt the system, but if you see effects, you know, in multiple assays then I think you have to consider. In a, in a given assay you'll see whether it's a pubertal or fish assay if you have a summary end point that's affected and it's corroborated by other end points in that assay then I think you can't necessarily ignore that, say you have the fish you have vitellagenic induction, you have gonadal, you know, pathology and you've got reproduction error, I think the context of that path

used even if you didn't see an effect in the pubertal or you didn't have corroborating evidence in the in vitro assay you'd still have to think, well, there's a potential, the context of that potential as to whether it is universal, comes in all vertebrates or whether it might be unique to fish or non-mammalian systems would be something that we'd have to kind of look and see, well, what evidence did you have emanating from the rodent studies.

DR. KULLMAN: Will this remain a qualitative assessment or will you try to make it a quantitative assessment?

DR. TOUART: The purpose of the Tier 1 is to be qualitative just to indicate a potential. The purpose of the Tier 2 is to do the actual concentration response, the dose response information and to really assess the adverse consequences, so once we see that there's a potential then we will go into the Tier 2 tests until, to try to get the quantification of that particular event.

DR. HEERINGA: Before we turn to the next question I was a little out of order. I wanted to make sure Dr. Furlow's arrived and give him a chance to introduce himself. David.

DR. FURLOW David Furlow, UC Davis by

way of Los Vegas and Atlanta, my laboratory focus is on thyroid hormone regulation of amphibian metamorphosis but also corticosteroid regulation of muscle mass in mammals as well so kind of multi species and multi hormones.

DR. HEERINGA: Thank you very much, David. Dr. Bucher

DR. BUCHER: John Bucher, I was wondering if you would just spend a minute talking about issues related to the guidance that EPA will give for dose selection and concentration selections for the in vitro and in vivo assays, general terms.

DR. TIMM: For the in vitro assays for the most part, John, we have specified a top dose, a limit dose and so for the binding assays, for the aromatase assays, for instance, and also I think for the H295R we would pick, go one millimole or perhaps ten micromolars as the top dose and run doses down probably logarithmically and that would be for the first run and then for the second run they could tailor things based upon what they saw. I'll let Les talk about the in vivo.

DR. TOUART: I think the context in the in vivos in terms like of our studies the context is I'm trying to establish the maximum power eight dose

and that would usually be defined as like a dose that doesn't affect less than ten percent weight reduction in the fish and frog. The context is that we want to evaluate a dose that's below a, you know, toxic level, generally using like a mentality type of end point if we have LC50, LD50 type of information, we'd be stepping down, you know, from that to get to a concentration where we would not likely see overt toxicities. One of the contexts of having multiple doses in the screens again because they're somewhat more qualitative and quantitative but having multiple doses allows us if the high dose, if we do get mortalities or other overt signs of toxicity than to lure concentrations but hopefully, you know, still suffice to be able to identify that we touched I guess concentrations that would still be relevant and that the effects we would see would be more likely endocrine mediated than non. I think the, part of that thinking is that for those materials where we are within toxic range we can at least can have any kind of responses and affect any of the endocrine endpoints but also the contexts that our other more traditional toxicity tests, you know, get the knowledge from a hazard assessment risk assessment perspective would be able to deal with those materials very effectively for the

purpose of, you know, dealing with endocrine disruption, and that context would be that these will likely affect that otherwise would be missed or at least be more pernicious in terms of the effects on growth development or reproduction.

DR. HEERINGA: Dr. Vandenbergh and then Dr. Chambers.

DR. VANDENBERGH: John Vandenbergh, I wonder -- well, I understand that it would be difficult to add any new protocols to the testing procedure at this point but I wonder about within the ones that have already been identified here, is there flexibility as far as the panel is concerned about tweaking any of the protocols or eliminating any aspects of them?

DR. TOUART: I guess from my perspective I mean that, we're open to that kind of advice or suggestion but I think the context of if there are unnecessary end points or end points that might otherwise be tracked or overload a particular assay, the context of you need additions I think those are possible as well as long, again, you know, it's the context of the analogy of the Christmas tree. You don't to have too many ornaments, you know, or it will topple over but you still want to have enough and also we want to be able to make use as much as possible of

the animals that we utilize so those end points you glean as much information for the use of the animal we want to make sure that we captured every piece of information that we can. In the context of other modifications in our view point what we're trying to do is establish some kind of a basis that in terms of a battery and any improvements, you know, modifications of an assay or other assays these can be folded in, you know, some of these may be considered like performance based type of a concept where if you have an assay like a different transcriptional activation type assay that may exist or a different, you know, recombinant cell line or something like that, you know, but if it performs the same function, you know, as long as it can demonstrate that it gives the same information or perhaps better information so much the better and the same for the in vivo assays if there's a way of getting the same information and it will use those, you've kind of established this kind of a reference then anything else that comes along that's as good or better or gives the same information quicker, cheaper, whatever, then that would also be, you know, up for consideration. It's just I think right now we're limited in what we see and if the panel's aware of some things that are probably up to speed or ready for prime

time then we're willing to, you know, consider those as well.

DR. TIMM: Just one add on note. The validation requirement is a substantial one and so there's probably a lot of stuff out there, there may be things that might even be better than what we have but they haven't been validated, at least for right now we can't use them, we would have to go through a validation process and of course we're working with OEC, OECD to validate some other things that are not quite ready yet.

DR. VANDENBERGH: May I follow up quickly, is there any plan for review of the program at some intervals in the future?

DR. TIMM: I'll be happy to speak to that point. As part of that review back in 1999 by the SAP, they recommended a number of things, they said this was a really ambitious program and they said, you know, don't just go full boar, that you should take fifty to a hundred chemicals and then full stop, look at what results you've obtained on those fifty to a hundred chemicals and re-evaluate your battery in light of that information and prune it if there's some things in there that provide unnecessary duplication, substitute some things out if there's better technology

that has been validated for some assays but that's exactly what we intend to do.

DR. HEERINGA: Dr. Chambers?

DR. CHAMBERS: I'd like to follow up on Dr. Bucher's question of a minute ago, Mr. Timm. The limit doses that you're talking about in the in vitro assays, are those based on toxicity levels or solubilities or anything like that or just arbitrary levels?

DR. TIMM: They were basically consensus levels but that's the idea that you would run it first time and go up to the limit dose if you could. But you clearly have to look for precipitate, make sure that in fact you're not exceeding the limits of solubility. Then if it's a cell based assay you're also interested in cytotoxicity so that would also limit your concentration so we don't expect the assay to always be run up to the limit dose but that's the first thing to start with, check for solubility, check for cytotoxicity if that's applicable and then make your second and third runs accordingly.

DR. HEERINGA: Yes, Dr. Lasley.

DR. LASLEY: Is there accommodation or flexibility for emerging new mechanisms of action in terms of endocrine disruption?

DR. TIMM: Not on this seventy three chemicals but down the road, clearly what EDSTAC said is hey, this is a rapidly changing science, that EPA will need to look at other mechanisms of action, other hormone systems in the future.

DR. LASLEY: What about hormones that interact with your EAT hormones, are they to be included now or would you delay that?

DR. TIMM: We are pretty well set on the battery of assays that we have and we really, you know, we can't add to those, we've got deadlines to get the program going, we've got the battery we've proposed, we would like your comments on that and as Les indicated, you know, if there are endpoints within assays that you don't think really add to the value of the battery or add to that value of that assay, we would like your recommendations on those. If you think that among the suite of things that we have, we should put more in, that there's something there that's final, I want to hear that. If you think that we've got needless redundancy and some assays should come out then we want to hear that, but it's, it's really focused on the battery we proposed.

DR. LASLEY: Still what I get from that is this is a reductionist point of view; in other

words you would tolerate reducing your battery but not adding to it?

DR. TIMM: What you could certainly recommend is some other areas but they would be really recommendations for research. At this point they would not be recommendations for inclusion in the battery at this time.

DR. HEERINGA: Dr. Cooke.

DR. COOKE: Joe Cooke, what combination of positive results would trigger a Tier 2 testing?

DR. TOUART: Again, the way of the others and one could go through lots of permutations, I think one would have to look at the nature of which assays were positive, if it's a single assay, you know, the severity or the magnitude of the change in the end points that were associated and again which assay I think for the apical assays, you know, one's going to place more weight on those than one places say on the in vitro assays. I think the in vivo tend to trump in vitro if in that context but to try to identify like if a single end point it is affected, you know , if it's a severe response I think it may warrant, you know, some further investigation. Whether, you know, that's a clear trigger for Tier 2 or just, you know, something else is maybe something that could be debated but most

cases an experience that we have is for some of the more significant end points if those are the type that there's usually corroborating information and other end points to suggest that those are real benefits, not just a, you know, say a false positive, you know, kind of a context and that's part of the strength of having multiple assays and stuff, but I think if it's a single end point and a single assay one would have to look at that with a little more caution than when one's looking at multiple hits in multiple assays.

DR. HEERINGA: Gary Timm?

DR. TIMM: Yes, if I may add, in the, some of the examples that Les ran through in his talk what you could see is some characteristics that indicated, gee, this chemical needs because it was negative in vitro but it was positive in vivo assays, it clearly needs metabolism to be active, it's one of the metabolites, it's active, so, you know, you, there's no way to assign points to assays and add up the points and magically if you hit a certain number it goes to Tier 2. It, you have to use judgment and part of the reason for having the battery is that you get a picture as to what's going on and you can really hypothesize what sort of stuff might be happening here and looking through mammalian assays, looking at your

fish assays, looking at your in vitro assays decide whether in fact you've got something that's a concern or it's not a concern.

DR. HEERINGA: Dr. Isom.

DR. ISOM: When we look at the battery and the objective that you state is to identify the potential and not to I guess elucidate mechanism. And when you look at the total battery, the in vitro assay's a little more mechanistic I guess you could say and the more complex in vivo assays will give you the same information plus additional information so I'm a little confused then why we have that overlap and why it is stated that we're not interested in mechanism if you just pointed out by running the battery we'll have some idea of the mechanism.

DR. TIMM: I think we're interested in mechanism, but I don't think that one can conclude that because you've seen say estrogen receptor binding that that is necessarily the mechanism by which a chemical is acting. I remember a case, a number of years ago, where yes, it was an estrogen receptor binder but in fact they found out when they ran some more experiments that indeed it also regulated aromatase. Now with our Aromatase, recombinant aromatase inhibition assay we're never going to see that. Hopefully we would see that

in the H295R and I think it would expect to see that but to really nail down mechanism you really have to have a...you have to formulate a hypothesis which I think you could with the assays we have but you need to confirm that hypothesis in some additional follow up experimentally designed studies, sometimes this will give you the mechanism but I don't think you can always guarantee that what you see is going to be conclusive proof of, that that's the mechanism of which it operates.

DR. HEERINGA: Dr. Brown.

DR. BROWN: Terry Brown, I just have a question about the practical implication of the assays, how many laboratories will be running these assays, how will they be selected, what kinds of, you know, expertise will be required within various laboratories to evaluate particularly in the in vivo assays where you have a constellation of end points where many of them may lend themselves to different interpretations based on the expertise of the individuals who are looking at the histology or other aspects of the assays.

DR. TIMM: Histology may be one of the limiting factors here. Of course selection of the laboratories will be the responsibility of the

regulated industry because what these test orders do is that they will require the industry to test compounds and typically several companies who manufacture or who register a chemical as a pesticide will band together to jointly sponsor the testing, it could be done in their own in-house laboratories, if one of them has a toxicology laboratory that can do these tests or they could go out to the commercial sector and use one of the commercial laboratories. One of the things that we plan on doing is to develop some data to answer your question because we have not yet since we, since only recently the protocols were even nailed down we have not gone out and done a survey to see how many laboratories there are but that's something we clearly want to do.

DR. HEERINGA: Dr. Touart.

DR. TOUART Just a follow up I think one of the elements of the validation was to evaluate transferability of protocols and part of doing it in the laboratory investigations were to collect laboratories at least in employee laboratories and with the protocols to determine, you know, how well they were able to follow that and then to adjust the guidance in that context. In some cases, for some of the endpoints that are a little bit more, I guess

difficult than others like histopathology, I mean, we went to great efforts to develop guidance documents to explain things, you know, very, very clearly so I think there's a lot of guidance, but I think as Jerry points out there's still questions in terms of the numbers of laboratories and the lab capacity for doing some of the, you know, the assays that we're talking about in terms of the number of compounds in the first group.

DR. HEERINGA: Dr. Furlow and then Dr. Delclose.

DR. FURLOW: It's just to follow up on that, so one of the inter-laboratory validation concerns at least I think in the pubertal assay was perhaps measurements of TSH for instance and each lab may use a different kit, et cetera, and the EPA in the documentation said that you guys didn't feel that that was your place or in your purview to sort of be the in house hormone assay department, but couldn't there be some contract lab that's designated that could in fact coordinate these things so each lab isn't doing all those assays?

DR. TOUART: I'm not sure if there would be a single lab say that would be given sometimes a prize of being able to manage all that but I think that the context for the assays and some of those types of

end points is to have some performance material in terms of what one's expected so if one uses a particular type of hormone kit versus somebody else that they're still able to be able to differentiate, you know, and to demonstrate that kind of context. If in terms of lab capacity situation, if the industry wants to double up or at least to have specialized centers that can do work, I guess that's something that would be within their volition to try and do, but I think from the context what we want to try to do is provide test method guidance and performance criteria for those methods so that those would be, you know, we would have confidence that they would be conducted and be acceptable when the data presented.

DR. HEERINGA: Gary Timm.

DR. TIMM: In addition, I mean government is supposedly in the business of breaking up monopolies and not creating them so to specify that someone must use a particular kit is something we wouldn't do and OECD under their guidelines clearly would not do that as well so I think that's the reason Les says the approach we would take would be to try to set the performance criteria so that we might even, if a validation were conducted by someone and a number of different kits met the criteria, certainly there's a

way to inform people that these have met it and so that these they can choose from but clearly we would not specify one particular approach.

DR. HEERINGA: Dr. Delclose.

DR. DELCLOSE: Barry Delclose, I was just wondering if you envision this is this going to be a yes or no, goes through Tier 1 and you know, send it to heaven or to hell or is there a purgatory so that if you got you say in vivo assays would trump in vitro, if there were, say, two in vitro assays are positive and all of your in vivo assays are negative, are you just going to stop there and say, well, it looks like this is probably not a concern or a low level concern. Would you be concerned about things like thorough characterization of that metabolic profile in young animals, for example, maybe there's a difference in the way the compounds metabolize so I know you made mention in the in utero fly-tational that there's a possible Tier 1 point five, is there envisioned for something like that? You've got enough concern here. I would imagine that the industry would do that anyway, would look into it further if that kind of situation came out in research.

DR. TIMM: We won't have a real Tier 1.5 but I suspect if there's a real conundrum that as you

said industry will probably conduct a special study probably in consultation with us if they can formulate a hypothesis of what might be going on and can design such a study that would be probably something that we would both be interested in.

DR. HEERINGA: Dr. Touart.

DR. TOUART: Just a follow up. Just normal regulatory process, you know, there is the ability for industry to do like a rebuttal or something, I mean if we come in with our interpretation of the Tier 1 say, you know, this triggers Tier 2 but the industry would have a different perspective, they could provide some information or evidence to indicate why, you know, they felt that we over interpreted or misinterpreted, you know, the information so there's that potential context but again I would hope that the logic and rationale that we would be using in our weight of the evidence and the findings that we would have would be sufficiently supportive in the case that that would kind of establish what we need to be doing next.

DR. HEERINGA; Other questions from panel members with regard to the presentation. Dr. Denver?

DR. DENVER: I have a couple of

questions. Actually the one relates to a follow up of the question that was just asked and that is being able to detect endocrine disruption at early developmental stages so early development tends to be very sensitive to disruption and I don't know that any of these assays are specifically designed to address that question. If that's been considered.

DR. TIMM: Back when EDSTAC was deliberating, one of the things that they talked about to use low doses, they talked about in utero or in ovo exposures and of course there was always the desire to have that in utero or in ovo part of that assay being a screen and they don't look much like screens, they're, to have enough end points in there to do the kind of job you want they become rather laborious and complicated but also the consensus of the committee was that you probably will not have an endocrine disrupter that does something only in the developing animal and shows no signs at all at puberty or so the idea was that you would have these higher dose level studies performed in the pubertal animal or in the fish repro, I mean you do really have here a different life exposure stage in the fish reproductive study so that by that combination the thought is that you would catch most everything of real interest.

