VIDEOCONFERENCE MEETING

STATE OF CALIFORNIA

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

DEVELOPMENTAL AND REPRODUCTIVE TOXICANT

IDENTIFICATION COMMITTEE

ZOOM PLATFORM

TUESDAY, DECEMBER 14, 2021

10:00 A.M.

JAMES F. PETERS, CSR
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APPEARANCES

COMMITTEE MEMBERS:

Ulrike Luderer, PhD, MPH, Chairperson

Patrick Allard, PhD

Diana Auyeung-Kim, PhD

Carrie Breton, PhD

Aydin Nazmi, PhD

Isaac Pessah, PhD

Irva Hertz-Picciotto, PhD

Tracey Woodruff, PhD

STAFF:

Lauren Zeise, PhD, Director

Dave Edwards, PhD, Chief Deputy Director

Carol Monahan Cummings, Chief Counsel

Marlissa Campbell, PhD, Staff Toxicologist, Reproductive Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

Vincent Cogliano, PhD, Deputy Director, Division of Scientific Programs

Julian Leichty, Special Assistant for Programs and Legislation, Proposition 65 Implementation Program

Ling-Hong Li, PhD, Staff Toxicologist, Reproductive Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

APPEARANCES CONTINUED

STAFF:

Francisco Moran, PhD, Acting Chief, Reproductive Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

Yassaman Niknam, Phd, Staff Toxicologist, Reproductive Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

Martha Sandy, PhD, MPH, Chief, Reproductive and Cancer Hazard Assessment Branch

ALSO PRESENT:

Allegra Kim, PhD

Suzanne Hume, CleanEarth4Kids.org

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PROCEEDINGS

WELCOME AND OPENING REMARKS

DIRECTOR ZEISE: Good morning, everyone, and welcome to this December 2021 virtual meeting of the Developmental and Reproductive Toxicant Identification Committee. I'm Lauren Zeise. I'm Director of the Office of Environmental Health Hazard Assessment.

Today's meeting the Committee is going to be considering two major items. First, the male reproductive toxicity of perfluorononanoic acid, or PFNA, and its calls. And then secondly perfluorodecanoic acid, PFDA, and its salts. We'll also have updates and a consent item.

So the meeting is being transcribed and recorded. The transcript will be posted on our website. And now I'm going to ask Elizabeth Marder who's handling the logistical aspects of this meeting to go over how the public can better best participate in the meeting. It's a reminder of logistics from the meeting agenda that went out earlier. So turn it over to you Elizabeth.

DR. MARDER: Good morning. Just a reminder that individuals who wish to make an oral comment at today's meeting are asked to do two things. First, please join the Zoom webinar and second please fill out a speaker request card. Information on how to join the Zoom webinar

is shown on the slide that is currently being presented. Now, go to bit.ly/registerDARTIC2021, which I am currently putting in the chat as well, and register for today's Zoom webinar. You will receive a link to join the webinar at the end of the registration process. And if you provided a working email address, you will also receive an email with a link to join the webinar.

Information on how to access a speaker request card is also shown on this slide. Go to bit.ly/OEHHADARTIC2021 and request to speak on a specific agenda item. It is requested that your Zoom display name match the name you use to fill out the speaker request form. Individuals who have not submitted a speaker request card may also indicate their wish to make an oral comment by using the raise hand function when requested by the Chair. That's in the Zoom control panel.

I'll also add briefly that as to -- as regards closed captioning, for those joining the Zoom webinar, artificial intelligence-, AI, generated subtitles and a full transcript can be displayed. The subtitles will be visible to those joining the listening and viewing only broadcast. Thank you.

DIRECTOR ZEISE: Thanks, Dr. Marder.

Now, I'm going to introduce the Panel, the Developmental and Reproductive Toxicant Identification

Committee. And as I introduce you, if you could raise your hand.

So we'll start with Dr. Patrick Allard, Associate Professor at the University of California, Los Angeles, Institute for Society and Genetics.

Dr. Diana Auyeung-Kim, Executive Director, head of gRED Non-clinical Operations, GNO, safety assessment at Genentech.

Dr. Carrie Breton, Associate Professor of Population and Public Health Studies, Keck School of Medicine, University of Southern California.

Dr. Hertz-Picciotto, Profess or Epidemiology and Chief, Division of Environmental and Occupational Health at the University of California, Davis.

Our Committee Chair, Dr. Ulrike Luderer, Professor of Medicine, School of Medicine, and Director Center of -- for Occupational and Environmental Health, University of California, Irvine.

Dr. Aydin Nazmi, Professor of Food Science and Nutrition at California Polytechnic State University, San Luis Obispo.

Dr. Isaac Pessah, Associate Dean and Distinguished Professor, School of Veterinary Medicine, University of California, Davis.

Dr. Tracey Woodruff, Professor, Developmental

Obstetrics, Gynecology and Reproductive Sciences and Director of Program on Reproductive Health and the Environment, University of California, San Francisco.

Okay. So welcome, panel, and thank you for your participation today.

Now, I'll introduce OEHHA staff starting with Dr. Dave Edwards, Chief Deputy Director. This is Dr. Edwards first meeting with us. Carol Monahan, Chief Counsel. Dr. Vince Cogliano, Deputy Director for Scientific Programs.

Now, from the Reproductive Cancer and Hazard Assessment Branch, Dr. Martha Sandy, Branch Chief, Dr. Francisco Moran, Acting Section Chief of the Reproductive Toxicology and Epidemiology Section. And then other staff of the Reproductive Toxicology and Epidemiology Section that the Committee will be hearing from today, Dr. Ling-Hong Li.

DR. LING-HONG LI: Good morning.

DIRECTOR ZEISE: Dr. Melissa -- Marlissa Campbell, Dr. Yassi Niknam, and then finally Dr. Allegra Kim, who summarized the epidemiology studies when she was with OEHHA, and she will also be presenting.

And then from the Proposition 65 Implementation Program, Julian Leichty, Special Assistant for Programs and Legislation, Esther Barajas-Ochoa, and Tyler Saechao.

Okay. Now, I'll ask Carol Monahan Cummings,

OEHHA Chief Counsel, for some introductory remarks about Bagley-Keene and other legal issues related to participation in today's virtual meeting.

CHIEF COUNSEL MONAHAN CUMMINGS: Good morning. It's good to see everybody here today. I just had a few points I wanted to make. First, please feel free to ask me any questions at any time during the meeting. I'll be here the whole time. If I do have to step away for any reasons, Senior Staff Counsel, Kristi Morioka will cover for me. So there will be an attorney here all the time.

Please remember that all discussions and deliberations for this group need to be conducted during the meeting, not on breaks, lunch, or with individual members of the Committee on- or offline, including via phone, email, chats, or text messages.

At today's meeting, you'll be considering two chemicals for potential listing. OEHHA takes no position regarding whether a chemical should be listed, though staff are available to answer questions or locate information, if you need it.

The Governor appointed you because of your scientific expertise to be the State's qualified experts on the reproductive toxicity of chemicals. There's no need for you to feel compelled to go outside that charge. For example, you need not consider whether a warning may

be required for an exposure to a chemical, or any other consequences of the listing.

This Committee can consider human, animal, mechanistic, or other data in deciding whether a chemical has been clearly shown through scientifically valid testing, according to generally accepted principles, to cause reproductive toxicity. If you need for information, need more time to consider the evidence or discuss it further before you vote on a listing, there's no requirement that you make a decision today. For example, you may table the decision and take it up again at a future meeting.

Feel free to ask clarifying questions of me or the other OEHHA staff during the meeting. If we don't know the answer to your question, we'll do our best to find it and report it to you.

Do you have any questions?

Okay. Thank you.

DIRECTOR ZEISE: Thanks, Carol.

And now I'll turn the meeting over to our Chair, Dr. Ulrike Luderer.

CHAIRPERSON LUDERER: Thank you very much, Lauren and Carol. And good morning and welcome to all the Committee, members of the public who are joining today.

CONSIDERATION OF PERFLUORONONANOIC ACID (PFNA) AND

ITS SALTS AS KNOWN TO THE STATE TO CAUSE REPRODUCTIVE TOXICITY(BASED ON MALE REPRODUCTIVE TOXICITY)

CHAIRPERSON LUDERER: We're now ready to move on to our -- the first of two main agenda items, and that item is consideration of the perfluorononanoic acid, or PFNA, and its salts as known to the State to cause reproductive toxicity based on male reproductive toxicity.

So I would like to, having introduced the agenda item, now turn the floor over to Deputy Director for Scientific Programs, Dr. Vince Cogliano, to begin.

STAFF PRESENTATION

DR. COGLIANO: Thank you, Dr. Luderer. Good morning everyone.

I'd like to endorse Lauren's welcoming remarks, especially our appreciation for your service as experts on this Committee. You have an important role in bringing current science to bear on decisions to benefit the health of all the people of California. We know you're here as a public service, and so to assist you, OEHHA has summarized the scientific evidence that you will consider. I'll turn the screen over to Dr. Martha Sandy, Chief of our Reproductive and Cancer Hazard Assessment Branch who will introduce the staff presentation.

Martha.

DR. SANDY: Thank you, Vince and welcome and good

morning to everyone.

Let me provide some background on the process by which perfluorononanoic acid, or PFNA, and its salts was given a high priority and selected for listing consideration. PFNA was brought to the DART IC for consultation and prioritization last year, in 2020, and this Committee recommended that PFNA be placed in the high priority group for future listing consideration.

OEHHA selected PFNA and it salts for consideration for listing, and in March 2021, OEHHA solicited from the public information relevant to the assessment of developmental and reproductive toxicity. No information was received on PNF -- PFNA and it salts.

OEHHA has focused its current review of PFNA and its salts on evidence of male reproductive toxicity. This information is summarized in the hazard identification document released in October 2021, which also includes information on PFDA and its salts, which will be discussed in a separate agenda item today.

The hazard identification materials on PFNA and it salts provided to the DARTIC for your consideration include the hazard identification document, the references cited within it, one additional epidemiology study, and a revised Table 4.1, which has been updated to include that study, and public comments received on the document.

I will now ask Dr. Pancho Moran, who is currently serving as the Acting Chief of the Reproductive Toxicology and Epidemiology Section, to begin the staff presentation. And I will note that we will take a break part way through the staff presentation to provide the Committee an opportunity to ask questions of clarification.

Dr. Moran.

(Thereupon a slide presentation.)

DR. MORAN: Thank you very much, Dr. Sandy. Good morning, everybody.

Is the slide visible for everybody?

DR. MARDER: Yes, it is.

DR. MORAN: Just checking.

Okay. So good morning. We will present an overview of the evidence of the male reproductive toxicity of perfluorononanoic acid, PFNA, and it salts. This presentation will be a brief overview of the data reviewed in the hazards identification document. Due to time constraints, this presentation is not able to cover every finding discussed in the HID. I would like to acknowledge that this work was a group effort from the staff in the Reproductive Toxicology and Epidemiology Section.

NEXT SLIDE

DR. MORAN: Here is an outline of this presentation. We will start by presenting introductory

information on chemical structure, use, occurrence and exposure, and the systematic literature review approach that we implemented.

Next, we will summarize key pharmacokinetic information for PFNA.

We will then present a brief summary of the male reproductive toxicity data for PFNA and its salts, starting with data from whole animal studies, followed by findings from human epidemiological studies.

An overview of mechanistic data for PFNA will then be presented, followed by a summary of those data.

The concept of the key characteristics of male reproductive toxicants will be presented, together with the key characteristics of endocrine disrupting chemicals.

We will conclude the presentation with a summary of the animal and human data.

NEXT SLIDE

DR. MORAN: PFNA and its salts are a perfluorinated organic compound with surfactant properties that belongs to a group of chemicals that collectively are called per- and polyfluoroalkyl substances or PFASs. The chemical structure of PFNA is shown on this slide, where PFNA has a fully fluorinated nine-carbon chain.

NEXT SLIDE

DR. MORAN: PFASs, including PFNA, have been used

to make products resistant to stains, grease, soil, and water. PFNA and its salts have been used in fluoropolymer manufacturing, and PFNA has been detected in Cosmetic products. There is limited information on the production and emission of PFNA and its salts.

NEXT SLIDE

DR. MORAN: PFNA is a global pollutant of air, water, soil and wildlife, and is present in the environment. Level of PFNA in Californians has been documented in several studies conducted between 2010 and 2019 by Biomonitoring California with high detection frequencies.

NEXT SLIDE

DR. MORAN: OEHHA conducted literature search on the developmental and reproductive toxicity of PFNA and its salts. We used HAWC, Health Assessment Workspace Collaborative, as a tool for multi-level screening of literature search results. Then we focused on literature relevant to male reproductive toxicity.

NEXT SLIDE

DR. MORAN: Here we have a summary of the screening of the DART literature for PFNA with a particular focus on the literature relevant to male reproductive toxicity. Studies identified as providing general information on PFNA are shown here on the blue

box, while the studies relevant to male reproductive toxicity are highlighted by the red boxes. Note that for the human epidemiological data, the study designs were also identified.

NEXT SLIDE

DR. MORAN: PFNA is well absorbed and binds to serum proteins. It is widely distributed throughout the body, and in human tissues PFNA was found principally in brain, – which indicate that it can cross the blood-brain barrier, and in kidney, with lower levels in lungs and liver. PFNA was detected in semen, cord serum, fetal tissues, which indicate that it can cross the placenta and in breast milk. PFNA is not known to be metabolized in animals or humans, and the excretion is mainly through urine and feces, but a small amount are also found in nails and hair. The half-life of PFNA ranges from several years in humans to a few month in rodents.

Now, Dr. Ling-Hong Li will present data from whole animal studies.

NEXT SLIDE

DR. LI: Good morning. I will present an overview on the data available from whole animal studies on PFNA.

NEXT SLIDE

DR. LI: This table shows five groups of male

reproductive outcomes that we have data on PFNA. These are the outcomes that are generally used to assess male reproductive toxicity in animal studies. They include organ weights and histopathology, sperm production and quality, hormonal evaluation, reproductive performance including fertility, and development of the male reproductive system.

In the next few slides, I will present data on PFNA from animal studies on each of these five outcomes.

Next.

Next, please.

NEXT SLIDE

DR. LI: We found studies on PFNA in rats, mice, and zebrafish from the literature search. All studies in rats treated the animals by gavage. Six to ten pubertal or young adult -- adults per group received the treatment daily for 14 or 28 days. Similarly, studies in mice treated the animals by oral gavage. Three studies treated the prepubertal mice, one for 90 days, and two for 14 days. Two studies treated pregnant mice during gestation and then either evaluated the testicular effects in neonatal mice or examined developmental landmarks in male offspring. The study by Zhang et al. treated zebrafish with PFNA in fish tank water for a total of 180 days.

Next.

NEXT SLIDE

DR. LI: Reproductive organ weights were measured in studies in rats and mice. In rats, the NTP study included five groups of rats treated by daily gavage of PFNA from 0.625 to 10 milligram per kilogram per day. Organ weights were measured in the control and three lower dose groups. Epididymal weight was significantly reduced in these three lower dose groups dose dependently. The significant reduction in epididymal weight seen at 0.625 milligram per kilogram, the lowest dose used in the study, occurred in the absence of any effect on body weight. Testis weight was also significantly reduced in this study.

Next.

NEXT SLIDE

DR. LI: In mice, two studies measured testis weight following exposure beginning at 25 days of age. Reductions in testis weight were observed in both studies, but these reductions did not reach statistical significance. Measurements were conducted on relatively small numbers of mice per treatment group in these studies. The large reductions in testis weight in pubertal mice treated with PFNA could be biologically meaningful.

Next.

NEXT SLIDE

DR. LI: Histopathological evaluation was performed in studies in rats and mice. In rats, in the NTP study, germ cell degeneration, interstitial cell atrophy, spermatid retention, and epididymal lesions were observed in PFNA-treated rats. As shown from these histopathological photos taken from the NTP study report, you can clearly see dying germ cells, missing layers of germ cells, and a diminished cluster of Leydig cells.

Next.

NEXT SLIDE

DR. LI: In the studies by Feng et al., pubertal rats were treated for 14 days with PFNA at doses slightly higher than those used in the NTP 28-day study. The authors found germ cell degeneration and sloughing of the seminiferous epithelium. Using the TUNEL labeling methods, the authors found increased number of spermatocytes and spermatogonia in apoptosis. These investigate -- investigators also observed histological lesions in Sertoli cells.

Germ cell degeneration has also been observed in multiple studies of pubertal or adult mice treated with PFNA. In the 90-day study, the authors found germ cell degeneration in adult mice treated with 0.5 milligram per kilogram of PFNA.

Next.

NEXT SLIDE

DR. LI: Sperm parameters were measured in the control and three lowest dose groups in the 28-day NTP study in rats and in the 90-day study by Singh and Singh in mice. In the NTP study, epididymal sperm count was significantly reduced in a dose-dependent manner in the second and third lowest dose groups. In the study in mice, sperm number, motility, viability were all significantly reduced in the high-dose group.

Next.

NEXT SLIDE

DR. LI: The hormonal effects of PFNA were assessed in rats, mice, and zebrafish. Statistically significant reduction in serum testosterone levels was found in rats and mice. In the 28-day NTP rat study, serum testosterone was measured in the control and three lower dose groups, and a significant reduction was seen. In the 14-day rat study by Feng et al., 2009, serum testosterone was significantly reduced in the high-dose group. In mice -- in mice, serum testosterone was significantly reduced in the 90-day study in adults in the high-dose group, and in pubertal mice in a 14-day study in both dose tested.

In zebrafish, serum testosterone was

significantly increased at the low dose, but not at higher doses. Significant reductions intratesticular testosterone levels were also found in pubertal mice at both doses tested following a 14-day exposure.

Next.

NEXT SLIDE

DR. LI: In addition to the effects on testosterone level, increased serum levels of MIS and estradiol, and decreased serum level inhibin B were found in rats. There was no effect on serum FSH or LH in rats.

Next.

Next, please.

NEXT SLIDE

DR. MORAN: Slide 19, ling-Hong.

DR. LI: There are two studies that evaluated the effects of PFNA on reproductive performance.

In the study in mice, the authors reported a significant reduction in litter size following mating of unexposed females with male mice at the end of the 90-day dosing period, but the detailed information on the study design was not presented in the paper.

In the 180-day study in zebrafish, the authors reported a significant reduction in egg hatching rates at the lowest and highest concentrations. However, both female and male fish were exposed to PFNA making it

difficult to determine if this effect was male-mediated.

Next.

NEXT SLIDE

DR. LI: There are two studies in mice assessed the effect of PFNA on development of the male reproductive system following prenatal exposure.

In the study by Das et al., the authors found significant delays in preputial separation in prenatally exposed male mice in the low and high dose groups.

In the study by Singh and Singh, the authors treated pregnant mice from gestational day 12 to birth and then examined the testis in neotal mice on postnatal day 3. Major findings from this study will be presented in the next two slides.

Next.

NEXT SLIDE

DR. LI: The authors reported no effects on testes weight or histology. However, based on these histopathological pictures the authors presented in the paper, as shown on this slide, the diameter of seminiferous cords from PFNA-treated mice appear to be smaller. These apparent histopatho -- histopathological changes suggest reduced Sertoli cell population.

The authors reported a 20 to 30 percent reduction in the testis weight, which did not reach statistical

significance. Since neonatal testis mainly consists of proliferating Sertoli cells, the reduction in testis weight in neonatal mice indicate reduced number of Sertoli cells.

The authors found testicular protein level of PCNA was significantly reduced. Taken together, these findings suggest inhibition of Sertoli cell proliferation after prenatal exposure to PFNA. Proliferation of Sertoli cells in neonates determines the number of Sertoli cells in adult animals and each Sertoli cell can only nurture a certain number of germ cells in the adult. Reduced Sertoli cell proliferation in neonates could result in reduced sperm production in the adult. Therefore, effects on Sertoli cell proliferation during the perinatal period need to be considered carefully.

Next.

NEXT SLIDE

DR. LI: In the same study, the authors found intratesticular testosterone levels were reduced. Testicular levels of several proteins involved in key steps in steroidogenesis were also reduced. Testosterone production by Leydig cells in neonates is critical for germ cell development.

That concludes my presentation on PFNA.

DR. SANDY: All right.

DR. MORAN: Thank you, Dr. Li.

Now, we have time for questions from the DARTIC members, if they decide to.

CHAIRPERSON LUDERER: All right. DARTIC members if you if anyone has any questions, please use the raise hand method or you can also wave your actual hand. So I see that Dr. Pessah has a clarifying question. I'll start with you and I see that others also do.

COMMITTEE MEMBER PESSAH: All right. Thank you. I was wondering, did any of the studies you just presented provide quantitative levels of the PFNA in the serum or in the tissues?

DR. LI: The NTP study -- I'll take -- I'll try to respond and will go back to check on the NTP report more carefully, come back to you, Dr. Pessah. The NTP study measured serum levels of PFNA in serum. And I don't think that they measured the PFNA levels in testicular tissues.

