VIDEOCONFERENCE MEETING

STATE OF CALIFORNIA

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

DEVELOPMENTAL AND REPRODUCTIVE TOXICANT

IDENTIFICATION COMMITTEE

ZOOM PLATFORM

TUESDAY, DECEMBER 12, 2023

10:00 A.M.

JAMES F. PETERS, CSR
CERTIFIED SHORTHAND REPORTER
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APPEARANCES

COMMITTEE MEMBERS:

Ulrike Luderer, MD, PhD, MPH, Chairperson

Patrick Allard, PhD

Diana Auyeung-Kim, PhD

Laurence Baskin, MD

Carrie Breton, PhD, MPH

Suzan Carmichael, PhD

Aydin Nazmi, PhD, MSc

Isaac Pessah, PhD

Charles Plopper, PhD

STAFF:

Lauren Zeise, PhD, Director

Dave Edwards, PhD, Chief Deputy Director

Carolyn Nelson Rowan, Chief Counsel

Faye Andrews, PhD, Reproductive Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

Esther Barajas-Ochoa, Analyst, Proposition 65 Implementation Program

Erin Delker, PhD, Reproductive Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

Amy Gilson, PhD, Deputy Director, External and Legislative Affairs

APPEARANCES CONTINUED

STAFF:

Kannan Krishnan, PhD, Acting Deputy Director, Scientific Programs

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

Yassaman Niknam, PhD, Reproductive Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

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

Kiana Vaghefi, Environmental Scientist, Proposition 65 Implementation Program

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PROCEEDINGS

DIRECTOR ZEISE: Good morning, everyone. I'd like to welcome you to this year's meeting of the Developmental and Reproductive Toxicant Identification Committee. The meeting is being held virtually. My name is Lauren Zeise. I'm Director of the Office of Environmental Health Hazard Assessment, or OEHHA. OEHHA is a Department within the California Environmental Protection Agency and is the lead agency for the assessment of health risks posed by environmental contaminants.

So our main agenda item today for -- is the consideration of Bisphenol S, or BPS, for listing as a female reproductive toxicant under Proposition 65. After the BPS agenda item, the Committee will also take up a consent item on the section 2700 -- 27000 list of chemicals for which testing has been required, but is inadequate. This is different from the Proposition 65 list. And then for the final agenda item, staff will present updates on various Proposition 65 regulatory and other activities.

So we'll be taking a 45-minute break for lunch around noon and we'll take a short 15-minute break sometime in the afternoon.

This meeting is being recorded and transcribed.

The transcript will be posted on OEHHA's website.

So regarding public comment, there's going to be an opportunity for public comment on the bisphenol S item. People who wish to comment are asked to join the Zoom webinar. Information on how to join the Zoom webinar is shown on the slide. Go to bit.ly/registerdartic2023 without spaces. So that's how you register for today's webinar. And you'll receive a ink to join the webinar at the end of the registration process. If you provided a working email address, you'll receive an email within the link -- with the link.

So those of you joining by CalEPA webcast will be able to watch the meeting, but you do need to join the meeting by Zoom webinar to speak to provide comment. So when you're requested by the Chair, individuals would raise -- would queue to provide oral comment by using the raise hand function. It should be on the bottom of your screen. When your name is called to speak, you will unmute yourself and then comment. If you would like to present slides and you have not already sent them, please email them now to p65public.comments@oehha.ca.gov. So public comments will be limited to five minutes per commenter.

Okay. So with that item done, we'll move to introducing the DART -- the members of the Committee. So

I'm pleased to introduce the Committee members that are present. Dr. Irva Hertz-Picciotto is not able to join us today.

So Committee, as I introduce you, please turn on your camera, state your name, position, and affiliation.

So first, Dr. Patrick Allard.

COMMITTEE MEMBER ALLARD: Good morning, everyone. My name is Patrick Allard. I'm a professor at UCLA with a specialty in molecular biology and reproductive toxicology. Happy to be here.

DIRECTOR ZEISE: Thank you, Dr. Allard.

Dr. Diana Auyeung-Kim.

COMMITTEE MEMBER AUYEUNG-KIM: I'm Diana Auyeung-Kim. I'm the Executive Director at Genentech with a speciality in developmental and reproductive toxicology and reproductive and development.

DIRECTOR ZEISE: Thank you, Dr. Auyeung-Kim.

Dr. Laurence Baskin.

Hi, Dr. Baskin. You'll want to unmute. Can you?

COMMITTEE MEMBER BASKIN: Yes. Hi.

DIRECTOR ZEISE: Great.

COMMITTEE MEMBER BASKING: Laurence Baskin. I am from UCSF. I am chief of pediatric urology and a surgeon scientist with an interest in developmental congenital anomalies.

DIRECTOR ZEISE: Thank you, Dr. Baskin.

Dr. Carrie Breton.

COMMITTEE MEMBER BRETON: Hello. I'm Carrie Breton. I'm a professor in environmental health at USC in Los Angeles and I'm an environmental epidemiologist.

DIRECTOR ZEISE: Thank you, Dr. Breton.

Dr. Suzan Carmichael.

Dr. Carmichael, you'll want to unmute.

COMMITTEE MEMBER CARMICHAEL: Hi, everybody. I am a professor of pediatrics and OB/GYN at Stanford University and I am a perinatal epidemiologist.

DIRECTOR ZEISE: Thank you, Dr. Carmichael.

Our committee chair, Dr. Ulrike Luderer.

CHAIRPERSON LUDERER: Good morning, everyone. I'm Ulrike Luderer. I'm a professor of environmental and occupational health in the Program in Public Health at the University of California, Irvine. And my area of research is reproductive and developmental toxicology.

DIRECTOR ZEISE: Thank you, Dr. Luderer.

Dr. Aydin Nazmi.

COMMITTEE MEMBER NAZMI: Good morning, everyone. I'm Aydin Nazmi. I'm an epidemiologist and professor at Cal Poly San Luis Obispo.

DIRECTOR ZEISE: Thank you, Dr. Nazmi.

Dr. Isaac Pessah.

COMMITTEE MEMBER PESSAH: Good morning. Isaac Pessah, professor of toxicology emeritus, UC Davis.

DIRECTOR ZEISE: Thank you, Dr. Pessah.

Dr. Charles Plopper.

COMMITTEE MEMBER PLOPPER: Charles Plopper, professor emeritus at UC Davis, area of developmental and cellular toxicology.

DIRECTOR ZEISE: Thank you, Dr. Plopper.

So welcome, Committee. We really appreciate the time you're talking today to provide your advice and judgment at this meeting. So now I'd like to introduce the OEHHA staff and invite them also to turn their cameras on as I introduce them.

Dr. David Edwards, Chief Deputy Director.

DR. EDWARDS: Good morning.

DIRECTOR ZEISE: Okay. Welcome.

Carolyn Nelson Rowan, Chief Counsel.

CHIEF COUNSEL NELSON ROWAN: Hi. Good morning.

DIRECTOR ZEISE: Dr. Kannan Krishnan, Acting Deputy Director for Scientific Programs.

DR. KRISHNAN: Hello. Good morning.

DIRECTOR ZEISE: And now from the Reproductive and Cancer Hazard Assessment Branch, Dr. Martha Sandy, Branch Chief.

DR. SANDY: Good morning.

DIRECTOR ZEISE: Dr. Francisco Moran, Section Chief of the Reproductive Toxicology and Epidemiology Section.

DR. MORAN: Good morning.

DIRECTOR ZEISE: And now I'll introduce staff from Dr. Moran's section that will be presenting today.

Dr. Faye Andrews.

DR. ANDREWS: Good morning.

DIRECTOR ZEISE: Dr. Erin Delker.

DR. DELKER: Good morning, everyone.

DIRECTOR ZEISE: And Dr. Yassaman Niknam.

DR. NIKNAM: Good morning, everyone.

DIRECTOR ZEISE: Okay. And now from our Office of External and Legislative Affairs and Proposition 65 Implementation Program, Dr. Amy Gilson, Deputy Director for External and Legislative Affairs.

DR. GILSON: Thanks for joining.

DIRECTOR ZEISE: Kiana Vaghefi, environmental scientist.

MS. VAGHEFI: Hello.

DIRECTOR ZEISE: Ester Barajas-Ochoa, analyst.

MS. BARAJAS-OCHOA: Good morning.

DIRECTOR ZEISE: And now I'd like to turn it over to Carolyn Rowan for some introductory remarks about Bagley-Keene and other legal issues related to

participation in this virtual meeting of the Committee.

CHIEF COUNSEL NELSON ROWAN: Good morning. Thanks Lauren. I just have a few reminders for the group before we get underway today. First, a reminder that the Bagley-Keene Act applies to this meeting. So please remember that all discussions and deliberations for this group need to be conducted during the meeting, not on breaks, at lunch, or with individual members of the Committee, and that's on or offline. So it includes phone, email, chat and text messages.