DR. DENVER: Well, I think that I would beg to differ with that characterization because I think that it's clear that early developmental stages can be perhaps more sensitive to endocrine disruption than pubertal stages. Also it's also important to point out that the actions of hormones can differ dramatically between early developmental stages and later stages. In fact you can have just the opposite effects of some hormones during early stages versus later stages.

DR. TIMM: I would not dispute either of the two points that you made. I guess that what I would still say is that how likely is it that you're going to have an effect of a compound on development where you see absolutely nothing in any of the other assays in the battery. There may be such a compound. If you know of one, we'd like to know about it, but that is our... that's currently our view based upon what we've seen.

DR. HEERINGA: Let's be sure we don't drift into our recommendation from the discussion but go ahead.

DR. DENVER: Yeah. Well, I guess I'm just trying to understand how if one has say a positive result in an in vitro assay but all negative results in

in vivo assay, then does one consider that that could have some effect say at a different developmental stage? I think that was the question that was posed here earlier, right, so would an in vitro result but not an in vivo result trigger further investigation of the potential?

DR. TIMM: I guess the... you know, we tend to look at statistical significance as a yes/no, and maybe in this case what one would want to look at is look at all your end points, you've got a, some very strong signals in vitro but you had some end points that came up in vivo and they were .08, didn't make .05, maybe you'd say hey, there is really something going on here, but in general if you can't get anything in vitro or in vivo rather in these assays, why would you expect to see something in vivo in the two generation assay for instance.

DR. HEERINGA: Dr. Touart had some contributions.

DR. TOUART: I just, this was a follow up to the earlier question in terms of early development in context as far as a component like that in the battery but typically what I think what can occur with changes or exposures that may occur like in ovo or embryo it may not be manifest until that

individual reaches adulthood or maturity and the context of that is you're talking a much longer term assay than even some of our apical assays already which twenty one days or so can be considered a pretty long time for a screen and so that's the context of considering and we do have some early development but they're not earlier, I mean it's like pro metamorphosis in amphibians but again those are for thyroid endpoints not for other, you know, reproductive type effects. There is a component in the fish short term reproduction assay if you were to collect the eggs that you're counting in terms of what's being produced, those can be kept and hatched and end points collected which would be really effectively the beginning of your mostly generational task but we felt that from the screening context as Gary pointed out, that for most of the kinds of compounds that we are aware of we still are able to get some, you know, catch in terms of the assays that present but I think again it would be, it would be difficult for us, it's just a positive in the in vitro and all the in vivos were negative in kind of a firm negative in context to think well, to go into a multi generational study again at the screens are tested at fairly high levels, you know, the context that these are lucky to, you know, still manifest it

would be a difficult sell for us.

DR. HEERINGA: Dr. Denver.

DR. DENVER: So my other question goes to the broad goals of the screen which are to identify compounds that disrupt the endocrine system not only in humans but also in wildlife, and in some cases those goals appear to be separable, for example, in case of the fish reproduction screening assay I think you mentioned that that was intended to be specific to fish, although it can identify some estrogenic or potentially anti-androgenic compounds but what I was curious about is the amphibian metamorphosis assay considered to be an assay that would identify compounds that would affect amphibia and how would the EPA use the information from all these different assays to assess the risk to humans versus wildlife or are those two considered together?

DR. HEERINGA: Dr. Touart?

DR. TOUART: I didn't mean to imply that the fish is for fish and that the frogs were for frogs, I think that the context of these are established kind of the bookends of the file and the fish and form what we see on the mammalian side as well as on the mammalian side when flora when we see in the fish. If we get corroboration between the fish and rodent or the

frog and rodent I think the indication is that this is something that is more, you know, universally manifest through vertebrates and we would anticipate that birds or reptiles or really any, any class of bird primate or what not would, would likely also be affected if we see in just one if just the rodent is positive but we don't see a positive in the fish say for compound, or in the frog the context it could be a metabolic difference that could explain that in terms of the mammal couldn't metabolize it into an active form and vice versa if the situation was reversed in the fish, so those are some things that we'd have to take a look at but if the...there were several end points that were affected within a given group but we didn't see it in the other taxonomic group, then that potentially could affect how we treated the Tier 2s in terms of which group might get looked at but the general sense would be that it would have been flagged as a potential and given that we have different life stages in terms of a pubertal rodent versus a say a reproducing adult in the fish or larvae in the frog. The indications are that a different life stage may also be affected, so the fish and frogs are to work in combination with the mammalian assays and we're really not considering them a mammal, the fish, or the frog, they're just sort of models that

are being utilized in interpreting and that some of those models have... give us better insight and better resolution for effects that may be occurring in one case versus another.

DR. HEERINGA: Dr. Zoeller

DR. ZOELLER: Tom Zoeller. To maybe rephrase that a little bit is Tier 2 tailored to the specific kind of profile of effects that you see in Tier 1 or if Tier 2 is triggered it's the entire tier?

DR. TOUART: Well, most likely it would be the entire Tier 2 because for a lot of this there's an exposure component and the context is that if everybody's exposed then there's a potential we would only take a look at if there was exposure information did it indicate that the particular compound because of its manufacturing or use, there's really just a subset that's exposed, maybe it's just, you know, humans in an indoor type situation and there's not anticipation for fish or wildlife that would have any substantial exposures then be retained just on the mammalian side and vice versa if a material's released, but, but it's hard to contemplate a particular compound if it's out, it's out, everybody's going to get exposed in some fashion in terms of if it gets into the water fish would have it, the fish would be up the food chains or

what not, so other wildlife as well as humans would be affected in that context. I mean, the drinking water is coming from, you know, those sources of supplies would be of concern so there's, you know, there's a potential would be more likely is that one might focus on given end points in the Tier 2 to say well, everything seems to be indicating that this is a potent estrogenic type compound, so we want to pay more attention to those modalities and ramifications that would be more related to estrogen and maybe not spend as much time on something say like thyroid type end point necessarily, the context being that if you have limited choices in terms of what assays you're doing, whether you're going to look for a given hormonal measure or a histopathology type measure you may want to make some of those type of choices based upon what kind of mechanism you think might be involved, but again the Tier 1s aren't really defined mechanisms, in some cases we've got stronger indication than in others but I think it would still be a little bit dangerous for us to try to form any conclusions from the Tier 1 data per se if that helps.

DR. HEERINGA: Other questions of clarification from the panel. Dr. Delclose?

DR. DELCLOSE: Barry Delclose, this is a

pretty simple question I think. In the OECD guideline for the uterotrophic assay it gives a choice between the immature and the castrate, and indicates that the immature is preferred for animal welfare concerns, it also indicates that it's responsive perhaps less sensitive but the response is to a broader range of end points because of the HPG acts as an impact, but that's not listed among your HPG responsive assays.

DR. TIMM: I think if we were having the uterotrophic done for us, we would probably request that it be done using the subcutaneous route in an animal, however if a uterotrophic assay exists, and it's done according to the OECD guidelines we certainly would accept those data. There's the Mutual Acceptance of Data Treaty that we are party to back in 1983 and so we would use those data and make an evaluation on that basis.

DR. DELCLOSE: So your guideline is not, is not necessarily the OECD guideline?

DR. TIMM: No, no, we accept the OECD guideline but when we are, I mean even with the OECD guideline we could say hey look, you know, do it according to the OECD guideline but our preference is to have you do it this way and they may come back and say hey look, you know, we would prefer to do it this

other way and we could get into the discussion and they may prevail but that's generally the case, if the OECD guideline allows a wide range of things and we have our preferences we'll let our preferences be known.

DR. HEERINGA: Seeing no additional questions at this point, we'll have a chance to return. I think we're going to hear public comment, we'll have a chance to interact there, and before we move on to the charge questions we'll certainly let Gary Timm and Dr. Touart open it up for a few additional clarifications if things have come up, but at this point I'd like to thank you very much for the presentations this morning. As I mentioned before the break, I think the materials were very well organized, very efficiently presented, I think that's helped considerably so at this point in time, I think I would like to move to the period of public comment and for the audience my aim would be that we will run, since we started at nine we'll run 'til about 12:30 and then take a break for lunch at 12:30.

Public commenters, a number of people have registered prior to the meeting, with Jim Downing the designated Federal official and have been given ten minutes or extended periods for further presentation, and I would like to make sure that they hold to that as

closely as possible and I'd also like to mention as I did this morning that if there's anyone else in the audience who has not registered for a public comment, but would like to make a public comment, please see him during the noon hour and I think you'll find the agenda here for you to make a short public comment.

At this point in time I'd like to invite up our first public commenter and that's Dr. Christopher Borgert who is with Applied Pharmacology and Toxicology, Incorporated. Dr. Borgert.

There's a public commenter mike unless you have a presentation, either one would be fine. That's great.

DR. BORGERT: While she's getting that up I'll first of all, thank you for taking these public comments and mention that I worked with the American Chemistry Council in the past on issues related to the endocrine screening program. My comments are however my own, I tend to stray far from the farm and won't hesitate to do that at this juncture.

Thank you very much. Okay, we've got it. I was a member of the EDSTAC Plenary Committee Screening and Testing Work Group as well. My perspectives differ a little bit, I was always one of the folks who was advocating a more streamlined

approach in trying to do something, one thing perhaps well before we got so ambitious to develop a program that was maybe unwieldy. I want to point out that FQPA mandated screening and those are supposed to be cost effective but we really can't evaluate cost effectiveness unless we know the public health or environmental problem that the program is supposed to address so I hope the Agency will define that health problem so that we can measure it and then measure the cost effectiveness of this program once it's underway. Currently the predictive power of the battery and the assays themselves can only be evaluated in the context of known positives or negatives.

I'm going to talk about the in vitro assays specifically, these are generally faster and cheaper, usually considered better suited for screening, but at this point I would say that none of the proposed in vitro screens are really fully validated and standardized and so they're not really ready. We need better in vitro methods or at least to shore up the ones we have to best fulfill the goals of replacing and reducing and refining whole animal studies so let's get into the assays themselves, I'm just going to hit a few high points, the estrogen receptor binding assay or the estrogen receptor

transcriptional activation assay probably only assays that could actually be said to fulfill the mandate to test for estrogenic activity in humans, I don't think the program can really go forward without one of them, but they are not fully validated and standardized so far. For instance there's still questions about which cell line or rat size cell should be used, there's questions about how chemicals that would denature proteins would behave in this assay, we're going to use a radial label requiring method or non radial label that will cut costs and disposal, this assay of course doesn't differentiate agonists from antagonists and their other limitations that we know about but there are some aspects of validation and standardization yet to complete and I think those are essential. We could replace that perhaps with the transcription activation assay which will differentiate agonists from antagonists, would satisfy the three r's if done in a helo immortalized human cell line but we have got some problems, if you peel back the onion skin and look at the validation data that we wonder what is a positive response. For instance, the positive control ten percent gave a thirty percent false positive rate in this assay so I think some better criteria need to be established for an acceptable PCX value whatever that X

is. Again, cytotoxicity and protein denaturation need to be, we need to decide how to evaluate those based on the siferase assay and it's really open to some confounding so that would need to be worked on and many of these in vitro assays we talk about the limit dose being used but nonetheless if you're going to really interpret the response some other doses are useful and important but how will that be evaluated in such an assay, will it be expert judgment, I would favor certainly stricter statistical criteria. The validation, OECD's validation was done in Japan, it was a few labs, just a few chemicals, and much larger scope is needed so it wouldn't be recommended at this time as opposed to the binding assay which I think I would recommend if the validation can be completed.

The androgen receptor binding assay is probably the closest to being validated and standardized. It does minimize animal use but it does use animals. Minimizes the use because you can prepare a back cytosolic fraction but there's the biggest problem with that assay is you've got to resolve the issues of preparation of that rat cytosolic fraction. In the validation efforts some labs obtained fairly poor results when they used their own preparations.

They did much better when the

standardized cytosolic preparation was passed around the labs. This assay is sensitive for chemicals that bind strongly but less so for weak binders, again I won't go through all the other limitations, they're in my more detailed written comments on the slide but this would be my recommendation but again the validation needs to have the i's dotted and the t's crossed here, although this one is the closest.

The aromatase assay has some advantages, it's full in vitro method but again the standardization and validation are incomplete. There are only a few laboratories and a few chemicals evaluated while only one mode of action is evaluated. The assay functions only on inhibition, misses potential enzyme induction, again protein denaturence could give false positive results and other limitations so that would not be a recommendation of mine at this time. Finally the steroidogenesis assay again would satisfy the three r's because of a human 295R cell line standardization and validation again are incomplete and there are serious questions about transferability. CSV decided what culture median, what source of charcoal strip fetal bovine serum, other things you can read in my bullet point there, passage number, edge effects, et cetera. It's still unclear exactly which endpoints would be

included and what the performance standards are for those but those are sorely needed and also how cytotoxicity will be evaluated. The live dead method that's recommended by the Agency is somewhat questionable, the MTT method would be my preference. It's based on myochondrial function, gives a little more precursor idea of cytotoxicity than just live dead cells but it hasn't neither one of those has been adequately demonstrated. Again some of the same problems with the other in vitro assays that we know about but this a too premature stage of validation and standardization to be recommended at this time so I've been fairly quick in my comments, to move along and allow time for other speakers butt I think that these in vitro assays are key to the screening program and we really need to do a thorough job of validation standardization and seriously address at some point this question of what public health problem is this meant to remedy. That ultimately should be the performance standard. Thanks for your attention.

DR. HEERINGA: Thank you, Dr. Borgert. Just a second, any questions for Dr. Borgert based on his presentation? I would like to thank you very much. At this point I want to move to Dr. Sue Mardy of Dowell Chemical. Dr. Owens and Dr. O'Connor, I think we'll

try to load your presentations over the lunch hour, just to keep things flowing.

DR. MARDY: I too would like to thank the panel for the opportunity to present public comments. My name is Sue Mardy, and I'm a toxicologist with Dowell and I have been fortunate at Dowell to get first hand experience with a number of the Tier 1 assays and I'd like to share some comments on the male and female pubertal assays today.

Just to remind everyone that the pubertal assay designs, what these are, they start with weaning rats, females are exposed from day twenty two to day forty two by oral gavage, we also expose from day twenty three to fifty three also by oral gavage to look for changes in the age at puberty onset, vaginal opening in the females, prepubertal separation in the males. When vaginal opening has occurred with females you monitor estrocycline and then at necropsy what you're looking at is organ weights, you collect blood for serum hormone measurements and you do histology on selected organs. More specifically the assay end points you have two dose levels, one of which is an NTD which is designed to produce up to a ten percent change in term of body weights, age evaluated pre pubian onset, age at first estrous and regularity of estrous

cycles in the females and then at necropsy you're looking at pituitary, adrenal, and thyroid weights in both males and females, ovarian and uterine weight in females, testes, epididymis, ventral prostate, dorsilateral prostate, seminal vesicles, and other anion weights in the males. Certain hormones that are measured are T4 and TSH in both genders and testosterone in the males and then histopathology in the thyroid, ovaries, uterus, testis and epididymis.

Now what I wanted to address today were some issues in the pubertal assays with respect to assay specificity. To start with the specificity for detecting estrogen, androgen, and thyroid active agents, the assays require a number of apical end points which can be altered by both endocrine and non endocrine or systemic toxicity effects. There is an inherent variability in the age of puberty onset so the mean age of vaginal opening in the integrated summary reports varied over a three and a half day range. The mean age of puberty, preputial separation varied over a four day range and quite honestly this inter animal variability of puberty onset is really quite poorly understood. We do know that puberty onset can be altered by a number of factors other than just estrogen, androgen, and thyroid and I've listed a

number of those variables on this slide. Estrous cycles themselves are variable particularly at the initiation of the estrous cycle and this was shown in the multi chemical study, multi chemical study that was done in the integrated summary report where this had only two of fourteen females that showed estrous cycling, regular estrous cycling in the control group so it can be inherently variable just in the initiation of cycling. Target organ weights can be affected not only by endocrine active agents but also by stage of estrous cycle at the time of necropsy and changes in terminal body weight, so it may be difficult to determine when you see a positive in this assay whether or not you have estrogen, androgen, or thyroid active agents.