COMMITTEE MEMBER PESSAH: Thank you.

CHAIRPERSON LUDERER: All right. Dr. Breton.

COMMITTEE MEMBER BRETON: Hi. Thanks. In the last study that you were just presenting with the intratesticular testosterone levels, I was wondering whether they also looked at circulating testosterone or serum testosterone. And just it would be helpful if they

happened to have any correlation between the intratesticular levels of testosterone and the circulating levels. Was that looked at all?

DR. LI: No. They only presented data on intratesticular T level.

COMMITTEE MEMBER BRETON: Okay. Thanks.

CHAIRPERSON LUDERER: All right. Dr. Woodruff.

COMMITTEE MEMBER WOODRUFF: Hi. I was interested in the figures that you showed on your literature search and that were using HAWC for the literature search tool. So I don't recall the little spider diagram being in the document. So that's why I was asking for the slides. And I was wondering -- and maybe -- I'm going to talk about this in -- when we go on to the main comments. But I'd like to hear maybe at the end of the presentations your plans for using HAWC more completely in terms of being able to add in the quantitative information from the studies. I think that would be a great use to the committee for reviewing this. And are you making the literature review component in HAWC public?

DR. LI: Pancho, would you like --

DR. MORAN: Yes. Yes. Yeah, of course. No, at this point, we are not making it public, but probably we are in the processes of early attempts of using this tool for our systematic literature view. So we are in the

process of adding more features to it as convenient for our studies.

COMMITTEE MEMBER WOODRUFF: Right. But I guess I would encourage the State to make that literature space -- because you could make those projects public on HAWC. And I really think that that would be very useful as a public service tool.

DR. MORAN: Yes, I think we need to talk about that internally, because the Prop 65 may have some indication, some --

DR. SANDY: Yeah. This is Martha Sandy.

DR. MORAN: Yes.

DR. SANDY: So thank you, Tracey -- Dr. Woodruff for your comments and we'll take those under consideration, and we'll -- we can talk about this later.

COMMITTEE MEMBER WOODRUFF: Okay. Thanks.

CHAIRPERSON LUDERER: Dr. Hertz-Picciotto, did you have a question?

DR. MARDER: You're muted.

CHAIRPERSON LUDERER: You're muted.

COMMITTEE MEMBER HERTZ-PICCIOTTO: There we go. Okay. So this is actually sort of a broader question. And I'm not sure about this. So the products in which PFNA is used, do those also typically include other -- other perfluoro -- other PFAs? And I guess what I'm

really getting as is whether there's been any studies looking at mixtures in which PFNA would be one component? The concern being that we do always test things one by one. And as we know, that's not realistic and, you know, tends to -- it could be masking effects that are more a result of a mixture. So that's my question.

DR. SANDY: This is -- this is Martha Sandy. Thank you for that question. It's a good question. It's very difficult for us to find information on current use of PFNA. We do know from biomonitoring studies that we're exposed to it, but we -- and we focus on our hazard identification documents on testing of the chemical that we're considering.

So we have not focused on looking at mixture studies. And I don't recall that we found any. There may have been one. I'll turn to Dr. Moran, if he remembers anything.

DR. MORAN: Yes. As a common practice, we try to avoid in animal studies at the least mixtures, because, you know, we are trying to list one particular chemical, not a class, not a mixture. So when we run into those type of studies, we save it as a normal background information, but we concentrate on we can call it clean studies with one single chemical applied at the time.

On epi studies, of course, that is impossible, so

the answer is yes we are exposed to a mixture, but for animal studies, we concentrate on a single chemical at a time.

CHAIRPERSON LUDERER: Thank you. I'm not seeing any additional raised hands, so I believe we can go -- continue with the staff presentations.

DR. MORAN: Okay. Thank you very much.

NEXT SLIDE

DR. MORAN: So Next Dr. Allegra Kim will discuss epidemiologic studies of PFNA that examined male reproductive outcomes.

Dr. Kim.

You may be muted, Allegra.

DR. SANDY: Dr. Kim, if you're able to unmute yourself and talk, otherwise we'll go to plan B.

Elizabeth, can you see --

DR. MARDER: Dr. Kim is unmuted. She's asking for a moment, please.

DR. KIM: Hello. Can you hear me now?

DR. MARDER: We can. Thank you, Dr. Kim.

DR. KIM: I don't -- I don't know what changed, because I didn't anything since our test. Anyway, I apologize for that. Good morning.

As is common with -- can we go to the next slide, please.

NEXT SLIDE

DR. KIM: As is common with studies of the male reproductive endpoint, many studies of PFNA were cross-sectional in design. There were also some prospective cohort and case-control studies. A noteworthy methodological issue is that compared to two other PFASs, PFOA and PFOS, the measured PFNA concentrations in serum or plasma in these studies were very low – often close to 1 nanogram per milliliter.

In addition, the studies generally had low sensitivity, that is, within study populations, the differences between the lowest and highest PFNA concentrations within studies was small.

And finally, all population samples have multiple chemical exposures, and in particular, multiple PFAS exposures.

As in the HID, the findings I will report are statistically significant, unless otherwise stated.

Next slide, please.

NEXT SLIDE

DR. KIM: OEHHA identified 18 studies that examined male reproductive outcomes. The most studied outcomes were of male reproductive function, and I will talk more about those later.

There were also two studies that examined the

effect of maternal PFNA exposure on the developmental landmark anogenital distance in male offspring. The findings from these studies were inconsistent, with one study finding an increase and the other finding a decrease in anogenital distance. And there were two studies of prostate cancer or prostate-specific antigen as a marker for prostate cancer. These studies found no associations with PFNA.

Next slide.

NEXT SLIDE

DR. KIM: The next few slides will focus on outcomes of reproductive function, starting with reproductive hormones. Decreased serum testosterone levels were associated with higher PFNA concentrations in serum, plasma, or semen in several studies of younger boys and men. These findings were in studies of pre-pubescent boys aged 6-9 years old, in 13-15 year old boys, in young men being considered for military service with a median age of 19 years, and in men visiting a reproductive health center. In this last study, the association was strongest in men under 30.

Other studies, most of which have small sample size or low serum PFNA, with median concentrations near or below 1 nanogram per milliliter, reported no associations or inconsistent results.

No consistent associations were seen with PFNA and other reproductive hormones or related proteins.

Next slide, please.

NEXT SLIDE

DR. KIM: A cross-sectional study using data from the National Health and Nutrition Examination Survey, NHANES, reported that a doubling of serum PFNA was associated with a 16.3 percent higher concentration of thyroid stimulating hormone in males 12 to less than 20-years-old. Associations with other thyroid hormones were not observed.

Next slide, please.

NEXT SLIDE

DR. KIM: The study with the highest PFNA concentration reported a substantial and dose-dependent reduction in sperm concentration in the second and third tertiles, with a 25 percent reduction in the third tertile. A dose-dependent reduction in sperm count was also observed, although the reductions did not reach statistical significance. Findings on sperm morphology and/or sperm motility were inconsistent.

Next slide, please.

NEXT SLIDE

DR. KIM: Sperm DNA integrity was examined in two studies with contrasting population samples. Infertile

men were overrepresented in the study by Pan et al., and underrepresented in the study by Specht et al.

Effects were seen by Pan et al., but not Specht et al. These effects were an increase in the percentage of sperm with high DNA stainability, which is an indicator of the percent of sperm with immature chromatin and an increase in the DNA fragmentation index. In the one study to examine IBF -- IVF outcomes, no associations with adverse effects were observed.

I will now hand the presentation over to Dr. Moran.

NEXT SLIDE

DR. MORAN: Thank you, Dr. Kim.

So in this section, we will present an overview of the mechanistic evidence on the effect on the hypothalamic-pituitary-gonadal or liver axis, and the effect on the thyroid and thyroid hormones. I would like to emphasize that this is an overview of the mechanistic data, and I refer to the -- you to the HID for discussion for additional study findings.

NEXT SLIDE

DR. MORAN: I would like to start with a brief review on the physiology of the hypothalamic-pituitary-gonadal-axis. Gonadotropin releasing hormone, GnRH, produced by the hypothalamus,

stimulates the pituitary to release gonadotropins, luteinizing hormone, or LH, and follicle stimulating hormone, FSH, that will stimulate the gonads to produce gametes and hormones such as testosterone, inhibin B, and Müllerian inhibiting substance.

NEXT SLIDE

DR. MORAN: The effect of PFNA on reproductive hormones in humans and whole animals have already been presented.

For in vitro endocrine effects of PFNA, we have that in cultured primary Sertoli cells isolated from eight weeks old rat, PFNA exposure resulted in increased in Müllerian inhibiting substance messenger levels, and decreased inhibin B messenger levels.

In a mouse Leydig cell tumor line treatment with PFNA resulted in a concentration-dependent decrease in the production of progesterone.

NEXT SLIDE

DR. MORAN: In the next three slides, I will summarize PFNA's effect on the expression binding and/or activity of HPG related hormone receptors.

The studies in vivo, in mice, reported that PFNA reduced androgen receptor messenger levels and in male Zebrafish, PFNA reduced gonadotropin receptor messenger levels in the gonads.

Reduced estrogen receptor-alpha and beta messengers in zebrafish brain at the low and -- at the low dose and increased messenger levels at the higher dose, also reduced androgen receptor messenger levels in the brain and increased liver messenger levels for estrogen receptor alpha and beta. In rainbow trout, cyt -- liver cytosol, PFNA displayed very weak competitive binding with estrogen to estrogen receptor alpha.

NEXT SLIDE

DR. MORAN: In primary rat Sertoli cells culture, PFNA exposure resulted in a reduction in messenger levels of FSH receptor, and PFNA had no effect on androgen receptor messenger levels. In a Chinese hamsters ovary cell line PFNA had no androgen receptor agonist activity. However, PFNA did exhibit concentration-dependent antagonistic effects on dihydrotestosterone (DHT)-induced AR transactivation.

NEXT SLIDE

DR. MORAN: In MVLN cells, a human breast adenocarcinoma cell line PFNA had no effect on estrogen receptor transactivation. And in a separate experiment, PFNA was found to inhibit the estrogenic response to estradiol in a concentration dependent manner. In MCF-7 cells, another human breast adenocarcinoma cell line, co-treatment with estradiol and PFNA resulted in

downregulated expression of two estrogen responsive genes.

In a human embryonic kidney cell line, PFNA induced human estrogen receptor alpha gene reporter activity, by up to two and a half fold.

In Silico studies reported that PFNA is predicted to bind at the active site of human, mouse, and trout estrogen receptor alpha, and is predicted also to bind to the surface of the estradiol activated form of the human estrogen receptor alpha.

NEXT SLIDE

DR. MORAN: The studies in mice and zebrafish indicate that PFNA has effect on two regulatory proteins involved in early steps in steroidogenesis, the steroidogenic factor 1, SF1, and steroidogenic acute regulatory protein or StAR. PFNA reduced protein expression of SF1 in the testes of prepubertal mice and in mice exposed in utero and evaluated on postnatal day three. And also reduced StAR messengers and protein levels in the testes of mice in multiple studies. PFNA also increased messenger levels of StAR in zebrafish gonads.

NEXT SLIDE

DR. MORAN: Here we have a summary of the enzymatic steps involved in steroidogenesis. Sex steroids like progesterone, testosterone, or estradiol in the

gonads are produced from cholesterol in a series of sequential enzymatic reactions. There are two main types of enzymes in this pathway, first, the P450s highlighted by the red boxes and the hydroxysteroid dehydrogenase, HSDs, in the blue boxes. Remember this, because we will use this schematic representation to present data on the effects of PFNA on steroidogenesis.

NEXT SLIDE

DR. MORAN: PFNA reduced messengers and protein of P450 side chain cleavage enzyme in mice, and increased messenger in zebrafish. There were reduction in the messenger and protein expression of 3beta-HSD in mice and zebrafish.

NEXT SLIDE

DR. MORAN: For P450c 17, there were unclear effects in rats, and no effect on zebrafish. There was a reduction in messenger and protein expression of 17beta-HSD in mice, and increased messengers levels in zebrafish.

PFNA exposure resulted in an increase in aromatase expression in zebrafish and has no effect on aromatase activity in a human placental choriocarcinoma cell line.

NEXT SLIDE

DR. MORAN: Now, Dr. Marlissa Campbell will

present data on thyroid hormones.

Thank you.

DR. CAMPBELL: Can you give me the slide, Pancho.

NEXT SLIDE

DR. CAMPBELL: Thank you. Just a brief introduction.

The thyroid hormones, T3 and T4, are produced and secreted by the thyroid gland in response to the regulatory hormones, thyroid stimulating hormone, or TSH, from the pituitary gland, and thyrotropin-releasing hormone, TRH, from the hypothalamus. Once secreted, T4 can be converted to T3 or to reverse T3, represented as rT3, which is an inactive isomer of T3.

Circulating T4 and T3 can either be bound to carrier proteins or unbound, also called free. Measurements of serum thyroid hormone levels are typically referred to either as, "Free", which is the unbound only, or, "Total", which consist of the bound as well as the free.

Thyroid hormones regulate basal metabolic rate, as well as exerting control over growth, development, and differentiation of many cells and organ systems, including the testes. Thyroid hormone receptors have been identified on testicular cells and T3 binds directly to receptors on Sertoli cells. Binding of T3 to the

receptors on Sertoli cells activates gene transcription and protein synthesis, as well as Sertoli cell proliferation and differentiation, and is suspected to have a role in initiating spermatogenesis.

While there are contradictory reports as to how thyroid hormone acts on Leydig as well as Sertoli cells in testes, proposed mechanisms suggest a role for T3 in stimulating basal testosterone generation. Despite some inconsistencies among studies, as well as species differences, reviews of thyroid hormones in male reproduction and infertility found indications that short-term hypothyroidism in post-pubertal males can have adverse effects on outcomes of male reproductive toxicity, including sperm motility and semen volume.

NEXT SLIDE

DR. CAMPBELL: With PFNA, there was only one human study which looked at both outcomes of male reproductive toxicity and thyroid levels -- thyroid outcomes.

Serum samples from 857 human males were divided into age groups and the median PFNA levels in their serum increased with increasing age. No significant associations were found between PFNA and testosterone levels for any age group. Among the 12 to 20-year olds, the median PFNA level for that group was associated with a

significant increase in thyroid stimulating hormone.

In rats, in the NTP 28 drinking water study of PFNA, the lowest observed significant effect level for outcomes of male reproductive toxicity was higher than the lowest observed significant effect level for thyroid outcomes and those were both total and free T4 levels.

In zebrafish, PFNA induced disruption of thyroid hormone transport, metabolism, synthesis, and function. The authors suggested that an observed increase in transthyretin transcript in zebrafish could reflect induction of transcription due to competitive binding of PFNA. They proposed that PNFA could induce transthyretin transcription across species, and yet still produce opposite effects on thyroid hormone levels in rats versus zebrafish, in other words, the same mechanism could produce different apical outcomes.

In vitro, across several studies, PFNA bound to transthyretin and inhibited T4 binding. PFNA decreased proliferation of T3-dependent rat pituitary GH3 cells, with -- in the absence of cytotoxicity. An in silico molecular docking model found PFNA fit binding pockets of both TTR and thyroxine-binding globulin.

Overall, these results teased for a possible mechanistic relationship with PFNA -- between PFNA disruption of thyroid hormone function contributing to

observed male reproductive effects. While the available data are consistent with such a relationship, it cannot establish a cause and effect.

And turn back to Dr. Moran.

NEXT SLIDE

DR. MORAN: Thank you, Marlissa.

So now I'll present a summary of the mechanistic data for PFNA. We have the effects of HPG axis included altered hormone levels, such as reduced testosterone levels in male rat and mice and increased serum estradiol in male rat and zebrafish.

We have increased MIS, Mullerian inhibiting substance, messenger levels in rats and primary rat Sertoli cells, and decrease progesterone production in vitro. PFNA also induces changes in gene and protein expression of a number of enzymes and factors involved in steroidenic -- steroidogenesis in mice and zebrafish. PFNA interacts with estrogen receptors in several animals and in vitro models, and with the androgen receptor in vitro.

Affects gene and/or protein expression of some hormone receptors, such as decreased testicular androgen receptor in mice, decreased FSH receptor and LH receptor in rat -- in rat primary Sertoli cells and decreased brain estrogen receptor alpha and beta, and androgen receptor

also gonadal FSH and LF receptor, and increased liver estrogen receptor and estrogen alpha and beta in zebrafish.

PFNA interferes with thyroid hormones binding, serum levels, and function.

Now Dr. Yassaman Niknam will present a summary of the key characteristics -- characteristic of male reproductive toxicants and endocrine-disrupting chemicals for PFNA.

NEXT SLIDE

DR. MORAN: Dr. Niknam.

DR. NIKNAM: Good morning.

NEXT SLIDE

DR. NIKNAM: Recently, a set of key characteristics that are frequently exhibited by exogenous agents that can cause male reproductive toxicity, and another set that are exhibited by endocrine-disrupting chemicals, or EDCs for short, have been identified by scientific experts.

The key characteristics, or KCs for short, can encompass many types of mechanistic endpoints, and are not constrained to previously formulated hypotheses, allowing a broader consideration of multiple mechanistic pathways and hypotheses.

KCs are useful as a tool to identify, organize,

evaluate, and summarize relevant mechanistic data.

Next, please.

NEXT SLIDE

DR. NIKNAM: The eight KCs of male reproductive toxicants are shown on the left-hand side of this slide and the 10 KCs of EDCs are shown on the right-hand side. In the HID, we discussed the available mechanistic information in relation to these two sets of KCs.

Next, please.

NEXT SLIDE

DR. NIKNAM: The KCs shown here in bold are those for which there is applicable information from studies of PFNA and has already been presented by previous speakers.

Next, please.

NEXT SLIDE

DR. NIKNAM: And now here, this table summarizes the animal and human data for PFNA. All of the studies in animals were conducted by the oral route.

In rats or mice, there were reduced epididymal and testis weights. Histopathological changes were seen in the testis and epididymis, including interstitial cell atrophy, spermatid retention, germ cell degeneration, and other changes in rats, and germ cell degeneration in mice.

There were reductions in epididymal sperm counts in rats and mice, as well as reductions in epididymal

sperm motility and viability in mice. Serum levels of testosterone were reduced in rats and mice, and reductions in intratesticular testosterone levels were also seen in mice. Effects on the development of the male reproductive system included delayed preputial separation, reduced intratesticular testosterone, steroidogenic protein, and PCNA levels, which most likely indicate an inhibition of Sertoli cell proliferation in mice following in utero exposure.

In humans, a dose-dependent reduction in sperm concentration was observed in the study with the highest PFNA levels. Decreased serum testosterone levels were associated with higher PFNA concentrations in serum, plasma, or semen in studies of boys, adolescents, and younger men.

This concludes our presentation on PFNA.

Thank you.

CHAIRPERSON LUDERER: Thank you very much for those overviews.

COMMITTEE DISCUSSION

CHAIRPERSON LUDERER: I'd now like to ask the Committee if anyone has any clarifying questions based on the most recent presentations. So again just you can raise your hand either on camera or using the raise hand function.

Let me see. Sorry. I'm having a bit of an issue here scrolling through the...

DR. MARDER: There are no raise hands --

CHAIRPERSON LUDERER: Okay. I can't see any. Yeah. Thank you. I also don't see any, so then we can turn to the Committee discussion of PFNA. And so I'm going to now ask the discussants that were designated for each of the topic areas and we're going to start with the epidemiological studies. So I'd like to ask Dr. Hertz-Picciotto to begin discussing the epidemiological studies of PFNA.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Thank you.

So this is an interesting set of data. I think the summary that was given was actually very -- it was really quite excellent and, you know, I will sort of briefly go through the studies, but just really want to thank the staff for the work that you've done in going through and summarizing all of these data. I will say my overall assessment is I'm a little bit -- I'm underwhelmed by the epidemiologic evidence on PFNA and the -- many of the studies have really null findings. And that's, you know -- that's at, you know, levels that we are seeing in the actual populations. The studies are, as a whole, quite a few of them did adjust for a lot of the factors that one would want to adjust for, not all however.

And the -- so the anogenital distance -- you know, as pointed out, there were sort of opposite effects. Although, the effect that were in the increased anogenital distance actually seemed to be more -- seemed to be just limited to one quartile. And I'm not sure it really -- therefore, it really meant anything. So on balance, I see the evidence being more towards a decrease in anogenital discount, but again just two studies there.

For the sperm studies, and there were quite a few of them, the two that I think really stand out were the Pan study, which looked -- wait. Is it the Pan study? Sorry. Hold on. Let me see if I've got this right. Yeah, so the Pan study saw a number of outcomes that were sort of similarly showing decreased motility and decreased velocity measured in two different ways using curvilinear and straight velocity linear, and it also saw increases in DFI and in HDS. So this seemed to be -- and that was in the serum. In the semen, there was no association excepted for the increase in HDS. But that at least to me seemed to show sort of a bit of a coherent kind of set of results.