Next, the charge for this Committee has to do with listing -- with the listing of chemicals for purposes of Prop 65. And the Governor appointed each of you to serve as the State's qualified experts, because of your scientific expertise regarding the reproductive toxicity of chemicals.

You've been provided with a copy of the listing criteria that will guide your decisions today and a reminder that the decision to list is evidence based. And the standard is whether the chemical has been clearly shown through scientifically valid testing, according to generally accepted principles to cause reproductive toxicity.

So sometimes your comments you might hear information that goes to the impact of a particular

listing, for example whether or not a warning might be required or about impacts on certain sectors of the economy. And while that information is helpful in the general sense, it's not part of the criteria for this committee.

The Committee is allowed to and often does make decisions based entirely on animal evidence. The chemical that you are considering need not have been shown to be a human reproductive toxicant and you don't need to have information about whether or not human exposures to the chemical are sufficiently high enough to cause reproductive toxicity in order to list a chemical.

If you need more time to think about the evidence or discuss it further before making a decision. There's no requirement that you make a decision today. So please feel free to ask me or any other OEHHA staff clarifying questions during the meeting. And if we don't know the answer, we will do our best to find it and get back to you.

I'll be here the whole time. If I do have to step away for any reason, Staff Counsel Kristi Morioka will cover for me. There will always be an attorney here if you have any questions.

Any questions at this point?

Okay. Great. I'll pass it back to Lauren.

Thank you.

DIRECTOR ZEISE: Thank you, Carolyn.

Now, I'll turn the meeting over to our Committee Chair, Dr. Luderer.

CHAIRPERSON LUDERER: Great. Thank you, Lauren and Carolyn. And good morning and welcome to all the Committee members and all the members of the public who are joining today. We're now ready to move to the main agenda item for today, which is consideration of bisphenol S as known to the State to cause reproductive toxicity based on female reproductive toxicity.

So the first agenda item is going to be a staff presentation by Dr. Francisco Moran, Chief of the Reproductive, Toxicology, and Epidemiology Section. And so Dr. Moran, I'd like to turn the floor and the screen over to you.

(Thereupon a slide presentation).

DR. MORAN: Well thank very much, Dr. Luderer.

Let me start my sharing my

Okay. I hope you're seeing the full slides or -- not the notes, yes?

DR. SANDY: No.

DIRECTOR ZEISE: Dr. Moran, we're not seeing them in presentation mode.

DR. MORAN: Okay. One second. I'll change it.

DR. SANDY: That looks good.

DR. MORAN: And now, I don't see it. Well, you see it well, right? Okay.

Well, good morning. Let me provide some background on the process of which bisphenol S, or BPS for short, was brought to you today.

BPS was brought to the DARTIC for consultation and prioritization in 2020, and this Committee recommended that BPS be placed in a high priority group for future listing considerations. OEHHA selected BPS for consideration for listing, and in March 2022, OEHHA solicited from the public information relevant -- relevant information to the assessment of developmental and reproductive toxicity.

Information received at that time has been reviewed and considered by OEHHA in the course of preparing the October 2023 hazard identification document on BPS, which is focused on the evidence of female reproductive toxicity.

This document was released for public comments. No public comments were received.

The document and the references cited within it were provided to the DARTIC for consideration in the Committee's deliberations at today's meeting.

NEXT SLIDE

DR. MORAN: Here is the outline for today's presentation. Due to time constraints, this presentation is a brief overview and will not cover every finding discussed in the hazard identification document. 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 the chemical structure for BPS and its structural analogy with BPA.

NEXT SLIDE

DR. MORAN: BPS is used as a color developer in thermal paper, to make some types of hard plastic, and synthetic fibers for clothing and other textiles. BPS may be -- also be used to make colors last longer in some fabrics and it is a common replacement for BPA.

As reported by the U.S. Environmental Protection Agency, the national BPS aggregated production volume ranged between one million and 10 million pounds per year. Some increases in BPS productions are associated with regulations on BPA. For example, in 2011, California passed a law that banned the use of BPA in baby bottles. And this was followed by a similar ban by the FDA in 2013. Since that time, manufacturers have been gradually replacing BPA with BPS in consumer products.

As such, exposure to BPS has increased. BPS has been detected in cash register receipts, food, personal care products, and environmental samples. Studies from Biomonitoring California show detection frequencies between 64 and 77 percent in the California study.

NEXT SLIDE

DR. MORAN: Using a systematic approach, we conducted literature searches on the developmental and reproductive toxicity of BPS. In addition, there was a data call-in from March 4 to April 18, 2022. The last comprehensive search was in July 2023. We used HAWC, the Health Assessment Workspace Collaborative, as a tool for multi-level screening of literature search results. For this document, we focused on literature relevant to female reproductive toxicity.

NEXT SLIDE

DR. MORAN: Absorption of BPS by the oral route is rapid. Dermal absorption is slower compared to the oral route. It is distributed throughout the body and likely undergoes enterohepatic circulation. It has been detected in human cord blood, amniotic fluid, breast milk, and placenta. In rat, it has been reported in several tissues with consistently high concentrations in the liver and kidney. BPS undergoes metabolism by conjugation, both glucuronidation and sulfation, and by hydroxylation, after

oral and dermal exposure in animals and humans. It is excreted in urine in animals and humans exposed via the oral and dermal routes. It is also excreted in the bile, feces, and breast milk. The half-life in humans has been reported as approximately seven to nine hours and in rodents half-life ranges from three to 12 hours.

NEXT SLIDE

DR. MORAN: Now I will present the evidence on BPS and Female Reproductive Toxicity from studies in animals.

NEXT SLIDE

DR. MORAN: I will discuss the findings in animals exposed to BPS in this order: effects on the ovary, uterus, hormones, reproductive performance, mammary gland development, and puberty onset.

NEXT SLIDE

DR. MORAN: But first, I would like to start with a brief description of the biology involved in follicle and oocyte maturations in the ovary. Here is a diagram summarizing the events during follicle development, which starts with the germ cells -- germ cells in a nest or cyst and the breakdown of the germ cell nest starting just after birth in mice and in the second trimester in human pregnancy.

The primordial follicles that have been -- have

three possibilities: follicular atresia, maintenance of primordial status or transition to growth phase. Alterations in the process of germ cell nest breakdown include increased loss of germ cells by formation of multi-follicle oo -- multi-oocyte follicles or germ cell death by apoptosis.

NEXT SLIDE

DR. MORAN: And here is the oocyte and follicle development process from the primordial to the antral follicle ready for ovulation. This process take about three weeks in mice and about four months in humans. And on the lower panel, there is a schematic representation of the oocyte going through the first meiotic division, a process that takes about 12 hours in mice and around 36 hours in humans.

NEXT SLIDE

DR. MORAN: Now, I will present the effects of BPS in the ovary. Before I continue, I would like to mention that most of the effects reported here on the female reproductive toxicity of BPS are dependent on the doses, the time and duration of the exposure, and the timing of assessment. Most studies used the oral route of exposure. Here, I will indicate the studies using other routes in italics next to the citation as needed. Finally, all data presented are statistically significant

unless otherwise described.

NEXT SLIDE

DR. MORAN: BPS has effects on ovarian structures including germ cells, follicles, granulosa cells, and corpora lutea, and on oocytes including meiotic progression and structural damage, such as spindle malformations.

NEXT SLIDE

DR. MORAN: Alterations in the -- BPS effects in the ovarian follicle development include: alterations in the timing of germ cell nest breakdown in several studies in mice; decrease in the number of primary, secondary, and antral follicles in mice and hamsters and decrease in preovulatory follicles in chickens; increased number of secondary follicles in mice exposed at a higher dose; and increased number of atretic follicles in mice, rats, and hamsters.

NEXT SLIDE

DR. MORAN: In the ovary there was a decreased number of granulosa cell layers in mice and a decreased number of corpora lutea in rats and hamsters.

NEXT SLIDE

DR. MORAN: BPS effects on oocytes in mice include: accelerated meiotic progression, that is the distribution of oocytes in different stages of meiosis was

altered compared to controls; and increased number of abnormal oocytes, damaged oocyte structure, and chromosome spindle damage and spindle malformations.

NEXT SLIDE

DR. MORAN: Ovarian effects in Zebrafish include effects on oocytes, such as alterations in oocyte maturation, and increased oocyte degeneration, also reduced gonadosomatic index was observed in females treated either as adults or as embryos.

NEXT SLIDE

DR. MORAN: Now, I will present the effects of BPS in the uterus.

NEXT SLIDE

DR. MORAN: BPS in the rodents -- in the rodent uterus include morphometric changes, such as narrowing of the uterine cavity, reduced endometrial area, and increased number of uterine glands in mice, and histological effects such as the presence of squamous metaplasia and increased cell vacuolization in rats. There was alteration in relative uterine weights in rats at different doses.

NEXT SLIDE

DR. MORAN: Now, I will present the effect on the endocrine system.