Specificity, we've talked a little bit already about the negative control chemical that was used in the pubertal assays which was 2-chloromethyl benzene. This agent affected both males and females in the pubertal onset assay. Delayed vaginal opening in the females as well altered a number of thyroid endpoints. In the males it delayed preputial separation, affected androgen dependent organ weights and testis histopathology, Now it's possible that this was simply a poor choice for a negative compound but it

still leaves the author the question as to whether or not this assay is prone to non specific effects resulting in a high frequency of false positives. In the assay recommendation paper that recently was released, it said although a toxic negative chemical has not been identified, several chemicals positive for one of the mode of acts...one of the modes of action have been found to be negative for other modes of action evaluated in this assay, and this concept is difficult to apply consistently across the data set of data in the integrated summary reports for the pubertal assays. For example, if you consider phenobarbital which was used as a positive thyroid agent in a number of Tier 1 assays, phenobarbital was detected in the male pubertal assays for effects on reproductive end points not effects on thyroid so obviously this case you wouldn't report that phenobarbital would be a negative control for thyroid end points in this assay. That's a difficult concept to uphold across the data set and in addition it still doesn't answer the question as to whether or not systemic toxicity by non endocrine active chemicals might produce positive assay results.

The maximum tolerated dose that's been proposed for the pubertal assay is a dose level that

would be considered to be at or just below the maximum tolerated dose would be a dose that caused the body weight changes of no greater than approximately ten percent of the mean for the controls. We believe that body weight decreases that are approach or equal ten percent are not appropriate for the pubertal assay maximum tolerated dose. These are effects of body weight on the organ weight end points to the female pubertal assay. This was a feed restriction study that was done by Susan Laws at the EPA. For this study again it was feed restriction using the female pubertal assay design. There were no chemical treatments and what I did was under the terminal body weight column you can see changes in terminal body weight with increasing magnitude of change. Two percent, five percent, twelve percent, twenty percent, and what you can see is that a twelve percent change in terminal body weight affected pituitary weights, adrenal weights, and ovary weights. On the maximum tolerated doses designed to be less than equal to ten percent. We don't know exactly what they'll do to assay end points, but we do know that somewhere in between five and twelve percent there's a significant change in the number of organ weights that we measure.

This is the body weight effects on the

male pubertal assay and this there are more feed restriction studies from which to draw so I've listed those in the left hand column. Terminal body weight changes, you can see there about the fourth column from the left and I've put them in ascending order for the magnitude change in body weight. Again no chemical treatment, just feed restriction through the pubertal assay design and what you can see is that at a four percent change in body weight there was already a significant change in pituitary weights, up an eleven percent change in body weight there was a significant change in epididymal weights, ventral prostate weights, seminal vesicle weights and dorsilateral prostate weights which I didn't list up there. So you can see somewhere again in between where we see a four and eleven percent change in body weight we have a number of significant changes in organ weight end points. I should also mention as well that there are a number of assay end points from the pubertal assays which hadn't been looked at for whether or not body weight effects might impact them from, for instance level A9 which has never been addressed with respect to body weight changes nor have initiation of regular estrous cycles been looked at.

On statistical analysis the integrated

summary reports state that body weight mediated differences in organ weights can be alleviated by evaluating organ weights on a relative weight basis. However this analysis is not permitted according to the pubertal assay designs. What's recommended is a covariant analysis, covarying with body weight at weaning. Now this isn't appropriate with respect to statistical practices in that you have a covariant that is not affected by treatment. However, we know that body weight affects a number of these end points and it doesn't account for body weight effects, terminal body weight effects on organ weights. There has been a recommendation in the past that covariant analysis can be done with terminal body weights, Muscale and Torry have made that kind of recommendation. I myself am not a statistician but I would implore this group to consider whether or not there might not be an alternative statistical approach.

So in closing I just want to say that the end points, a number of the end points in the male, female pubertal assays are apical and do have some inherent variability, so it may be difficult to determine whether chemicals have estrogen, androgen or thyroid effects. The assays have not been adequately validated for specificity, there's no evidence that the

assays are specific for just endocrine acting materials. We have no negative control data using a negative control chemical. Ten percent change in terminal body weight is likely to alter assay end points, we've seen this with feed restriction data and we don't feel that those statistics that are recommended for the assays are going to adequately consider changes in terminal body weight with respect to these organ weights.

We'd like that the MPD be reconsidered for these assays. The problem with this is that the assays may produce a high rate of false positives which is my final slide, so it's the impact of false positives with the pubertal assays. Well, the pubertal assays are designed to detect modes of action that are not readily detected by a number of assays. Because they're in vivo they can detect activity of metabolites that you won't see in in vitro assays. Positive results for pubertal assays may be difficult to refute and could trigger Tier 2 testing. Tier 2 testing is going to be costly, resource intensive, and is going to use large numbers of animals. Multi generation study costs half a million dollars on average and uses more than twenty seven hundred rats. Compare this with the other Tier 2 tests which include studies on fish,

birds, and amphibians, and that's going to be a lot of animals that are going to be used. And costs will easily exceed a million dollars per chemical. We also face product deselection in between generated Tier 1 results and being able to follow through with full Tier 2 testing. Thank you.

DR. HEERINGA: Thank you, Dr. Mardy. I would ask the panel at this point. Are there are any questions? Dr. Chambers?

DR. CHAMBERS: Dr. Mardy, one of your earlier slides said there was some variability in the days required for vaginal opening, preputial separation I think, is that what you said? Were you meaning between different laboratories or among the same animals in the same laboratory test?

DR. MARDY: You can see within the same...that happens to be, that study data that I showed you is across laboratories but within the same laboratory from time to time, you can see that variability and there's a very nice data set that was published by Merck which included thirty five studies in which they looked at puberty onset and it showed exactly the same span for males and females so even within the same lab.

DR. HEERINGA: Thank you very much, Dr.

Mardy. At this point in time I would like to do a little check here. I tell you what, rather than getting too far out of order with the agenda, we are at 12:00 and we have a number of public commenters I think, several, who need to load their presentations so what I'd like to do is call a lunch hour at this point and ask that we...let's reconvene at 1:15, that gives everybody a little over an hour for lunch and at that point in time we will continue with the public comments.

What I would ask though is that anyone who does have a presentation is scheduled for public comment this afternoon that you coordinate with Dr. Madden to make sure that your presentation is loaded on the presentation lap top so thank you, everybody and we'll see you at, what did I say, quarter after, well, 1:15.

(**WHEREUPON**, recess was taken for lunch.)

DR. HEERINGA: We'll get back underway, please. Welcome back everyone to the afternoon session, our first day meeting of the FIFRA Science Advisory Panel on the topic of the Endocrine Disruptor Screening Program Proposed Tier-1 Screening Battery. At this point in time we are in the process of the period of public comment, and we have heard prior to

the lunch hour from Dr. Chris Borgert and Dr. Sue Mardy. And I believe at this point we are up to Dr. Owens who is here from Procter and Gamble.

DR. OWENS: Let me see if this works. Okay. Somebody needs to underline this, pretty please. Just to -- I'll start off with the first slide which did say Willie Owens of Procter and Gamble Central Product Safety. There's also in a sense some transparent disclosures, and that is I've worked with the OECD since 1999 as an industry member of their validation management group, and that is for the Hershberger, the uterotrophic, et cetera. Did not conduct any of the laboratory work but was heavily involved in writing the reports, the peer review for the uterotrophic. I actually was secunded to the OECD and had a pleasant stay in Paris for a year. I have also here in the US been a member of the FACA, et cetera. So I'm interwoven with a number of the assays et cetera, particularly the uterotrophic and the Hershberger that I will speak about we hope.

DR. HEERINGA: We'll take a moment until Charlene gets that brought up.

DR. OWENS: Certainly. Just trying to make the best use of time here.

DR. HEERINGA: There we go.

DR. OWENS: All right. Now that we've done the formalities. We'll move down. The basic views here for this presentation is not one of complaint. It's one of good news. It's both of the assays, the uterotrophic and the Hershberger have been through an international validation program. They are found to be satisfactory. They have complied with all of the criteria in the OECD Guidance Document 34, which addresses validation, et cetera. So I'll be giving you some background. I think there's also some learning points in some of the questions and comments I heard this morning.

Basically the rationale for the assays is extremely straightforward. The estrogens and the androgens, of course, and actually regulates specific male and female target tissues. Fortunately, in most cases this growth is specific. It's rapid. There is a significant percent change or magnitude of response, and particularly with the male tissues, if your dissection is good, you can keep control of the coefficients of variation. So quite frankly, you can, with a den of only six animals per group, get very good results. Also the assays were developed actually in the 30s. The original Hershberger itself was without the muscle complex. It's been refined in the

pharmaceutical industry. It's also been adapted to antagonist in both cases. It's also been adapted in both cases to specific inhibitors, that is for aromatase. You take the immature animal with the ovaries still present. You coadminister an aromatizable androgen and with a given inhibitor, and you can get a nice dose response based upon uterine weight. Also there were public health studies for both of these assays in the 60s, massive inter-laboratory study, over 700 compounds for each assay, the problem being that most of these were steroidal compounds. They weren't commercial compounds.

Now quickly, the program with the OECD, there were videos and dissection guides, figures, et cetera. Roll over here to the dissection guide for the Hershberger. The test protocol was sent out to a number of laboratories in international settings, primarily because English as a second language issue. The statistical powwow was analyzed thanks to Joe Haissman, and then the protocols were normally tested in a phase 1 with open compounds. From there a dose response with weak positive commercial compounds was carried out in phase 2. Then in phase 3, there were coated substances. Since working with coated substances to avoid bias and subjectivity in the

laboratories, there's a criteria under Guidance Document 34 which should be applied flexibly. Negatives and the same dose for all of the weak positives in phase 2 were used in phase 3 basically so could address the other criteria - are the laboratories as a whole getting the same result over time.

Now in the utero-trophic, and we've got an exhibit, the issue that has come up again and again of dietary phytoestrogens was addressed.

Briefly the overview of the utero-trophic. There are a number of elements that were standardized in the left column, and recalling this was intended as an international test guideline and also as a screen. There were a number of parameters that were allowed to vary, but for example, both Wistars and SD's, Sprague Dawleys were in the validation program. And these were also co-analyzed.

Now top line on the left is the epi needle estradiol and to assure indeed we were working with a set of weak compounds, you can see where they sit on the overall dose response curve. The VPA, and that was the OP prime of DDT. Going on, you'll see here a comment from this morning. You can see the immature gavage and SC the adult ovex SC at three and seven days. There was a great deal, shall we say, of

controversy about whether the ovex was indeed superior or not. The bottom line is it's equivalent. The power differences come down to the third decimal place; so they are basically equivalent. You'll also see gavage in SC were run, and there quite frankly the DDT and the methoxychlor were more potent if administered orally. The others if SC. The guideline in reference to the comments that Gary made this morning, the guideline basically indicates in guidance that you should use the relevant route of exposure. Now personally and in context of my employer for animal welfare, frankly choosing a default SC is a misuse of animals. It's not ethical. Use the relevant route of exposure of the dose is straightforward. So I would urge you to think about that point quite carefully.

The Hershberger linger on -- you'll see the dose up at the top of the slide -- is the weaker antiandrogen in a number of the assays. What you have here is the dose from phase 2 in four labs on the X-axis and the coated same dose from 10 laboratories in phase 3 on the Y-axis. What I've done is here are the means, across, and we've plotted them. So the body weight decreases slightly. Then there's the glans penis. Cows is Cowper's glands, the muscle complex, ventral prostrate, and seminal vesicles coagulating

gland. Okay. That approximates the response of each of these tissues with the other antiandrogens in terms of a decrease. And I admit, like you, I was quite surprised by the R-square.

Now one of the elements that, quite frankly, was mentioned this morning is what about in-utero exposures. In validations programs are what's called, rightly or wrongly, a predication model. It's really a correlation or correspondence test. How well does the screen do against higher tier assays? What you have here are the five weak positives, and there in the central column is the utero-trophic minimal effective dose by oral gavage. And over on the right-hand column you have, either from a pubertal type assay, an in-utero type assay, or a full multi-gen de-correspondence of the doses. You will see that for this phenol-A at the bottom there is not. Remember we're working with three administrations with necropsy on the fourth day; other toxicities, quite frankly, don't come through. And so you can actually press these animals well above the, what I call a normal MTD, and in this case the so-called estrogenicity doesn't come through in the multi-gen.

Now I'll put this down. Now importantly here on the Hershberger, here the in-utero effects are

drastic and irreversible for antiandrogens, and it deserves close examination. You can see its minimal effective dose and, quite frankly, relatively sensitive endpoints in terms of anogenital distance, and retained nipples in the male animals, and where you've had in-utero exposure in the proper window in the dams. The net result is, quite frankly, compare the screening data, compare the higher tier data. And I think in a question that was asked this morning is you can see if indeed there is a lack of sensitivity in the screen or there is a good correspondence in the screen? And that's why the criteria to make this comparison is part of a validation program for Guidance Document 34. It is, I will point out, something absent in many of the other assays. But I think you can see its power and its utility.

Now the phytoestrogens to try and explain the slides and the procedures is here are the data for non-phenol in the intact in the tiers. It's a relatively high dose of 250. That will exceed the MTD in longer assays. The diamonds are the means. Those bars are not SDs or SEs. They are actually the upper and lower 95% confidence levels. Remember you've got an N06, and if the lower part of the bar exceeds a value of 1, indeed you are statistically significant.

These are from dietary assays on the phytoestrogens. Here you'll see that, one, the means do not decline despite as phytoestrogen levels increase. Neither is there an absence of sensitivity as the phytoestrogen levels increase.

In another set over on the far right at about 85, there was one laboratory that did with other issues. So an apparent decrease in a test guidelines. Therefore, there is a ceiling on the phytoestrogens in the uterotrophic assay in order not to lose any sensitivity in the assay, but, again, the issue has been addressed.

Again, as I begin to close down here, these on the table are the various criteria in Guidance Document 34, paraphrased mind you. Both the utero and the Hershberger have complied with all of them. I would urge you to go forward and ensure that other tables are self-instructed, so you know if the assays presented to you are in full compliance and what is the rationale if something is lacking. Finally conclusion, the EDSTAC battery quite frankly is seriously outdated. It was conceived in 1996. The uterotrophic and Hershberger are fully validated. They're accepted for EA and Anti-A. They are also even now in use by Europe, Japan, and others. There is also a significant

data set approaching a 1000 compounds for the uterotrophic in Japan. I can also assure you that industry in Europe has also run a number of compounds according to these guidelines. It would be inappropriate to repeat data. So how to use and acquire these data is one of the things that confronts you to minimize animal use. On the thyroid, there is also 28 and 90-day repeat dose. We also have the reach legislation in Europe where many of the assays, higher tier assays, are going to be de facto. And do you use the data available to see if you need to screen. Then finally highly specific, small-M, in-vitro can proceed. How do you arrange a battery that is fully compatible with the RRR's in terms of animal use? And finally I would argue that complex animal-intensive assays such as the pubertal shouldn't be used in a default routine requirement pending other available information. In other words, the battery itself should not be a default checklist but appropriate to the information that's available, both at higher tier and existing tiers, lower tiers, for a compound. Thank you. And in interest of time, any questions?

DR. HEERINGA: Any questions for Dr. Owens? Yes, Dr. Eldridge.

DR. ELDRIDGE: Uh, Chuck Eldridge, Lake

Forest. Um, Dr. Owens, what would be your opinion of using the uterotrophic assay to identify estrogen antagonism as well as agonist.