And then the other one was the Ma study, which was highlighted in the presentation as well -- and I'm having trouble finding it on my list -- the table here. Let's see. Yes -- which saw also a reduced sperm

concentration and that was in the IVF cohort. So it's a different -- it's a very specific population. But I think it's still valid to be looking at these findings in special populations that may, for one reason or another, be more susceptible. So that -- I thought that that was, you know, a finding that was definitely worth paying attention to.

The other finding that I found very impressive was the DNA methylation, the epigenetic study by Leter, which saw changes, in fact increases, in a couple of the -- like in the LINE-1 and also in the SAT-alpha, and a decrease in the flow cymetric -- flow cytometric assay using -- looking at the digital global methylation assay.

And these were part -- male partners of pregnant women, so the -- it was not actually having -- seemingly not having a clinical effect on fertilization, but nevertheless it -- these were -- seemed like important indicators to take into account.

And then, you know, other studies -- most studies -- most of the other studies looking at morphology really did not see anything. However, the Lewis study saw a greater proportion with normal morphology and a lower proportion with a coiled tail. So that -- there's really not much on morphologic outcomes in terms of sperm morphology.

The last outcome that I think was notable were several studies looking at a PSA, prostate specific antigen. And these studies actually -- one of the studies did find higher concentrations of PFDA actually in prostate cancer versus controls, but there was actually no association that they found with PSA.

Another study which came up with very -- quite null results on PSA seemed really quite flawed in that their adjustment for age was extremely crude. I mean, it was, you know, less than -- 49 and lower and 50 and above. And, you know, they had -- they had a very wide age range, including -- it was a very large study with thousands of men and they certainly could have done a much more precise parameterization of age. And, of course, age would be associated with both PSA and could be associated easily with -- with the -- with PFNA, because of the time trends in different perfluorinated substances, so that you would tend to have a longer period of some of the other PFAs for the older men. So I think it's pretty inconclusive around the PSA.

And I think that concludes the outcomes that were looked at epidemiologically. The hormone levels I think I mentioned, we have a little bit of data. And, you know, it is striking that animal studies seem to -- well, I'm not here to summarize that, but I do think we need to, of

course, look at all of the data as OEHHA has reminded us on previous occasions. But I would say that overall, the epidemiology is not very compelling around male reproductive outcomes, although there are some indications that I have pointed out, particularly related to some of the epigenetics and some of the other sperm characteristics like motility, but not enough replication of those findings to be -- to be -- to say something truly definitive.

CHAIRPERSON LUDERER: Thank you very much, Dr. Hertz-Picciotto. Next, I'd like to ask Dr. Breton to discuss the epidemiological studies as well.

COMMITTEE MEMBER BRETON: Sure. Thank you. I -- I'll start by saying, you know, that I agree with much of what Dr. Hertz-Picciotto said. I would like to reiterate that I think for the -- just in summary that I think that the outcomes of anogenital distance and the cancer related outcomes generally have very few studies and they're very either null or quite inconclusive, in general, so there's really, I think in my mind, no evidence in support of those outcomes at this point in time.

The ones that have more data and more evidence I think are both in the area of the hormones a little bit, and then with the sperm function and motility. So I think that I'll start -- I just had a couple of additional

comments I wanted to make about a few of the studies.

The hormone -- the testosterone data in general I found, you know, it's very mixed from the studies that we have. But one of the ones -- the studies that I sort of gave a little bit more weight to was the study -- the 2020 study by Cui. So it has a reasonable sample size of 650 and it's actually the same study population as the Pan study that looked -- that was like the year before that looked at the sperm, so -- which I'll talk about later too.

And I think -- so this study did show decreased testosterone in serum and also in semen. And so what I liked about these two studies was they were the only studies that looked at levels in the semen in addition to serum, and saw similar results and I thought that that was fairly powerful that you're looking more in the target tissue in humans and you're seeing some effects of the PFNA. So I wanted to mention that.

And then -- but, you know, the other -- the other new study when it comes to hormones though that is conflicting, of course, is the Espinosa study, which was that later study that was added. And this has a really big sample size of more than 2000. It's -- you know, it's a higher exposed population, but it's boys. So it's -- the age range is very different in that -- you know, that

may play a role in the effects that we either do or don't see. So they did not observe any effects on testosterone levels in that population, although they did for PFOA. So, you know, it looked at other PFOS, but -- or specifically PFNA, there was no evidence for an association.

So again, I'm left -- you know, the data on the testosterone is pretty mixed. And by itself, I would have a hard time saying there's real conclusive evidence, but I think in parallel with other evidence in vitro and animal models, you know, that we can discuss down the line, you know, that also paints a bigger picture.

So I think of all the outcomes actually the sperm function and motility studies probably have the greatest level of support from the epi literature. And Irva mentioned the two studies that -- like the Ma study as well as the Pan study, which I think, again, I was -- I really liked the -- liked those two because there's a lot of things that are different about them, but they are showing some consistency and results.

And again, the Pan study, because they had both semen and serum levels that showed consistency and a strong sample size, I thought that was good. The caveat there was that it's a mixture of men who are infertile and not -- fertile and infertile, and so I thought, you

know -- you know, sort of it's a mixed bag, but with the Ma study it's an IVF population and it's -- they were all couples who were coming to the clinic, because there was female infertility, so not male infertility by my read of the paper. And so that I thought was a good separate population of fertile men and you're seeing the same result even though the sample size was a lot smaller. So those were the two I think I wanted to point out with regard to sperm function.

And I guess my summary is that of all -- sort of all the epi literature to date, we probably have some evidence for reduction in sperm quality taken as a whole, particularly when you look at the parallels in semen and plasma or serums, and that they -- but it's really a mixed bag on testosterone and the other outcomes. There really have been very few studies and most of them have either been null or mixed results.

And I think that's about all I have.

CHAIRPERSON LUDERER: Thank you very much, Dr. Breton. Now, we're going to move on to the animal studies, so I'd like to ask Dr. Auyeung-Kim to discuss the animal studies.

COMMITTEE MEMBER AUYEUNG-KIM: Hi, everybody.

Yeah, I wanted to echo the sentiments of others that the OEHHA staff really did a really good job of

summarizing the animal study data. I think it was very helpful to look at the data in a chart-like manner, rather than just discuss it. It definitely -- it was good to breakdown the effects that were seen in the papers.

So the NTP studies -- there was an NTP study where male rats were treated with -- treated with PFNA for 28 days. And so a full sperm cycle in a rat is generally about, you know, 50 -- it's about 56 days. And so a full histologic -- histopathology was conducted in these studies, but it was associated at the higher doses in the study with deaths and at the higher doses, meaning five and 10 mg per kg, as well as some liver and immunotoxicology effects.

So essentially because the testes is presumed to be a target tissue for PD -- PFDA, it was evaluated further in this -- in this -- it was evaluated as a target tissue for this -- in this study.

And so, you know, as mentioned in the -- as mentioned in the summary previously, you did see some decreases in testes and epididymal weight, about 1.25, and lower testosterone levels compared to controls, and then some effects on the -- histologically on the epididymis and testes, so -- and so this -- in this study, I guess one of the issues I had was that it was -- we only eval -- it was only evaluated -- the histo time points were

evaluated only one time point, and that was not a full sperm cycle. And so, you know, it's likely that with continued dosing that it would -- we would continue to see the same effects. And whether there would be recovery if there was no dosing, that was not able to be determined in this study.

Another study Feng et al. looked at the ultrastructural effects of PFNA on male reproductive organs and found that there was altered structure of the Sertoli cells. And this study, the animals were dosed for only 14 days at 1, 3, and 5 mg per kg. And so ultrastructurally there were -- there were impacts on the Sertoli cells and sperm, and essentially, you know, demonstrated that PFNA treatment for these 14 days can lead to the damage of specific secretory functions of the Sertoli cells, and -- let's see, then two additional rat studies were investigated on the hormones. And this is in the Hadrup paper as well as Feng. And it essentially showed that the plasma concentrations of testosterone were -- was -- were decreased.

Let's see. And then in the -- and then we move on to like the mice studies where essentially in these studies, we looked at -- or Das looked at PFNA in pregnant mice, and so you're -- the study looked at the development effects of the male pups. And so it's not a direct male

reproductive tox -- not of the direct effect on male reproductive toxicity.

And then the other mice studies conducted by the Singh lab treated prepubertal mice, and so they looked at the spermatogenesis, steroidogenesis, and fertility, and the effect of gestational exposure in the development of neonatal mice testes, and essentially did see some effects at the higher dose levels.

In one of the studies, male fertility was looked at and where unexposed females were mated with exposed males. And at the 0.5 mg per kg dose only five of the seven unexposed females were impregnated, whereas at the lower dose levels and the control, all the females became pregnant. And so whether this is part of variability is unknown.

And then there was also the zebrafish where essentially the zebrafish were exposed to different levels of PFNA for 180 days and led to a decrease in male GSI index, and increases in both -- and unlike in the rodent studies, it's all increases in testosterone levels in those studies.

So while the studies on their own do indicate that PF -- PFNA can be a male reproductive toxicant and impairs spermatogenic process, I think we need to look at the studies carefully. Each study has some deficiencies

that make it difficult to indicate that it's a clear reproductive toxicant, based on what information was provided.

The main issue I have is that the reproductive effects are observed at extremely high doses that are essentially thousands fold higher than what is seen in California residents. I think it was Dr. Pessah that asked earlier about the serum concentrations. And so both the NTP studies and the Hadrup paper both looked at serum concentrations.

So in the NTP studies toxicity was observed at 1.25 mg per kg per day, which had a plasma concentration of approximately 161,000 nanograms per ml. And the Hadrup study, the plasma concentration in rats administered the lowest dose, which was 0.0125 mg per kg per day was 396 nanograms per milliliter. And so this is in the range of the same exposure when taken into effect what the dose is.

And then in the HID that was sent to us, the Cali -- the range of PFNA that is observed in California residents ranged from 0.20 -- 0.205 in the California -- nanograms per milliliter in the California Regional Exposure Study, and that was published in 2019, and then -- and then the high end was 1.15 nanograms per ml in the Firefighter Occupational Exposures Project, and that was a 2010-ish study.

Oh. So I see -- okay. I see Dr. Woodruff's comments on not supposed to make --

COMMITTEE MEMBER WOODRUFF: Yeah, I'll just say it. I just thought -- maybe Carol can weigh in. I thought we weren't supposed to consider dose exposure levels when we're making our evaluation of the hazard, is that right, Carol, can you clarify?

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah. Generally, that's true. You shouldn't consider the current dose that a person might be receiving, but I think you can certainly look at the doses in the studies.

COMMITTEE MEMBER WOODRUFF: Right. Right.

COMMITTEE MEMBER AUYEUNG-KIM: So based on my calculations, we're looking at doses that are approximately 150,000-fold higher than what may be observed in humans in California.

Additionally, I think that there is a very limited number of studies that did not have an overlap in the male reproductive evaluation. You had the NTP studies that was a repeat dose that looked at histologically and sperm parameters, but then -- and then in the remaining studies were mice studies that looked at more development exposure in younger mice.

And then the reproductive performance details are limited in the one study in -- conducted in the mice and

the zebrafish studies, both males and females were treated. And so -- and then lastly, the doses that affects were seen were also associated with other significant toxicity, such as decreased food consumption, body weight also, and overt liver toxicity or immunotoxicology in the animal models that were tested.

As such, I do not believe that the animal studies reviewed clearly indicates that PFNA is a male reproductive toxicant. I think that you know there are some further evaluation -- well, a further evaluation would be helpful to see -- to determine whether it's a reproductive toxicant -- male reproductive toxicant.

CHAIRPERSON LUDERER: Thank you very much, Dr. Auyeung-Kim.

Dr. Woodruff, would you like to discuss the animal studies next?

COMMITTEE MEMBER WOODRUFF: Yes. Sure. Thank you very much. And thank you for that summary and thank you to the staff for the summary. I want to start off by saying that I looked at the animal studies and I -- before I give my comments, I want to note that in terms of the sensitivity of animal studies in order to see an observational health effect, I'm talking about an apical endpoint, and I -- that there's been a lot of good literature, including reviews by the National Academy of

Sciences that find that while we have good concordance between animal and human studies humans are often much more sensitive in terms of their response -- their dose response than animal studies.

And there's a lot of good reasons why animals are not a very sensitive model for human health effects, including the -- some of it has to do with the exposures that occurred. Do they -- do the exposures during sensitive time periods of development. Also, Dr. Hertz-Picciotto raised the issue about cumulative exposures. Animals are individually dosed and it doesn't really -- it does not reflect the fact that there are multiple exposures occurring, so you would not expect a response at a similar level in humans.

And then I also want to comment on the statistical approaches that these studies often use. They typically compare individual doses to the control and don't look at the dose response. So while it's true that you may see more quote statistically significant responses at higher levels, there's often a dose response curve. And I'm going to talk about -- and those -- and so not all the data are modeled in this. And as you can observe trends in the data, it may not be -- but that is not necessarily statistically analyzed in studies, though that would be observed if they had done that. But it does

limit the statistical power if they only are looking one dose at a time.

And let me see. All the studies that -- as we noted, there are six animal studies. The OEHHA staff reviewed all the studies. Also, I want to note that there also can be strain and species differences in sensitivity. So some species are more responsive to exposures. Mice tend to be more sensitive to exposures than rats for example. In the studies, animals studies of PFNA, we saw exposures on rats, mice, and one in zebrafish. And they tended to evaluate more apical outcomes, so as was noted by the presentation, testicular weight, prepubertal separation, epididymal weight, sperm parameters, heads, viability, number, and testosterone.

But I really was -- I thought we had a lot of good information on exposures to PFNA in animals and responses in the biological mechanisms that are related to either male reproductive health or development male reproductive health effects. So I thought that made the information much more powerful.

I want to note that while there's six mammalian studies, four of them were published by the same authors, Singh and Singh, and they were all published in 2019.

I also went through the studies individually and evaluated the study quality, because -- and this is where

I feel it would be helpful from the OEHHA staff to look at what's been going on at EPA in terms of giving us better information about the parameters -- the methodological parameters that can contribute to internal validity in the studies.

And so I want to note that pretty much the studies -- so the NTP study, the Das study, the Singh Laboratory studies, and the -- looking down here -- the Zhang study, which is in zebrafish, and the Feng study which is a male -- I'm going to leave that one out, because they didn't report about whether they randomized or not. But the NTP, the Das, the Singh studies, and the Zhang studies all reported purity of the substance. They had high purity of the substance for exposure. They all randomized their study, the -- in the experimental design. It was not reported whether they did blinding of exposures and outcomes, so that makes the study a little more difficult to interpret. They did have complete outcome data and selective reporting and they didn't identify any other biases in the studies.

I want to note that the -- I looked at the studies a little bit differently, because there were two of the studies that were focused on prenatal exposures. And I think this is really important when we're thinking about comparing it to the human epidemiological evidence,

because we would expect that the prenatal period will be a more sensitive period for exposures to any particular chemical, and we can look at PFNA in this case, because it could affect male reproductive development. And as you see, there's been a lot of studies that have evaluated the relationship between exposure and different biological mechanisms that can contribute to male reproductive health effects.

So the prenatal exposure studies were the Das 2015 study and the Singh 2019 study. I think what was nice about these two studies was that we saw -- actually, they're both in mice, one in CD-1 mice and the other in male Parkes mice. And they looked at different aspects of male reproductive development. Their gavage doses were in the similar range, as was actually all the studies that were conducted in animals studies for PFNA.

And they -- the Das study evaluated the prepubertal separation in males, and the Singh study looked at testes testosterone, and testes weight. And I know that there was a question earlier about testes testosterone. And I wanted to just say that that has been evaluated as a relevant male reproductive health effect. And I would refer that question to the National Academy of Sciences report on their systematic review of prenatal phthalate exposure in male reproductive health effects,

which also looked at testosterone in fetal testes.

So I want to note that in the Das study that there was no statistically significant effects in maternal body weight indicating that there's no effect of treatment on the mother, but they did see a difference in a dose-dependent manner on prepubertal separation. What I really liked about this study is that modeled the dose response and they calculated a benchmark dose at the five percent response level. And the benchmark dose at the lower end was 1.3 milligrams per kilogram day.

Now, I just want to note that this is a good example of where all the data were used to analyze the dose response and that they saw a response that's pretty much at the -- kind of -- I would say looking at across these dose response more at the lower end of the dose response across all the different animal studies.

I did also want to note that when you do the benchmark dose analysis, that allows -- because you're using all the data in the dose response, it allows a more sensitive evaluation of the statistical -- of the response level in animals. And so if you just analyze one dose at a time, it would be hard to see a five percent response, but because they're using all the data, they're able to see this five percent response, which is at a relatively -- which is at the bottom end of the dose

response range. And if you look at the graph in the paper itself, you can see that there's a dose response in the preputial separation, but that if you just compared one dose at a time, you would not see this five percent response. So I feel like this makes the data very compelling. And I just noted already what the methodological features on this study are.

And then -- so the other study that looked at prenatal exposures and response is the Singh study. And they also looked at -- let's me see, I'm just -- sorry. I have of files open here. And the Singh study looked at testes testosterone and testes weight. They evaluate it during gestational exposures and then looked at postnatal day three. And they also saw effects on testosterone and a trend in declining testes weight. And I think in this one, there was -- let me look at this one. Yeah, so it's -- that's -- so I did -- those are the two prenatal exposure studies.

And then the rest of the studies, as Dr. Auyeung-Kim noted were down in adulthood, though not during the full cycling of sperm, and the NTP study, the Singh study 2019, and -- sorry, I lost all my pages. And then the Zhang study, which is the -- in zebrafish.

And I want to just briefly mention the sperm quality, because this also is in relationship to the

epidemiology studies. So the NTP study, the Singh 2018, looked at sperm motility, viability, and number. On the NTP study they looked at epididymal sperm count. And I want to note that in the same 2019 study, that the body weight -- there was no effects on body weight of the animals at the different exposure levels, but there were effects on sperm, fertility, and proteins linked to sperm production. So I thought that was important to note.

There also was a dose response relationship between the exposures and the proteins and enzymes involved in testosterone biosynthesis including the StAR protein, and the SF1. And that histological, which I'm not an expert in histology, but that was presented by the study staff.

I just want to talk a little bit about this NTP study, because the other endpoint that was evaluated in the animal studies, that was more easy to -- easily compared across the studies was testicular effects, so testes weight, relative testes weight, and the gonadosomatic index, which is the gonad mass as a proportion of total body mass index. And I thought this was very important, because as Dr. Kim mentioned, that there can be some changes in body weight across the exposure groups, so it's important to look at the -- so what I did was look at the testes or the epididymal

weights as a proportion of the body weight.

So in the NTP study, there was, just looking at this here, a change across the body weights, but when you look at the -- and to be fair, I didn't have all the data for this, but just roughly looking at testes to body weight proportion, you see kind of a dose response decline, which you did also see for the ratio of the epididymal weight to the body weight. So I thought that was an important indicator. And finally, this effect was also observed in the zebrafish.

So I'm not going to -- I want to also mention that -- many of these studies also looked at -- I think someone else is going to talk about this -- exposures as it relates to the various biological mechanisms. And so I thought that the -- I think that one reason that it's -- to me, the study was -- data was very compelling if you consider the fact that the studies are reasonably high quality and that if you look at the -- when they evaluate the dose response, that you do see these dose response effects and the fact that you see a relationship to these upstream biological indicators, including the proteins and enzymes that are part of testosterone synthesis.

And I just want to comment briefly on the epidemiological evidence, because it was not as robust as we would in general maybe look for, if we were in the area

of -- in certain areas. But I just also want to say that -- reemphasize the point about the sensitivity of the epidemiological evidence to observe these types of effects. The exposures are less and they can be less sensitive due to that. And so you would need a lot of people to see an effect. And so it's sometimes really good to have heterogeneity in findings because more -- it has studies that are more sensitive and able to pick up effects that we wouldn't see across the general population.

And so that could include also study populations that are having infertility issues, because they may be more sensitive to effects of -- or more responsive to effects of these chemicals like PFNA. And then also developmental exposures, and just wanted to comment on the -- on the -- when we say null findings, I think what sometimes we're -- we need to distinguish between what's a statistically significant -- what we have marked as a statistically significant association and an effect level where the confidence bound includes one.

And I did note in these epidemiology studies on the -- like this Lopez-Espinosa study that there was a relationship with some of these markers like -- well, testosterone and IGF, sometimes the confidence limit crossed the zero mark, but I think along with the animal

study, that gives us a good constellation of evidence for PFNA.

So I'm done.

CHAIRPERSON LUDERER: Thank you very much, Dr. Woodruff.

I'm going to now turn to the mechanistic studies and ask Dr. Allard to begin the discussion of those studies.

Dr. Allard.

COMMITTEE MEMBER ALLARD: Yeah. Thank you very much. Can you hear me well?

CHAIRPERSON LUDERER: Yes.

COMMITTEE MEMBER ALLARD: Yeah. Okay. Good.