NEXT SLIDE

DR. MORAN: BPS effects on the endocrine system including decreased levels of gonadotropins in rats, mice, and hamsters. And in zebrafish, BPS exposure resulted in down-regulation in the expression of the gonadotropin releasing hormone, or GnRH, and follicle stimulating hormone subunit beta genes in zebrafish brain tissue. Changes in progesterone levels in several animal models were mixed in direction depending on the dose, time of exposure, and the time of assessment. There was an increase in progesterone receptor expression in the mammary gland in mice exposed during gestation and lactation.

NEXT SLIDE

DR. MORAN: There was a decrease in estradiol level in serum in mice and hamsters, in plasma in rats and ewes, and in urine in mice at several BPS doses, while other studies observed increased serum estradiol level in mice. There was an increase in estrogen receptor alpha expression in the mammary gland in mice.

NEXT SLIDE

DR. MORAN: In zebrafish, there was an increase in plasma and whole body estradiol levels, as well as an increased vitellogenin, which is a marker for estrogenic activity. Decreased estrogen receptor alpha and vitellogenin messenger levels were also reported.

NEXT SLIDE

DR. MORAN: Among the effects of BPS on androgens, there were increased serum testosterone levels reported in three studies, two with gestational exposure and assessed on postnatal day 28 or at nine months, and one with exposure from birth to postnatal day 60, and increased plasma testosterone levels in rat was reported in two studies. While in one of the gestational exposure studies in mice, there was a decrease in testosterone level on postnatal day 35 after exposure to a relatively higher BPS dose.

NEXT SLIDE

DR. MORAN: Now, I will present the effects on reproductive performance.

NEXT SLIDE

DR. MORAN: BPS effects on reproductive performance were reported in various animal models. The rodent estrous cycle is a well-characterized four-to-five-day cycle that includes key events such as the preovulatory gonadotropin surge during proestrus. Irregular estrous cyclicity in mice, after exposure during gestation, resulted in several days in estrus and diestrus at all doses tested in mice. And in adult rats after gestational and lactational exposure, there were longer estrous cycles with increases in the number of days in

diestrus.

Other effects includes: reduced fertility in rats; decreased fertilization at 10 micrograms per day and increased fertilization at 100 kilograms -- micrograms kilogram per day in mice exposed during puberty; decreased in vitro fertilization rate of oocytes from in vivo treated female mice and decreased blastocyst development rates were also observed; there was also decreased mean number of implantation sites in rats; and in a separate study, an increased rate of post-implantation loss in female rats exposed from premating to parturition and beyond.

NEXT SLIDE

DR. MORAN: BPS effects on placenta include: a decreased ratio of spongiotrophoblast to giant cells area in mice treated for two weeks prior to mating and through gestational day 12.5; a Non-significant increase in placental weight at gestational day 120; and no effects on other placenta parameters in sheep treated with BPS by a subcutaneous injection from gestational day 30 to 100.

NEXT SLIDE

DR. MORAN: BPS on reproductive performance were also reported in non-mammalian species. In zebrafish, there was a decreased number of eggs during the seven-day spawning period and lower hatching rate and there was an

increase in time to hatching of embryos. Another study reported altered female spawning behavior. In C. elegans, BPS exposure was associated with a dose-dependent increase in embryonic lethality and a decrease in brood size.

NEXT SLIDE

DR. MORAN: Now, I will present the effects of BPS on Mammary gland development. Gestational and lactational exposure to BPS in mice resulted in alterations in the development and retention of terminal end buds, development of alveolar buds, and other effects on mammary gland cell proliferation and growth.

NEXT SLIDE

DR. MORAN: Specifically for growth of terminal end buds there was a dose-dependent increase in the number of terminal end buds on postnatal day 20 and at three months with no effects at puberty. Larger terminal end buds area increased average size of terminal end buds and increased terminal end bud-like structures.

NEXT SLIDE

DR. MORAN: BPS effects on mammary glands alveolar -- bud in mice include an increased number of alveolar bud and increased incidence of intraductal hyperplasia, increased incidence of mixed cell inflammation at three and 14 months of age, and non-neoplastic lesions, and lobuloalveolar hyperplasia.

NEXT SLIDE

DR. MORAN: Other effects -- other effects of early life exposure to BPS on mammary gland development in mice include: decreased mammary gland cell proliferation on postnatal day 24 and increased cell proliferation in adulthood at the same dose; there was a decrease in ductal area; increases in the mammary gland developmental score on postnatal day 20, 35, and 56 at various doses; and reduced volume of lobules and increased volume of adipose tissue.

NEXT SLIDE

DR. MORAN: Now, I will present the effects of BPS on puberty onset.

NEXT SLIDE

DR. MORAN: BPS exposure during gestation in mice resulted in delayed puberty onset at 20 micrograms per kilo per day and earlier puberty onset at a lower dose of 0.5 micrograms per kilo per day. There was a delayed puberty onset was observed in rats exposed perinatally while other studies with similar dosing strategies reported no effects on pubertal timing.

This is the end of this section. Thanks

NEXT SLIDE

DR. MORAN: Now, Dr Yassi Niknam will present the relevant mechanistic data.

DR. NIKNAM: Thank you Dr. Moran. In this section, I will summarize the findings from in vivo and in vitro mechanistic studies of BPS. I will focus on findings relevant to BPS effects on ovarian development and maturation of oocytes, effects on the placenta, and effects on the endocrine system.

NEXT SLIDE

DR. NIKNAM: In vivo mechanistic data relevant to ovarian development and maturation of oocytes include changes in chromosome alignment and spindle formation in mice and C. elegans. Alterations in the expression of genes related to ovarian development and oocyte maturation in mice, chickens, and zebrafish were also reported. Other effects such as oxidative stress in rodent ovaries and apoptosis in mouse oocytes and the germline of C. elegans, and epigenetic effects in mice such as altered histone and DNA methylation in oocytes were observed.

In addition, there were changes in signaling pathway genes in mouse oocytes, including Notch2 and Jagged1, which are important in germ cell nest breakdown and primordial follicle assembly.

Lastly, lipid profiles were altered in zebrafish ovaries affecting lipids which are important in providing energy for oocyte development.

NEXT SLIDE

DR. NIKNAM: In vitro studies of oocyte maturation demonstrated abnormal germ cell nest breakdown in cultured newborn mouse ovaries incubated with BPS. This was blocked by co-incubation with tamoxifen, a selective estrogen receptor modulator with the potential for both ER agonism and antagonism, which suggests an estrogenic mode of action for BPS.

In pig ovary cumulus oocyte complexes, or COCs, incubated with BPS, there was a concentration-dependent decrease in oocytes reaching metaphase 1 and a failure to resume meiosis, with no effect on viability.

NEXT SLIDE

DR. NIKNAM: Exposure to BPS in vitro caused alterations in spindle morphology and chromosome alignment in cow metaphase 2 oocytes without altering the proportion of oocytes entering metaphase 2. In pig ovary cumulus oocyte complexes, there were alterations in alpha tubulin assembly with a number of consequences, including concentration-dependent decrease in the number of tubulin filaments, retention of oocytes in the germline vesicle stage, arrest in metaphase 1, and spindle disorganization at all concentrations.

NEXT SLIDE

DR. NIKNAM: BPS also had effects on follicular cell communication and proliferation. The figures on this

slide show the relationship between theca and granulosa cells and the oocyte in follicles at different stages of development. In human and sheep primary ovarian theca cell cultures, BPS caused an increase in theca cell gap junction intercellular communication. Additional experiments indicate that BPS modulates gap junction intercellular communication in these cells through the MAP kinase pathway and partially through the PC-PLC pathways. These pathways are important for proper cell proliferation. In adult sheep ovarian granulosa cells, BPS caused a decrease in cell proliferation. BPS increased Cx37 mRNA levels in cultured cow ovary cumulus cells, but not in cumulus oocyte complexes. Cx37 is one of the connexins that regulates gap junctional cellular communication.

NEXT SLIDE

DR. NIKNAM: In vivo mechanistic data on the effects of BPS in the placenta come from studies in mice and sheep. In mouse placentae, BPS caused alterations in the expression of several genes, including calmodulin, which plays an important role in calcium signaling. In the same study, there were changes in fatty acid levels and alterations in neurotransmitters that control autocrine and paracrine signaling in the placenta. In sheep placentae, BPS caused alterations in the level of

proteins related to the proper function of placentomes, in binucleate cells, which are important in proper maternal circulation and feto-maternal exchange, and in levels of fusogenic genes that are important in placental cell fusion.

NEXT SLIDE

DR. NIKNAM: In vitro mechanistic studies utilized the placental human cell line HTR-8/SVneo, derived from extravillous trophoblast cells and the human trophoblastic 3A placental cell line CRL-1584. In BPS-exposed HTR cells, there was an increase in cell proliferation, which was mediated through the ER and ERK1/2 pathways. Inhibition of ERK1/2 phosphorylation by BPS induced secretion of the inflammatory cytokines, IL-6 and IL-8.