DR. OWENS: In the case of the antagonist, the OECD has written a guideline. You will note that I had up there E-A and Anti-A. The peer review noted that only a single potent antiestrogen had been used. The data was reproducible across labs. No evidence of CD problems, but, quite frankly, were absent a battery of weak antiestrogen commercial chemicals to take the assay forward in terms of a true validation program. There is nothing on paper that would say it cannot be used, and, in fact, in the phase-1 report if you want to refer to it, all of the data is there on the antiestrogen phase-1, again, a single potent compound for a shakedown of the protocol. Protocol was adequate. So in terms of you have a green light to proceed, but the question is, is do you have actual data in hand established to say that it is valid for that use. Therefore, it is not in the test guideline. It is only in an associated guidance document with the OECD. There are no technical barriers. There is the regulatory requirement for validation that would be the hurdle. Let me add though, that confronts any of the other assays also is

there are a very limited number of antiestrogens, so all of the other assays are in the same boat.

DR. ELDRIDGE: Thank you very much.

DR. HEERINGA: Thank you very much, Dr. Owens. At this point in time I would like to invite up Dr. John O'Connor, who is with Dupont Haskell Global Centers for Health and Environmental Sciences.

Dr. O'Connor?

DR. BECKER: Mr. Chairman, it's Rick Becker with ACC. If we could just switch, that might be better.

DR. HEERINGA: Sure.

DR. BECKER: Thank you.

DR. HEERINGA: No problem.

DR. BECKER: But now we close --

DR. HEERINGA: Yeah. Close the computer.

DR. BECKER: Sorry.

DR. HEERINGA: Why don't you leave the computer open, I think.

DR. BECKER: Well that was good. Thank you, Mr. Chairman. Thank you, again, to the EPA for this meeting, and thank you for allowing us to provide our comments at this time. This is a critically important issue obviously from a chemicals industry perspective. Just to note that we supplied written

comments late last week and appreciate the, you know, this was up against the deadline. I wanted to thank the EPA for receiving those comments and sending those out quickly so that the Science Advisory Panel members had a chance to have those before the meeting. I particularly appreciate that. So as we go through some of these slides, if there's additional information that we just cover very quickly, I would refer you to those written comments.

I want to talk to day briefly about the 15-day intact male, adult male assay for the Endocrine Disruptive Tier-1 Screening Battery. I'm a toxicologist with the American Chemistry Counsel, a trade association that represents the commodity chemical manufacturers in the United States. I've been working with that organization for almost, more than nine years now, and have been engaged with the endocrine program since the time that I joined.

You've seen a slide like this similarly in the presentation from the EPA staff, so I won't go into great detail on this. But just to note that the EDSTAC recommendations here did include, in fact, I'll turn to screening battery number one, an alternate to the recommended screening battery, and that alternate included the adult male assay. And then there was also

an alternate too that was discussed earlier. Well as folks have indicated, since that time a significant amount of laboratory has gone on to develop the methods, to evaluate them, to standardize them, and to validate them. And so things have changed as to be expected in a scientific endeavor like this. So where are we at today? Well in March, just recently in this year, the EPA has proposed this new screening battery, which differs, as was pointed out, significantly from the EDSTAC recommended battery. On the left-hand-side I've listed the EPA's newly proposed screening battery. The ones in red there are the assays that I particularly want to talk about, the contrast or compare to the intact adult male rate assay. And so what we want to talk about today, what I'd like to at least introduce today is a recommendation to streamline and appropriately focus the tier-1 screening battery, which would substitute the intact adult male assay for those outlined in red on the left-hand-side. For the steroidogenesis assay in lieu of the pubertal female assay, in lieu of the pubertal male assay, and in lieu of the amphibian metamorphosis assay.

I think ever speak has talked about the societal attributes. So I won't go into great detail other than to say that the fact is that if these assays

can be sensitive and specific at the same time, that's the most desirable attribute that we can have, as well as cost effective and quick. So I think there is this issue about low-false negatives, but we also want to make sure there is an acceptable degree of false positives. If that sieve is too wide and everything comes through as a positive, it really doesn't help distinguish the true substances that you need to go on and focus on verus those that are of lower priority. So the study design of the intact adult male rate assay is depicted here; young adult animals, 10-week old, 15 per group, three dose groups plus a control, and the test substance is administered orally for 15 days. The endpoints to focus on here are, to point out, is the hormonal battery, a comprehensive hormonal battery, serum hormones, testosterone, THT, estradiol, prolactin LH, FSH, T3, T4, and TSH coupled with organ weight and focused histopathology, testis, epididymis and thyroid. And, if necessary, biochemical preparation of the padded microsome for later evaluation, if needed. Of course you're working with the intact animals, so you have the intact hypothalamic pituitary testis axis. You've seen similar slides like this, so the X indicates those areas in which interactions can be detected and measured in this assay.

One of the real advantages of this assay is it is a mode of action screening assay. It focuses on mechanistic endpoints, non-apical endpoints, and so the advantage with this is that you can get a profile of responses that would be predictive of a particular mode or mechanism of action. So just as shown here, if you've looked at the substance, it would be an estrogen receptor agonist. The ASG would either be not changed or decreased, no effect on thyroid, decrease in testosterone, no effect or decrease in E2, prolactin increased, LH and FSH plus or minus, and then TSH and T4 no change. And so you can use this profile then to help you identify what particular mode of action of your unknown substance is, and this will be very helpful, I think, in trying to further understand and interpret the tier-1 screening battery as you go forward in trying to integrate the results from the various screens and tests.

The EAC, the endocrine active compounds, that have been evaluated in this assay during its develop standardization and validation are listed here. I won't go through every one of them, but just to point out that it's a full range of endocrine active substances. Estrogen agonists, androgen agonists and anti-agonists, as well as progesterone-receptor

agonist, antagonist, dopamine agonist, antagonist, thyroid hormone agents, steroidal genius inhibitors and also aromatase substances.

We've also evaluated in this assay an allyl alcohol, which is a negative control chemical. We heard earlier today some discussion about the failure of the EPA studies to evaluate or to come up with a negative control chemical in pubertal assays. In this came, and similar to what was done earlier in the other multimodal assays, specificity is usually evaluated with substances of known mode of action and then following that particular pathway, in looking at expected responses and making sure you don't get the unexpected responses. So you don't get androgen responses when you're testing an estrogenic agent and vise versa, but I think it's also important, as Dr. Marty pointed out, to look at negative control chemicals, in particular when we're going to be testing substances with unknown activity in fairly high doses. It's important to have that degree of specificity, and so allyl alcohol was one chemical that was tested as a negative control chemical and shown to be negative in this assay.

We have, in addition, initiated just this year some ongoing studies to continue with and

complete the validation studies of this assay. Two testing laboratories each will be testing four substances, allyl alcohol as a negative control, DE71, iopanoic lactone, and of course allyl alcohol is the compound that was tested earlier. So when these studies are completed by the end of this year, then we'll have a better data set in which to evaluate of the negative test article, negative androgen control chemical as well as some thyroid-monitored chemicals and aromatase inhibitors.

So there's some advantages to using the adult male assay in lieu of the pubertal assays in tier-1 screening. It's a mode of action screen. It's comprehension, sensitive, and specific. It's apical of evaluating many different modes of action in a single assay, focusing on mechanistic endpoints not apical endpoints. So it's focused on trying to evaluate EAT activity specifically. It allows for that profile interpretation that I mentioned. I think it's sensitive and specific enough for the purposes of tier-1. As indicated here, it will reduce the numbers of animals needed. The pubertal tests require male and female, two dose levels plus a control, 15 per each, and this is 15 animals in those groups in a control. The other thing which was also mentioned this morning

is this mode of action assay, as indicated here, you can add additional endpoints if needed as you go forward because it is really focused on evaluating the serum hormones or other hormone activities.

So I'll end with that and say thank you very much. I appreciate it.

DR. HEERINGA: Are there any questions for Dr. Becker on his presentation from the panel? Thank you very much, Dr. Becker. Is Dr. O'Connor a go at this point? John O'Connor, again, as I mentioned from the Dupont Haskell Global Centers for Health and Environmental Sciences. Dr. O'Connor?

DR. O'CONNOR: Well I thank you very much. I appreciate the opportunity to talk today in hear comments. And what I want to do is basically add to what Dr. Becker has talked about with respect to the 15-day intact adult male rat assay. And I've been at Dupont for 18 years now and working in the endocrine screening and working with endocrine disruption issues relative to Dupont Chemicals for about the past 10 or 12 years. And so a lot of the work that I've done has actually been related to the pre-validation work for the intact adult male rat assay.

So, again, I don't want to spend any time on this. I just want to point out that really

what I'm going to do is I want to just give you some examples of some of the aspects of the 15-day intact adult male rate assay that we feel make it a more attractive assay to include in a tier-1 screen than the pubertal assays, and how we perceive this kind of replacing those two assays. Again the study design as Dr. Becker has already discussed, and, again, the key points here are we do have organ waste and histopathologies similar to what is used in the pubertal assays. The real difference is that we have this comprehensive hormonal assessment, and there is limited hormonal endpoints that are evaluated in this pubertal animals, specifically for the thyroid. But the comprehensive hormonal battery here allows us to really differentiate mode of action, which again can be valuable for setting up tier-2 tests, and I'll give an example of that later on.

So this table, I apologize for the small font, but essentially this summarizes the compounds that have been evaluated in the intact adult male assay. You can see there are over 25 chemicals. The testing laboratory is indicated there. On the right side it basically indicates whether it has been or has not been detected successfully in the intact adult male rat assay. You will see there are two piloted and read

there are no, and just to point out that both those chemicals were not run as MTD. So it is unclear if it would be detected if they ran them up to the MTD. The other thing to point out again, allyl alcohol, the negative control chemical that we ran that is a hepatic toxin. We did detect as a negative chemical in this assay, and I will show that data in a little bit. The two chemicals that are highlighted in the gray represent the two chemicals that were part of the EPA's sponsored validation effort of the intact adult male rat assay. So one of the real negatives for this assay has been that it hasn't gone through the amount of actual validation work that some of the other assays have, but there has been, as you can see here, quite a bit of pre-validation work that has been done.

Again, as Dr. Becker indicated, we are in the process of doing some additional validation through some ACC sponsored work. EPA has been involved in discussions around laboratory selection and chemical selection for these. So, again, we continue to generate data for validation of this assay we move forward.

Just a comparison to the pubertal assays. I'm not going to say that this is a 100% complete list of chemicals that have been run in

pubertal assays, but it should represent a majority of them for sure. Again, what's indicated here in red are chemicals that were not identified they say to a red note in those assays. And then, again, I'd like to point out, as Dr. Marty has pointed out earlier, 2-chloronitrbenzene at the bottom, which was actually identified as an endocrine active chemical even though it was ran as a non-endocrine active control chemical.

So one of the big issues with one of these, with the assays, in particular pubertal assays, is really trying to define the MTD, and as part of the pre-validation work, an experiment was run to essentially look at where body weight effects in the intact adult male rat assay will cause endocrine-like effects or cause effects on the endpoints of the intact male assay. I don't want to go through this in detail, but as you can see, this line summarizes the organ-like data and the next two summarize the hormonal endpoint data. Where if you targeted MTD in the intact adult male rat assay that was no greater than 10% difference in final body weight at the end of the 15-day test, you can see that there are no effects on any of the endpoints that would be evaluated. And, again, the next two slides show the serum hormone data, which should, again, by targeting that 10% difference in

final body weight, you don't have any effects due to secondary body weight.

One of the other questions and one of the other criticisms of the intact adult male rat assay has been the fact that for the most part its dogma is that the immature animals are much more sensitive for detecting endocrine active chemicals than mature animals, but unfortunately there's not a lot of good literature out there to really compare that within the context of one study design. So what we did was ran an experiment to really compare immature and mature animals within the context of a single study, in this case using a 15-day duration by oral route. Again, in a 15, same exact study designed for the intact adult male rat assay but with immature animals and mature animals at the same doses so we can compare the responses and get an idea of what the sensitivity differences might be.

We tested this with six chemicals as listed down on the bottom here. I just want to show a few chemicals. So this is the data for Vinclozolin. On the left side are the immature animals. On the right side are the adult animals. The age indicates that day at which they went to necropsy. Okay. So at 53 days of age is equivalent to what is used for the

pubertal male assay. And if you look at the bottom, so this is looking at epididymis, seminal vesicle and prostate rate after treatment with Vinclozolin for 15 days. You can see that between the immature and the mature animals, the responses are really pretty similar. There is not a whole lot of difference there.

If you look at the serum hormone data, again, you see a similar pattern there in that biologically the responses, the sensitivity between the mature and immature animals are pretty similar. Okay. And then looking at data for phenobarbital, in this case looking only at the thyroid hormone data, the thyroid weight was increased in the mature rats but was not increased in the immature rats. But essentially, again, what you see is that there aren't a whole lot of differences between the immature and mature animals.

Now, again, I didn't show all the data for the six chemicals, but what, to summarize all six chemicals, essentially this is the same pattern that you see for the immature and the mature animals when you run them within the context of the single study design, in this case with necropsy occurring on day 53 days of age for the immature and day 84, 84 days of age for the mature animals.

This data is the data from the allyl

alcohol study, and, again, the importance here is that, you know, as we develop these assays for detecting endocrine active chemicals, we need to be certain if we run a negative chemical that is expected to be a non-endocrine active chemical, that it will, in fact, be identified as a non-endocrine active chemical. And, again, if you look here in this study, we did achieve MTD of 90% final body weight in the high dose level. There were no effects on organ weights. There was no effects on histopathology, although that data is not shown. There were a few effects on the serum hormone levels, specifically a decrease in testosterone and DHT at the high dose and has sporadic effects on prolactin at the 40 mg/kg/day dose. But essentially we consider this a negative compound based on the fact that we weigh the organ weight and histopathology data higher than the hormonal data. So in the case here where we have just a single statistically significant change at high dose only, we would not necessarily consider that a positive response. But, again, keep in mind that with this assay, as any other assay in any tier-1 screening battery, you are considering the weight of evidence from all of the assays.

And then finally I'd like to end by going over a brief case study with flutamide,

ketoconazole and finasteride. I don't want to show, I'm not going to show you actual data. This has been published. In this case we're looking at flutamide, which is an antiandrogen, finasteride, which is a 5-alpha-reductase inhibitor, and ketoconazole, which is a testosterone biosynthesis inhibitor. Well if you look at the data, and, again, this is just a summary of the data, but if you look at the organ weight data alone, and, again, this is similar to what would be done in the pubertal assay, you can see that all three of these test substances decrease the androgen-independent tissue weights. So if you're looking only at organ weights, you cannot differentiate mode of action. However, if you couple that with the serum hormone data, in this case looking at testosterone DHT, FSH, and LH, you can actually determine the mode of action by the hormonal pattern. And, again, I'm not going to go into looking at this, but suffice it to say that if you sit down and think about the biology, the responses do make sense and are consistent with the specific modes of action for flutamide, ketoconazole, and finasteride. So, again, this is the value of a mode of action screening battery. It can allow us to tailor those tier-2 tests to more effectively identify those endocrine active chemicals.

So for data interpretation, again, the key here is that the high dose level should not exceed an MTD, and for the adult animals it's fairly easy to define that MTD, again, in contrast to the pubertal animals where it's much more different. The organ weight and histopathology data is weighted more than the hormonal data, again, because the hormonal data is inherently more variable. But, again, when you're looking at the data from any tier-1 screen assay, you need to consider the weight of evidence from all of the tests in that screening battery itself.

And then finally, this the proposed tier-1 screening battery again that we're recommending, which in this case we're replacing the intact adult male rat assay replaces with the pubertal male and the pubertal female assay. And that's it.

DR. HEERINGA: Thank you very much, Dr. O'Connor. Questions on this? Dr. Zoeller?

DR. ZOELLER: Thomas Zoeller. So I'm a little bit confused. In the last talk the proposal was to kind of replace the amphibian metamorphosis and the two pubertal assays with this assay, and now I'm not sure that I understand whether you were --

DR. O'CONNOR: Clearly, what I'm referring to is specifically the intact male assay, so

I was referring to the pubertal assay. But, again, one of the strengths of this assay is manipulability to protect these thyroid-modulating chemicals, and, again, that's why in the current foundation we've added two additional thyroid-modulating chemicals in there. So the belief is, yes, it could, it could also replace the --

DR. ZOELLER: So being close wind, for example, was one that was put through this assay. It showed a decrease in T4 but no increase in TSH, and the interpretation was if TSH didn't go up, it doesn't really affect the system.