Yeah. So a host of mechanisms were mentioned in the hazard identification document. Personally, I would not actually call them mechanisms as much as molecular endpoints. And so what I really to do is sort of weave the animal studies -- the outcome from the animal studies and some of the sort of molecular information that we have, and really try to get at biological plausibility. So I did take into account doses for some of the stuff that I will mention. But just in the sense of trying to see what is realistic to expect in terms of mechanistic effect and how that could indeed be happening in our animal studies.

So, you know, looking at the levels mentioned that I found in human serum, for example, we're talking about levels within the nanomolar range. So again, this is not necessarily a decision point, because we are on the hazard identification side of things, but this is more as a way to sort of guide the molecular analysis that I performed.

So my reading of the -- my starting point was really reading through the animal studies and the human studies of course. And what sort of emerged, again from my perspective, was really an effect on testosterone production. There's some conflicting data, but the -- in my mind, the weight of evidence led was in the direction of a decrease in testosterone production. And this was supported by the NTP 2019 and the Singh and Singh 2019 A and B.

And again, you know, the human studies, there was a little bit more inconsistency, but I thought that overall the data there was perhaps more compelling. And this is supported at the molecular level by several molecular outcomes that were mentioned. So there's a decrease in production of StAR, of TSPO, of CYP11A, of CYP17 and 17-beta hydroxysteroid dehydrogenase, HSD, that are well known to be involved in the steroid -- I'm going to stumble on that word -- in the production of steroids

and steroids hormones.

So there's an alignment of, you know, the decrease in outcome with the enzymes that are necessary for the production, including -- so what's interesting about StAR, for example, is that it's very early in the steroidogenesis pathway. And so, you know, you would expect a pretty -- really profound effect on steroid hormone production.

So now -- this is not necessarily getting to a molecular initiating event. I think that was the part that was a little bit frustrating about reviewing actually both chemicals is that we don't really know biochemically how PFNA could be causing a lot of these things. And perhaps this is, you know, often happening in toxicological studies.

So for this I actually turned to, as I usually do in those meetings, I turn to the ToxCast data and really try to parse out the plethora of different sort of molecular tests and assays that would run PFNA, which ones really sort of seem to rise to the top and could be explaining these -- these molecular events, such as decrease in testosterone production.

And really the one that's happening at the lowest level that was tested in the nanomolar range is an effect on the farnesoid-x-receptor, FXR, which is known to

respond to bile acids, but there's an increased connection between bile acids and steroid hormone production. And this was beautifully reviewed in a paper published Garcia et al. in 2019. And through molecular analysis with knockouts, we actually know that actually both elevated levels of FXR, but in the K -- in the knockout, you know, elevation of FXR, we know that this is a dramatic impact on sperm production and on sperm quality as well.

So it seems that PFNA acts as an antagonist of FXR, so it would align with what we know from the knockout, and that it's AC50 is actually relatively lower within the human range of 41 nanomolar. So I thought that this was to me sort of encouraging in terms of potential molecular initiating events.

And it's also important to note that, as was noted in the hazard identification document, that PFNA also seemed to bind the estrogen receptor, at least alpha, although at higher level than it binds the FXR receptor, but still within the nanomolar range. So we're in the high nanomolar. We are about 700 nanomolar for AC50 for the estrogen receptor alpha. But, you know, this is something that is a lot more different than any other receptors that PFNA seem to bind at a much higher level than that. So, for me, these things are potential of course molecular initiating events, but at least there's

biological plausibility behind the effects mentioned and detected in several studies on testosterone.

The thyroid hormone mechanism was to me less convincing, and honestly after reading through it I decided to not necessarily investigate that that much further, because again, I did not think that this was very convincing.

But I do want to sort of wrap up my rather brief comments by saying that I did find the tables for 441 and 442, so the characteristics tables, on male reproductive toxicants and on endocrine disruption to be extremely helpful, in sort of, you know, having a good platform to then decide are we really talking about an endocrine disruptor, are we really talking about a male reproductive toxicant? And I think the -- it doesn't really necessarily resolve all the ambiguity, because you don't have to necessarily hit every single one of the key characteristics to be a reproductive toxicant, but what does it mean if you hit only one out of all the many ones that were mentioned, or two, or three, or four have -- you know, where do you land at the end, at least in your mind, in what is a reproductive toxicant or an endocrine disruptor.

Nonetheless, using this table and looking at the molecular endpoints that support some of the -- these key

characteristics, I ended up leaning towards PFNA, indeed having the biological mechanisms and the biological plausibility, to indeed be a male reproductive toxicant.

And I'll end here for my comments.

CHAIRPERSON LUDERER: Thank you very much, Dr. Allard.

And we'll then turn to Dr. Pessah to continue discussing the mechanistic studies. Dr. Pessah.

COMMITTEE MEMBER PESSAH: Thank you. I took a rather different approach. I thought about biological plausibility with regard to the face validity of in vitro and in vivo studies in particular the animal studies. And I appreciate Dr. Breton's comment that given the variability and oftentimes contradictory results from the epidemiological studies that the animal studies really do need to have -- if you are to interpret them as either supportive or not supportive of plausibility, that they need to have face validity and productive -- predictive value.

I just want to point out that if you do a calculation on the highest blood levels in the studies that have been done, serum levels, and I always think of concentrations, if you want to look at mechanisms, as molar concentrations. So I converted the nanograms per liter, the geometric means in the firefighters to a molar

concentration, and they are relatively low, about 1.5 to 2 femtomolar, that's 10 to the minus 12.

Now, if we go to the NTP study, where, in fact, I thank Dr. Li for pointing out the table, Table 20, they actually do a good job of converting the milligrams per kilogram per day dosing to a molar concentration in plasma. And if you'll see at two and a half mg per kg per day, you're saturating the pharmacokinetics of PFNA. In other words, you're up around 800 micromolar plasma levels at two and half mg per kg per day, and you double that dose and you're pretty much saturated.

You have to realize that many of the results that have been presented here from animal studies, whether they be the sperm or blood outcomes require doses certainly of two and a half or above mg per kg per day. There is a relationship that the longer the exposure is, there's slight shift to the lower concentrations for the LOEL -- or the minimal -- no sorry, NOEL.

So what does this mean? It means that all the animal and most of the in vitro studies, which were done in mid-micromolar to high micromolar in many cases up as high as 300 to 500 micromolar are orders of magnitude, six orders of magnitude higher than those observed in the highest exposed population.

What does that mean mechanistically? Many of the

targets that have been looked at, and Dr. Allard really did a great job at pinpointing two endpoints, two targets that seem to be influenced by nanomolar PNF[SIC] -- PNFA[SIC], and those may be sort of the benchmark sort of points of departure where you can say, well, here we're getting very close -- or at least closer, but you still have to realize we're at least three orders to four orders of magnitude above, higher, than what you find in the highest exposed population.

What does that mean in terms of receptor occupancy? Well, for the FXR and the ER receptors, these receptors, their ligands are in the subnanomolar. And so if you're talking about 10 nanomolar PNFA[SIC], which is again two to four orders of magnitude higher than what you find, there is no possibility that they can interact with those targets, given the difference in their affinity, even over long periods of time as these receptors are signaling with their endogenous molecule.

So I found that the animal studies and the biochemical studies at the very start lack face validity, because there's no uncertainty factor that you can put in that would give you six log units. So with that, I also looked at the quality of some of these studies, in particular Singh and Singh, and I found that there was no control for bias, in other words, there was no blinding

for the histological evaluations. The histological evaluations were not quantitative, rather qualitative, although they did have some strengths. And those strengths were pointed out in terms of sampling and sample design.

I also want to point out that many of the outcomes in Singh and Singh and, for example, Feng et al., they did not actually show dose response relationships. They either showed a threshold effect, where the lowest concentration had a maximal effect and the higher concentrations maintained that effect, so they didn't have a no effect. And if they did, it was also reduced. It wasn't zero. And in some of the outcomes, it was non-monotonic. In other words, the low concentrations produced a statistically significant effect, but as you escalated the dose, it actually disappeared.

And I sort of wonder why that might be. There was no real rationale or explanation for that. And the zebrafish tend to be more sensitive. But when you're working with such high concentrations, if you think about zebrafish in a pool of water, there's only one place that those aqueous doses, if in fact they are soluble, because there's some uncertainty whether the solubility in water can even be attained at the concentrations used, that they would accumulate in the zebrafish over the 90 days. So it

would have been really reassuring if they had measured the internal doses in the zebrafish and gave a bioaccumulation factor for their experiments.

So with that, I'm going to stop. Thank you.

CHAIRPERSON LUDERER: Thank you, Dr. Pessah.

We now have time for a little bit of Committee discussion. I know that we're scheduled to have a lunch break at 12:00, so I wanted to ask staff if -- can we go a little bit longer with the discussion now or do we want to hold it to five minutes and then break for lunch?

DIRECTOR ZEISE: No, you can break for lunch when you find a good breaking point. That's okay.

CHAIRPERSON LUDERER: Okay. Wonderful. Thank you.

So then I'm going to open up the -- to full Committee discussion. So please let -- again use the raise -- hand raising option to indicate that you wish to speak and see if any other Committee members have comments about these studies, epidemiological, animal, or mechanistic studies.

Let's see, Dr. Breton.

COMMITTEE MEMBER BRETON: Hi. I just had a question actually about what you sort of just alluded to, which was that there might -- that there wasn't dose response associations shown in some of the animal models,

but I -- I guess I had been given the impression that from the summary slide and also from Dr. Woodruff that there were evidence -- there was evidence of dose response in some of the animal studies. So I was just wondering if we might discuss that a little bit more.

COMMITTEE MEMBER PESSAH: Sure. I must have missed something, but I did go to Singh and Singh and Feng et al., and if you look at some of those measures, they really aren't dose response. They just -- you have an effect at the lowest level and those effects are maintained as you escalate the dose. And then in some measures, they disappear at the higher levels. And so they don't really account for that very unexpected concentration effect or dose effect relationship.

I know that some persistent organic pollutants do show a non-monotonic, but they always show a no effect level that's more of U shaped dose response. Here, I don't think that some of these measures had a U shaped dose response.

COMMITTEE MEMBER WOODRUFF: Yeah, I just want to comment on this. I made this comment about Das study. So the Das study -- and again, it's a little bit influenced -- its's somewhat influenced about how they analyze the data, right. In an epidemiological study, they take all the data and then they create a beta

estimate with all the data. That's not how a lot of these animal studies are evaluated. They look at each dose, and then they compare it to the control. So it's a little bit like a quartile -- you know, a quartile analysis or something like that.

So the Das study is a good example, where you saw this prenatal exposure, and then -- and the prepubertal separation that you can see in the graph is increasing. And when they analyzed all the data together, they did a benchmark dose model. And what that is is they analyzed the data. They create a dose response, and then they look at the exposure level that relates to a certain percent in decrement. So, for example, in this case, it would have been a five percent decrement. In the -- just the length -- the time to separation. So what that means is that often when you're looking at the animal studies, there's a lot of reasons that they appear let -- they may not -- it could be that there's less sensitive measures of the relationship, and therefore you don't see a response.

So I just want to address this NOEL issue, because this has come up a lot in the toxicological literature. That response -- that means the no observed effect level. And we've done an analysis of the data -- the dose response data from animal studies and comparing it to the no observed effect level. The no observed

effect level is highly driven by methodological design, because they basically take the control and then each of the exposures, and then they say, oh, well, this one is not statistically significant, but there could still be an elevated effect. That must be a no observed effect level.

That does not mean there is not an effect at that exposure. In an analysis, we did a benchmark dose that EPA used for reference dose has found that NOAEL, which is typically in the range of one to 10 percent response, and it's because it's very difficult, given that there's not very many animals per dose, to see responses in that lower end of the dose response range.

So I think we have to analyze -- this is why I feel like it would be super helpful if OEHHA would take the data from the animal studies and put it into HAWC, so we could look at the dose response levels together. It's quite -- it's quite standardized now within that process. And I think that would help us see -- like you can in these epi studies, like this Lopez -- this Lopez study, you can see quite well what's going on at the different dose levels and the responses. So that's what I want to say about that.

CHAIRPERSON LUDERER: Do we have any other comments from other panel members?

COMMITTEE MEMBER PESSAH: But, Dr. Woodruff, I

think you mentioned a benchmark dose of 1.3 mg per kg per day as one outcome.

COMMITTEE MEMBER WOODRUFF: Yeah, it's in this -- yeah, that's right.

COMMITTEE MEMBER PESSAH: And that would correspond to about a 350 micromolar plasma level.

COMMITTEE MEMBER WOODRUFF: Okay. But we're not suppose to consider what the exposures are in the human -- in the California population when deciding the hazard, right?

COMMITTEE MEMBER PESSAH: No. No. I understand that, but I think 350 micromolar to observe a benchmark dose in an animal study is unrealistic and probably does not have face validity, which weakens its usefulness for prediction.

COMMITTEE MEMBER WOODRUFF: I guess I don't quite -- face validity. I mean, I just want to just also -- I mean, the levels at which we're seeing reference doses now being set for these perfluorinated chemicals are quite low, so I know OEHHA said for PFOA set an acceptable daily dose of -- what am I -- I did this, because I was interested in this -- it was like 0.45 nanograms per kilogram day.

You know what, EPA just came out with a reference dose for PFOA based on a systematic review of the PFOA

evidence. That's 1.5 times 10 to the minus 9 milligrams per kilogram day. I mean, that's -- you know, basically, they're saying that there is levels, very, very low, at which we're seeing potential health effects from these exposures. So I guess, I'm not -- and that's based on animal studies and human studies that are at higher levels. Also, the human studies are at relevant exposure levels. So I think we have to look at the human data in conjunction with the animal data, in conjunction with the mechanistic data. So that's what I feel is important.

CHAIRPERSON LUDERER: And I think another thing that I wanted to again add too, which I think several of the panelists brought up, was that the -- there -- the dosing intervals were relatively short in these studies, but there did appear to potentially -- that it looked as though with longer dosing, there -- that there was a tendency maybe for more -- greater effect. Obviously, that was with different studies.

COMMITTEE MEMBER WOODRUFF: Right. Right.

CHAIRPERSON LUDERER: And obviously in humans, I mean these were very persistent chemical -- this is within the body. It has a long half-life and measured in, I think it was, years we heard. So that, you know, we see bioaccumulation with these chemicals. So I think that's another thing to keep in mind. Do we have any other

comments, or questions, or is maybe everyone --

DR. SANDY: I see two hands up, Dr. Breton and Dr. Allard.

CHAIRPERSON LUDERER: Yes. Okay. Thank you. I had to circle back through the other page.

Dr. Allard.

COMMITTEE MEMBER ALLARD: Yeah. I just redid my calculations just to be extra clear. And we're spending a lot of time talking about dose when perhaps we shouldn't be. But I did sort of land on the human serum levels corresponding to the nanomolar range, which to me is interesting to consider, thinking that the in vitro endpoints that were measured are also performed at the nanomolar range.

So again going back to the question of biological plausibility and thinking about there could be.... But I did hear what Dr. Pessah said in terms of the endogenous ligands being able to bind at much lower levels. So that's something to consider, but at least we are talking about something where the human doses and some of the in-between points were generated using -- there's overlap. That's the bottom line.

COMMITTEE MEMBER WOODRUFF: Can I ask you a question about that. If you have multiple chemicals, though, in this low range all computing say, in this case,

for the receptor, wouldn't that then mean that you would have the effect of PFNA could be happening, you know, at -- even at the low level, but because there's other chemicals that we're all exposed to, that could influence the sensitivity?

COMMITTEE MEMBER ALLARD: I think it's possible, but we -- right, there's no competitive assays that were performed, and so we are -- we're sort of getting further and further away from -- you know, it's basically --

COMMITTEE MEMBER WOODRUFF: No, not here, but in other examples for another chemical and another receptor finding.

COMMITTEE MEMBER PESSAH: For PCBs, it's been demonstrated --

COMMITTEE MEMBER WOODRUFF: Right.

COMMITTEE MEMBER PESSAH: -- that there's additivity and sometimes more than additivity. So, yes, but that -- I don't think that was in our data set, you know, to consider others. And that goes back to Irva's point that exposures are more complex than just PNFA -- PFNA, so...

CHAIRPERSON LUDERER: Dr. Breton.

COMMITTEE MEMBER BRETON: I just wanted to say one other thing that Trace -- that Dr. Woodruff brought up, which was that I forgot to mention when I was

summarizing the epi studies, which is that -- especially I think for the testosterone results, several of the studies that showed no association did, in fact, have non-significant trends in that direction. So coupled with the fact that generally the sample sizes are low and the PFAS levels -- or the PFNA levels are often on the lower end, or don't have a great dynamic range that we probably are butting up against statistical power issues for some of the studies.

I didn't do a post-hoc power analysis for any of then, so I can't tell you, you know, with certainty, but I would say -- I would be willing to bet that at least for some of them, that's also -- you know, it's unfortunate in that it's -- but it's playing a role in some of the studies that showed effects. Some of the -- I think some of the studies that were from China and Taiwan tended to have some of the higher PFNA levels too. So I think that's sort also sort of supporting this notion of this confluence of, you know, how high are the levels, what's the dynamic range of the levels, and what's your sample size.

And so -- so some of the non-significant trends, I do think lend some support. Obviously, it partly depends on where you sit on your interpretation of statistical significance a little bit, but I just wanted

to mention that.

CHAIRPERSON LUDERER: Thank you.

Dr. Hertz-Picciotto.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Yeah. Again, I'm -- I think that the context of these exposures is really important. And the -- you know, I started out thinking, oh, the mechanistic data was really strong, and that that makes up for kind of the -- you know, particularly some of the in vitro studies in particular seemed very overwhelmingly -- you know, there's something going on here. And, of course, I was thinking well, I don't know how these doses are. And Dr. Pessah really did a great job of pointing out that they are really at astronomically high levels in some cases.

And then, you know, the animal studies, yeah, I feel like we -- when you put it all together, there's this feeling that there is something there. The epi has not been the strong enough studies, or the large enough studies, or the sensitive enough populations to necessarily pick it all up. But if we're only talking not about risk assessment here, but about hazard identification, does -- do -- does PFNA pose a hazard? And that means that at some level, it could be causing male reproductive toxicity.

And so I feel like that's the question to ask.

And I also want to just point out that the issue of interactions -- okay. I understand that the way it -- when the Proposition was -- of 65 was passed, it was all about one-by-one chemicals, but we are -- we are in a phase in -- I mean, and even OEHHA has a lot of effort now on cumulative risk. And I think that we -- there are many situations where at fairly low exposure that itself -- all by itself would be unlikely to cause the outcomes that you're interested, in combination with other similar kinds of chemicals, or chemicals that may act on a different juncture in some sort of a progression of steps that leads to the final clinically observable outcome, can often be -- the effect can be amplified many fold by the context in which these exposures may occur.

And I'm saying that not just -- I mean, I started out earlier in the day talking about, well, other PFAs, but I'm thinking about other repro -- male reproductive toxins that are out in the environment as well that may operate through similar mechanisms, through other junctures on the same mechanistic pathway and so forth.

And that -- that this -- this situation that we live in, where the number of chemicals is in, you know, the tens of thousands on a daily basis, and that some of those are now reproductive toxins that maybe have shown more compelling data, I find it hard not to -- not to take

that mindset as we evaluate PFNA as an individual added compound in this mix.

It does not seem to be benign. That's -- that seems to me to be clear, but the definitiveness and how strong an impact in real-life situations is what does seem to be at question. So I think that's -- I think that's still fair enough and within our role here as members of this panel to put that in -- that context in place. And if I'm totally wrong and it is off-base for hazard identification, okay.

CHIEF COUNSEL MONAHAN CUMMINGS: Can I just really quick --

CHAIRPERSON LUDERER: Yes, Carol Monahan Cummings.

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah. I just wanted to say that Prop 65 is unique and perhaps odd in the way that it addresses these kinds of exposures, because it really does look like -- look at a single exposure to a single chemical and doesn't look at cumulative effects. And so I totally understand the scientific context you're talking about, but I don't think that you can consider that directly and say, you know, since everybody is exposed to lots of these chemicals, adding this one in could cause an effect. So I think you really need to look at this individual chemical and its

exposures and effects to the extent you can, based on the evidence you have.

Hopefully, that's helpful

CHAIRPERSON LUDERER: Any other comments?

I think that given that the dose -- that there are clear evidence of dose response for some of these endpoints, both, you know, testosterone and some of the other male reproductive endpoints, as Dr. Woodruff and others have noted, that -- and that we are to focus on evidence of effects on reproductive -- male reproductive endpoints, not necessarily taking into consideration the human concentrations, I think based on those data, we can say that there's clear evidence of male reproductive toxicity, and from multiple species, and in multiple studies. So I'm just curious what other -- if other panel members have different thoughts on that who haven't had a chance to talk yet?