In the CRL cell line, BPS altered ABCB1 promoter activity. ABCB1 encodes for an important placental transporter, P-glycoprotein. The P-glycoprotein transporter extrudes its substrates from the trophoblasts back into the maternal circulation protecting the fetus from xenobiotics.

NEXT SLIDE

DR. NIKNAM: Relevant in vivo data on endocrine system effects include altered levels of hormones after BPS exposure in various species. Here is a brief summary

of what was previously presented in detail by Dr. Moran.

NEXT SLIDE

DR. NIKNAM: In vitro studies demonstrated decreases in progesterone in human and sheep granulosa cells, increase in progesterone in human -- in the human-derived -- human adrenal-derived cell line H295R and no changes in progesterone secretion in cow theca cells. Androgen levels were altered in human H295R cells, including decreases in testosterone, androstenedione, and DHEA. However, there were no changes in androstenedione secretion in cow theca cells.

NEXT SLIDE

DR. NIKNAM: Estradiol was decreased in human and some sheep granulosa cell studies; increased in cow and some sheep granulosa cell studies; and, in a study with human H295R cells, there were no changes in estradiol or estrone levels.

NEXT SLIDE

DR. NIKNAM: As presented earlier, BPS induced changes in steroid hormone receptor expression in vivo. These effects included increased progesterone and estrogen receptor alpha expression in mammary glands of mice. There was also a decrease in ER alpha mRNA levels in zebrafish.

NEXT SLIDE

DR. NIKNAM: BPS affected gene and protein expression of the estrogen receptor in vitro. There were decreases in mRNA expression levels of ER alpha in oocytes and ER beta in pig ovary cumulus-oocyte complexes. However, ER alpha and beta protein levels were increased in pig oocytes. ER alpha and beta mRNA and protein levels were increased in mouse ovaries.

In addition, ER beta mRNA levels were increased in human Ishikawa cells without an increase in ER alpha levels. In sheep granulosa cells, ER alpha and beta gene expression were increased. In cow cumulus-oocyte complexes, there was an increase in anti-Mullerian hormone receptor mRNA levels.

NEXT SLIDE

DR. NIKNAM: A set of key characteristics that are frequently exhibited by exogenous agents that cause female reproductive toxicity and another set that are exhibited by endocrine disrupting chemicals have been identified. 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 SLIDE

DR. NIKNAM: Here are the KCs of female reproductive toxicants. In the document, we have also presented KCs of endocrine disrupting chemicals. We will be focusing on four KCs of female reproductive toxicants with examples of relevant data for each.

NEXT SLIDE

DR. NIKNAM: KC 1 alters hormone receptor signaling; alters reproductive hormone production, secretion, or metabolism.

Over 40 in vivo and in vitro studies presented in the document report findings relevant to KC1. These include changes in gonadotropins, progesterone, estradiol, and testosterone and other androgens, and alterations in mRNA and/or protein expression of receptors for estrogen, androgen, and progesterone.

NEXT SLIDE

DR. NIKNAM: KC2. Chemical or metabolite is genotoxic. In pig ovary cumulus-oocyte complexes, BPS treatment resulted in spindle disorganization, chromosome misalignment, and increased aneuploidy in oocytes.

NEXT SLIDE

DR. NIKNAM: KC8 Alters direct cell to cell interactions. In human and sheep primary ovarian theca cell cultures, BPS increased theca cell gap junction

cellular communication. In cow ovary cumulus cells, there was an increase in connexin 37 mRNA expression, while in cultured mouse ovaries, there was abnormal germ cell nest breakdown. This process involves cell-to-cell interactions mediated through the ER and JNK pathways and cell adhesion proteins such as E-cadherin.

NEXT SLIDE

DR. NIKNAM: And lastly, KC10, alters microtubules and associated structures. In cow oocytes, there were alterations in spindle morphology and chromosome alignment. In pig ovary cumulus oocyte complexes, there was a decrease in the number of tubulin filaments, and in mice, there was incidence of spindle malformation. There was spindle disorganization and chromosome misalignment in oocytes from pig ovaries and in C. elegans.

Now, we will break for clarifying questions from the DARTIC members.

Thank you.

NEXT SLIDE

CHAIRPERSON LUDERER: Thank you very much Dr. Moran and Dr. Niknam for those presentation. I will look for raised hands from any of the panel members who may have questions.

Let's see. I'm not seeing any raised hands at

the moment. So not seeing any clarifying questions from the Panel, I will then turn the presentation back to Dr. Moran who will introduce our next presenter.

NEXT SLIDE

DR. MORAN: Yes. Okay. Thank you, Dr. Luderer. Now, DR Faye Andrews will present data on female reproductive outcomes examined in epidemiologic studies of BPS.

Dr. Andrews

DR. ANDREWS: Hi, everyone. My name is Faye, as Dr. Moran introduced me. I'm one of the reproductive epidemiologists on the team and I'll be walking you through the growing body of research of reproductive outcomes examined in epidemiological studies of BPS.

NEXT SLIDE

DR. ANDREWS: This figure shows the number of studies published by year for BPS. The previously discussed animal and mechanistic studies, shown in gray, were published from 2013 to 2023. Overall, studies on associations between BPS and human outcomes, shown in blue, are more recent. The first human study was published in 2018 and the evidence base is growing. Over half of the included human studies were published between 2020 and 2023.

NEXT SLIDE

DR. ANDREWS: Before discussing the results of the emerging research on epidemiologic studies, we'd like to note several points.

We included studies that used cross-sectional, cohort, and case-control study designs. Across studies, limits of detection varied for BPS. Similarly, the proportion of samples with detectable levels of BPS ranged widely, from as low as 15 percent in one study to greater than 95 percent in others. This resulted in different ways researchers analyzed BPS exposure, sometimes continuous or categorical, limiting comparability between studies.

Almost all studies included measurement of multiple bisphenols, not just BPS, and on several occasions, other chemical exposures were measured. We noted in the written document when mixture analyses were used and when correlations between BPS and other bisphenols were reported. We note here that correlations between BPS and other bisphenols were generally low, in the range of 0.1 to 0.3.

NEXT SLIDE

DR. ANDREWS: I'm going to take a step back and show in this graph the percent of samples with BPS detected in epidemiologic studies we reviewed.

On the Y axis, each individual study is listed in

the order of sample collection dates with the most recent sample collection dates at the top of the graph and the oldest at the bottom. We have also listed on the Y axis the limit of detection in nanograms per milliliter for each study.

On the X axis is the percentage of samples, either serum or urine, with detectable levels of BPS, which varied across studies. The limit of detection, or LOD, which is listed here, for BPS varied across studies from as low as 0.002 to as high as 0.20. Three out of the 23 studies had less than 30 percent detection frequency, where almost -- most studies were above 70 percent detection frequency.

This blue arrow is indicating the introduction of BPA regulations globally beginning in 2011. As manufacturers have phased out BPA, BPS has become more common, likely due to its replacement of BPA in some consumer products.

NEXT SLIDE

DR. ANDREWS: There were a wide range of female reproductive outcomes examined in the included studies.

Outcomes examined in two or more studies included gestational diabetes, polycystic ovary syndrome, or PCOS, thyroid hormones measured during pregnancy, and sex steroid hormones measured during pregnancy and in young

females.

There were a number of other female reproductive outcomes examined in one study each.

NEXT SLIDE

DR. ANDREWS: First trimester BPS exposure was associated with gestational diabetes in two studies and with fasting plasma glucose in one study. The first prospective cohort study from China reported higher odds of gestational diabetes with tertile 2 BPS exposure compared to tertile 1. Similar direction and magnitude of association between the highest and lowest tertile of exposure was observed, although this estimate was not statistically significant. Associations were stronger for those pregnant people with a BMI greater than or equal to 23 kilograms per meter squared or for pregnancies with a female fetus.

Next, a nested case-control study in California reported higher odds of gestational diabetes for tertile 2 and tertile 3 of BPS exposure compared to tertile 1 exposure. This study stratified by race/ethnicity and reported that associations were strongest for those who identified as non-Asian/Pacific Islander, which included the race/ethnicities of White, Black, Hispanic or other. No elevation of odds with changes in BPS exposure were observed for those who identified as Asian or Pacific

Islander.

Finally, here we discuss -- finally, a prospective cohort in China reported no significant associations between BPS and gestational diabetes, but researchers did report BPS was associated with higher fasting plasma glucose and that was stronger for pregnancies with a female fetus.

NEXT SIDE

DR. ANDREWS: Here we will discuss two case-control studies of PCOS and the associations with BPS.

First, a case control study in China reported higher odds of PCOS, first with continuous BPS as the per unit increase in BPS was associated with higher odds of PCOS. Similar associations and a dose response was seen when BPS exposure was analyzed in quartiles.

Second, a case-control study in Poland reported analyses stratified by tertiles of BPS exposure. This study did not report the rationale for analyzing associations between BPS and PCOS within tertiles of exposure, and the distribution of PCOS cases across tertiles was not reported, making the data from this study more difficult to interpret.