DR. O'CONNOR: Yeah. If you look at -- many materials will decrease T4 due to transient decreases in T4 due to liver enzyme. Specifically, if you look at the data for this assay, of the 27 or so chemicals that have been evaluated here, if you look at T4 as a marker, I think there was only one chemical that was run in this assay during the validation or pre-validation that did not increase T4. So, again, that's why it's important to look at the way that evidence is not reliable and stable such as T4 or even just T4 and TSH. It's really important to rely on all of the thyroid endpoints that are available. So it's critical to have that histopathology to thyroid weight

as well as the thyroid hormone.

DR. ZOELLER: So I guess I -- I mean, I'm still -- there's a couple things that I don't understand. One is why would T4 go down if TSH doesn't up, and it's probably not transient because it's at least a 15-day sort of assay and in the other example where exposure is longer, TSH still doesn't go up. So I don't think we really understand what's happening in those kinds of situations to just make the kind of default, you know, using this idealized model that if T4 goes down, and if that reduction is important, then TSH would go up. If TSH doesn't go up, then it's not important.

DR. O'CONNOR: Right.

DR. ZOELLER: And so to just kind of finalize that, in these mammalian assays there's no measure of thyroid hormone actions. There's no tissue-related thyroid hormone action. The amphibian metamorphosis assay is really the only that does that, and there is a number of differences between amphibians and mammals that might be important to consider in a weight of evidence. So it seems to me that if you really eliminate many of the -- there's really only three assays that touch on the thyroid, and if you eliminate two of them or three of them and replace that

with a single assay, you're really losing a lot, at least it seems to me. And I guess my question is how do you respond to that?

DR. O'CONNOR: Right. So I think when I talk about these transient effects, really what I was talking about is the fact that if you look at long-term studies, most of the materials that we've tested in the pre-validation effort do not produce thyroid tumors in long-term rodent studies. So really that's, when I say they're transient, they don't result in tumors in long-term rodent students, and, therefore, for all effective purposes we would expect them to be negative for thyroid, thyroid disruption. With respect to the frog metamorphosis assay, I would agree with you that there's certainly a difference in what the assays are designed to do, but I believe for the most part that if you look at not only the intact adult male rat assay but the pubertal assay, they're also relying on these thyroid endpoints, thyroid weight, histopathology and hormone levels. I don't know that there are chemicals that have been run in the frog metamorphosis as we would necessarily expect to miss in these other assays. So, again, to me it goes back to the fact that if you detect it as an endocrine active chemical, specific for thyroid, regardless of how you're picking that up, is

it really important in a tier-1 screen as long as you're picking it up as a thyroid-modulated chemical? But, again -- so that's kind of how I would respond to that.

DR. HEERINGA: Other questions for Dr. O'Connor? I would like to thank you very much. Again, thank you to everyone. The quality of these presentations all around has been very good. At this point I'd like to as Dr. Lisa Ortego, who is representing CropLife America to come forward, please.

DR. ORTEGO: Good afternoon. I need to add my thanks to the thanks of everybody else, to EPA and the panel for allowing us to make public comments on the assays and batteries today. I'm going to continue discussion with the comments on the amphibian metamorphosis assay and it's inclusion in the tier-1 battery.

I want to add some questions. I want to follow what John O'Connor was just talking about was do we need a frog assay in tier-1? First of all some questions that are really pertinent to tier-1 assay. Is it rapid and is it cost effective? The frog metamorphosis assay requires 21 days in order to run, and if you run a control in four doses, which was what was previously proposed, it takes 400 vertebra and

about 100,000 US dollars to perform that test. Now I do understand that the current proposal by EPA is to drop one of the doses, so that would bring it down to about 320 animals and about $80,000 to perform.

Does it identify the mode of action, which is important for us in tier-1? Can it identify a thyroid-active substance? The thyroid histopathology in the amphibian metamorphosis assay does indicate a mode of action, but the endpoint is already available from the rodent assays that are proposed for the tier-1 in addition to the intact male, which a number of colleagues are proposing to replace pubertals.

Are the results straightforward to interpret? Thyroid histopathology is by a qualified individual. Rodent development effect can be confounded by compounds that cause sublethals for growth and development, and lab variability also has been an issue, especially with developmental stage.

Does the inclusion of this assay address concerns regarding animal usage? Well, because of the large number of animals and the fact that the data that's collected, or at least the very specific data that's collected is available for some of the other assays, I would say no. It seems a bit redundant to have such a large, a lot of animals required and a

costly assay to be included when other rodent assays already in tier-1 might fly compounds as thyroid active.

I put together a table, and, unfortunately, you're not going to be able to read a lot of it. But essentially this is to compare the thyroid activity in a rodent and in the amphibian assays. At least the compounds for which equivalent data is available, and here I have primarily focused on thyroid histopathology. And I have included the compound PTU, T4, and phenobarbital. Perchlorate also is available across rodent and amphibian assays, and there was a presentation earlier today that did show that thyroid histopathology was consistent between rodents and amphibians for that chemical. As you can see, we've got a test guideline 407 which is actually an OECD rodent guideline, the intact male assay, the female pubertal, the male pubertal, and then the amphibian metamorphosis assay histopathology. And as you can see if you read across for PTU, all of these assays were positive for thyroid activity, and it picked up essentially the same histopathological finding. For T4, in the scope of these validation exercises only the TG407 use T4, but it picked up, it's pathology picked up on a thyroid active material, where

as the amphibian assay histopathology was not quite as conclusive. For phenobarbital, that caused a little bit of trouble in most of the assays. In some cases they don't think the MTD was achieved. In the frog assay as well they had contradictory response there. In the intact male, it would have flagged thyroid as positive, not necessarily based on histopath but based on thyroid hormone findings and thyroid weight. So you can see that phenobarbital wasn't necessarily a good test model for this particular one. And as I mentioned, perchlorate also was equivalent across all the assays. This information is presented in, I think, in more detail in written comments that you should have available.

I wanted to mention too, this morning we talked a little bit about iopanoic acid and the very interesting finding of its asynchronistic development in frogs. Well iopanoic acid had not been tested in any of these rodent assays for validation. So I can't give you a comparison of that. What I know is it can cause thyroid hormone changes in humans, whether the rodent assays will pick that up I can't answer cause it hasn't been tested. It's a little bit unfortunate we don't have a larger suite of compounds that were tested across all the assays to do a more full comparison.

So continuing with some questions about inclusion in tier-1, is the method sensitive? Yes. Amphibian metamorphosis assay does appear to be sensitive, especially for the strong agents. It has not been as effective for the weak agents. Substances like benzophenone-2 may also have been tested at doses that resulted in non-specific toxicity based on findings in the test itself and in some fish data that indicates that those concentrations may have been a little high.

We can also say that we have not tested enough substances to determine the limits of sensitivity and specificity for this assay. We need more weakly acting thyroid substances and more non-thyroid toxicants to be evaluated to really understand the limitations and the strengths of the amphibian metamorphosis assay. I do have a concern that if we're looking at endpoints strictly based on growth and development, that we may have potential to flag a lot of false positives with the amphibian metamorphosis assay. And as you've already heard, for us to trigger tier-2, and my understanding from this mornings conversation was we'd probably be doing everything in tier-2 based on positive tier-1 findings. We're talking about millions of dollars and lots, and lots,

and lots of animals. It's an avian two generation assay. It's a fish two generation assay. It's an additional rat reproduction assay. So there's lots of work into it too.

Has it been sufficiently standardized? Well the results can be confounded, and they're inconsistent; sometimes within labs, and not specific for a lab, and across labs. And it's thinking that animal dye and iodine content in the water may play a part of this, but it's not fully understood how those factors will effect a response to a testing agent. So we really need some more work to really define the conditions in which the assay should be performed across laboratories, not just what the minimal dye and iodine should be but a real understanding of how those components with interact with the test substances or effect the results of the test substances. Also in some experiments the animals were developing and reached metamorphic climax already by day 21, which almost makes the data un-interpretable.

We need performance criteria for growth and development, and there are some performance criteria that are proposed in the integrated summary report. But I think they don't go far enough. I think we need to have more performance criteria for development

throughout the assay, so what stage should controls be approaching plus or minus day seven as opposed to just what the minimum they should be at, at day 21. That would help us a little bit more than interpretation for synchronicity of the development of the eggs.

As I mentioned, overt toxicity can confound specificity of the assay, and we need clear recommendations about how to determine when that toxicity is interfering with our interpretations. I also heard this morning that mortality will be the clear indicator for overt toxicity, but we all know, sublethals can also affect growth and development. And if we can have a growth and development effect trigger a positive finding, I see the potential for a lot of false positives here.

It's a challenging assay to run. We've had some experience with it at our own laboratories, and without sufficient standardization, it's going to be difficult to transfer this to laboratories to be able to run this regularly, reliably for all the compounds that must be screened.

So to summarize, I want to say there's been a lot of very good work on this assay. It has come a really long way. You see clear improvements from phase-1 validation, to phase-2 validation, to phase-3

validation. However, I don't think it's quite there, and I'm really concerned about the conclusion in tier-1. It is a long assay, as I mentioned it is 21 days. It requires a lot of animals and a lot of money to perform. It doesn't seem to give us a lot more information than the rodent assays, and if you're using tier-1 to say the compound is thyroid active, we can probably get that with another test. It doesn't provide, it doesn't work well for the weakly active agents, and we have a little trouble with consistency between and within laboratories. We need some additional work to address husbandry, and we need to understand the weak thyroid substance activity and what negative thyroid substances are going to perform like in this assay. Concern again about the false positives, and you heard about the cost for tier-2. However, that said, we don't want to ignore the amphibians in this screening program. What the recommendation or what we would like to do is look at not including the amphibian metamorphosis in tier-1. Relying on the rodent data to trigger thyroid activity, and then doing, in tier-2, using amphibians to do a quantifiable risk assess, or data that can be used in a quantified risk assessment to fully evaluate the impact in the environment. Thank you.

DR. HEERINGA: Thank you, Dr. Ortego. Questions from the panel members on amphibian assay from Dr. Ortego and her presentation? Yes, Dr. Furlow.

DR. FURLOW: Just one question. So just to reiterate what Dr. Zoeller was saying earlier, actually in my work it's gratifying to hear that you think that the mammalian xenopus is identical in terms of thyroid responses and things like that. So that's actually kind of nice. The trouble is that there isn't really a receptor-based screen in any of these assays besides the amphibian metamorphosis, which was pointed out. Are you aware, do you know of industrial or other kinds of efforts to have say a thyroid hormone receptor binding assay, transactivation assay? If those things were in place conjunction with the intact rodent assays, would that make you feel more comfortable, I guess, with this kind of an approach?

DR. ORTEGO: I guess I just want to clarify that I'm not saying they're identical. I'm saying that the data that we have so far suggests that the rodent assays would have picked it up. Admittedly, we've not tested as many of those as we should across the animals, and to my knowledge are not on a receptor binding assay there. But if the rodent assays will flag it in tier-1, then do we need to do frog testing

in tier-1, or can we do more thorough frog testing in tier-2, which is what the plan is? And I do think there's a roll for the frogs in tier-2, just not sure about tier-1. I think we need more data to make that call. I would like to see us postpone that until we had more data.

DR. HEERINGA: Dr. Cooke?

DR. COOKE: Gerald Cooke. The water that the frogs are in, where did it come from and is it tested?

DR. ORTEGO: The dilution water that's used in the assays, it comes from a, it's usually local water that may be reverse osmosis treated and then media added to it in order to make the animals thrive and be happy. It is only screened usually for the standard pesticide screen and an organic toxicant screen. So there is a screening process. Anybody who does GOP work is screening their water for those kind of contaminants, and a lot of the laboratories use local water that they further purify and then maybe modify with amendments for the animals to thrive.

DR. HEERINGA: Further questions for Dr. Ortego? Not seeing any, I'd like to thank you very much, Dr. Ortego. At this point I'd like to ask Dr. Reinhardt Fischer representing Bayer CropScience to

come up.

DR. FISCHER: Thank you very much for listening to my comments. My name is Reinhardt Fischer. I'm with Bayer CropScience. I'm involved for the last 12 years with the endocrine screening and testing question. My background, I'm an ecotoxicologist by training, and I'm responsible for human and environment risk assessment at Bayer. To make the challenge a little bigger for you, I'm not showing some of the slides that are in your presentation, so you have to be on your toes.

Background for my comments, I'm looking at the proposed EP80 assay because there's also an OECD design that is a little bit different. The differences are outlined on your slide number 11 in the very back of your document. Also I have to state that currently there's no information available on how the results actually will be used within the screening battery. We believe that the assay by itself should provide the data that can be clearly interpreted as either positive or negative for endocrine disruption because we believe that not only some agencies in this world, but especially the public, will use positive results as positive results for the compound.

This slide is basically the core of my

comment. The study design is scientifically sound, but we believe in its current design it does not fulfill the requirements for a screen for endocrine disruption. And the two key points for that are that as admitted by all the researchers that have been involved in the validation process, some of those endpoints are not specific for endocrine disruption. They could be caused by other pathways, but then the second point is that any significant effects in one or more of these endpoints should be considered a positive response. So even if fecundity alone is affected, that would be considered a positive response, and as a result the compound would be regarded as a potential endocrine disruptor.

Is the basic design of the assay a screen, a rapid screen? It's definitely not rapid. The exposure duration alone is three weeks. You need a two to three week pre-exposure period plus you need a time for the range finder test because normally you don't have the results for the species that you, that you need. So you need additional information to be able to perform it. Just as it's not a short test, it's also not a cheap test. It involves a lot of labor to run a flow-through system, so that long period of time requires a lot of labor. It is expensive including the analytics,

the histopathology, the plasma-6 steroid analysis. So it could easily run up to $100,000 for this compound. One of the options could be to focus on the OECD endpoints alone. In doing so there may be the option of a reduction in test duration and in cost therefore.

I'm flipping now to slide number seven. The question asks, has the test method been sufficiently standardized? It's a question that is really open to debate, and a question that depends on what level of resolution. You're looking at the validation results. If you just want to see if all of the labs found some positive result at some concentration levels, then, yes. It has been validated. If you want similar results at similar levels, then you will see that there was considerable variability within the test results. And on the other hand the variability of histopathology and of the sex steroids measurements cannot really be assessed because both of them were only performed by one laboratory each.

And then, you know, kind of minor point, some of the technical aspects need to be revisited, some of the quality criteria for the test.

Is the test for its intended purpose sensitive? Yes. It's definitely sensitive. Actually there is no negative in the test. All the test

chemicals that were tested in the validation program including in the validation program at OECD were positives in this test. It found all chemicals, so it's definitely sensitive, but it's not very specific depending on what endpoints you include. And it's also, because of that, not predictive. And I admit here, you know, that really depends on the evaluation method. If it states there that any effect would be a positive result as an endocrine disruptor, then it is not sufficiently predicted for endocrine disruption. It may be good for a good productive toxin. But it is reproducible, although it may need some more work to reduce the variability.

Overall the design, in our opinion, goes beyond the requirements of a screen for detecting potential endocrine disruption. The biological test procedure has been adequately demonstrated. It works from the biology of the test. Some of those endpoints, I mentioned the histopathology and the sex steroids measurements, they have not been validated so far. Some of the protocol parameters need to be verified. Someone asked this morning, is there sufficient laboratory capacity. We believe there probably is not when you're looking here in the first round of the priority chemicals alone, 73 chemicals to be screened.

There may not be sufficient laboratory capacity for this type of exercise. And finally, last not least, we recommend to go with the OECD test methodology and focus on the vitellogenin and secondary sex characteristics alone to make the assay more handle-able. With that, I thank you for your attention and for your consideration.

DR. HEERINGA: Thank you, Dr. Fischer.

Dr. Lasley has a question.

DR. LASLEY: On your last point, could you tell me how many, if any of the positives were not picked up on the OECD but were picked up with the other parameters?

DR. FISCHER: I think all of the positives would be picked up by the OECD as well when you go to that level.

DR. LASLEY: Then according to what's been tested, there's no advantage to the additional parameters.

DR. FISCHER: Not for the purpose of this, of this screen. You know there could be additional parameters when you look, as across all assays and miss any reproductive effect somewhere. But for that there's no guidance developed so far, so if the test should deliver a result by itself, then you,

you really should eliminate questionable endpoints.