Okay. Dr. Breton, did you want to say something else or is your hand up --

COMMITTEE MEMBER BRETON: Oh, I did actually. I was just going to sort of say that Dr. Allard had also mention when he was summarizing how their -- you know, these key characteristics and was sort of questioning how many of them one needs to actually check off to sort of have sufficient evidence. And I just wanted to comment

that like sort of my own interpretation when I was going through all of this was that -- and from a snippet from the epi literature was -- like I was under the -- under the impression that basically as long as you hit one, you know, you had sufficient evidence. So I was kind -- I kind of wanted to raise, because I was curious what other people thought --

COMMITTEE MEMBER WOODRUFF: Well, but --

COMMITTEE MEMBER BRETON: -- about that too, you know.

COMMITTEE MEMBER WOODRUFF: I see Patrick shaking his head, but --

COMMITTEE MEMBER BRETON: I know. I know. See so that's why I wanted to ask.

COMMITTEE MEMBER WOODRUFF: But I'm sorry, if you affect -- if you look at the -- look just look at how the NAS looked at -- did their systematic review of phthalates and male reproductive effects, they just looked -- they looked at fetal testosterone levels and anogenital distance, and that -- and that's just one -- right, that's one of these key character -- that's a key characteristic, and that's -- and that was -- that evidence was sufficient to say it was a presumed male reproductive toxicant, so --

COMMITTEE MEMBER BRETON: Right.

COMMITTEE MEMBER WOODRUFF: -- I feel like if you

affect testosterone levels --

COMMITTEE MEMBER BRETON: Yeah.

COMMITTEE MEMBER WOODRUFF: -- that's an upstream effect that's going to affect multiple downstream apical endpoints. Though I agree with you, it's interesting, because usually we'd be like, oh, we're just arguing about whether there's a particular mechanism of effect. But I agree that the key characteristics allow you to think about a constellation of mechanisms, which is true that probably chemicals can influence multiple pathways.

COMMITTEE MEMBER BRETON: So for my -- like for me, I was looking at even just with the epi literature and so even --

COMMITTEE MEMBER WOODRUFF: Right.

COMMITTEE MEMBER BRETON: -- the epi literature is -- you know, it's, you know, not as -- I think we've all said it's not as strong as we would have liked. I still would have concluded that, you know, it at least showed some evidence of the impaired sperm, and, you know --

COMMITTEE MEMBER WOODRUFF: Oh, right.

COMMITTEE MEMBER BRETON: -- and sperm from that perspective, and that that in and of itself was enough to actually meet the definition of male reproductive toxicity. So that's why I was kind of asking the question

about sort of how many of these things does one feel like you have to have to have sufficient evidence. And sort of posing that question, you know, to all -- to all of the members.

CHAIRPERSON LUDERER: Dr. Hertz-Picciotto. I see your hand is raised.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Yeah. And I agree with Carrie actually, you know. And in fact looking back at the Lopez-Espinosa paper, just looking at the graphs they showed, you can see there's -- there are some pretty strong trends around testosterone.

And another endpoint that I just want to ask, because I don't think I heard anybody else mention it and I wondered exactly what its connection was with male reproduct -- or reproduction, and that is insulin growth factor 1 that was steady in that -- in that, and in the same table next to the testosterone. So could someone explain to me what its relevance is for male reproductive toxicity? I thought it was -- it was very strongly -- and it was in boys and girls, which was kind of interesting too. Clearly associated with PFNA in that study.

CHAIRPERSON LUDERER: Dr. Allard, did you have your hand raised?

COMMITTEE MEMBER ALLARD: But that was not in response to that question.

CHAIRPERSON LUDERER: Okay.

COMMITTEE MEMBER ALLARD: That was --

(Laughter.)

CHAIRPERSON LUDERER: Okay.

COMMITTEE MEMBER ALLARD: -- to follow-up to Dr. Breton's. That's why I lowered my hand.

CHAIRPERSON LUDERER: Okay. I wasn't sure.

Anyone want to comment on that?

COMMITTEE MEMBER WOODRUFF: Well, I think they said in the paper it was related to that, but I -- it's true. It wasn't in the pathway present nor am I not that familiar with it either, so maybe OEHHA can comment on it.

CHAIRPERSON LUDERER: That's what I was just going to ask.

(Laughter.)

COMMITTEE MEMBER WOODRUFF: I'm sorry. Don't want to take away your power as the Chair.

CHAIRPERSON LUDERER: No, no, no, that's -- thank you.

COMMITTEE MEMBER WOODRUFF: -- But I also had that same question. I was like, oh, that's really interesting.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Oh, okay. So there's a paper -- I should have done a check myself. There's a paper, testosterone increases insulin like

growth factor 1 and insulin like growth factor binding protein. It's a 1995 paper in the Annals of Clinical and Laboratory Science. So I guess it's the other direction that it could have been via the testosterone increase in that study.

CHAIRPERSON LUDERER: Thank you.

Dr. Allard.

COMMITTEE MEMBER ALLARD: Yes. I'm re-raising my hand now.

(Laughter.)

COMMITTEE MEMBER ALLARD: Yeah. No, I agree with you, Dr. Breton, and comments that were made as well by Dr. Woodruff. It sort of depends on the endpoint, right? It seems like some endpoints are so incredibly clearly reproductive toxicant, if you had any of them that, then it's okay. But like under -- I think it was under the reproductive toxicant, DNA damage was mentioned. And for me, it depends where the DNA damage is caused, right?

It's also that when you look at the data, some of it is stronger, some of it is a little bit weaker. And so, you know, it's weighing the strong endpoints, the weak endpoints, where do they fit in that table, and that's where, for me, it becomes a bit murkier. So I feel more confident with this in overlap, at least that's the way that I personally use that table. When there's an overlap

of several endpoints and that at least, you know, it's not just one, but several of them. And some of them are stronger. And then that gives me more confidence, but I guess we can all use that table differently depending on how we go about it.

CHAIRPERSON LUDERER: Yeah. And I think that was -- I agree with that point that was just made about the it depends on which cell -- whether it's in cells or tissues of the male reproductive system when you're looking at the key characteristics. So I think that's an important point to keep in mind.

Dr. Kim, I think you had -- Allegra Kim, you had a comment about the IGF, I think, maybe.

DR. KIM: Yeah. I think that we -- if I -- you know, I'm just -- I'm a little fuzzy on this right now and apologize, but I think we talked about whether or not to highlight that and determine that it wasn't really closely enough related to male reproductive toxicity that we -- that we didn't want -- so that we didn't want to highlight it, that it's more of a developmental issue, but we didn't really discuss it for that reason.

COMMITTEE MEMBER WOODRUFF: Can I ask you a question, because I'm just looking at the document now and it talks about it for the animal studies insulin-like growth factor hormone receptor 1, hormone receptor 2 in

the mice studies and in the -- so is it different hormones or --

DR. SANDY: So this is Martha Sandy. I think Dr. Ling-Hong Li may have something he wishes to add.

DR. LI: Hi. Actually, I wanted to clarify a few issues on the NTP study not on insulin.

DR. SANDY: Okay. Well, Ling-Hong before you do that -- so I guess we'll say that, you know, there's always more for us to learn. In the role of the insulin growth factor and male reproductive toxicity, we're still -- we're still learning. So I guess we don't have any answers for you. I apologize. I don't know if you'd wish to hear just a little perspective on the NTP study from Dr. Li or not at this time.

CHAIRPERSON LUDERER: Sure, Dr. Li, would you like to go ahead?

DR. LI: Yeah, I wanted to clarify a few issues regarding the NTP study. The NTP study actually is really a stand-out NTP high quality study. That study included five doses, 0.625 to 10 milligram per day. And it only measured 13 parameters at three lower doses 0.625, 1.25, 2.5. A the lowest dose, there was no effect on body weight. It was already significant effect on epididymal weight.

If you look at several endpoints, including

histopathology incidence, most parameters are dose dependent. I want to point that out.

Secondly, in male reproductive toxicity testing, organ weight and histopathology in many cases are the most sensitive parameter for male reproductive toxicity. In testes weight, usually people use - as well indicated in the guidelines, EPA guidelines - absolute testicular weight has always been used not as a relative. The same with the epididymal weight. If you look at the NTP data, I think the effect is clear. Certainly about the treatment duration, NTP study treated animals 28 days. That's about half of one full cycle of spermatogenesis in rodents. If you expose the animals longer than that, so what would you expect?

Normally, you would expect effective dose -- effects at lower doses. And actually, the study by Singh et al. the non-NTP study supported that, you know, postulation. That study exposed the animal for 90 days, then you observed the effect at 0.5 milligram per kilogram per day.

And so those are things I wanted to point out for you to consider.

Thank you.

CHAIRPERSON LUDERER: Thank you. I know Dr. Pessah you had your hand raised for a while.

COMMITTEE MEMBER PESSAH: That's okay. So just to follow up on Dr. Li. I do agree with what you're saying, but there are dose response or concentration effect relationships, and then there are very weak ones or ones that require you to use your imagination. And I want to point out that at 2.5 mg per kg per day, you're literally saturating all PK parameters. You're at saturation. At 5 mg per kg per day, you're not escalating the internal concentration. You're saturated.

At the lowest dose, you're having only a few biological outcomes and so really you have one data point to fit the dose response extrapolation. That to me is weak at best and you can't build an adverse outcome pathway when the doses required in an animal study are completely orders of magnitude off from human exposure.

We try to correct for this with uncertainty factor, but just take a look at the math. The numbers are six orders of magnitude above. So let me ask you a different question. What if you were to pick five outcomes at random, at random, and put them in this dose escalation study that the NPT[SIC] did and what's the probability that because of nonspecific effects, those five parameters would show you the same trends.

In toxicology, really the concentration and the dose do make the poison. And here, you're escalating a

dose in the saturation range where the body can't even deal with it. It's saturating all the PK values, you know, processes, I should say. So that's where I'm coming this, in terms of how strong the animal data is to support what I feel is, yes, suggestive data in the epidemiological study.

If that's enough, then I'm on board, but you can't say that these animal studies have predicted value. I'm sorry.

DR. LI: I have no comment. Thank you.

CHAIRPERSON LUDERER: All right. Any additional comments from the panel members?

Okay. I'm not seeing any raised hands and we're almost at 12:30, so I will let us take a lunch break. And I'll ask Carol Monahan-Cummings to give a Bagley-Keene open meeting law reminder and then also ask the staff how long we should take for our lunch?

CHIEF COUNSEL MONAHAN CUMMINGS: This is Carol.

DR. MARDER: I just wanted to say, sorry, quickly that Dr. Pessah's hand is still raised, Dr. Luderer.

CHAIRPERSON LUDERER: Oh, I was assuming that -- okay. Thank you.

DR. SANDY: This is Martha Sandy. In terms of lunch break is -- would 40 minutes be the right amount of time you think?

CHAIRPERSON LUDERER: That would be fine. Do we see any objections from anyone?

Okay, 40 minutes. So that would be at 10 after 1:00.

Carol Monahan Cummings.

CHIEF COUNSEL MONAHAN CUMMINGS: Right. There's just -- I just want to remind you what I said earlier about not discussing these issues outside of the meeting, and that includes phone calls, texts, chats, all of that. So maybe just chat about something else during lunch.

Thank you.

CHAIRPERSON LUDERER: Okay. Thank you, everyone. And enjoy your lunch and we'll see you back at 10 after 1:00. Bye-bye.

(Thereupon a lunch break was taken.)

AFTERNOON SESSION

CHAIRPERSON LUDERER: Okay. Welcome back. I hope everyone had a good lunch. I'm looking -- I'm not sure if everyone is back yet. Maybe you can show -- turn your cameras so we can tell. Thank you.

All right. I think we have maybe a few panel members still missing.

DIRECTOR ZEISE: Maybe wait an additional five minutes, Ulrike and have everybody turn on their calendars -- their cameras or three minutes.

Let's see, Dr. Woodruff and Dr. Hertz-Picciotto I think are not back yet. Give it just another minute or two for everyone to be back.

I was saying, please, if you're a panel member, just if you could turn your camera on just so I can see who's off here.

Thank you.

DR. MARDER: We're just waiting on Dr. Hertz-Picciotto.

CHAIRPERSON LUDERER: Dr. Hertz-Picciotto I think is still missing.

Well maybe to give a little bit of time, I noticed in the chat that there was a power outage at -- and did somebody want to say something about that, one of the staff members?

DR. MARDER: Sure. Just mentioning that we actually had a two alarm fire at the local power substation just a few blocks from here in downtown Sacramento. You may hear some sirens still behind me --

CHAIRPERSON LUDERER: At the CalEPA headquarters.

DR. MARDER: -- at the CalEPA headquarters.

CHAIRPERSON LUDERER: I think we can maybe go ahead and get started, even though -- I'm not sure. I don't think Dr. Hertz-Picciotto is back yet.

CHIEF COUNSEL MONAHAN CUMMINGS: I think it would be best to wait for her.

PUBLIC COMMENTS

CHAIRPERSON LUDERER: So our next agenda item is public comments on the PFNA. That will be limited to five minutes per speaker. And, Dr. Marder, can you maybe start showing the public comment housekeeping slide that shows the URL to the speaker request form. And as a reminder, I just wanted to let people know that if you would like to make a public comment, please go to the URL shown on the slide on the screen right now and fill out a speaker request card.

Alternatively, you can also click on the Zoom webinar raised hand icon to indicate that you would like to speak. And just wanted to ask Julian if we have received any speaker request cards.

MR. LEICHTY: We have not received any.

CHAIRPERSON LUDERER: Do we have any speaker request cards, Julian?

MR. LEICHTY: No, we have not. Can you hear me?

DR. MARDER: We can hear you.

CHAIRPERSON LUDERER: It looks like you're unmuted, but we can't hear you.

DR. MARDER: Julian did mention that there were no cards.

COMMITTEE MEMBER AUYEUNG-KIM: It sounds like it might be Dr. Luderer is having technical issues with hearing, because I could hear the --

CHAIRPERSON LUDERER: If there are no speaker request cards, then Elizabeth, are there any raised hands?

DR. MARDER: There are no raised hands at this time.

CHAIRPERSON LUDERER: Oh, I cannot -- I see in the chat that you're asking if I can hear you and I cannot.

All right. Headphones that might be better. Okay. Do we have any public comments?

DR. MARDER: There are no speaker cards and currently no hands are raised, Dr. Luderer.

CHAIRPERSON LUDERER: Okay. So we have no comments at all at the moment.

Okay. So I've just -- since we've checked several times for public comments, then I guess I think we can close the public comment period and we can move on to our committee discussion and the decision on PFNA and its salts.

COMMITTEE DISCUSSION AND DECISION

CHAIRPERSON LUDERER: Do any of the Committee members -- would any of the Committee members like to comment before the vote?

Okay. I don't see any raised hands. All right. Then will -- is everyone ready to vote? Okay. I see nodding heads, thumbs up. All right. So then I'm going to read the question before the Committee, that is, has perfluorononanoic acid, PFNA, and its salts been clearly shown through scientifically valid testing, according to generally accepted principles, to cause male reproductive toxicity?

And now I'm going to call each of your names and ask you to vote yes, no, or abstain on this question. And I'll go in alphabetical order.

So Dr. Allard?

COMMITTEE MEMBER ALLARD: Yes.

CHAIRPERSON LUDERER: Dr. Auyeung-Kim?

COMMITTEE MEMBER AUYEUNG-KIM: No.

CHAIRPERSON LUDERER: Okay. Dr. Breton?

COMMITTEE MEMBER BRETON: Yes.

CHAIRPERSON LUDERER: Dr. Hertz-Picciotto?

I think you're muted still.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Yes.

CHAIRPERSON LUDERER: Thank you.

And Dr. Luderer, I also vote yes.

Dr. Nazmi?

COMMITTEE MEMBER NAZMI: Yes.

CHAIRPERSON LUDERER: Dr. Pessah?

COMMITTEE MEMBER PESSAH: No.

CHAIRPERSON LUDERER: Dr. Woodruff?

COMMITTEE MEMBER WOODRUFF: Yes.

CHAIRPERSON LUDERER: All right. So I have counted two no votes and the remaining votes are yes, so that's six yes votes by my count. Anybody got a different count in the staff members who were also keeping track, I assume?

DIRECTOR ZEISE: We count six as well.

CHAIRPERSON LUDERER: Okay. All right. So six or more yes votes are required to add a chemical to the list, and six is -- six is -- because six is the majority of appointed members. So the result then is yes to add PFNA. The panel votes as a majority to add PFNA to this list.

CONSIDERATION OF PERFLUORODECANOIC ACID (PFDA) AND ITS

SALTS AS KNOWN TO THE STATE TO CAUSE REPRODUCTIVE TOXICITY

(BASED ON MALE REPRODUCTIVE TOXICITY)

CHAIRPERSON LUDERER: Okay. The next item is the consideration of PFDA, perfluorodecanoic acid and its salts as known to the State to cause reproductive toxicity based on male reproductive toxicity again. So I would like to turn the floor over to Deputy Director for Scientific Programs Vince Cogliano to begin.

(Thereupon a slide presentation.)

DR. COGLIANO: Thank you very much, Dr. Luderer. Once again, to assist you, OEHHA summarized the scientific evidence that you will consider for PFDA. So I'll turn the screen over to Dr. Martha Sandy to introduce the staff presentation.

DR. SANDY: Thank you, Dr. Cogliano.

So perfluorodecanoic acid, or PFDA, was brought to the DARTIC for consultation and prioritization last year in 2020, and this Committee recommended that it be placed in the high priority group for future listing consideration. And OEHHA selected PFDA and its salts for consideration for listing. And in March of 2021, OEHHA solicited from the public information relevant to the assessment of developmental and reproductive toxicity. No information was received on PFDA and its salts.

OEHHA has focused its current review of PFDA and

its salts on evidence of male reproductive toxicity. As I mentioned earlier, this information is summarized in the hazard identification document released in October of 2021. The hazard identification materials on PFDA and its salts provided to the DARTIC for your consideration include the hazard identification document, the references cited within it, and public comments received on the document.

We will take a break part way through the staff presentation to provide the Committee an opportunity to ask questions of clarification, just as we did with the previous item. And I will now ask Dr. Pancho Moran to begin the staff presentation.

Thank you.

DR. MORAN: Well, again, thank you, Dr. Sandy.

Let's see. Okay. Can you see the slide?

Yes.

So good afternoon. Now, as the introduction said, we are presenting a brief overview of the evidence on the male reproductive toxicity of PFDA and it salts.

NEXT SLIDE

DR. MORAN: And here is the outline on the presentation on PFDA. It is similar in structure to our earlier presentation on PFNA.

NEXT SLIDE

DR. MORAN: PFDA is a perfluorinated organic compound with surfactant properties that belongs to a group of chemicals that are collectively called PFASs. The chemical structure of PFDA is shown on this slide. PFDA has a fully fluorinated ten-carbon chain.

NEXT SLIDE

DR. MORAN: PFASs, including PFDA, has been used to make products resistant to stains, grease, soil and water. PFDA has been found in cosmetic products. No data were available on production of PFDA or on emissions.

PFDA is a global pollutant of air, water, soil, and wildlife, and is persistent in the environment. Level of PFDA in California has been documented in several studies conducted between 2010 and 2019 by Biomonitoring California with high detection frequencies.

NEXT SLIDE

DR. MORAN: Similar to what was presented for PFNA earlier today, OEHHA conducted literature searches on the developmental and reproductive toxicity of PFDA and its salts. We used HAWC as a tool for multi-level screening of literature search results. Then we focus on literature relevant to male reproductive toxicity.

NEXT SLIDE

DR. MORAN: This is a summary of the screening of the DART literature for PFDA. The studies identified as

providing general information on PFDA are shown here in the blue box and the studies relevant to male reproductive toxicity are highlighted in the red boxes.

NEXT SLIDE

DR. MORAN: PFDA is well absorbed, binds to serum proteins and is widely distributed throughout the body. In humans, PFDA is found primary in brain with lower levels in lungs and kidney. PFDA is also detected in semen, cord serum, fetal tissues, and breast milk. PFDA is not known to be metabolized in animals or humans and the excretion is mainly through urine and feces with small amounts are found in nails and hair. The half-life for PFDA ranges from several years in humans to a few months in rodents.

NEXT SLIDE

DR. MORAN: Now, Dr. Ling-Hong Li will present data on animal studies.

NEXT SLIDE

DR. LI: Thank you, Pancho. I will present an overview of the data available from whole animal studies on PFDA.

NEXT SLIDE

DR. LI: This slide lists the studies of PFDA relevant to male reproductive toxicity found through our literature search. There are studies in rats, mice,

hamsters, guinea pigs, and zebrafish. The NTP study is part of a set of 28-day gavage study on seven different PFAS chemicals.

In the case of PFDA, adult SD rats received FDA -- PFDA by daily gavage at five doses ranging from 0.156 to 2.5 milligram per kilogram, as shown here in the first row of the table. All other studies shown in this table, which were in rats, mice, hamsters, and guinea pigs, used a single IP injection of PFDA, and these doses were considerably higher than the daily doses administered in the NTP study. In these single-dose studies, the animals were sacrificed at varying days after PFDA injection, as shown on the last column in the table.