Within the first tertile of exposure, BPS was analyzed as a continuous variable, and the per unit

increase in BPS was associated with higher odds of PCOS. Similar direction and magnitude of association was seen in tertiles 2 and 3, although these estimates had wide confidence intervals and were not statistically significant.

NEXT SLIDE

DR. ANDREWS: Five studies examined thyroid hormones during pregnancy, usually measured during early pregnancy, and associations with BPS. Many of the same thyroid hormones were measured across studies with multiple comparisons. Generally, findings were mixed with regard to the direction of associations and the statistical significance of associations.

NEXT SLIDE

DR. ANDREWS: A prospective cohort study in Puerto Rico reported higher levels of BPS in pregnancy was associated with lower corticotropin releasing hormone, and with no associations observed for sex hormone binding globulin or SHBG, estriol, progesterone, or testosterone.

A case-control study located in China reported that women in the control group, or in other words women who did not have PCOS, BPS was associated with higher levels of testosterone.

There were two cross-sectional studies using NHANES data from 2013-2016 of female girls and

adolescents. Wang et al. 2021 reported quartile 2 vs quartile 1 BPS associated with higher testosterone to estradiol ratio and non-linear associations for free androgen index and SHBG and BPS, respectively.

Neither studies reported associations with BPS and other sex steroid hormones.

NEXT SLIDE

DR. ANDREWS: Now, I'll summarize recent publications from 2020 to 2023 of other female reproductive outcomes, each of which has been examined in one study each.

A cross-sectional study measuring antimüllerian hormone, or AMH, and diminished ovarian reserve at an infertility clinic reported lower AMH and higher odds of diminished ovarian reserve for women with higher levels of BPS.

A cohort study reported lower gestational weight gain associated with higher levels of BPS.

A case-cohort study of Black women reported women who were fibroid free at baseline had lower risk of uterine fibroids with increased levels of BPS over 60 months of follow-up. Alternatively, those with existing fibroids at baseline had a 4.1 percent increase in existing fibroids with higher levels of BPS exposure.

A cohort study of female adolescents reported

delayed onset of menstruation associated with higher levels of BPS exposure.

And lastly, a case-control study reported higher odds of unexplained recurrent miscarriage and higher BPS exposure, which was stronger for those women over 30 years old.

There were several other outcomes examined in one study each, though these were not associated with BPS. These included maternal kisspeptin levels, time to pregnancy, gestational hypertension, endometriosis, and risk of infertility.

This concludes the presentation on human studies of BPS.

NEXT SLIDE

DR. ANDREWS: I'll move forward to the summary of evidence for BPS on the ovary.

As stated earlier, the effects of BPS are dependent on the doses, the timing, and the duration of the exposure and timing of assessment. Exposure to BPS resulted in alterations in germ cell nest breakdown and subsequent follicle development. In addition, there were effects in the number of granulosa cell layers and the number of corta -- corpora lutea. There were accelerated meiotic progression and damage to the oocyte including spindle malformations.

Mechanistic data included observations of altered tubulin assembly, spindle malformations, chromosome misalignment, and aneuploidy. Effects on the follicular cell interactions and proliferation were also reported.

In humans, higher BPS levels were associated with higher odds of diminished ovarian reserve in one small study and PCOS in two studies.

NEXT SLIDE

DR. ANDREWS: Here is a summary of the evidence for the effects of BPS on the uterus and placenta.

BPS effects the rodent uterus -- excuse me. Let me start that again. BPS effects in the rodent uterus include morphometric changes, such as reduced uterine cavity and endometrial area. There were increased numbers of uterine glands in mice and histological alterations such as squamous metaplasia and increased cell vacuolization in rats and the effects on uterine weights in rats at different doses. There were decreased implantation sites, increased post-implantation loss, and altered ratio of placental cell types in mice.

Mechanistic data included changes in placental gene and protein expression, including those involved in autocrine and paracrine signaling

In humans higher BPS exposure was associated with higher odds of recurrent miscarriage, increased growth of

existing uterine fibroids; and no associations with endometriosis, time to pregnancy, or risk of infertility.

NEXT SLIDE

DR. ANDREWS: And finally, here is a summary of the evidence for the effects of BPS on hormone levels and hormone receptor expression. These include a decrease in gonadotropins in various animal models, and changes in progesterone levels in rats, mice, sheep, hamsters, and zebrafish.

There were decreased estradiol levels in mice, rats, sheep, and hamsters, while other studies observed increased serum estradiol levels in mice at different times and doses. There were also increased testosterone levels in mice and rats and a decrease in testosterone levels in mice at relatively higher doses. There were also effects on gene and/or protein expression of several hormone receptors.

No associations between BPS exposure and progesterone or estradiol in humans were seen, although there were changes noted in AMH, which is associated with ovarian reserve, and corticotrophin-releasing hormone. There were inconsistent thyroid hormone associations and higher testosterone in adult women in one study. Two studies reported higher odds of gestational diabetes within specific groups of their populations and one study

reported the delay in onset of menstruation.

NEXT SLIDE

DR. ANDREWS: Thank you very much. This concludes our presentation.

CHAIRPERSON LUDERER: Thank you very much, Dr. Andrews, and Dr. Niknam, and Dr. Moran. We now have time for clarifying questions from the members of the Committee. So please raise your hand if you have any clarifying questions and I will call on those who raise their hands.

I am not seeing any raised hands.

Oh, Dr. Baskin, you have the floor.

COMMITTEE MEMBER BASKIN: Fantastic presentation and thank you for the accuracy and the thoroughness. Just for clarification, all these studies which is our preview relate to kind of developmental issues, and we don't really touch on cancer at all. And I'm wondering in your research whether there was any cross-over, whether you saw studies that related both to cancer as well as developmental problems?

CHAIRPERSON LUDERER: And you're referring to epidemiologic studies specifically, Dr. Baskin, or any of the studies?

COMMITTEE MEMBER BASKIN: Well, also some of the animal studies too when you -- there seems to be a bit of

cross-over. And it seems like it was nicely kind of partitioned out.

DR. MORAN: Right. Let me start by answering in our systematic literature search, we concentrate, you know, on the developmental and reproductive search as keywords in the search. So normally, we don't get cancer -- much cancer data, but from now and then, there are some publications that I cannot retrieve. I cannot say much now, but it is not in our -- it's not in the result of our search.

COMMITTEE MEMBER BASKIN: Thank you.

DR. MORAN: Yes.

CHAIRPERSON LUDERER: Other questions from Committee?

Dr. Sandy, did you want to comment on that as well?

DR. SANDY: Yes, just to expand on what Dr. Moran has said. We did not see in the literature that came up in our -- in the search any reporting of cancer in either the animal studies or the epidemiologic studies.

CHAIRPERSON LUDERER: And this is maybe a clarifying question for the staff. I assume that the search is set up the way it is because that would be more under the purview of the Carcinogen Identification Committee, is that correct?

DR. SANDY: That's correct.

DR. MORAN: Yes.

CHAIRPERSON LUDERER: Thank you.

Any other questions from Panel members or comments from staff?

All right, not seeing any.

Then we'll now then move on to hear from the individuals designated as initial discussants and so we're going to start with the epidemiology studies, then move on to the animal studies, and then finally the mechanistic studies.

So for the epidemiology studies, we have Drs. Breton, Carmichael, Baskin and Nazmi. And so we will go in that order start -- beginning with Dr. Breton.

COMMITTEE MEMBER BRETON: Okay. So just to clarify, since I'm starting this off, you just want me to review sort of my thoughts and opinions of these various studies?

CHAIRPERSON LUDERER: Yes.

COMMITTEE MEMBER BRETON: Okay. Under the categories. So I'll do that.

I think I just want to -- I'll just start by saying, you know, it was a very nice epidemiologic review. And just as a reminder, there were a total of 23 epi studies looking at the effects of BPS on the female

reproductive system.

I think I will start by talking about the studies that looked at BPS in association with gestational diabetes. These were three different studies and they all, in general, found supportive evidence of an association for higher risk of GDM or GDM-related biomarkers, in association with BPS. And in some cases, these effects actually might -- were found to be exacerbated in sensitive subgroups including obese women, pregnancies with a female fetus, and in non-Asian races.

And so I wanted to just speak to a couple points about each of the studies themselves, because I think there were some consistencies that I found quite interesting. And the Tang et al. paper, which was in 2021, had a population or sample size of about 600 and used some nice methodological statistical approaches using Bayesian kernel machine regression, or BKMR, as well as G-computation analysis to look at mixtures of bisphenols. So as you heard before, a lot of the -- a lot of the studies -- the epi studies looked at mixtures of different BP -- not just BPS alone, but in combination with some of the other bisphenols.

And so one of the key points is that their analysis showed a non-linear dose response. So it was a -- there was an inverted U-shape dose response, which I

think is important to keep in mind. And in the mixtures' models, BPS had one of the second highest weights of the bisphenols, in terms of what was potentially driving the association with associations of the bisphenol mixtures and GDM risk. And then in a totally, you know, different population, in the Kaiser Permanente cohort, which was the Zhu et al. 2022 paper, that can -- was also like a nested case control study and it had a smaller sample size of about 333, but also used BKMR modeling approach and also showed a non-linear response, so an inverted U relationship with gestational diabetes.