DR. HEERINGA: Thank you very much, Dr. Fisher. At this time I'd like to invite up Dr. Steven Levine from Monsanto.

DR. LEVINE: I'd like to first start off by thanking the panel as well. And, again, I'm Steve Levine, and I'm a ecotoxicologist and a science fellow at the Monsanto Company and had the opportunity to be involved with providing guidance as well to EPA between 2004 and 2006 as a member of the EDMBAC. What I'm going to talk to you about today are recommendations and considerations for the tier-1 battery.

So I'm going to over a little bit of ground that Gary covered earlier today, and I'm going to talk a little bit about the origins of the endocrine disruption screening program. And as you heard earlier, it really came into fruition through the enactment of two pieces of legislation. FTPA and an amendment to the Safe Drinking Water Act in 1996. So what the FTPA stipulated was that EPA must screen pesticides for estrogenic effects that may affect human health. The EPA must use appropriate validated test systems, much of what we're talking about today or other scientifically relevant information to assess the potential for endocrine activity. And EPA can conclude

that other effects beyond estrogenic effects could be included, and we're talking about androgen thyroid and the potential effects on steroidogenesis as well. The Safe Drinking Water Act amendment allows EPA to screen drinking water contaminants to which substantial numbers of person are exposed.

So you can see that the program is very large, and to help EPA develop the program, they brought in a number of advisors, as we heard earlier, academics, industry, governmental organizations to develop the framework, and that was broken up into three primary pockets, which was priority setting, screening, and if activity was identified in screening, you could be triggered to be go on to testing. We also heard that the approach on priority setting changed, and it went from using biological effects information such as the results from high throughput screening to a qualitative exposure-based analysis on relative exposure. We heard about those four routes of exposure.

I'd like to talk a little bit about selection criteria for assays in the tier-1 battery, and those assays should be motive-action based to identify specific types of endocrine activity. These assays should be broadly predictive. In other words

they should have the appropriate sensitivity and specificity. They should produce data that can be clearly interpreted as being either positive or negative to the best of the abilities, and attempts should be made to minimize type-1 and type-2 error rates. We heard about a biased towards minimizing the type-2 error rates or the false negatives, but we don't want to do that at the expense of dramatically increasing type-1 error rates. There are currently about 10 assays in the EPA proposed tier-1 battery. If each of those assays has a 5% to 10% false positive rate, when you look at the chance of finding one false positive in that battery, the percent is relatively high. It can be as high as 50% to 60% percent depending on how many assays are included in the rate or the chance of a false positive.

As we have just heard, the assays should be relatively inexpensive, quick and easy to perform to really meet the requirements of a screen.

I would like to talk a little bit about assay validation as well as battery validation, and through your deliberations over the next two to three days, you're going to be talking a lot about whether or not each of the individual assays are validated. And two of the criteria you're going to have to evaluate

against are relevance as well as reliability with relevance being can the assay identify a compound that has potential endocrine activity? And the other one is reliability. Are assays reproducible within a lab and between labs? Are they robust and are they portable?

This is an important to make. Assays in a proposed battery were not tested during the validations with the same standard set of core test substances, and this was something that Ed Stack had spoken to in their guidance document, the relative importance of this. Because with this head-to-head comparison, it's difficult to select the optimal battery. It's difficult to make apples to apples comparisons of sensitivity and specificity among the assays looking at similar endpoints, and they felt that the assay could not be considered to be properly validated unless you had this same standard set of core substances tested across the battery.

Another point that I want to make before moving on is the insufficient number of negative compounds that have been tested during method validation. Many of the compounds that go through testing ought to be negative. Therefore, it is very important to challenge each of the assays in the battery with a sufficient number of negative compounds

to really have a good understanding of specificity.

Another important topic that's been talked about earlier this afternoon is dough setting, and the reason for this is because dough setting takes a considerably greater importance in the screening program than in traditional toxicity testing. The purpose of screening is to identify the potentials that interact with the endocrine system not merely to identify adverse effects. So it's critical to assure that systemic toxicity or a stress response is not confused with genuine endocrine mediated effects, and this is particularly important because many of the mammalian assays are pushing the doses up to or above potentially the maximum tolerated dose.

Feedback that EPA specifically asks for in the FR Notice was comments on the limitations of the assays in the proposed battery, and as you've heard through the course of the afternoon, key limitations of the proposed battery are related to the number of assays that include equal endpoints. And concern stems in large part from the lack of specificity of several of the endpoints in these assays, and the concern again was generated out of the high probability of getting false positives at an exceptionally high rate out of these assays. Some of the examples that we've heard

talk about earlier this afternoon included the pubertals, the fish reproduction screen, and the amphibian metamorphosis assay. In the second slide I talked about FQPA and some of the mandates under FQPA, and some of the wording was that other scientifically relevant information could be used to assess the potential for endocrine activity. It is important to point out to the folks in the panel here that many of the compounds in the draft screening list of 73 have undergone extensive testing that's capable of detecting effects, endocrine-mediated effects. We're talking about higher tier apical tests. So this is a time to urge EPA to be flexible in determining which, if any, screens need to be performed based on the availability of functionality equivalent data, and we heard from Willie Owens about the prediction model and very similar predictions between uterotrophic Hershberger results from rat 2-gen studies. So there's good alignment there.

Another topic we've heard a fair amount today is use of a weight of evidence approach to determine the results of tier-1 screening, and the FR note is for the meeting we're attending today. EPA laid out the relevance and the importance of using this weight of evidence approach, and the rationale for that

is the battery will likely produce a database with a unique ray of results for individual compounds. So we really need a weight of evidence approach to evaluate this type of information, the quality of the information in a way that explicitly addresses the qualitative differences in the information. So I'm asking that EPA develop standardized and transparent recommendations, taking it to the next step to apply weight of evidence approach to determine if tier-2 testing is necessary. Ideally it would be nice to have this guide, these recommendations before tier-1 screening initiates and certainly before tier-1 screening is completed.

This is a variation of a slide you've seen earlier today in presentations. On the left column we're seeing the assays that can potentially be in the tier-1 battery. In the center column is the March 2008 EPA proposed tier-1 screening battery, and a recommended tier-1 screening battery on the far right column. One of the major differences between these two screening batteries is using the intact male to replace steroidogenesis, the pubertal male and female as well as the frog metamorphosis for reasons that you've heard earlier today. Another difference is the recommendation for only using ER binding assay versus

the ER binding in the transcriptional assay. The ER binding assay can detect agonist as well as antagonist. The ER transcriptional assay can only detect agonist. So the recommendation is for that ER binding assay. Additionally we just heard a presentation on the fish reproduction screen, and the recommendation there is to harmonize with the OECD protocol, which looks at two endpoints, which are very predictive of endocrine activity, vitellogenin induction as well as changes in secondary sexual characteristics.

So to close, I just wanted to provide some additional rationale for this recommended tier-1 screening battery. The proposed battery is mechanism of action based. It's efficient and satisfactory in evaluating estrogen, androgen, and thyroid activity, as well as potential effects of steroidogenesis. It is believed to have greater predictively, and that is driven by the greater specificity of the proposed assays to include in this battery. It maximizes interpretation while minimizing the chance of type-1 error rates, and overall it has a decreased complexity which is consistent with a screening battery. So with that I'll end and take any questions.

DR. HEERINGA: Thank you, Dr. Levine.

DR. LASLEY: I'm Dr. Lasley. You

indicated that the estrogen transmission assay could not measure antagonist. I assume you meant hasn't been validated to.

DR. LEVINE: Yes. Yeah. That's correct. It has not been validated to detect antagonism. I think there may be plans to build on the validation looking at agonist.

DR. HEERINGA: Dr. Brown?

DR. BROWN: Terry Brown. So if you were in a position of having to assign weight of evidence, some kind of quantifiable way of assessing that, can you express any perspectives on that?

DR. LEVINE: Yes. Let me start with, we did hear some comments on that earlier today. Clearly in-vitro assays cannot be the gatekeepers. Likely would assign greater weight to the in-vivo assays over the in-vitro assays. But that would be the high level of guidance. Clearly we're going to have to interject expert judgment, and I would really point towards some of the guidance that's been developed for similar weight of evidence framework, specifically the type of weight of evidence framework that we use for immunogenicity and carcinogenicity testing. A lot of these rules for weight of evidence have been, have been really pushed on and discussed to a great extent with

developing frameworks for those endpoints.

DR. HEERINGA: Dr. Vandenbergh?

DR. VANDENBERGH: Yes. I wonder if you could explain to me how the adult male 15-day test replaces both the male and the female pubertal test, especially the female test. We have an animal that cycles. Males don't cycle. That's the basic difference between the sexes, and how do you, how do you look at the effects on cyclicity if you don't make any measurements?

DR. LEVINE: This is -- being a screening assay and being mechanism-of-action-based, I guess I would refer back to that approach for screening. I'd refer -- being a mechanism-of-action-based approach, I guess there would be some questions with the pubertal assay and Estro-cycle in whether the duration of the current study is sufficient to really, really assess that.

DR. HEERINGA: Thank you, Dr. Levine. At this point I'd like to ask our next public commentor, Dr. Ellen Mihiech. Hope I'm pronouncing the last name correctly. Okay.

DR. MIHIECH: I've learned over the years not to worry about that too much.

DR. HEERINGA: I need my earring aide.

DR. MIHIECH: I too want to thank you all for being willing to sit here and listen to all of us. I appreciate that. My name is Ellen Mihiech, and I'm an environmental toxicologist/risk assessor. I have been an interested observer and a stakeholder since the inception of this program in 1996. I've also been an active participant because I am on -- I'm a representative to the OECD echo validation management group. So I've been very involved in accepting the studies from the environmental side.

What I wanted to talk about, it's very similar to what Dr. Levine talked about and that's that the purpose of the screening battery and what is this purpose? We've got lots of studies that we've looked at. We've learned a lot over these years, and I do commend the efforts of the EPA, and industry, and others that have been involved, academics, in where we've taken the science here, and I think it's been very good. But we do have certain requirements for doing this, and that is that it has be validated. It has to be specific, and we have to keep in mind that this thing that you're talking about today is the screening level. We don't have to answer every question. That would be something that we would be doing more in the risk assessment part of this. This

is just screening. It's not hazard even. It's just screening. It's do we trip a trigger. So it's to identify substances with a potential to act, and act on one or more of the components of the endocrine system. And with this we've got to be sure that we can say we have things that act, and we have things that don't act. And I hope that you've heard through the talk these last few talks that we've got some issues there. We've got a lot of these studies that are these screens that have a tendency to probably hit just about everything, and there are some pretty significant ramifications if that happens.

So I know it's been mentioned by a couple of speakers, but all these experts that have spoken to you so far have been part of a group that have provided very detailed written comments. And I really would encourage you to be sure to look at those because it's people that have taken the time to be part of the design of these studies, whether or not these studies are standardized, validated. What are the strengths? What are the weaknesses? And these are people that have been involved since day one, so they know the progression of how things have gone, and the pitfalls that we've fell into, and those that we've climbed back out of. So I think it's very important

for you to give that some time to read that.

And, unfortunately, again, having been involved for so long on this that there are things that we're looking at that do lack specificity and will not lead to interpretation; that are consistent with what the EDSP objectives are, and that's to screen and identify things that interact with the endocrine system. And, again, I want to just bring up the three that I think are the most problematic, the pubertal assays, the amphibian metamorphosis, and I'm specifying this here as EPA's proposed fish reproduction screen because it is different than the OECD screen that we've worked for a really long time to get moved forward within the OECD. So problems with the pubertal assays. I think as Dr. Marty said earlier, you see that there is definitely some issues with specificity on this assay. They're apical endpoints that respond to both endocrine and non-endocrine modes of action, and that's a problem because if they do that and we can't adequately address that or piece that out, then everything that trips a trigger here is going to go on to tier-2; and we've heard that already too.

There is only two dose levels, and the high dose has to be at the NDT to be interpreted as adequate, and I think you've seen from the data that

was presented earlier that pretty much nothing with the 5% to 10% decrement in body weight gain is going to be a negative. Nothing is.

With the amphibian metamorphosis assay, we really haven't shown specificity, except for maybe the histopathology endpoint there. This is one that's been very central in activities within the OECD also. It has had a lot of work that's gone on with it. Again, there's apical endpoints in this assay that respond to both endocrine and non-endocrine modes of action. And I think as Dr. Ortego brought up earlier, I think it's pretty clear that, again, when you think about the fact that this is a screen and we just want to trip a trigger, we just want to say should it go on or shouldn't it go on, the data today is showing that the rat studies would do it. And it is extremely expensive. It is extremely complex, and it uses an awful lot of animals. So I encourage you to think about that. I know the question came up earlier is this a, are you here to add things to it, or keep it, or take it away. It is probably keep it or take it away, but it is to really think about. What do we need that's going to answer the question about is this compound one that should be further evaluated in the two generation studies that are going to be part of

tier-2, which you don't get to talk about today too much I guess.

And the third, the third assay, the fish reproduction assay, again, the thing that I really hope that we can think about here is that within the OECD, which you act as a signature to, there is an assay that is moving forward. It has gone through peer review, and there have been a lot of very focused fish people sitting around tables that have said the vitellogenin and the secondary sex characteristics are good ones. They can tell it's a lot, and the other components that are in the current EPA reproduction screen are not as validated. There's a lot of play in the data, a lot of inconsistencies, and maybe in the future these could be things that we might consider. But today it's not and that's why OECD has dropped those from the current fish screen, the 21-day fish screen that OECD is going forward with. And it's the same kind of thing. Right now with all the apical endpoints in the fish study, there is very few that are going to be negatives.

You've seen this over and over. Just again, we need to keep in mind what types of methods and what these screens need to be, relevant, reliable, sensitive, specific, graphic, cost effective, all those things, and they need to cover estrogen, androgen, and

thyroid responses without a lot of redundancy. Again, if we, if this is a battery that's validated, then we should be comfortable in saying if we have a compound it will be tripped if it's going to effect the estrogen, androgen, or thyroid. And we need to think about utilizing animal tests wisely.

So the recommended battery that Dr. Levine just talked about was ER binding. Currently the transactivation is not validated. Maybe in the future it could be something that we could consider as being useful to the uterotrophic. The intact male, I think that is something that I know you guys are -- I don't believe it's something you're considering, but I hope you do think about it because it has been, a lot of compounds have been tested in it. It combines a lot of different endpoints, and it's been very useful. The fish screening assay, but I'm recommending the OECD screen assay, the AR binding, Hershberger, again the intact male, and the OECD fish screening, and then for thyroid the intact male.

In closing, I just want to say remember that we need to find that appropriately focused tier-1 battery that's for screening, that's going to be finding the compounds but is' going to be mechanistic based and will inform for tier-2 testing. As Dr.

Levine said, we need to think about the weight of evidence and how we're going to use the data. It was very troubling for me to see the materials you got with the fish reproductive screen that said any positive in any of those endpoints is a positive. That just goes counterintuitive to what this program was about, which was to look at estrogen, androgen, and thyroid. And we need to make sure that it meets within the regulatory authorities, that there's communications about the use of the data and it's consistent agency wide. So that, again, this is screening. It's not hazard. It's certainly not risk and that we need to be very careful about what we, how we use the data. Thanks.

DR. HEERINGA: Thank you, Dr. Mihiech. Questions? Yes, Dr. Kullman.

DR. KULLMAN: Hi. I think the last three speakers have some pretty valid points with trimming the assays. One of my concerns with your points though is that if we trim this too much we're going to lose some of the inherent redundancies that we have in these assays which allows us to make a defined definition of what some of these compounds might be doing. So I agree maybe we can trim some of the fat, but to trim it too much may really dent the ability to make a definitive answer on some of these.

DR. MIHIECH: I certainly understand that and I agree. I guess, again, if we had say, for example, a thyroid assay that was just perfect that didn't have a lot of the other problems associated with it, then maybe it would be okay to say all right we'll do a specific thyroid assay in the intact rat. But the problem we run into is if you think about, for example, the amphibian metamorphosis assay, if you really go in and look at the data from all the of the validation efforts that have gone on across the globe, because it's been a global program, some of those endpoints are just not there yet. I'm not saying they couldn't be, but they're not there. And so I would rather error on the side of assay that I can trust than one that I'm not going to understand how to answer the question.