Next.

NEXT SLIDE

DR. LI: Reproductive organ weights were measured in several studies. In the NTP study in rats, reproductive organ weights were reported for the control and three highest dose groups. Epididymal weight was reduced in a dose-dependent manner with statistically significant reductions at the two highest doses. Also, in the NTP study, testes weight was significantly reduced at the highest dose. Reduced testes weight was also observed in rats treated with a single IP injection of 50 milligram per kilogram PFDA in the study by Olson and Andersen, and

at the highest dose in a study by Bookstaff et al. In the study by Bookstaff et al., reduced weights of seminal vesicles and ventral prostate were also found in rats in all three dose groups, 7 days after PFDA injection.

Next.

NEXT SLIDE

DR. LI: In the studies in mice, hamster, and guinea pigs, the authors stated the testes weights were reduced, but the paper did not report the actual data on testes weights.

Next.

NEXT SLIDE

DR. LI: Histopathological evaluation in studies of rats, mice, hamsters, and guinea pigs were found.

In the NTP study in adult rats, PFDA caused histopathological changes similar to those induced by PFNA, including interstitial cell atrophy, spermatid retention, germ cell degeneration, and epididymal lesions. The NTP report did not include any pictures to show PFDA-induced histological changes in the testes or epididymis. As shown in this table, the incidence of interstitial cell atrophy was significantly increased at the two highest dose levels. Increased incidence of spermatid retention was also significantly increased at the highest dose. Four out of 10 animals in the highest

dose group had germ cell degeneration and epididymal lesions, but the increase did not reach statistical significance.

Germ cell degeneration was also observed in rats in the study by George and Anderson, 16 days after a single IP injection. The rat study by Bookstaff et al., which examined the testes 7 days after a single IP injection, did not report any changes in the testes, but they did observe epithelial atrophy of the seminal vesicles in the high dose group, epithelial atrophy of the ventral prostate in the mid and high dose groups.

Next.

Germ cell degeneration was also reported in hamsters 16 days after a single IP injection and in guinea pigs 14 days following a single IP injection. No testicular changes were reported in mice 28 days after a single dose. Photographs of histopathological changes in the testes of hamsters were included in the study report. As shown on this slide, the left panel shows cross-sections of seminiferous tubules with multiple layers of germ cells. The right panel shows seminiferous tubules from hamsters received a single IP injection of PFDA. You can see PFDA treatment caused diminished layers of germ cells in the testes.

Next.

NEXT SLIDE

DR. LI: Sperm parameters were measured in the NTP study in rats in the control and three highest dose groups. PFDA reduced the spermatid counts in the testes, but it was only statistically significant in the 2nd highest dose group. Epididymal sperm count dose-dependently, with -- reduced with a significant reduction, about 30 percent, at the highest dose.

Next.

NEXT SLIDE

DR. LI: Finally, the NTP study found that PFDA reduced serum testosterone levels at the two highest doses. Reduced serum levels of testosterone and dihydrotestosterone were also observed in the rat by Bookstaff et al., following a single IP injection, in the mid- and high- dose groups.

In zebrafish, PFDA exposure had no effect on testosterone levels, but altered the ratios of E2 to T and E2 to 11-ketotestoerone in the second highest dose group. There was no effect on LH levels in rats in the study by Bookstaff et al.

That concludes my presentation on PFDA.

NEXT SLIDE

DR. MORAN: So now, you have time for questions.

CHAIRPERSON LUDERER: Yes. Yes. I was just

about to say that.

DR. MORAN: Good.

(Laughter.)

CHAIRPERSON LUDERER: So now, I will ask the Committee members if they have any clarifying questions regarding those two presentations? So again, you can raise your hands on camera or using the raise hand. I see Dr. Woodruff.

COMMITTEE MEMBER WOODRUFF: Thanks. I was just interested in the -- you mentioned the structural similarity to PFNA and if you had looked at any in silico studies that compares the two chemicals?

DR. LI: Pancho, do you want -- do you want me to answer or you wanted to --

DR. MORAN: I didn't look for that precisely, but we do have some in silico data that -- regarding the interaction with hormone receptor. It will come later in the mechanistic section, but I think --

COMMITTEE MEMBER WOODRUFF: Oh, okay. That's fine. That's fine. Thanks.

DR. MORAN: Yes. Okay.

CHAIRPERSON LUDERER: Thank you.

I'm not seeing any other raised hands from the panel members, so I think we can move on to the next staff presentation.

NEXT SLIDE

DR. MORAN: Yes. And that would be Dr. Kim again who will discuss epidemiologic studies of PFDA that examine male reproductive outcomes.

Dr. Kim.

NEXT SLIDE

DR. KIM: Thank you.

The PFDA studies are a subset of the PFNA studies, so the study designs are the same, and the same methodological issues mentioned for PFNA apply here as well.

Also of note, within each of these studies, the PFDA serum and plasma concentrations were often lower than the PFNA concentrations. As in the HID, the findings I will report are statistically significant unless otherwise stated.

Next slide, please.

NEXT SLIDE

DR. KIM: OEHHA identified 11 studies that examined male reproductive outcomes in association with exposure to PFDA. These included the same outcomes as for PFNA. In the next few slides, I'll present the findings on indicators of reproductive function.

Before I do that, I will briefly touch on the findings from the two studies that examined the effect of

maternal PFDA exposure on the developmental landmark anogenital distance in male offspring. The findings were inconsistent, with one study finding an increase and the other finding a decrease in anogenital distance.

In the one study of prostate cancer, PFDA exposure above the median was associated with a 40 percent increase in the odds of prostate cancer, which was not statistically significant. For men who had a close relative, such as a father or brother, who had prostate cancer, the risk was greater, with an odds ratio of 2.6. However, because blood samples were collected after the cancer diagnosis, the possibility of reverse causation could not be excluded.

Next slide.

NEXT SLIDE

DR. KIM: Now I'll focus on outcomes of reproductive function, starting with reproductive hormones. Lower serum testosterone levels were associated with higher serum PFDA in a study of 13-15 year old boys. A non-statis -- excuse me. A non-statistically significant association with lower testosterone was also observed in a study of young men being considered for military service with a median age of 19 years. In a study of men whose partners had tubal infertility, PFDA was non-statistically significantly associated with higher

testosterone. No association was observed in another study. No consistent associations were seen with PFDA and other reproductive hormones or related proteins.

Next slide.

NEXT SLIDE

DR. KIM: The study with the highest PFDA concentrations and variability reported a substantial and dose-dependent reduction in sperm concentration in the second and third tertiles, with a 24 percent reduction in the third tertile. A non-statistically significant reduction in sperm count was observed in the third tertile. This study also reported a non-significant decrease in the percentage of sperm with normal morphology in the second and third tertiles.

Another study reported an increase in the percentage of morphologically normal sperm.

Next slide.

NEXT SLIDE

DR. KIM: The largest study that looked at sperm motility, by Pan et al., reported decreases in the percent of progressively motile sperm, and in straight line velocity. A non-statistically significant reduction in progressive motility was also -- was observed in another study as well.

Sperm DNA integrity was examined in two studies.

In the study by Pan et al., in which infertile men were overrepresented, both serum and semen PFDA were associated with increases in percentage of sperm with high DNA stainability. Semen PFDA was also associated with an increase in the DNA fragmentation index. Another study, by Louis et al., reported no associations with these measures of sperm DNA integrity. In the one study to examine IVF outcomes, no associations with adverse effects were observed.

I will now hand over the presentation to Dr. Moran.

NEXT SLIDE

DR. MORAN: Okay. Thank you, Allegra.

So in this section, we will be presenting an overview of the mechanistic evidence on the effect of PFDA on the Hypothalamus-pituitary-gonadal axis, and the thyroid hormone.

NEXT SLIDE

DR. MORAN: So effects on reproductive hormones in humans and whole animals were presented earlier. Regarding other endocrines effects observed in vivo, we have the PFDA upregulated transcription levels of the aromatase CYP19A gene in the gonads of male zebrafish.

In vitro studies on PFDA exposure reported a decrease in aromatase activity observed in human placental

choriocarcinoma cell line, and in studies in isolated rat Leydig cells exposed in vitro, PFDA inhibited hCG-stimulated testosterone secretion.

NEXT SLIDE

DR. MORAN: In MA-10 cells, there was a concentration- and time-dependent decrease in hCG-stimulated progesterone, and decrease in pregnenolone secretion in the absence of cytotoxicity, also a decrease in messenger and protein levels of the mitochondrial translocator protein, TSPO, was reported.

PFDA had no effect on steroidogenic acute regulatory protein, StAR, levels, P450 side-chain cleavage activity or mitochondrial integrity.

In another mouse Leydig tumor cell line progesterone production was decreased in a concentration-dependent manner, and these effects were seen at lower doses than those that reduced mitochondrial membrane potential. In a human adrenocortical carcinoma cell line, PFDA had no effect on estradiol, or testosterone level, or on the estradiol/testosterone ratio.

NEXT SLIDE

DR. MORAN: In the next three -- in the next three slides, I will summarize PFDA's effects on the expression, binding and/or activity of HPG related hormone

receptors.

There was an increased expression of estrogen receptor alpha and beta in brain of male Zebrafish. PFDA induced a concentration-dependent increase in plasma vitellogenin levels in male rainbow trout.

In vitro PFDA induced human estrogen receptor alpha gene reporter activity by up to two and a half-fold in a human embryonic kidney cell line, and in rainbow trout liver cytosols, PFDA has shown to competitively bind weakly to estrogen receptor alpha.

NEXT SLIDE

DR. MORAN: In a human breast adenocarcinoma cell line, PFDA had no effect on estrogen receptor transactivation. In a different study in MVLN cells, PFDA was found to inhibit the estrogenic response to estradiol in a concentration-dependent manner. In another human breast adenocarcinoma cell line, MCF-7 cells, co-treatment with estradiol and PFDA resulted in downregulated expression of two estrogen receptor responsive genes.

NEXT SLIDE

DR. MORAN: In a Chinese hamster ovary cell line, PFDA had no androgen receptor agonist activity, but PFDA did exhibit concentration-dependent antagonistic effect on dihydrotestosterone-induced androgen receptor transactivation.

In sil -- in silico studies determined -- were determined that PFDA is predicted to bind at the active site of human, mouse, and trout estrogen receptor alpha. Modelings also predicts that PFDA can bind to the surface of the estradiol activated form of the human estrogen receptor alpha.

NEXT SLIDE

DR. MORAN: Now, Dr. Marlissa Campbell will summarize the data related to thyroid hormones.

NEXT SLIDE

DR. CAMPBELL: In the NTP 28 drinking water rat study of PFDA, the lowest observed significant effect level for effects on outcomes of male reproductive toxicity was higher than the lowest observed significant effect level for thyroid outcomes, which in this case was free T4 levels.

Additional in vivo rat studies looked at thyroid outcomes following acute doses of PFDA, but did not also evaluate potential outcomes of male reproductive toxicity.

Overall, single doses were -- by the IP route were associated with reduced total, free plus bound, T3, T4, and rT3. The addition of supplemental T4 only partially restored total T4 in the animals that had also been given PFDA. A higher single doses of PFDA led to increased serum T3 and reduced T3 uptake by serum thyroid

binding proteins.

Across several studies in vitro studies, PFDA in -- decreased proliferation of T3-dependent rat pituitary GH3 cells with no increase in cytotoxicity. PFDA inhibited binding of labeled T4 to human TTR, and displaced labeled T4 from binding sites on rat serum albumin. An in silico molecular docking model found that PFDA fit into the binding pocket of TTR.

And again overall, these results tease possible mechanistic relationships between PFDA disruption of thyroid hormone function and a contribution to the observed male reproductive effects. And the available data, while consistent with such a relationship, could not establish a cause and effect relationship.

Now, back to Dr. Moran.

DR. MORAN: Thank you very much.

NEXT SLIDE

DR. MORAN: So now in summary we have the effect of PFDA on the HPG axis includes:

Altering hormone levels, plus reduced plasma testosterone and dihydrotestosterone, and has no effects on LH levels in male rats; increased plasma ratios of estradiol/testosterone estradiol to 11-ketotestosterone in zebrafish; and decreased hCG-stimulated pregnenolone, progesterone in mouse Leydig tumor cells and testosterone

secretion in isolated rat Leydig cells.

PFDA reduces the levels of messenger and protein for mitochondrial translocator protein, TSPO, in vitro.

Induces upregulation of aromatase in male zebrafish gonads and brain and decreased aromatase activity in vitro in the human placental carcinoma cell line.

PFDA interacts with estrogen receptor in fish and in vitro in multiple human cells line, and with the androgen receptors in vitro.

PFDA affect gene expression of some hormone receptors, such as increased brain estrogen receptor alpha and beta in zebrafish.

PFDA interferes with thyroid hormone binding, serum levels, and function.

NEXT SLIDE

DR. MORAN: Now Dr. Niknam will present a summary of the key characteristics of male reproductive toxicants and endocrine-disrupting chemicals for PFNA.

NEXT SLIDE

DR. NIKNAM: Good afternoon.

The KCs shown here in bold are those for which there is applicable information from studies of PFDA and has already been presented by previous speakers.

Next slide, please.

NEXT SLIDE

DR. NIKNAM: And now this slide summarizes the animal and human data for PFDA. In rats exposed by the oral or IP routes, there were reductions in epididymal and testes weights. Reduction in seminal vesicles and ventral prostate weight were also seen in rats treated by the IP route.

The NTP study found clear histopathological lesions in rats that are similar to those induced by PFNA. Changes were seen in the testes and epididymis, including interstitial cell atrophy, spermatid retention, germ cell degeneration, and other changes in rats. Germ cell degeneration was also reported in hamsters and guinea pigs treated by the IP route. These histopathological findings are consistent with the effects of PFDA on reduced organ weights, sperm count, and reduced serum testosterone levels in rats.

In humans, a dose-dependent reduction in sperm concentration was observed in the study with the highest PFDA levels. Decreased serum testosterone levels were associated with higher PFDA concentrations in serum, plasma, or semen in studies of adolescents and younger men.

Next slide, please.

NEXT SLIDE

DR. NIKNAM: If you would like to see how the animal data for PFDA compares to that of PFNA, here is a table for comparison. There are a number of similarities and findings on organ weights, histopath -- histopathology, sperm parameters, and testosterone levels.

And this concludes our presentation on PFDA.

Thank you.

DR. MORAN: Thank you, Yassaman.

CHAIRPERSON LUDERER: Thank you. Thank you for the presentations. And now we have time for some more clarifying questions from Committee members. So again, please raise your hands to indicate that Dr. Breton.

COMMITTEE MEMBER BRETON: Sorry. Yes, I just had a question on that. I like that last table of comparisons, but I just wanted to clarify the -- in the animal experiments, they're different animal experiments that did PFNA versus PFDA, right, because like it -- when we get to the epi studies, like a lot of them are actually the same study that look at both chemicals, so -- but for the animals, because I just want a clarification that they're different, right?

DR. SANDY: Yes. They're completely different studies in the animals.

COMMITTEE MEMBER BRETON: Completely different studies and study designs.

COMMITTEE MEMBER WOODRUFF: Well, no, but aren't -- isn't the NTP studies all done -- because it's all in one report, right, so isn't it done --

DR. SANDY: The NTP -- you're correct, Dr. Woodruff.

COMMITTEE MEMBER WOODRUFF: Because on my --

DR. SANDY: The NTP --

COMMITTEE MEMBER WOODRUFF: -- because there's --

DR. SANDY: Yes. Yes. The NTP studies were -- they tested seven different PFASs, yes. And it's all reported in two different publications, yes.

COMMITTEE MEMBER BRETON: Thank you.

CHAIRPERSON LUDERER: Any other clarifying questions from panel members?

Dr. Hertz-Picciotto.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Just on that very last thing about the NTP, did they -- they didn't dose them all simultaneously -- like all of the chemicals simultaneously, they like did them sequentially, or did one and then cleared the system and cleared them out or what --

DR. SANDY: I believe they were done at -- I don't know if they were done at the same time. They were -- they're different experiments of testing and using different doses. And I'll turn to Dr. Li if he recalls if

that information is in the document anymore in the NTP report.

DR. LI: Similar to just said is what I saw. And we don't know whether they did one experiment in October another one in December, and -- but -- and the designs for all the groups were very similar, I would say. Beyond the animal doses, everything was almost identical. Yeah.

COMMITTEE MEMBER HERTZ-PICCIOTTO: But they were different --

DR. KIM: Different sets of animals. They were different sets of animals.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Different sets of animals. Okay.

DR. LI: Difference between species of animals, but in different groups. Yeah. Each chemical included multiple groups of animals.

CHAIRPERSON LUDERER: And there was a vehicle control group for each chemical?

COMMITTEE MEMBER WOODRUFF: Um-hmm.

DR. SANDY: That's correct. So you can think of them as a set of experiments, but they're each separate experiments and use --

COMMITTEE MEMBER WOODRUFF: But I -- oh, go ahead.

DR. SANDY: -- using different dose levels.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Different animals.

DR. SANDY: And different animals for sure, yes.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Okay.

COMMITTEE MEMBER WOODRUFF: But the same species and strain?

DR. SANDY: Correct.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Yes.

COMMITTEE MEMBER WOODRUFF: And I just want to say that looking at the PFDA, there -- while they're slightly different doses, the doses overlap, right, with the PFNA, so they have control. They both have 0.625, 1.25, and 2.5.

DR. SANDY: Correct.

COMMITTEE MEMBER WOODRUFF: Right. Okay.

CHAIRPERSON LUDERER: All right. Do we have any other clarifying questions from panel members?

I'm not seeing anymore raised hands at the moment, so then we can move on to Committee discussion if there aren't anymore clarifying questions.

COMMITTEE DISCUSSION

CHAIRPERSON LUDERER: So again, we're going to go through the discussion of the different topic areas by the primary discussants and then we'll have full Committee discussion after that. So we'll start again with the

epidemiology studies and start with Dr. Hertz-Picciotto.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Sure. Am I muted? No. Good

CHAIRPERSON LUDERER: No.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Okay. So just let me open up my notes here, so I can see them.

So I think that the -- you know, as with the animal studies, there's a lot of similarity, and, in fact, in some cases, this -- many cases, the same investigative looked at PFNA and PFDA, and often PFOS and PFOA as well. And again, similar kinds of findings.

One of the -- one of the studies that looked at anogenital discount, actually I think I may have misreported as being for PFNA, but they actually -- the results that had the opposite -- the increase at the third quartile in anogenital distance was actually the PFDA and not the PFNA, so just a clarification on that. But again, the other -- there's really essentially no data really on AGD.

The more abundant literature again is in the sperm quality and hormones. And here, the -- there's, you know -- there are just a few really strong studies. I think again it's the Pan study, the Cui study, and the Ma study. I'll just say something about some of the other studies have -- many of them have fairly null findings,

including this study, the Specht study, and there's a few others, Toft also, looking at this cohort in Ukraine, Greenland, and Poland that tended to have not much -- not much found at all. And those studies were not -- they were -- they were null studies, and they were -- they actually did a pretty good job of controlling for a lot of factors like, you know, BMI, and age, and, you know, self-reported genital infections, and testicular disorders, and semen spillage, and a lot of other things. So they were reasonable studies with null findings.

And then when we come to the stronger studies, one of them -- actually, another strong study I do want to mention is the one on the DNA methylation. And that study -- actually, that study -- sorry, I thought that that had done PFDA, but I'm looking at it again and it looks like that was -- that was not true. So, yeah, that was -- that was just PFNA as far as I can tell here.

Okay. So then moving on to the three really much stronger ones -- where is it? Okay. And Lopez-Espinosa, I also did not see a PFDA analysis in that, but I'll go back and check again, because I thought I had seen that.

Okay. Scrolling down. Pan. Here we go. So this is -- this one -- the PFDA -- why am I -- I need my glasses or something. Something I thought I saw yesterday and this morning is not showing PFDA, and I thought I just

looked at that again.

What is going on?

One moment.

Well...

CHAIRPERSON LUDERER: Well, if you're having trouble finding your documents, would you like us to go on to Dr. Breton and then have --

COMMITTEE MEMBER HERTZ-PICCIOTTO: Well, actually, yeah, I did find it.

CHAIRPERSON LUDERER: Okay.

COMMITTEE MEMBER HERTZ-PICCIOTTO: So PFDA actually does show very similar findings as the PFNA, which is that there's -- there's -- there seems to be increases in both the DNA fragmentation index and in the high DNA stainability findings, not -- nothing really significant in the concentration or -- but there is some trend towards the decreased concentrations. And, in fact, looking at the -- at the actual trends, there actually do seem to be trends in the motility that are very clear, and, in fact -- and Similarly for the -- yeah, the motility, and DFI, and HDS. So those do seem to be in -- showing up again a kind of consistent pattern in that particular study.

The other study - let me move my cursor here - was the Cui study. And again, my notes -- and when I'm

looking at also the table from OEHHA are a little less clear than I thought they were.