So these two different papers showed very consistent results with gestational risk expose -- sorry, gestational diabetes risk and suggested non-linear dose responsE -- or non-linear relationships. And then the other strength of that study was that the samples were collected before gestational diabetes diagnosis. So I think that's just one of the strengths of the cohort design.

And then the last paper was actually a lot larger, the sample size had about 1,800 participants. And although they didn't see overall associations with GDM, they did see strong associations with biomarkers such as fasting plasma glucose, which, of course, is used to diagnose GDM. And I think the one limitation though is

they didn't actually look for non-linear relationships. So given that the other papers did see this evidence of a U-shape curve, Zhang paper didn't look for that and they may have missed a relationship as a result.

So those are sort of, I guess, my comments or thoughts about gestational -- the evidence for gestational diabetes.

Okay. The next topic would be -- or next outcome would be the evidence looking at thyroid -- thyroid-related hormones. And there were five papers in total, four that were cohort studies and one that was a nested case control. And I think part of the challenge with this body of literature is that -- and this was alluded to before in the presentation is that all of them seemed to evaluate or to observe slightly different relationships with different thyroid hormones. So it's sort of a mix of results broadly speaking within the context of thyroid hormone data.

One of the studies -- I would say the case control study in particular I had some concerns with. So I just want to share those, that for starters, the frequency -- the percent of samples below the limit of detection was actually really high in this study, so at 73 percent. So that really limited their ability to do much in terms of statistical analysis. They had to dichotomize

their analysis and to basically, you know, present or not present in the participants. In addition, it was a nested case control that was enriched from pre-term births, which complicated some of, you know, the interpretability of the findings. And generally, they did not observe very many associations.

The other four studies are cohort studies. And I think probably the most consistent results are either significant or non-significant, but suggested increases in FT4. So that was observed in several of the studies. Again complicating I think some of the interpretation -- interpretation from some of these papers is the fact that there is some suggested evidence for non-linear relationships, and many of the studies didn't actually look for that.

So for instance, let's see, it was the Huang paper in 2022. So that was a paper that -- conducted in China. It had a reasonable sample size of about 500. And the nice thing about it was that, you know, it had a good -- a good amount of -- their samples were detected above the limit of detection. And so while they looked at -- they looked at thyroid hormones by tri -- by trimester and then they looked at tertiles of exposure, they also conducted a restricted cubic splines analysis, which showed some evidence for suggestion of a non-linear

relationship between BPS and free T4, or FT4, which when they looked in other modeling strategies, that relationship didn't necessarily come to light.

So I think, in sum, you know, for me, the literature on thyroid hormones is pretty mixed and a bit limited, but those were some of the strengths and weaknesses of those papers.

And then there were -- so for sex hormones in pregnancy, there were only two studies. There was not much supportive evidence. There was just the one that found some evidence with a relationship for CRH and kisspeptin. So generally, not a lot of data for sex hormones in pregnancy.

Similarly, for sex hormones in young girls, there's pretty limited, maybe you could consider it emerging, evidence from three studies in total looking at different sex hormones. I think the strongest associations -- or sorry, some consistency in associations had to do with estrogen. Again, I think -- there are some studies that looked at evidence for non-linearity and suggests that there may also be a U-shaped curve specifically for BPS and some of the sex hormones. So I think that's important to consider that for studies that only look at linear associations they may be missing some relationships.

What else did I want to say about that?

Well, so in the paper by Hu et al. in 2022, this was -- had a pretty fairly large sample size. They looked at relationships in several different ways. They had single analyses that showed decreases in several of these hormones. And then they used BKMR models and evaluated non-linearity of the response, and again showed that BPS in this mixtures analysis had one of the strongest weights basically in the model in relationship to estrogen. And then, you know, there were still some limitations, I think, with that study in the sense that there was some lack of clarity around effects being stronger in pre-puberty or puberty periods. And then, yeah, so sort of like the information on pubertal stage was a bit limited.

And -- okay. And then the last thing like -- I think the last thing that I wanted to cover was the evidence related to polycystic ovary syndrome. This -- for BPS, there are two studies and they all -- both showed an increased association for PCOS with higher BPS exposure. And I think, you know, what was nice is that although these were two very different studies, they're both case control studies, one was smaller than the other, and they did slightly different approaches in their statistical methodology, but they both found fairly

consistent elevated risk for PCOS.

And in the Zhan 2023 publication in particular, they really tried to look at and address this question through multiple different approaches. And every which way they did it, they saw very strong and consistent results. So their overall logistic regression model showed elevated odds for PCOS in single pollutant models. And then if when they looked by quartiles, there was a clear sort of evidence for a trend, that was statistically significant sort of -- so increasing tertiles, increasing risk.

And then when they used a mixtures-based approach, which looked at BPS in the context of other bisphenols, they also found that that BPS effects that actually had the strongest weight in driving that mixture's effect for PCOS. So I thought that although they were only two studies, they were quite consistent in providing some evidence for effects for an increased risk on PCOS.

And then as mentioned before, there are a couple other outcomes that have been looked at just on one study here, one study there. So I think that what I just covered with GDM, PCOS, and some of the hormones are really where the strength -- the largest strengths for evidence in -- for the effects of BPS currently exist in

the epi literature.

And I think that that's pretty much my summary, my take on the literature.

CHAIRPERSON LUDERER: Thank you very much for that summary and those -- the examination of the epidemiological literature, Dr. Breton.

So next, we'll hear from Dr. Carmichael.

COMMITTEE MEMBER CARMICHAEL: Hi, everyone. And thank you, Dr. Breton, for that excellent summary. I think rather than going through, you know, each outcome since Dr. Breton has just done that, I just wanted to primarily highlight a few major concerns I had that are methodologic. And due to these concerns, I think it's difficult to discern any -- very much, if any, conclusive evidence from the epidemiologic literature. So exposure assessment, most of the studies used a single spot urine sample. And the validity for a single sample understand -- to help us understand the actual individual level exposure and potential impact on disease mechanisms is difficult.

As noted in some of the background information from OEHHA, the half-life is just seven to nine hours. It's a non-persistent chemical. And there's considerable interindividual variation in metabolism. And there are studies looking at, you know, consistency or variability,

up levels in serial measurements. And they support sort of the concern about what you can do with a single sample. So there's some concerns there.

Temporality is another major issue that concerned me, because really in order to determine cause and effect, it's really important that the samples be measured before the outcome occurs. And as Dr. Breton pointed out, the GDM studies were a particular exception to that. They did measure -- clearly measure the samples before the outcome occurred, but a number of the studies did not. For example, a cross-sectional study would have measured the sample and the outcome at the same time. There were also several studies that essentially inherently had a prospective design, but when it came to their sampling, they were actually more of a cross-sectional design in that the sample and the outcome were measured in the same sample for example. So that would apply especially to, I think, the thyroid and sex hormone studies.

And some even had sort of a reverse collection, so, for example, the PC -- the two PCOS studies. I wrote down also the miscarriage, endometriosis, fecundability, infertility, they knew the outcome before the measurement of the sample.

And then just to point -- just to reiterate what's already been said, exposure assessment there was

some variability. One concern that was pointed out was the level of detection for BPS varied widely across studies, as did the percent of samples below the LOD. So I think it was pointed out that there were at least three studies that had I think it was greater than 70 or so percent of samples below the limit of detection, but there were also three studies by the -- for the -- in the -- that used data from the Generation R study in the Netherlands. And their first, they used measure -- this one actually did have multiple samples, but I guess they varied in the extent to which that was -- that data was capitalized on, but their second and third trimester data had 70 and -- around 70 and 80 percent of samples below the limit of detection.

And let's see. And then it's just -- it's hard when there are so few studies for any specific outcome. And I think that really the ones that Dr. Breton pointed out, the exceptions to that would be the GDM with three studies that had -- seemed to have solid designs in a lot of ways and then the thyroid studies.

And I think those were -- those were the main points that I wanted to make.

Thank you.

CHAIRPERSON LUDERER: Thank you, Dr. Carmichael.

Let's see, next we'll turn to Dr. Baskin for some

comments on the epidemiological studies.

COMMITTEE MEMBER BASKIN: Not a huge amount to add from Dr. Carmichael and Dr. Breton. I think the two areas where there was the most evidence that BPS certainly has a deleterious association would be the diabetes study -- the diabetes studies -- the three diabetes studies, two from China, one from California. As Dr. Carmichael pointed out, this -- there was confounding variables in the sense that there's only one measurement, and also the focus wasn't necessarily on BPS. They were measuring BPA as well. But there was -- they were prospective and there was a clear association.