DR. HEERINGA: Thank you, Dr. Mihiech. At this point in time I would like to call up -- and I'm going to guess at the last name here -- Christy Stoic, Stoic. Stoit is representing the Physician's Committee for Responsible Medicine.

MS. STOIC: It's just Stoic just like you announced it. I don't have a handout or a Powerpoint presentation. I just have some notes that I'm going to read. I'm going to make copies of those and pass them out to you tomorrow, so you will have

that in hard copy.

So PCRM is a group of physicians, scientists, and lay persons who advocate good nutrition, preventative medicine, and apical research, including use of alternatives to animals and toxicity testing. I would also like to turn your attention to a set of written comments submitted by Dr. Katherine Willet of People for the Ethical Treatment of Animals, which, along with PCRM and several other stakeholder groups. I just want to make a few short points today. The first point is I hope the EPA will take this as constructive criticism, but it was really hard to prepare for this meeting. Not only were there tons and tons of documents, but they weren't all in one place. I wasn't really sure which documents to focus on. They weren't on the public docket in advance of the deadline for written comments. There was a really nice summary that was made available after the deadline for the written public comments, so the technical review document; that's what I'm talking about. And some of the documents in the peer review lists and such on the EDSP website were different than what was presented here today, so I'm just asking to maybe next time try to get everything ready at least before the written comments are due.

So although many stick holders including my organization have at one time petitioned or urged the agency to implement the EDSP more quickly, the information presented here leaves me with the conclusion that the first tier, as proposed, are not ready for implementation. Battery assays, for example, the transcriptional activation assays are not validated. Some of the assays EPA included in the battery are not yet validated. There are also serious problems with the validation of some of the assays. It's not the time now to offer expediency over scientific validity. This program is too significant, too important, and it's going to have a huge impact. Instead, we believe EPA should endeavor to adopt a program more in line the original intent of the statue. First and foremost detect estrogen-like effects in humans using very rapid priority setting in-vitro mechanistic screens. Further tier screening and testing will be contingent on a clear stepwise process.

It's inconvenient for the agency to adopt a more flexible approach to assay validation for this program. This is not appropriate. It does not follow that because these assays are not replacing another assay, they should be subject to less vigorous validation. If anything, the validation process should

be more rigorous because neither the biological flexibility or some of the assays nor determining a positive and negative cause is immediately apparent. One limitation of many of their validation studies in our view, especially for the apical in-vivo assay, is there is a posse of chemicals tested in the validation sites that are toxic but not endocrine disrupting. This would essentially be relevant as we've heard in assays such as the amphibian metamorphosis. By not validating the assays using these compounds, the important evaluation of specificity of the assay is being left out.

And finally we would like, we recommend the agency set an acceptable level of false positives before it begins evaluating the battery instead of after the fact, and it's equally important to minimize the potential for false positives.

DR. HEERINGA: Thank you very much, Ms. Stoic. Any questions from the panel? Thank you very much.

MS. STOIC: Thank you.

DR. HEERINGA: At this point I'd like to call forward Dr. John Gordon who is here representing Xenobiotic Detection Systems in Durham, North Carolina.

DR. GORDON: Hello. I'm John Gordon

from Xenobiotic Detection Systems, Director of Research. I'd also like to thank the panel for allowing us all to come here and speak with you today. Here we go. Today I'd like to talk to you about the Lumi-Cell ER assay developed by our company for estrogenic testing of endocrine disruptors. Well about the assay, it is a transcriptional activation assay, which we've heard a little bit about today. It's a stable transcrepant cell line from a BG1 human ovarian carcinoma cell line and is implemented in a high-throughput format. This should be a very important aspect, especially post validation in that EDSTAC has identified some 78,000 compounds that they'd like to screen. And if an assay is not in a high-throughput format, that is going to take a considerable amount of time and expense to screen these compounds. So it should be an important aspect in any assay validation.

I'm going to skip the next couple of slides. I think we all understand transcriptional activation. Assay validation. The assay was started with an SBIR grant from NIHS in 1997. The system was given a high-priority validation from Sacaton in March of 2004. In April of 2004, the final report from the SBIR funding was given to ICCVAM. This included a study of over 125 compounds with both agonist and

antagonistic data. In March of 2004, it was approved for an international validation study with ECVAM, JaCVAM, and ICCVAM. In July of 2006, we completed a US Protocol Standardization Study also known as the US Pre-Validation Study. And in March of 2007, we did start the International Validation Study with ICCVAM, JaCVAM, and ECVAM. ECVAM was doing the study in house. It is for Italy. JaCVAM was doing the study with the Yusha Corporation in Japan and XDS is doing the study for ICCVAM.

Don't really look at the date on this study. It's just to give you an idea of what we can do for you as far as data. We can give you the compound and case number of course. I give you the EC50 and then we give you the relative induction to EC50. It gives you prioritization for those compounds, say beta-estradiol or to pesticide. In this instance it's chlordane, and what's your relative potency to chlordane. So it gives you a little bit of an idea of how to prioritize your efforts.

Now I'd like to spend a little time talking about the data that's been produced with the Lumi-Cell ER assay. This about a dozen compounds that are currently on your list of 73 that you want for validation of your studies that's already been

completed. This is some pesticides from ICCVAM, just a list of 78 compounds that we published in the paper two to three years ago, several compounds, well known endocrine disruptors nothing, no surprises here at all. This is actually the data from the US Protocol Standardization Study performed by ICCVAM. We had several. The one in pink on the left there is beta-estradiol, which is the standard, and it included two other strong endocrine disruptor compounds, ethinyl estradiol and dedes as well as several other weaker active compounds for the study. And this is just the summary of the agonistic portion of the study. This is a slide that several people might be interested in. We heard several speaks talk about the lack of validation of antagonistic data for transcriptional activation studies. This is the data from the US Protocol, antagonistic study performed by ICCVAM. The line on the left portion of the screen is the standard curve of raloxifene and E2. We've also included such compounds as dibenzo-(a, h)-anthracene, tamoxifen and several others, flavone for instance. We did something else with this study as well. We also looked at cell toxicity or cell viability with the study, which is very valuable for any antagonistic study that's being done. If you notice some of the lines are different

colored, portions to the line. In yellow, which doesn't show up very well there unfortunately, is damaged cells which will not viable, and the red portion were dead cells. This viability was conducted through both a visual inspection and using a CellTiter-Glo from Promega. The cell toxicity was evaluated by two methods. So we do know whether or not there, you see an antagonist response or cell death in this case, which is very important. If you take a look at the one line that come down. It looks like a perfect antagonistic line if I hadn't colored it in yellow and red. You'd see a false positive there if we didn't take into account the cell viability; that's a very important aspect to the study. And obviously the previous transcriptional activation assay that everybody is talking about hasn't been validated for antagonism. This one has gone through US protocol standards for antagonism and is currently involved in the international study with ECVAM, JaCVAM, and ICCVAM study.

This I threw in there. We not only do compounds, individual compounds, we also look at formulations. When we're doing these studies. This happens to be a sunscreen formulation study we did looking at various sunscreens bought off the shelf and

looking at their estrogenic potential. We also did, we do a classification system, which may be very helpful to this committee in prioritizing efforts. These are but a few compounds that we've tested, some strong and weak compounds, and what we did was, first thing I did was I looked at, well where's the background? That's the bottom blue line you see there. That's the background of the system. The red line represents the EC50 for beta-estradiol, which is the light blue line. It's the second to the left is the estradiol, and then the top light blue line represents 100% expression for estradiol. So we classify these as group A being about 100% estradiol, B being between 50% and 100%, C being between 0, background, excuse me, not 0, background and 50% of estradiol expression, and with D being below background or negative, non-detects. We further will take a look at this data and notice a few groupings that we can see here. You see the group to the left is your steroids pharmaceutical products kind of grouped together, and then there's a gap in the middle. There's very few data coming up, and then you take a look to the right. There's a big group of compounds that come up as the weak actives. I shouldn't say weak. I should say less active, and even within that group, there's two groups. If you take a look at the

-- sorry I don't have a pointer -- there's an upper portion and then a lower portion. You'll see two distinct groups of chemicals that are coming through. So we decide, we divide that into various groups as well and get those classifications, class 1, 2, 3, and 4. This will allow. This system classification will allow for prioritization going on to step 2 in the same group, group C class 4 shouldn't be given the same priority going onto to phase 2, as it would be class 3 or a group A class 1. This system of classification will really help refine the process of moving onto phase 2, tier 2, tier 2, pardon me, before it moves on.

And then a little summary here. It's a transcriptional activation assay. I don't believe that there is a TA assay included in the upcoming pesticide study. I could be wrong about that, but I don't believe there is one. It's very sensitive to test compounds less than 1 part per trillion. Wide range of both agonistic and antagonistic studies for IC and EC50 determinations. It has been through the US Protocol Standardization and is currently going through the International Validation Study for both agonistic and antagonistic studies, and it meets requirements mandated by EPA and ICCVAM for tier-1 screening assays in that it satisfies the 3 R's that we're all very

familiar with.

And I thank you for your attention. I'd like to take any questions on Lumi-Cell.

DR. HEERINGA: Thank you, Dr. Gordon. Yes. Dr. Lasley.

DR. LASLEY: Dr. Lasley, UC Davis. I noticed on your antagonist response where you're testing them against E2. There's an initial agonist afoot.

DR. GORDON: Correct.

DR. LASLEY: But then when you get to the classification, you're testing these alone and see only an agonist effect. So how do you separate these two different qualities when they're both in the same chemical?

DR. GORDON: Well the antagonistic assay is actually anti-activation assay. We activate it with E2 and then use varying concentrations of the compound --

DR. LASLEY: No. I understand that. But if initially --

DR. GORDON: Mm-hmm.

DR. LASLEY: -- in the presence of a strong agonist you get an agonistic, an antagonistic effect.

DR. GORDON: Yeah.

DR. LASLEY: But alone you get an agonistic effect. How do you separate those two properties if they happen to be in the same chemical?

DR. GORDON: Some chemicals are going to have both effects. In fact, if you look at the antagonistic data, you do see dibenzo-(a,h)-anthrocene is a biphasic compound. It has both agonistic and antagonistic qualities within the assay.

DR. LASLEY: So it depends upon the concentration of the strong agonist; is that right?

DR. GORDON: Correct.

DR. HEERINGA: Dr. Denver.

DR. DENVER: What is the timeframe of your assay?

DR. GORDON: Twenty-four hours.

DR. DENVER: So you could potentially be having some non-ER dependent effects in the assay given that long timeframe?

DR. GORDON: Non --

DR. DENVER: Right. I mean, if there was a compound affecting the expression of some other gene then could it impact your reporter?

DR. GORDON: Sorry. I did skip over that. This, this is the normal transcriptional

activation of, within the system. We added in the ERE right in front of the luciferase gene.

DR. DENVER: Right. I understand this, but what I'm saying is that given that length of time, the reporter could be influenced by other things that are changing in the cell?

DR. GORDON: It's possible but we have not observed any non-congruence with other, with other studies. In fact, the US Protocol Standardization showed 100% congruence with foreign data, with the agonist. Only 75% congruence with the antagonistic, but the previous studies it was compared to did not take into account cell liability.

DR. DENVER: Okay. And just one other question. Have you corroborated the transactivation data with any, any endogenous test regional response genes in the cells, or do you find in activation do you see enough regulation of known estrogen response changes of these different chemicals?

DR. GORDON: I don't believe we've done those studies. We have done some studies comparing it to the uterotrophic assay and several other in-vivo assays.

DR. HEERINGA: Dr. Eldridge and then Dr. Brown.

DR. ELDRIDGE: Eldridge of Wake Forest. I was going to ask, is this ER alpha in the cell.

DR. GORDON: It has both alpha and beta.

DR. ELDRIDGE: That's what I was going to ask you, or can you, can you create a construct of just data for example?

DR. GORDON: I'm sure you could create one. Yes. The receptor is an endogenous receptor, so it has both alpha and beta.

DR. ELDRIDGE: So the cell already has --

DR. GORDON: Correct.

DR. ELDRIDGE: receptors?

DR. GORDON: Correct. It's an endogenous receptor. So I'm sorry. I didn't cover that.

DR. HEERINGA: Does that cover your question, Dr. Brown? Okay. Dr. Belcher?

DR. BELCHER: Yeah. In what ERE are you using this?

DR. GORDON: I'm sorry. What?

DR. BELCHER: The ERE, the promoter.

DR. GORDON: The promoter?

DR. BELCHER: Yeah.

DR. GORDON: What's it from?

DR. BELCHER: What, yeah, which ERE.

DR. GORDON: Oh, it's a Bitta Jong.

DR. HEERINGA: Dr. Lasley?

DR. LASLEY: Lasley, UC Davis. Have you looked to see what other receptors are in here or the ability of this cell to metabolize toxicants?

DR. GORDON: No. I'm sorry. We haven't really looked at other receptors. It's a very slow-growing cell system. It does not have a very high metabolic rate at all.

DR. LASLEY: Right. But, but you could do PCR and find --

DR. GORDON: Yeah.

DR. HEERINGA: Just a note. If we're going to talk, you got to come up. Okay. Please come up and introduce yourself.

DR. CLARK: George Clark from Xenobiotic Detection Systems. Sean and I do this work together, and I did most of the cell characterization work. So he may not be aware of it, but this particular cell has functional receptors for EGF receptor, and ER receptor, an AH receptor, and it does, those have been characterized in previous work. So it's an interesting cell to say the least; that's why we selected it.

DR. HEERINGA: Why don't you remain just a second, Dr. Zoeller.

DR. ZOELLER: So how stable are these responses over passage?

DR. GORDON: They've been stable for, I believe, 10 or 15 years, 10 years?

DR. CLARK: We started this work in 1997 with the SBR grant, so it's been since there. It's almost 10 years.

DR. ZOELLER: Another example, I guess I'm a little more familiar with his MCF7 cells that within a lab over even, you know, a certain number of months you can lose sensitivity to estrogen for reasons that maybe we don't fully understand, but you've never seen this kind of variability through passage number?

DR. GORDON: No. We've gone through several generations of the cells, over 20 passages and never seen a drop in signal. Kept QC charts for positive controls and all this, and everything falls within acceptable ranges. And this is in accordance with ICCVAM.

DR. HEERINGA: Are there questions from the panel members?

DR. GORDON: I'm sorry. We also don't keep cells up very long, about three months and they go down.

DR. HEERINGA: Dr. Gordon, Dr. Clark.

DR. CLARK: Well thank you. I just wanted to correct --

DR. HEERINGA: No problem. I just want to make sure we get your name for the record.

DR. CLARK: George CLARK, President of Xenobiotic Detection Systems.

DR. HEERINGA: Thanks a lot.

DR. GORDON: Thank you.

DR. HEERINGA: Thank you very much, Dr. Gordon and Dr. Clark. At this point in time I'd like to take a short break, and we have two more public presenters, each who have registered for 10 minutes. And let me just check. We have one other additional presenter who was not on my original agenda. So let's take a 15-minute break and come back here just before 3:30, and we'll complete the period of public comment after the break.

DR. HEERINGA: Okay. Welcome back everyone to the final part of our afternoon session of the first day of our meeting in the FIFRA Science Advisory Panel on the topic of the Endocrine Disruptor Screening Program Endocrine Disruptive Screening Program Proposed Tier-1 Screening Battery. This afternoon we've been engaged in the period of public comment, and we have additional public commentors to

complete the afternoon. And at this point in time I'd like to invite up Dr. Jennifer Sass, who represents the National Resources Defense Counsel. Jennifer?

DR. SASS: Good afternoon and thank you for the opportunity to present public comments. My comments for other people in the audience should also be docketed, if not already, then soon. They have been submitted, and I think that panel members have paper copies. I won't be reading through all of them word for word. I'm going to be summarizing them, but first a bit of an introduction. My name is Jennifer Sass. I'm a senior scientist in the Health and Environment Program with the National Resources Defense Counsel. It's an environmental non-profit, and I'm located here in Washington. My background is toxicology, and molecular biology, and developmental biology, but a lot of these comments were written by my colleague who is in our San Francisco office, Dr. Sarah Jensen. She is an MD, PhD, and also Master's in Public Health, and her specialty is actually endocrine and reproductive talks. So her and I worked on these together, and in a way I'm representing her here in Washington. So I can -- if there are any questions that are very specific or anything where I begin by saying, "That's a good question," that's probably the clue that I can get back

to you if you'd like after talking with Sarah.