(Laughter.)

COMMITTEE MEMBER HERTZ-PICCIOTTO: Oh, yeah. Here it is. The -- oh, that's also PFNA. That was looking at serum and sperm. Sorry, I thought one of them was PFNA and one was PFDA. So maybe Cui did not do the PFDA and I'll -- I can check that and actually maybe Carrie already has the answer to that as well.

And again, Ma also I'm not seeing PFDA in -- except that it was highly correlated with PFNA. So I think there may be some issue of being able to distinguish which of those actually was playing a role.

Okay. All right. Well, I'm going to say that there's similar outcomes, and maybe a little less actual epidemiologic data at hand to evaluate the PFDA. And maybe Carrie can clarify, because I'm actually a little big confused at the moment as to whether I misread some things when I was preparing my notes.

COMMITTEE MEMBER BRETON: Chair, I can go now, but I -- and I'll say sort of right now I think you were right that Pan and Ma both did also look at PFDA, but I don't believe Cui did, so -- so -- but they did see -- but Pan and Ma both saw similar associations as you said, so they found PFDA associated with several semen quality

indicators. So lower semen quality indicators. And for Ma it, was lower sperm concentration.

So, yeah -- so I'll just so I guess pick up and start with these and say there were four studies that looked at some aspects of semen quality. And I think all four of those showed some associations with generally poor semen quality. And this included the Pan and Ma studies that we just -- that I just mentioned and then two others. And I think of all the data that we have to work with, which in general is a slightly lower body of epi work than for PFNA, that these four did find some fairly consistent associations with lower semen quality parameters.

The other -- the other associations with testosterone that were also looked at I think there were five studies evaluated in this -- with testosterone, and most of them did not find associations with testosterone. There was one that did and this was by Zhao et al., in 2016. And this was a study that had about -- so I should say all of these studies on average have sample sizes that range from 100 to 200 or 250 tops. So part of I think the problem with the epi literature is that they're generally small studies.

The Zhao study that showed an association had the highest -- some of the highest PFAS levels -- or sorry, PFDA levels. And they comment in their discussion

actually that compared to Americans, because this is a Taiwanese study, that they had much higher levels than Americans -- than seen in Americans, but those effects were observed in adolescents. So they were 13- to 15-year old boys.

So the other studies, two of them were done by the same author, one in 2009 in a sample size that was only about a hundred. And it was so -- their PFDA levels were so low that they actually couldn't even do the analyses completely the way they wanted to. And so then they, I think, a couple years later come back with a study that has higher -- a sample size of 250 instead, and they start to see some non-significant associations with test -- lower testosterone. But again -- you know, and then the Ma study, which also had only about a hundred, had no observed associations with testosterone.

So I think suffice it to say there's not a lot of evidence for testosterone at this point in the epi literature. I don't think that it means that there's not an association so much as we're probably not super well powered to be detecting some of these associations, so I was sort of leave it as the jury is still out from the human literature.

And so I think the hormones and the semen quality were the two sets of data that we had I think a descent

number of studies to at least start thinking -- start evaluating. And as Dr. Hertz-Picciotto said, the anogenital distance ones are -- it's the same two studies that were -- also looked at the PFNA and that really have no evidence that's compelling.

And then the cancer studies also don't -- there was really only one and I think it was not -- there was nothing much to show for that.

So of all of the -- of all of the outcomes that I think that the data are most suggestive for sperm quality.

And that's all -- I think that's all I have.

CHAIRPERSON LUDERER: Thank you, Dr. Breton.

Next, we'll move on to the animal studies, starting with Dr. Auyeung-Kim.

COMMITTEE MEMBER AUYEUNG-KIM: All right. So for the animal studies, so the NTP study, as mentioned before, it is very similar to the study design for PFNA, and where they dosed different sets of animals, so it's a -- different sets of animals by oral gavage repeatedly for 28 days. This is definitely the most robust studies of the set for PFDA, in the sense that it included clinical, hormonal, and histological evaluation. Similar to PFNA, there was statistically significant decrease in body weight, testes, and epididymal weight. Testosterone concentrations are also decreased starting at 0.625 mg per

kg per day.

And they also had -- hold on. And then also epididymal weights and sperm counts were also statistically significantly lower, starting at 1.25 mg per kg per day and went up to 2 -- and was also lower in 2.5 mg per kg per day.

The remaining rodent studies that were treated for PFDA were all acute studies where the animals received a single dose by IP injection and were evaluated for several days after exposure. And so in these studies, I mean, there were really a lot higher doses than what was studies in the NTP study, where the highest dose in the NTP side was 2.5 mg per kg per day, these started out at 50 mg per kg per day and went higher.

So in the single dose toxicity study published by Olson and Andersen, this was a 1983 study, they were dosed with a single dose level of 50 mg per kg per day. And what they saw was a decrease in testes weight and it was presumed to be due to tubular atrophy and continued to frank necrosis on day 16. And so the histo data was referenced to another study where the rats were treated with 100 mg per kg per day, so it did not -- the histo data was not actually in this particular study.

A similar study design to the Olson and Andersen paper, there was another study publish by George and

Andersen in 1986. That histopathology was conducted and the evaluation continued -- or the animals were -- had received a single dose and were sacrificed on days 4, 8, 12, 16, and 30, so over a period of time. In this study, atrophy and degeneration of the seminiferous tubules were observed at day 6 and remained severe at day 30.

In the Bookstaff study, they evaluated the effects of PFDA on the androgen status of sexually immature rats. These rats were treated with either 20, 40, or 80 mg per kg PFDA single dose by IP injection and evaluated for seven days. The histologic evaluation indicated that the testes were normal and -- hold on -- histopagically -- histopathological evaluation of the testes indicated that there was normal spermatogenesis and no histologic changes, so this differs from the George and Andersen paper that histologic changes were observed. The difference was -- could potentially be the time frame of the evaluation being day 7 versus day 8. That's what they postulated in the study. And then also the strain of rat used was different. In the latter study, the Bookstaff study, it was a Sprague-Dawley rat and then the previous paper was in Fischer 344 rats. So it could be a strain difference. Most likely, if anything, it may be a strain difference.

The remaining mammalian study was published by a

single lab, Van Rafelghem, in 1987 where acute toxicity studies were conducted in rats, hamsters, guinea pigs, and mice. These studies also had a single dose of PFDA by IP injection and they were sacrificed sometime between two weeks or one month after treatment depending on the species.

And so rats were treated with a single dose -- at a single dose level of 50 mg per kg and sacrificed on day 16. Hamsters were treated with 50 to 500 mg per kg per day -- mg per kg and sacrificed on day 16. And guinea pigs were treated from 125 to 175 mg per kg and sacrificed on day 14. And note in these studies, the number of animals in the study -- per each group ranged from three to five. It was -- for rats, it was five; hamsters, it was four; guinea pigs, it was three. Oh, and then for mice, it was 10 per group were treated with 150 to 250 mg per kg and sacrificed two weeks longer on day 28.

And so the results were mixed in these species and the rat results were similar to the George and Andersen paper discussed previously where there's seminiferous tubule degeneration was observed in the surviving animals for the hamster, which was mid-dose 100 mg per kg, and the guinea pigs, which was at the mid-dose, of 150 mg per kg, but not at the low or surviving high dose in the guinea pig study, and these were also to a

lesser severity than that seen in rats.

However, no histological testes effects were observed in the mice any doses through day 28, but the paper indicated that there was slight reduction in testicular weight compared to the controls at day 28 in the mice. So some study details in this set of studies from the -- from this lab was not disclosed calling into question the quality of the study.

And then the last study was in zebrafish and 10 eggs were exposed to a range of PFDA from of all -- 0.1, to 10 mg per liter from -- for one day post-fertilization to 120 days post-fertilization. The fish were sampled from replicate tanks and then essentially it was determined that significant -- there was significant increases in E2, testosterone ratios and E2 to 11-KT ratios in the male zebrafish with no effects on the plasma E2, testosterone or 11-KT levels. There were no studies available on the effects of PFDA on male fertility in the reproduction study. And so that's it for the studies.

And in general, the study -- there was only one study of good quality, which was the NTP study. The other studies I struggled with again, because it was high -- super high dose levels, even higher than what we evaluated for the PFNA studies. In addition, they were single dose studies and dosed buy IP injection, which can impact the

pharmacokinetics of the -- of the compounds.

So essentially, my conclusion again for PFDA is that there's no clear evidence that it is a reproductive toxicant based on the animal studies that we reviewed.

CHAIRPERSON LUDERER: Thank you, Dr. Auyeung-Kim.

Dr. Woodruff, would you like to comment on these animal studies?

COMMITTEE MEMBER WOODRUFF: Yes. Thank you. Thank you to staff for their summary and Dr. Kim. I want to first start off by thanking the people who talked about the epidemiology studies and that they noted that -- and I'm going to focus on semen quality, because that seems to be the most responsive endpoint in the animal and the human studies, and that they noted that all four epidemiology studies showed association between PFDA with poor semen quality, which I had to go back and look at the papers. And that was often in a dose response fashion.

I also want to comment that PFDA is structurally very -- you know, pretty similar -- very similar kind of like two PFNA, so we would expect similar types of affects, even though I agree -- even though there is less high quality studies in the animal studies compared to what we had with PFNA.

However, we only need one good high quality animal data to make a decision per the guidelines of what

we -- for the Committee, and the National Toxicology Program study is a reasonably high quality study. I just note that they evaluated multiple -- as we discussed, multiple individual PFAS using the same experimental design, but slightly different dose levels. The same rats, same housing, same set up, but slightly different -- same type of rats, slightly different dosing regimes between the PFNA and the PFDA.

And I wanted to comment on the -- I agree that the IP studies were single injections of very high exposure levels, so it's very difficult to conclude anything from those, but I just wanted to note that you did have an opportunity to look at the comparison of the different species to each other in the Van Rafelghem study, because they looked in the same experimental design rats, hamsters, mice, and guinea pigs. And I think we see this commonly in studies in general that the mice look to be a little bit more sensitive to dosing than rats, hamsters, and maybe the guinea pigs. So I think that's commonly seen in toxicological studies and something we should keep in mind when we're looking at the results from the rat studies for NTP, again that -- and that humans would be more sensitive than that.

So when you compare -- since they used similar dosing regimes between the PFDA and the PFNA, when I

compared the response -- and I'm going to focus on the sperm quality, because that is the one that's most consistent with the human findings, the spermatid heads, the epididymal sperm counts, and the epididymal sperm count, the -- they -- you can look at the 0, 06.25, 1.25, 2.5, they can take out those various high doses, because -- and just focus the 0, the control, 0.625, 1.25, and 2.5 and you can see actually a very similar response -- dose response for those three sperm measurements between -- for both PFDA, which we just decided was a male reproductive toxicant -- I mean, PFNA, that's the one we decided on, and the PFDA. And if you look at it, the responses are very, very similar in terms of a decline in those sperm metrics across the 0, 0.625, 1.25, and 2.5 dose.

Again, the way that the analysis is done is that they do individual comparisons to the control and there's not a valuation of the trend, like there would be in an eval -- in an epidemiology study, though they do note, so -- but you can see a consistency between this and the epi studies. So I agree with there's less evidence, but there's -- in terms of studies that have been done, but the high quality studies are consistent in terms of effects on sperm with the human epidemiological evidence.

Thank you.

CHAIRPERSON LUDERER: Thank you, Dr. Woodruff.

Next, we're going to move on to discussion of the mechanistic studies and we'll start with Dr. Allard.

COMMITTEE MEMBER ALLARD: Yeah. Thank you. I have to admit that I did not really know where to start, looking at PFDA compared to PFNA at least. I did not really know what sort of endpoint to latch on to then look at the mechanistic basis for it. And I -- you know, I didn't want to be biased by the HID document too much. So, you know, I sort of felt that the testosterone endpoint was a little bit inconsistent across the studies. I had a hard time including all the IP data that was generated in mouse studies, because IP studies at a high level single injection I did not want to lean on that kind of data, so that's sort of, to me at least, left the field a little bit open as to what to -- what to look at.

Also, compared to -- and I know this is not comparative. We're looking at them independently. However, compared to PFNA, PFDA had a less penetrant effect or less pronounced effect on the host of different enzymes that are important for steroid hormone production. So they didn't see the same thing. For example, with StAR, it had no effect on StAR, however there was -- some studies had an effect on TSPO, but TSPO in itself is kind of controversial in some sense. The role of TSPO is still

not actually very, very clear. So I could not necessarily lean on that either.

There seemed to be an effect on aromatase, but that as well seemed to be inconsistent across studies. So in the end, I was sort of left again to turn to ToxCast, which told me that there's not a lot of in vitro, you know, like cellular assays that I elicited by PFDA below the lowest level of cytotoxicity, right? So what you would hope from those in vitro assays is that you detect something that's a clear signal that is way below or significantly below the cytotoxic level. You want to make sure that what you're looking at is indeed specific.

And again, we fall back onto the same receptor, FXR, as we did for PFNA. Although, the AC50 for PFDA was a lot higher. We're talking about in this case, you know, 500 -- I think it's 522 -- talking about all this from memory -- 522 nanomolar, yes, AC50 compared to PFNA, which was a lot lower. And the magnitude of the effect was -- yeah, of the effect was not even that strong. Although, it was actually a little bit stronger than PFNA.

So in the end, actually, I had a hard time sort of coming up with a potential model of action for PFDA. You know, there's some elements there, but just like I think we've already sort of heard from the human side and the animal study side, I felt that once you sort of

exclude the stuff that's perhaps a little bit low quality, which I -- you know, I think we may have different metrics on sort of what a good high quality study looks like, but you're not actually left with much to -- from my perspective to actually draw a model of mechanism of action for PFDA. So that's sort of unfortunately a little bit unsatisfactory, but that's what I'm left with in terms of concrete information.

CHAIRPERSON LUDERER: Thank you, Dr. Allard.

Dr. Pessah, would you like to discuss the mechanistic studies?

COMMITTEE MEMBER PESSAH: Sure. PFDA is a very different chemical from PFNA. They may look similar, but the two additional carbons and two additional fluorines increase the log P of the decanoic by tenfold. So it's solubility properties are quite different. If you look at the molecular area that the two additional fluorines contribute to the structure of the decanoic, it makes it a much bulkier molecule.

I am rather surprised that you can actually do studies at 50 or even 10 mg per kg IP. My guess is that they put it either in oil or in Tween. I know that many of the studies that were oral gavage used detergent, Tween 20 I believe, to make sure that they could actually keep the molecule in solution or at least in suspension. Given

the tenfold lower solubility of the decanoic, this may explain at a particular receptor, in this case, the one that Dr. Allard mentioned, that there would be significantly less potency, because for every squirt that you put in your biological preparation, much less of it will be in solution, predictably anywhere between nine- and tenfold less. And things have to be in solution to interact with receptors, so I think that may explain and it may be a trivial explanation.

So I still have tremendous concerns about the in vitro and animal studies, given the disparity between the concentration used in all of these animal studies, both in vitro and in vivo and the relevant concentrations detected in human beings, but that's -- I'll stop there.

CHAIRPERSON LUDERER: Thank you. Thank you, Dr. Pessah.

We now have time for additional Committee discussion. And again, please raise your hands if you would like to speak.

Dr. Allard.

COMMITTEE MEMBER ALLARD: Yeah, I guess -- it's more of a comment than a question, and it's sort of, you know, me thinking through what Dr. Woodruff said about we may not need a lot of studies. We just need one really high quality study, and really talking about the NTP

study, right? That's what we're all talking about. Is that really of all the studies that we've discussed and we've looked at, is that really the one study that's left at the end, once we use some sort of quality metrics in our minds about studies, human and animal?

COMMITTEE MEMBER WOODRUFF: No. No. No. I didn't -- I didn't comment on the quality of the epidemiological evidence.

COMMITTEE MEMBER ALLARD: Okay.

COMMITTEE MEMBER WOODRUFF: But the other studies -- I mean, just thinking about their dosing, you know, they have pretty -- they have an acute exposure at a very high dose. And I would say -- I mean, I did look at the -- at their -- the methodological features and some of these studies are very old. I think that also tends to influence -- you know, methodological quality has improved over time, but I mean they -- all of them, except for the Bookstaff study, said that they randomized. There was -- it was unclear about the blinding as Dr. Pessah has mentioned. They seem to have reported all their outcome in outcome data, so -- but I think it's the IP injection that makes it difficult to really compare it to the other studies that we've been looking at to me. It's very high and a single dose, so -- I don't know, Dr. Luderer. What do you think?

CHAIRPERSON LUDERER: I agree, the IP route with the single high dose, I mean, it's not a relevant route of human exposure. And it's -- And they're very high doses. So I agree with that. That was my assessment as well. Dr. Breton, did you have -- you had your hand raised.

COMMITTEE MEMBER BRETON: Yeah. I was just going to sort of say, but you guys kind of discussed it anyway, but I had -- also, I think it said somewhere in this set of instructions that we had that, you know, a single well done study is -- can be sufficient evidence for, you know, making a decision. So I had -- I recall that as well.

But I just wanted to clarify, but you already clarified that, yeah, the -- you weren't talking about epi studies --

COMMITTEE MEMBER WOODRUFF: No. No. No, I'm sorry. I did not mean to say that.

COMMITTEE MEMBER BRETON: -- being -- No, no, no, I know. And that's fine, because I was going to say that, you know, I think that there are -- you know, there were several consistent epi studies when it came to the semen parameter -- semen quality parameters that showed a consistent effect.

COMMITTEE MEMBER WOODRUFF: Right. I didn't --

CHAIRPERSON LUDERER: Thank you. Any other comments or questions?

Dr. Pessah.

COMMITTEE MEMBER PESSAH: I would love to see a study modeled after the NTP study down at about a hundred-fold lower dose range. I think that would be extremely important. I mean, I know we don't have that, but it's just a thought.

CHAIRPERSON LUDERER: I would agree. Thank you.

Are there any other comments, questions?

COMMITTEE MEMBER HERTZ-PICCIOTTO: Yeah, this is Irva

CHAIRPERSON LUDERER: Dr. Hertz-Picciotto, please.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Yeah. Hi. Yeah, I think one of -- as I was looking through those epi papers and actually comparing the PFNA and PFDA, it's -- in the cases where PFDA does show some effect, it seems -- you know, with regard to I think it's testosterone for example, it has a weaker effect, although it was significant. But the correlation between PFNA and PFDA was somewhere around 0.8, which -- and then in light of Isaac's comment about the solubility and the likelihood that the PFDA is actually getting, you know, into the tissues and to the receptors being lower, I think it suggests that there could be quite a bit of confounding, and that if you were to choose between the two, the PFNA

is the more -- would be the more plausible of the two.

Again, it doesn't say there can't be compounding effects of more than one, but obviously the ability to distinguish in an epidemiologic study with that high of a correlation would be very -- there would be very weak power to actually distinguish the two. And you would expect to see if one of them is actually the culprit, so to speak, that the other one would tend to come out looking like it also had an effect. Since these were single -- well, these were single -- the analyses were of them singly, even though the exposure was concurrent in the human studies.

So I -- just putting together, you know, some of this by -- you know, the chemistry aspect of it with the epidemiology, it's -- it makes me feel like it's weaker than I otherwise would -- might have thought.

CHAIRPERSON LUDERER: Or the animal studies we know, we don't have that confounding issue of the concomitant dosing that we have with the epi -- epidemiological studies unavoidably.

Any other comments or questions from the panel?

PUBLIC COMMENTS

CHAIRPERSON LUDERER: All right. Not seeing any, we can take -- move on to public comments, which will be limited to five minutes per speaker. And Dr. Marder,

could you please show the public comment housekeeping slide with the URL to the speaker request form.

(Thereupon a slide presentation.)

CHAIRPERSON LUDERER: Thank you.

So as a reminder, I just wanted to let people know that if you would like to make a public comment, please go to the URL that's shown on this screen and fill out a speaker request card. Alternatively, you can click on these Zoom webinar raise hand icon to indicate that you would like to speak. And I'll ask Julian, if we have received any speaker request cards.

MR. LEICHTY: We have not received any speaker request cards.

CHAIRPERSON LUDERER: Okay. Thank you.

Do we have any raised hands?

DR. MARDER: There is, in fact, one raised hand.

CHAIRPERSON LUDERER: All right. Then it looks like we -- that's our one public comment right now. So can we let that person speak or --

DR. MARDER: Yes, absolutely. Ms. Hume, I am allowing you to talk. You will need to unmute and you have up to five minutes.

MS. HUME: Thank you so much for everyone here. What a -- what a grueling process. Hats off to everyone here.

So dear committee. Hello my name is Suzanne Hume. I'm the Educational Director and founder of CleanEarth4Kids.org. We ask you to list PFDA as Prop 65 because of reproductive toxicity. Just like PFNA, studies show PFDA affects developmental toxicity. We're concerned about semen quality. PFDA is similar to PFNA, so it can be expected similar results, as pointed out in the national quality study using the same design, same types of rats and housing. We're concerned about sperm quality indicators, lower sperm concentrations, hormones, organ weight. I can go on and on. So we would like you to please protect us and add PDF -- or I'm sorry PDF -- PDFA to -- as Prop 65.