The polycystic ovary syndrome had two studies. And as I recall, the -- let's just grab those notes, the Zhan study from 2023 was the one that was most convincing, and the Jurewicz -- I'm not pronouncing that right, but the study from Poland showed less of an association. But they were both prospective as I recall, but they knew -- I mean, they basically had patients who had polycystic ovary syndrome who they were measuring as opposed to, you know, they basically knew the outcome.

The other studies were either a single study or in the case of the thyroid hormones during pregnancy was kind of all over the map, so I wasn't really sure what to make of it. So quickly summarizing the major concerns

were in the area of the association with gestational diabetes and polycystic ovarian syndrome with the BPS exposure.

CHAIRPERSON LUDERER: Thank you, Dr. Baskin.

Then next, Dr. Nazmi -- asking Dr. Nazmi if you have any additional comments you would like to summarize.

COMMITTEE MEMBER NAZMI: Yeah. Thank you very much. I'm going to attempt to just integrate and not be redundant. I agree with the points raised by the other Committee members.

Some of the limitations of this body of work, I think were pointed out really well by Dr. Carmichael. And I won't rehash. I will, however, comment really briefly on this body in terms of the criteria for -- pardon me, for causality that seemed more convincing. And in the way I read it, there is -- there is a fair amount of consistency. And in terms of, I think, specificity, perhaps we have a little bit less convincing evidence.

I do believe that the criteria for plausibility and coherence are relatively strong in this body of work. And so the way I read it, in summary, there is a little bit of mixed evidence depending on some of the issues previously raised in terms of measurement, in terms of study design. But given that it's a relatively emerging body of literature, the thing that is most resonating with

me is the criteria for consisting -- consistency and plausibility. That's all I have. Thank you.

CHAIRPERSON LUDERER: And can I just clarify -- I'll start with a clarifying question, Dr. Nazmi. So the evidence for coherency -- for coherence and plausibility, is that in a particular subset of those studies that you were referring to?

COMMITTEE MEMBER NAZMI: Summarizing the 23 studies -- the 23 human studies -- or hold on.

CHAIRPERSON LUDERER: I thought -- I was wondering if it was a particular -- one of the endpoints that you thought was more consistent.

COMMITTEE MEMBER NAZMI: Yeah. I think I'm referring more generally.

CHAIRPERSON LUDERER: Okay. All right. Thank you.

Are there any other -- any other questions or discussion from other committee members regarding those epidemiological studies. We're going to have more time for discussion later, but just -- since we've just heard the presentations of the epidemiological studies, are there any questions or comments from other members or from the -- Dr. Breton, Carmichael, Baskin, or Nazmi, other comments?

Okay. Not seeing any raised hands then at the

moment, we can move on to discussion or presentation of -- by the Committee members of the animal studies. And we'll start with Dr. Auyeung-Kim.

COMMITTEE MEMBER AUYEUNG-KIM: Hi. So thank you again for OEHHA staff for the preparation of the document as well as a nice presentation that was presented earlier in this meeting that reviewed the 43 in vivo studies that evaluated bisphenol S for reproductive -- as a reproductive toxicant -- a female reproductive toxicant.

So most -- as indicated, most of the studies that were conducted in this past decade, mostly the latter half as a result of the increased BPS used as an alternative for BPA. It is a very complex data set, because there was a big mixture of studies in different species, different time periods of exposures, you know, some of them being premating all the way to multi-generational different dose routes, oral gavage being the primary, but there was also some sub-q and IP injections.

A number of animals on studies varied from I think it was like six to 24 per group. And there were different dose levels. Some of them were in the estimated human relevant range to high doses as high as, you know, 1,000 milligrams per kilogram.

The research was conducted by academic labs with data published in high-impact peer-reviewed journals. And

then also some studies were conducted on behalf of the chemical companies at contract research organizations. So the -- in general, the data were of adequate quality for assessment.

And so, you know, with the different data sets, I'm going to talk in general that, you know, in some cases the results seemed to be mixed, you know, in the ovary weight, uterine weight, progesterone, testosterone levels, puberty onsets, estrous cycles, and mammary gland development. And that was largely due to, you know, the dose levels that were used, as well as the period of exposure and the different species.

And so, in particular, I think like I looked at, you know, some of the studies that were conducted by -- at the contract research organizations and which were also used by the European Food Safety Authority in their technical documents, where, you know, Sprague-Dawley rats were given BPS by oral gavage for 90 days. In this extended one generation reproductive toxicology study, the NOEL was -- the NOEL was determined to be 20 mg per kg, the lowest dose tested. And that was due to the increased post-implantation loss of the F1 progeny at the dose level of 60 mg per kg per day.

Also, for all like were in male and female, the F1 and F1B parent, there was a significantly higher rate

of intrauterine mortality to the 60 mg per kg. That was considered adverse. However, in a study conducted in the same lab in pregnant Wistar rats dosed at higher doses up to 300 mg per kg by oral gavage for a shorter duration from GD 6 to GD 19. And the evaluation was done at GD 20, there was no effects on the post-implantation loss. And that was the BASF study 2014.

But the same -- and the same was also seen in mice -- or also in mice, C57 black mice. They were given a lower dose of 0.2 mg per kg by oral gavage in a mouse study, and there was no effect. So, you know, it -- this is just an example of some of the things that we were seeing effects, but not consistent in all the studies.

And so I think, you know, based on the studies, you know, it was shown that BPS does -- in the animal studies that BPS is -- has been shown to affect the female reproductive parameters. In some cases, it may not be in the same direction, but it's clearly showing that there is some effect. And so -- and, you know, the data -- the conflicting data would be because of, you know, looking at the different parameters that were -- or the different species and dose duration.

So I think it does show some evidence that there is some effects in these animal studies.

CHAIRPERSON LUDERER: Thank you very much, Dr.

Auyeung-Kim.

Next, move on to Dr. Plopper to summarize his perspective on the animal studies.

COMMITTEE MEMBER PLOPPER: Okay. Thank you. Yeah. I won't go -- I have the same perspective as Dr. Auyeung-Kim, but I would say that I thought for some of the organs that were evaluated here, there was very clear evidence that exposure to BPS was having a very negative impact on their function. And the ones that I would -- I was most concerned about were the ones in the ovary, which -- of course, the ovary goes through at least four or five different processes of development up to generating oocytes that then can be fertilized and then the ovary has another phase after that.

There was an extensive number of studies there. And the response, as she pointed out, depends on when the exposure was and the doses. And the doses were quite range, that was my concern is they go off of milligrams and down to micrograms plus large amounts. But it seemed to me that there was clearly a negative impact on the development and maturation of oocytes from the very beginning, prenatally, to puberty, and then into reproductive phases.

And I would say there -- I was not too concerned about the fact that not all of these studies for the

uterus that did not show an effect on the weight. I thought that was a relatively general assessment and it wasn't surprising to me that there were no weight effects on some of them. But I thought it was interesting that the no effect studies that also did some analysis of the micro anatomy of the uterus found that there were quite a few marked changes, including some things that seemed like they might even be precancerous.

I think the same comments for the mammary gland, there were obviously disruptions there. It was a concern to me that we're finding the changes in such structure, numbers, and densities is terminal end buds and sometimes it was increased, sometimes less. And I think that just had to do with the dose and the exposure pattern. I thought it interesting that studies that did very limited exposure to the female -- pregnant female in gestation -- early in gestation had less effect than ones that did their exposure in the female -- in the mother, so that it would go to the fetus all the way through to parturition, or birth, or later.

I would say one other thing, there was -- I would not be concerned about the fact that there appeared to be no effect of this compound in sheep in ewes and I will say having dealt with ewes for a number of years as a -- in teaching in the veterinary school, they -- it's a ruminant

for one thing, and it has a very thick subcutaneous adipose band. And all these -- the studies use subcutaneous injections. And I guess I -- since they didn't measure what actual concentration or dose was in the -- either in the plasma or in the urine, it's not really clear. I would be very surprised if this compound actually made it out at a high enough dose or over a long enough term to have an effect.

The other thing to remember is that for rumens, the metabolism of the liver, which would be the breakdown organ for most of these compounds and the vascular pattern would be quite different, so -- than it is for non-ruminants. Otherwise, I felt that there was fairly consistent evidence that there were some reproductive organs that were definitely -- had -- that this exposure had a negative impact on. And I guess with that, I'll stop.

More questions or comments. It obviously had -- maybe we'll hear from the mechanistic folks, but it obviously had some negative impact on the critical hormones that were involved in the developmental process for the -- for the organ -- reproductive organs.

I don't know if that's -- that's probably sufficient. If somebody has questions, I'll be glad to answer them.

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

I'm not seeing any raised hands at the moment, so I'll go on and add my comments on the animal studies. I agree there was a very -- there was a wide variety of different model animals that were used, developmental stages when the dosing occurred, huge range of doses. And so I'm going to focus, sort of like Dr. Plopper did, on two kind of the groups of outcomes, one of which was the mammary gland studies and the other was the -- you know, kind of general, the ovary, the studies that looked at different ovarian endpoints.