So first of all some general comments. My organization, NRDC, has been pushing EPA to meet their deadlines, and we've even been helping them to make their deadlines. And we will continue to do so, so we have an overall concern that EPA has already and may continue to fall behind on their mandated deadlines to implement the Endocrine Disruptive Screening Program. And if the Scientific Advisory Panel agrees that the assays are relevant and valid, and if concerns can be met by the EPA easily, then we would hope that EPA could begin testing the initial list of chemicals using these assays on the current schedule.

Going to page two, we have some concerns about EPA's failure, that EPA's failure to implement the Endocrine Disruptive Screening Program in a timely manner has stymied both regulations and the work of public interest groups like myself. NRDC has an expertise and a long history of working on chemicals that are suspected or known endocrine disrupting chemicals, and we're often approached by legislatures, by other organizations who are interested in learning about the latest science and also about the public. And many of these people wonder what our federal government is doing to help protect the public's health

when considering the potential association between exposure to endocrine disruptors and human health conditions such as infertility, birth defects of the reproductive systems, reproductive organ cancers, or neurodevelopmental conditions. In addition, there is increasing public scrutiny and public concern for effects of chemical contaminants on waterways, reports of intersects fish in our nation's rivers, and concern about these kind of contaminants in the drinking water.

The Endocrine Disruptive Screening Program was intended to help define which chemicals could be capable of causing these effects and ultimately to provide information to protect the public's health so that regulators could spend their resources wisely to make decisions to regulate appropriately to protect public health. EPA has failed to do this, not only by delaying implementation of the testing program but also by refusing to regulate any chemicals that had been shown to be endocrine disruptors in the public literature. EPA instead has inserted stock language in all of its pesticide REDs, the re-registration eligibility decisions, and tolerance reassessments that indicate that they won't make a decision on the endocrine disrupting potential for a chemical until the endocrine disrupting screening

program has been implemented, even where robust data was available in the peer review public literature.

So EPA's failure to regulate pesticides that are established endocrine disruptors on the basis of these endpoints has resulted in continued exposures to vulnerable human and wildlife populations with perhaps adverse permanent or irreversible health effects.

Some specific comments on the tier-1, the fish reproductive screen. NRDC is very supportive of the fish reproductive screen. It is an important test for endocrine disrupting effects, and it is highly relevant to predicting potential risks relevant to human health. The fish assay proposed by EPA has undergone peer review, and it's found to be biologically and toxicologically relevant. For a screen for the hypothalamic-pituitary-gonadal pathways, for perturbing chemicals, particularly the antiestrogenic and antiandrogenic compounds. It is seen as appropriate for identifying endocrine disrupting chemicals, both for ecological reasons and for extrapolating to human endpoints and because there is a significant degree of conservation in the function of the HPG access across vertebrae, the tests are relevant to predict likely endocrine-destructing modes

of action in other vertebrae including humans.

Fish levels also offer advantages over mammalian models in that the length of the assay is shorter, less expensive as well as offering more efficient routes of chemical exposure and delivery in many cases. In my written comments I site to an article by EPA scientists who are here, Gerald Ankly and Rodney Johnson, who reviewed the use of the fish assays to identify wildlife in human endocrine-destructing chemicals. I sum up what they wrote in their article and also a number of other reviews, and they conclude that, "Both from ecological effects and species extrapolating perspectives, fish tests are an important component of the endocrine disruption chemical screening and testing programs. Partial and full life cycle tests with fish that are focused on key aspects of reproduction and development not only provide a basis for quantitative predictions of ecological risk of the EDCs to fish populations, but through consideration of endpoints that are sensitive and diagnostic for different classes of EDCs, serve as effective generalized models for identifying chemicals that affect specific components of the vertebrae HPG axis."

I have a personal interest in this because my

entire PhD and a good chunk of my post doc was using different fish assays, and every time I spoke I had to make the argument that they were relevant. I don't think I made it as strongly as their article, and that's why I'm citing them.

EPA should set consistent protocols and procedures for the tier-1 assays. There's a number of concerns that NRDC has, and to be honest, I think that every one of them has already been brought up, but I'll touch through them quickly. And they are in my written comments. We're concerned that EPA does not have a set of consistent procedures that apply to all assays regarding issues such as how doses will be chosen and the use of positive and negative controls. And I'm not actually going to go into detail 'cause that discussed earlier by the SAP here. There are some inconsistencies, and they will result in variability in data that will make it difficult to predict, sorry to interpret the results. Those ambiguities could be averted, we think, with some pre-thought. Also EPAs should clearly define the procedures that are going to be used to decide what doses are used in the tier-1 screens. As recommended by Ed Stack, they should run the tier-1 screens over a wide range of doses that include environmentally relative doses. Utilizing only

high doses in the tier-1 may risk observing effects caused by phytotoxicity or modes of action outside of endocrine-mediated effects, and also effects of high doses may be different than effects at low doses, which was also brought up earlier in discussion. And finally, there are some inconsistencies, we've noticed, in the use of controls in the tier-1 screening assays. There are several assays that don't list having positive controls, and as the Endocrine Disruptive Screening Program is initiated and each screen is evaluated further, a positive and a negative control should accompany each screen. These inconsistencies, we believe, can be quickly resolved by EPA before implementing the tier-1 screening assays without holding up the work.

We are also suggesting for consideration that future rounds should include mixtures. It's the way people are exposed, and it's also the way wildlife is exposed in real world situations. And so considering drinking water contaminants and mixtures was recommended by Ed Stack. I think that's all I'll say on that.

We're also very supportive of the idea that it's an open process, that as the science develops and as new assays and new approaches are validated; that

they be incorporated and that there be a period of review built into the process, and that EPA have that recommendation be made to it quite clearly so that groups like mind can hold them to it.

Thanks very much. Are there any questions?

DR. HEERINGA: Thank you, Dr. Sass. Any questions for Dr. Sass on her presentation? Thank you very much. At this point in time I'd like to invite forward Mr. Scott Slaughter, who represents the Center for Regulatory Effectiveness.

MR. SLAUGHTER: Thank you for letting me be here and talk. I'm Scott Slaughter and I represent the Center for --

DR. HEERINGA: Scott, would you use the microphone.

MR. SLAUGHTER: That working now.

DR. HEERINGA: Yeah.

MR. SLAUGHTER: Okay. Thank you. It will be better the second time. I'm Scott Slaughter, and I represent the Center for Regulatory Effectiveness. Any questions about CRE are probably best answered by our website, which is www.DCRE.com. In my case, next to last is least 'cause I'm assuming someone is coming behind me. I've been very impressed

with the presentations so far. My predecessors have been outstanding in explaining the criteria that are necessary for validating tests and discussing whether the EPA tier-1 battery meets those criteria. With regard to this question, we urge the SAP to consider the data Ecuadia and the ICCVAM validation criteria in review of the T1 assays. The EPA cannot use the T1 assays or require that any of the assays be performed by an outside body unless the record for each assay shows that the assay beats the DQA and the ICCVAM validation criteria. These criteria are set forth in detail in our written comments, and I won't repeat them here, except to note that influential scientific information like these assays have to meet especially stringent validation criteria. Just one example, EPA has to assure the reproducibility of each assay before it can require it to be performed.

We also urge the SAP to consider the three R's in their reviews of the tier-1 assays. EPA has to comply with the three R's. This is mandatory by statute. It is not discretion over the agency. EPA cannot use these assays unless the record for each assay shows compliance with the three R's. Finally, we ask the EPA to identify on the public record the agency's previous summation review for compliance with

the data Ecuadia and the ICCVAM validation criteria, and with the three R's. We've looked, and it's probably our fault, but we're having a hard time finding an adequate discussion in the record and identification in the record by EPA of its compliance with these requirements.

We thank you for the opportunity to present these comments, and we look forward to continuing discussion with the EPA. Are there any questions?

DR. HEERINGA: Thank you Mr. Slaughter, any questions? Thank you very much. We have one more individual present for public comment, and that is Dr. Katherine Willet who is representing People for the Ethical Treatment of Animals, PETA. Dr. Willet?

DR. WILLET: Thank you very much. I'd like to thank the EPA for allowing us to give our public comments. I'm Katherine Willet, and I am employed by PETA. We had recently submitted lengthy written comments on behalf of the animal protection community of North America. So for more details and examples, I'd urge you to look at those comments. I'm going to give a sort of 30,000 view, 30,000 foot view of our comments in just the introductory part. I'm actually not going to touch on the individual assays

much at all 'cause those have been thoroughly covered by people that are much more qualified than I am.

I do want to tell you a little bit about my background. For many years I was a developmental biologist. I worked with Zebra Fish, and I did teratogenicity assays in a pharmaceutical company. I also was beginning to work on endocrine screening. I did a vitellogenin assay with Zebra Fish. So I have some familiarity with the issues around screening and moderate throughput screening, and for the past year and a half I've been the representative of ICAPA to the OECD under the VMG, mammalian and VMG, non-animal.

So throughout my slide I put pictures of animals just to remind everyone who's doing the lion's share of the work in these assays. Of course my talk is going to be filled with critique. I hope it will be taken in the light in which it's meant, which is to be constructive.

There are some logical issues with reviewing this battery. I'm sorry the print is so small. First of all, not all assays have yet been peer reviewed. One has been thoroughly, has been completed validated by the OECD. However, that validation was not without its issues shall we say. Three others are in various stages of validation at the OECD and have

not yet been fully completed. I'm sorry. I'm using the list of assays that was in the federal register notice for this meeting, which is slightly different than the list that was presented here today. I did not include the transcription activation assay for the estrogen receptor.

The EPA released results of its own peer review of six assays between November of 2007 and February of 2008. So altogether we have no information regarding the validation state of the 9 or 12, I'm not sure how many, proposed assays there will be. And the agency also has not provided any explanation yet as to how the tier-1 data will be used, either for decision-making in terms of potential classification or whether or not triggers for tier-2, that sort of thing, or how the tier-1 is going to be integrated in the overall risk assessment process. So on the one hand, therefore, there is not enough information to comment with any confidence really on the battery as a whole, and on the other hand there's, in a way, too much information about each individual assay to adequately evaluate one time. We've been given a task to evaluate somewhere between 5 to 10,000 pages of material. It's kind of a lot.

So let's get down to the overview, the

organization of the tier-1 battery. Again, these numbers are a little off because they're approximate. The putative tier-1 battery contains four, possibly five in-vitro assays and eight or nine in-vivo assays, which is approximately a dozen assays. The tier-1 did not consider at all physiochemical data, existing toxicological data, or things like QSAR modeling. The relatively few in-vitro assays for tier-1 is very in-vivo heavy. It ignores a lot of the in-vitro assays that are in validation at OECD, for example the transcription activation. I'm glad that the ER transcriptional activation assay will be considered. And for some of these assays, the EPA is not using internally validated protocols, which is an issue in terms of being able to share data, applicability to other countries, and that sort of thing. For example, the OECD binding assays, which the EPA is involved in the international validation of, uses a human recombinant androgen receptor, which makes a lot of sense in terms of human relevance. Whereas the protocol that's being used by the EPA for the EDSP uses cytosol from the rat prostate. I understand the issue is primarily over intellectual property. However, I think intellectual property is going to be an increasingly important issues, especially with non-

animal or alternative methods, in-vitro methods, in the future and for screening programs when we really have to figure out a way to get around this as a block because it's going to continue to increasingly be a problem, I think, as the technology develops.

As I mentioned, the tier-1 battery is very animal intensive. I mean, potentially we talking -- I understand today we're talking about the first round, which is 73 compounds. But this program in general -- this potentially talks about 10's of 1000s of chemicals to be tested in approximately a dozen different assays. Thus, based on the numbers from the assays that we've been given, each chemical would use a minimum of 186 rats, 72 fish, and 320 frogs assuming no range finding, minimal controls, and each chemical is tested only once. This is an extremely unlikely scenario, and this could be the largest screening program ever proposed by the US. It requires enormous quantities of each chemical, especially the aquatic assays. If you've figure out how much the volumes are for those flow things, its staggering. Okay. These are all characteristics that are not really suitable for a screen of possibly 10s of 1000s of comments. A more official approach would be to take into account the physiochemical date and all preexisting toxilogical

data. This was mentioned before too. For many of the compounds, especially in the initial list, they are some of most highly test compounds on Earth, and there's a lot of very apical and animal-intensive tests that have already been done on these. All of this data should be considered before making a decision of whether or not a chemical needs to be tested. A more efficient approach would also contain an initial tier of completely non-animal mechanism-based assays. For example, QSARs and a broad spectrum of cell-based assays. This would also be consistent with NRCs recommendations for effective toxicity testing, referring to that NRC report that was issued last June I believe it was. And this would fulfill the intended purpose of the tier-1, which is to identify substances that have a potential to interact with the endocrine hormonal systems and would use many fewer animals, resources, and time. Such an approach is kind of familiar. It looks a lot like the OECD conceptual framework. Now I understand that the conceptual framework is not intended as a tier, but it does provide sort of a logical framework with which one could devise a more efficient process. And if you compare the EPA's EDSP tiers to the OECD conceptual framework, you'll see that the framework, the levels,

the early levels in the framework consist entirely of existing data, chemical properties, and an entire battery of broad spectrum in-vitro assays. I also realize that a lot of these are not yet validated. However, if one were to take a more logical approach to this, one would, I think, spend the resources, time, and effort designing something from the ground up rather than from the sides in. And then it's only in level three and level four that you get to the more extensive animal assays.

Okay. So in summary, really the EPA has yet to articulate what this vast catalog of data will be used for. This has also been mentioned previously. What is the human health issue that we're talking about here, and how will this data be applied to regulation? Will it have any effect on regulation of these compounds at all? We don't know that. It's far from clear that the EDSP will result in effective regulation. What is clear is that the design, the clinic design of the EDSP is based on expedience rather than some science, and right now as it stands is a waste of animal lives, if it proceeds as it is.

So I'm very heartened to hear that there will be a review and that the EDSP will be subject to modification change, updating, that sort of thing. I

very much encourage that to happen to keep up with modern science and what we've learned, and that's it for me. Any questions?

DR. HEERINGA: Thank you, Dr. Willet. Any questions for Dr. Willet on her presentation? Dr. Lasley?

DR. LASLEY: Lasley, UC Davis. To see if I understand you correctly, your proposing a system where information in the literature would be used as it is; is that right? I mean, that any published report on a chemical could then be used independent of its design or quality control. I mean, I don't understand how that would work.

DR. WILLET: Well what I'm saying is many of the compounds have already been tested in assays, for example, in a multi-generation assay development, especially the pesticides, and since they've already been in what would be considered actually higher than tier-1; if you already have that information, why do you need to go back and do a tier-1 battery n that?

DR. LASLEY: I was stating to quality control.

DR. WILLET: Okay. I think you should use all appropriate data.

DR. HEERINGA: Additional questions from the panel? Thank you, Dr. Willet. At this point in our proceedings we have completed the period of public comment, and we are now at just short of 4:00 p.m. What I am proposing to do is to adjourn the meeting for today. We would move on to the charge questions, but I think there's merit in having everyone have a chance to absorb the information that they not only came prepared with but what we've heard today. And that should give Dr. Furlow some rest from his redeye flight from California via Vegas and Atlanta, right? That's the most unusual set of connections to unintentionally go to Las Vegas. In any case, so I'd like to adjourn for today, and we'll resume tomorrow morning, and it is scheduled for 9 o'clock on the agenda. I can't change that, so we will start at 9 o'clock tomorrow morning; and we'll open with some review comments from the EPA scientific staff, and then we'll move to the charge questions. So have a good evening everything, and we'll see you tomorrow morning at 9:00 a.m. Panel members, if we could just meet in the breakout room here briefly to discuss organization of our comments and writing assignments.

(**WHEREUPON**, the meeting was adjourned at 3:58 p.m.)

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SUBMITTED ON March 25, 2008

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