We also ask that PFAs, PFAs, be regulated as a class. How many studies do we need, right, where we really just need one as stated before. The problem with PFAs, obviously, PFAs, is the cumulative effects. And when we are looking at these studies in isolation and not looking at cumulative effects and ongoing effects, what the public is actually facing, you know, with the products we're encountering every day, you know, these chemicals in our air, our water, our environment our food, and the fact that we're facing these multiple cumulative exposures on a daily basis, we really need to -- we really need to change things and look at these in a cumulative way.

So I have a lot I could say. It's been a really long day. And I apologize, I missed the earlier comments. If you had called my name, I'm not sure, on PFNA. I was called away during your -- the last part of your lunch break. But I just would like to say thank you so much to everyone today. You know, the whole purpose of having things listed as Prop 65 is to protect the public. And it's just -- it's just really so important what you're doing. And as, you know, these things will help other states, and, you know, the world.

So people look to California and we are looking to you for help. So thank you so much. We ask that you please list PFDA as Prop 65. Thank you from CleanEarth4Kids.org.

CHAIRPERSON LUDERER: Thank you very much, Ms. Hume.

Do we have any other requests for comments from the public that have arisen, speaker request cards or raised hands?

DR. MARDER: There are no other raised hands at this time, Dr. Luderer.

CHAIRPERSON LUDERER: Thank you.

MR. LEICHTY: And no speaker requests cards as well.

CHAIRPERSON LUDERER: All right. Thank you very

much.

COMMITTEE DISCUSSION AND DECISION

CHAIRPERSON LUDERER: Next, we'll move on then to the Committee discussion and decision on PFDA and it salts. Would any of the Committee members like to comment before the vote?

COMMITTEE MEMBER WOODRUFF: I have a question.

CHAIRPERSON LUDERER: Yes, Dr. Woodruff.

COMMITTEE MEMBER WOODRUFF: Yeah. I was -- I was interested in the -- this PBPK discussion that we've been having. And the data in the document show that the similar half-lives and that PFDA is distributed throughout the body. It's been measured in brain, lung, and kidney, crosses the blood-brain barrier, and the placenta, and been detected in fetal tissues, cord serum, and breast milk.

So I guess I was thinking that that -- I mean, it's certainly hitting target organs, I guess that's what I'm saying, similar to PFDA. So that was -- I just wanted to note that.

CHAIRPERSON LUDERER: Thank you, Dr. Woodruff.

Any other comments from other panel members?

Dr. Breton.

COMMITTEE MEMBER BRETON: I guess I would just say I'm struggling a little bit right now, because I'm

thinking a little bit about what Irva was saying and thinking about, you know, not having -- appreciate the high correlation between the two and, at least in the epi studies not being -- you know, because so far, we haven't seen any studies that have really tried mixtures approaches to -- which might provide some weight towards, you know, one over the other.

You know, so balancing -- I guess this is perhaps not a question, but just a commentary, because I'm just mulling over sort of those last comments and trying to balance that. You know, since it seems like, to some extent, the epi literature is actually some of the stronger literature here than the -- at the moment, than the -- than some of the animal or in vitro, unless -- yeah. Just my comment.

CHAIRPERSON LUDERER: Thank you.

Any additional comments or questions before we move on to the vote?

Okay. Not seeing any, then we -- is everyone ready to vote? Anyone not ready to vote?

Okay. All right.

COMMITTEE MEMBER HERTZ-PICCIOTTO: I have a question.

CHAIRPERSON LUDERER: Yes.

COMMITTEE MEMBER HERTZ-PICCIOTTO: That is

maybe -- I don't know. I'm wondering -- and I maybe should have asked this earlier in the meeting, but we -- when we met last year, we put together some kind of priority list. And within the PFAS, we had multiple kinds of outcomes -- reproductive developmental outcomes. And I was just curious as to the choice of male reproductive toxicity being selected as the one to move forward with this year. And when I looked back over my notes, it wasn't -- it didn't seem clear to me that this was for PFNA and PFDA that this was the most -- I wasn't clear whether this was actually the most -- seemed to be the most sensitive or not among the various outcomes that we had looked at last -- as we did the prioritization, so...

DR. SANDY: I can try to address that. This is Martha Sandy. We selected all four of the PFAS chemicals that you prioritized last year and asked for relevant information from the public on all four. And we intend on looking at other endpoints and other chemicals in the coming year.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Okay.

DR. SANDY: And we selected these. We had a fairly short window of time to develop this document.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Um-hmm.

DR. SANDY: And so we selected the male reproductive endpoint and these two chemicals to bring to

you this year.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Okay.

CHAIRPERSON LUDERER: Thank you, both of you, Dr. Sandy and Dr. Hertz-Picciotto.

Any other comments from anyone?

All right. Then we can move on to the vote. The question before the Committee is, has perfluorodecanoic acid, or PFDA, and its salts been clearly shown through scientifically valid testing, according to generally accepted principles, to cause male reproductive toxicity?

So I'll now call each of your names and ask you to vote yes, no, or abstain on this question. So starting off with Dr. Allard?

COMMITTEE MEMBER ALLARD: No.

CHAIRPERSON LUDERER: Okay. Dr. Auyeung-Kim?

COMMITTEE MEMBER AUYEUNG-KIM: No.

CHAIRPERSON LUDERER: Dr. Breton?

COMMITTEE MEMBER BRETON: I'm going to abstain.

CHAIRPERSON LUDERER: Dr. Hertz-Picciotto?

COMMITTEE MEMBER HERTZ-PICCIOTTO: I'm also going to abstain.

CHAIRPERSON LUDERER: Okay. I'm going to vote no.

And next Dr. Nazmi?

COMMITTEE MEMBER NAZMI: Voting no.

CHAIRPERSON LUDERER: Dr. Pessah?
COMMITTEE MEMBER PESSAH: No.
CHAIRPERSON LUDERER: Dr. Woodruff?
COMMITTEE MEMBER WOODRUFF: I'm going to abstain.
CHAIRPERSON LUDERER: All right. So I count three abstain, and one, two, three, four, five no.
The staff agree with that tally?
DIRECTOR ZEISE: Yes.
CHAIRPERSON LUDERER: All right. So we have then five no votes and three abstain. And that's all we need to say, right? We don't need -- there's no requirement for a certain number of no votes and abstain votes, I don't think?
DIRECTOR ZEISE: That's correct.
CHAIRPERSON LUDERER: All right. Great.
Okay. Then thank you everyone.
Next, we will move on to the consent item. So this is an update of the California code of Regulations --
COMMITTEE MEMBER WOODRUFF: Can I --
CHAIRPERSON LUDERER: Yep.
COMMITTEE MEMBER WOODRUFF: Can I just make a comment about the documents?
CHAIRPERSON LUDERER: Um-hmm.
COMMITTEE MEMBER WOODRUFF: Yeah I just want to say, I appreciate all the work that goes into this and

that I also appreciate that you started to move towards a platform for doing your systematic searches that can be more transparent for us to look at with the HAWC program. And I want to -- I would like to see next year that you use the HAWC program to actually do the data extraction for these studies. I think having us be able to all look at the same data laid out as they do in HAWC, which has been, you know, implemented at EPA, National Toxicology Program, and not just ORD, but also in the Office of Water, and like their latest PFAS drinking water table, would actually be very useful for us and extremely time saving, so we don't have to -- we -- it will help us look across all these studies.

And I would encourage also that we consider looking a meta-analyses for these studies. I just will reference the PFOA drinking water document that just was released by EPA, which used the systematic review approach, and that HAWC data, and included a meta-analysis. And then by conducting the meta-analysis, it improved the statistical power to observe effects in the epidemiologic and the animal data. And I -- probably, you've seen this document, but their RRD is set I think at a lower level than the State of California is at a 1.5 times 10 to the minus 9 milligram per kilogram day. And it's based because they were able to do this meta-analysis

work and systematic reviews.

So I hope that the State of California will take the methods that EPA is using, which the National Academy of Sciences just commented on, and improve upon them, and make I think every -- it much more efficient for everyone and clearer to us in the public.

So thank you.

CHAIRPERSON LUDERER: Thank you, Dr. Woodruff.

Any additional comments?

CONSENT ITEM - UPDATE OF THE CALIFORNIA CODE OF REGULATIONS TITLE 27 SECTION 27000 LIST OF CHEMICALS WHICH HAVE NOT BEEN ADEQUATELY TESTED AS REQUIRED

CHAIRPERSON LUDERER: All right. So then we can move on to the update of the California Code of Regulations, Title 27, Section 27000 list of chemicals which have not been adequately tested as required. So as I said, this is a ministerial item. And the Committee is being asked to affirm changes in response to submissions from the Department of Pesticide Regulation and the U.S. Environmental Protection Agency.

So I'd like to ask OEHHA Special Assistant for Programs and Legislation, Julian Leichty, for the staff presentation.

(Thereupon a slide presentation.)

MR. LEICHTY: Thank you, Dr. Luderer. So this

slide indicates the proposed change based on information received from the California Department of Pesticide Regulation, the removal of triethylene glycol detailed in the staff report provided to the Committee.

And I will now turn things back to Dr. Luderer.

CHAIRPERSON LUDERER: Okay. So again, this is a consent, so I'd like to ask the Committee members if they are ready to vote or if they have any clarifying questions?

Not seeing any --

COMMITTEE MEMBER HERTZ-PICCIOTTO: I'm sorry. I may have missed a document. Was there the DPR document that's referred to in that previous slide for us? Was that sent around and I missed it, the triethylene glycol reported by the DPR?

MR. LEICHTY: Yes, there -- it was in the materials provided through the server.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Okay.

DR. SANDY: Julian, this is Martha Sandy, you might clarify as to what the document actually was.

MR. LEICHTY: It's -- oh, yeah, the document itself, yes, is a -- is a letter and it's within a staff report.

DR. SANDY: So just to clarify, Dr. Hertz-Picciotto, it's a letter received from the

California Department of Pesticide Regulation to OEHHA answering our question if this list need to be updated.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Okay.

DR. SANDY: And it's in the staff -- the staff document -- the staff report we sent to you.

COMMITTEE MEMBER HERTZ-PICCIOTTO: Okay. Okay. All right.

CHAIRPERSON LUDERER: All right. So does -- any other clarifying questions from the panel members?

Okay. Then we can -- I'm not sure what that was, but excuse me. So we can -- again, I want to say this is a consent. And so if we're all ready to vote, then I will read the formal question, which is, should section 27000 of the Title 27 of the California Code of Regulations be amended as indicated in the staff report? And I'll read everyone's name one by one and ask you to vote yes, no, or abstain on this question.

Dr. Allard?

COMMITTEE MEMBER ALLARD: Yes.

CHAIRPERSON LUDERER: Dr. Auyeung-Kim?

COMMITTEE MEMBER AUYEUNG-KIM: Yes.

CHAIRPERSON LUDER: Dr. Breton?

COMMITTEE MEMBER BRETON: Yes.

CHAIRPERSON LUDERER: Dr. Hertz-Picciotto?

COMMITTEE MEMBER HERTZ-PICCIOTTO: Yes.

CHAIRPERSON LUDERER: Dr. Luderer, yes

Dr. Nazmi?

COMMITTEE MEMBER NAZMI: Yes.

CHAIRPERSON LUDERER: Dr. Pessah?

COMMITTEE MEMBER PESSAH: Yes.

CHAIRPERSON LUDERER: Dr. Woodruff?

COMMITTEE MEMBER WOODRUFF: Yes.

CHAIRPERSON LUDERER: All right. So everyone voted yes, so that's 8 yeses, no noes, and no abstains.

STAFF UPDATES

CHEMICAL LISTINGS VIA THE ADMINISTRATIVE LISTING MECHANISM AND SAFE HARBOR LEVELS

CHAIRPERSON LUDERER: All right. Then moving on to our next item, which is staff updates. We're going to have staff updates on Proposition 65 listings, regulations, and litigation that have taken place since the last meeting.

So again, I'd like to ask Julian Leichty to make the presentation?

(Thereupon a slide presentation.)

MR. LEICHTY: All right. So since the Committee's last meeting, we have administratively added a reproductive toxicity endpoint, development toxicity to the listing of bisphenol A. And we have added two chemicals to the Proposition 65 list as causing cancer.

These chemicals are molybdenum trioxide and indium tin oxide

Next slide, please.

NEXT SLIDE

MR. LEICHTY: I'll now move to the chemicals currently under consideration for administrative listing, which are perfluorooctanoic acid, PFOA, tetrahydrofuran, 2-ethylhexyl acrylate, methyl acrylate, and trimethylolpropane triacrylate, technical grade.

And next slide, please.

NEXT SLIDE

MR. LEICHTY: Now, turing to safe harbor levels. Since the last meeting, four safe harbor levels have been adopted in regulation. No significant risk levels were adopted for p-Chloro-alpha,alpha,alpha-trifluorotoluene, dibromoacetic acid, dichloroacetic acid, and trichloroacetic acid.

And next slide, please.

NEXT SLIDE

MR. LEICHTY: Lastly, we have proposed safe harbor levels for one chemical, 1,3-dichloropropene for the inhalation and oral routes.

And now, I will turn the presentation to Carol.

NEXT SLIDE

OTHER REGULATIONS AND LITIGATION

CHIEF COUNSEL MONAHAN CUMMINGS: I don't know if you can see me. I can't see me.

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: So for other regulatory actions that we're taking currently, at your last meeting, I think we mentioned that we were in the process of adopting some additional warning methods for alcoholic beverages and we completed that rulemaking. And those changes were effective April 1st of this year. We currently have five other regulatory actions that are in one -- sorry, in one level or another of review, the first one being a regulation we have proposed that would set concentration levels for chemicals that are created through cooking or heat processing. And our first set of concentration levels are for acrylamide. We have internally completed the regulation and submitted it to the Office of Administrative Law for review. And we're hoping to have that approved and adopted within the next couple months.

We have also developed tailored warnings for cannabis and THC. There's actually four of them, depending on the way that a person would be exposed. And those are -- the regulatory process is complete on our side, and that has been submitted to the Office of Administrative Law for review also, hopefully to be

approved in the next few weeks.

We are also in -- still in the regulatory process for some changes to what we call the short-form safe harbor warnings for local exposures. This was part of our changes to the regulations back in 2016. We adopted a -- what we called a short-form warning that could be used on small packages, and parts, and things likes that. Over time, it became clear that that was being used on much larger packages, where the full warning could be included. And so we have proposed some changes to that regulation that would restrict the amount or the size of a label that could use the short form to -- currently that's proposed at 12 square inches and also a requirement that we -- that the business include the name of at least one chemical for which the warning is being given. And there's a slight change in the wording of the warning.

So we are currently in the second public comment period. We made some additional changes to the proposal and expect the comments to come in by mid-January, and then we can hopefully complete the process for that regulation within the next three or four months.

The last two are for, what we call, tailored warnings for exposures to particular chemicals. One is for exposures to acrylamide from foods. And it is a -- somewhat a departure from the way that we normally frame

our warnings for Prop 65, but it's partially in response to some litigation that I'll mention in a couple minutes in the federal courts about first amendment rights of businesses when the government is compelling commercial speech.

So we're trying to address some of the concerns that the appellate courts and the trial courts have identified for these two particular chemicals. Acrylamide is a little bit different in terms of what the concerns are. The argument is that acrylamide hasn't been shown to cause human cancer from any particular food and so the companies shouldn't have to provide a warning at all. We have changed our warning, as I said, to try and address the court's opinions, and we'll see what happens with that, but it has -- it was developed in response to the litigation.

The same thing for glyphosate, which is a pesticide that you may recall was listed some time ago and has been the subject of litigation for a long time and we have proposed a special tailored warning for consumer product exposures to glyphosate, again in response to some concerns that were articulated by courts.

So next slide.

NEXT SLIDE

CHIEF COUNSEL MONAHAN CUMMINGS: So the two cases

I had just mentioned that are in the federal court are the this National Association of Wheat Growers versus Bonta. It has to do with glyphosate warnings and First Amendment argument that the warnings should not be required, because it's only the IARC that has identified that chemical is causing cancer, and other agencies, including U.S. EPA, have said it is unlikely to be a human carcinogen.

And that is on hold currently, because we've proposed to adopt this special warning and the court is waiting to see if we complete that, and then to look at the new warning and see if it comports with the first amendment.

The same thing with Cal Chamber versus Bonta case. That case is on hold waiting for our regulatory process to be completed to see if the proposed warning will comply with what the court feels is needed to meet the First Amendment requirements.

This -- the case of Council for Education and Research on Toxics, or CERT, versus Starbucks has been in the courts of over 10 years. And most recently in the trial court, you may recall that we had adopted a regulation essentially saying that chemicals that are formed in coffee from the roasting and brewing of coffee don't require a warning under Prop 65, because there's some very special circumstances in this -- that's related

to the chemical mixture of coffee.

And so our regulation was actually used in this case as a defense, and was successful. So the CERT has appealed that decision to the court, and we have recently filed a brief in that case, amicus brief, defending our regulation and addressing some other issues. And so we'll see what happens in that case. It's still in the court of appeal.

Physicians for Responsible Medicine, this is a petition we had to list processed meats -- all processed meats under Prop 65 as carcinogens. We declined to list, and so there's a case pending in the trial court. And we're in the process of negotiating some discovery in that case. So it's pretty early to know how it's going to come out.

We actually resolved one case.

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: These take so long. But this one is the American Chemistry Council versus OEHHA. Many years ago, we had - I think it was in 2015 or earlier - we had listed bisphenol A for developmental tox -- as a developmental toxicant for a few days before we were ordered by the court to take it off the list. We litigated that case all the way up. In fact, the American Chemistry Council asked the State

Supreme Court to take the case and they declined. And so we were able to relist bisphenol A for developmental effects. It was already listed for female reproductive effects. So that's our current litigation. Does anybody have any question on either one of those?

Okay. Thank you.

CHAIRPERSON LUDERER: I don't see any questions. Thank you very much.

CHIEF COUNSEL MONAHAN CUMMINGS: Um-hmm.

SUMMARY OF COMMITTEE ACTIONS

CHAIRPERSON LUDERER: All right. So I'd next like to ask Dr. -- OEHHA Director, Dr. Lauren Zeise, to summarize the Committee actions today.

DIRECTOR ZEISE: Okay. So good afternoon. So the Committee found that perfluorononanoic acid, PFNA, and its salts has clearly been shown to cause male reproductive toxicity. The vote was six yes, two no, and so PFNA will be added to the Proposition 65 list.

The Committee declined to add perfluorodecanoic acid and its salts to the Proposition 65 list. It was a vote of five noes and three abstentions.

And then the Committee unanimously voted on the consent item to agree to the staff report on chemicals which have not been adequately tested as required to move -- remove one chemical from the list. And that is --

that are -- those are the Committee decisions.

And then just to close, I'd like to thank the public for attending the meeting. Much appreciated. We really appreciate it when you come and we also appreciate the input from the public earlier.

And then I'd like to also thank the Committee for your participation in this meeting. Really appreciate the extensive amount of time it takes to prepare for the meeting and then to take time out of your very, very busy schedules to come to the meeting. Really appreciate that. It's really a very great service to the people of California and so thank you so much for that.

And then I'd like to thank the staff for all the work to put this meeting on, to put the reports together, truly a huge amount of effort. So thank you very much to the OEHHA staff.

And then finally, I just want to wish everyone very Happy Holidays, and a healthy and safe -- healthy and safe holidays. Hope you get some time off to relax, and catch your breath, and wish you all the best, and we'll be seeing you in the new year.

And with that, I'll turn it back over to Ulrike.

CHAIRPERSON LUDERER: Thank you, Dr. Zeise. And I would really like to underscore and thank all the hard work that the staff put in to putting together this

documents for us.

(Applause.)

CHAIRPERSON LUDERER: It really makes our job that much easier, much easier. And I'd also like to wish everyone Happy Holidays to all the staff and also all the Committee members, and thank everyone for all their hard work.

So everyone take care and the meeting is adjourned.

(Thereupon the Developmental and Reproductive Toxicant Identification Committee adjourned)

CERTIFICATE OF REPORTER

I, JAMES F. PETERS, a Certified Shorthand Reporter of the State of California, do hereby certify:

That I am a disinterested person herein; that the foregoing California Office of Environmental Health Hazard Assessment, Developmental and Reproductive Toxicant Identification Committee was reported in shorthand by me, James F. Peters, a Certified Shorthand Reporter of the State of California, and thereafter transcribed under my direction, by computer-assisted transcription.

I further certify that I am not of counsel or attorney for any of the parties to said meeting nor in any way interested in the outcome of said meeting.

IN WITNESS WHEREOF, I have hereunto set my hand this 28th day of January, 2022.

JAMES F. PETERS, CSR, RPR
Certified Shorthand Reporter
License No. 10063