With the ovarian studies, there were a lot of different issues that I noticed with some of those studies that I kind of wanted to highlight, since I -- we haven't, I think, talked that much about some of the strengths and weaknesses. So the -- several of the studies at -- when they were measuring endpoints such as looking at meiosis in -- affects on meiosis in oocytes. They were using multiple oocytes from the same animal and there was no statistical adjustment for that, so that was something that I noted in quite a few of the studies.

They did not adjust of the fact -- for possible correlation within the dam coming -- the oocytes coming from the same animal. And many of the studies didn't

comment on randomization or whether the investigators were blinded among the ovarian studies, and as well as also noted in our document and many of the studies didn't comment on where the BPS came from or what his purity was.

Nonetheless, there were a few studies that even with those -- those issues related to the studies. There were -- there were some consistencies, in that multiple studies found effects of BPS exposure during gestation, the neonatal period, the pubertal period, and adult period on ovarian follicle development -- follicle initial formation, and the cyst break -- during cyst breakdown.

And I just wanted to highlight a couple of the studies. So the Nevoral et al. study is one was from 2021. That was a neonatal exposure and the other one pubertal exposure. I thought those didn't have in general some as many of the issues that I talked about. And they used doses that were in the range that were relevant to humans. So in the neonatal study, the lowest dose was 0.1 microgram per kilogram. And they used a route that was relevant. So drinking water exposure in both of their studies. They observe -- they dosed the females from the day of birth through postnatal day 15, so during lactation. And they observed effects on -- on the -- the maturation -- oocyte maturation with spindle mis-assembly and chromosomal misalignment, as well as decreased in

repressive histone mark H3K27me2.

And they also observed similar findings. That was when in vitro matured and they observed similar findings with in vivo matured oocytes. And there were also similar findings in the pubertal study by the same group with -- related to the -- here, they looked at follicle numbers. So in this study, they dosed pubertal mice and they also had a wide range of doses including some that were human relevant. And they found dose-dependent linked decreased primary, secondary, and antral follicle numbers. They also noted again increased spindle malformations and abnormal oocytes at several of the doses.

Then for the mammary gland studies, these were done by two groups. And those studies were, I thought, among the most -- the strongest studies in terms of how they were done. They didn't have the issues that I mentioned, you know, they did appropriate statistical analyses. If there was more than one offspring from the same dam, they adjusted for that for example in the Tucker et al. study. They clearly stated that they randomized the animals, that the investigators were blinded to treatment, and they used multiple doses. And all of those studies found some different effects because they were, I think, as Dr. Plopper mentioned, dosing during different

windows of mammary gland development.

But some of the things that I found particularly striking were the retention of the terminal end buds in adulthood in both the Tucker et al. study and the Kolla study, so from two different groups, as well as evidence of hyperplasia later in life and inflammation in the Tucker et al. study, and also in another -- the study by Kolla and Vandenberg.

So those were kind of -- you know, even though there were many more studies than that, those were the ones that I -- that's where -- those -- that's where I thought the data were the strongest within this data set. And so I will end there. I will then -- I'd like to see if there are any questions at all or comments from any of the Committee members relating to the animal studies.

Dr. Breton.

COMMITTEE MEMBER BRETON: Hi. Yeah, I just had two questions actually. And maybe I missed this, but is the -- would you say for the majority of the studies, was the dosing generally representative of human ranges like for most of them?

CHAIRPERSON LUDERER: The ones that I was focusing on, I would say yes. The ones that I -- you know, but there was -- as I said, there was a huge wide -- very wide range of doses from, you know 0.1 or less

than -- less than one microgram per kilogram up to, you know, hundreds, even a thousand, I think, milligrams per kilogram within the literature, because there were so many studies.

COMMITTEE MEMBER BRETON: Um-hmm.

CHAIRPERSON LUDERER: So -- yeah, so there was an extremely wide range of doses that were used. But some -- the lower ones were -- I would say are human relevant.

COMMITTEE MEMBER BRETON: The other question I had -- which I was struck by the comments about sort of different doses having sometimes opposite or different effects. And that made me wonder about again sort of a U-shaped dose response --

CHAIRPERSON LUDERER: Yes.

COMMITTEE MEMBER BRETON: -- that I think the human evidence is really --

CHAIRPERSON LUDERER: Yes.

COMMITTEE MEMBER BRETON: -- you know, I was really struck by that.

CHAIRPERSON LUDERER: Yes.

COMMITTEE MEMBER BRETON: And so is there -- did anybody do that in any of the animal studies?

CHAIRPERSON LUDERER: That was commented on in -- or not commented on, but observed in multiple studies. So some people commented on it and other studies it was

observed that it was not always the highest dose that had the greatest effect in general.

COMMITTEE MEMBER BRETON: Yeah.

CHAIRPERSON LUDERER: Yes, very much so.

COMMITTEE MEMBER BRETON: Thank you.

CHAIRPERSON LUDERER: See if other -- if Dr. Plopper or Dr. Auyeung-Kim have a different Perspective on that, but -- Dr. Pessah, I see your hand is raised.

COMMITTEE MEMBER AUYEUNG-KIM: I don't have a different perspective.

CHAIRPERSON LUDERER: Whoops. Yes.

COMMITTEE MEMBER AUYEUNG-KIM: I just want to say I didn't have a different perspective than what you provided, Dr. Luderer.

CHAIRPERSON LUDERER: Thank you. Okay. Thank you.

Dr. Pessah.

COMMITTEE MEMBER PESSAH: So, you know, one of the things that I'm trying to sort of get in my -- straight in my own mind in the mechanistic studies was how the concentrations used to elicits effects relate to whole animal burden levels, either in plasma, serum, you know, urine.

CHAIRPERSON LUDERER: Um-hmm.

COMMITTEE MEMBER PESSAH: And I looked at a

number of the animal studies and I couldn't find anyone that measured them. Did you find anyone that measured the levels?

CHAIRPERSON LUDERER: Not -- no, not that I can recall. It's possible that there were some. Dr. Plopper, I see you turned your camera on.

COMMITTEE MEMBER PLOPPER: No. I was just going to agree with him. One of my big concerns and I -- that I should have brought up was the fact that we don't really know what these concentrations are that are actually circulating when they're -- when they're used. And like Dr. Luderer said, some of these are what you would consider a sledge hammer into the studies that I'm used to dealing with. And I think that is one of the big concerns. And that's why I brought it up for the -- especially for the sheep, because I really don't think that there -- to say that there was a negative effect in sheep, I would want to know just exactly what was circulating.

And I think it would have been strong, if that had been the case for all of these animal studies, because then we would have been able to address these issues like what looks like the U-shaped response. It's very strange to run through a very detailed study and depending on the dose, and the time frame, you can get a different response

looking at the same time course. So I just wanted to say, no, there weren't any, and that was a big disappointment.

COMMITTEE MEMBER PESSAH: Thank you.

CHAIRPERSON LUDERER: Okay. Let's see. I'm not seeing at the moment any other raised hands, so I think then we can turn to the mechanistic studies. So segue to Dr. Allard and Dr. -- and then Dr. Pessah.

Dr. Allard

COMMITTEE MEMBER ALLARD: Yes. Thank you, Dr. Luderer. So I just want to frame how I'm going to approach this review here, which is that my goal was not to compile every potential set of evidence and relate all potential modes or mechanisms of action that BPS has been implicated in, but instead what I try to do is use an adverse outcome pathway framework and consider the studies that I felt aligned with each other into a biologically plausible mode or mechanism of action. And so of the studies that were presented and also, of course, looking outside of the studies present in the hazard identification document, what -- there were four points I wanted to make.

And I'll start with the first point, which was really the set of evidence that I found to be the most compelling, and that's -- and that was already mentioned on the -- on the animal evidence review that we just

heard, which was the effect on meiotic maturation. So again that to me is the strongest set of evidence in terms of a mode of action of BPS. I think the mechanism of action is still a little bit unclear. But the reason why I felt it was so compelling is because, first, there were many studies that showed a failure to complete meiotic maturation. Not all studies agreed on that, but overall I felt -- well, I saw that many studies reported this arrest during meiotic maturation. So as oocytes transition between prophase I and metaphase II.

This was associated with an abrasion in spindle morphology and chromosomal alignment, which is very concerning, because that can be the genesis for aneuploidy. And what was really not worthy as part of the studies was the fact that these effects were observed. We were just talking about concentration, but these effects were observed sometimes at very low surprisingly low BPS concentrations often in the nanomolar range or even lower. And so in this Campen et al. study with cow oocytes. They even went to down femtomolar levels.

The other part that was noteworthy to me was the fact that these effects were described in multiple species. We also heard some concerns about that on the animal side by cow, pig and mouse, when performing these studies. And I should say that these studies are -- I

think most of them are in vitro studies. So you led those -- you collect the ovaries, you release the oocytes, and then you can add the compounds in these arrested oocytes and then monitor what's happening to them.

But, you know, this is -- this is a tractable system to look at the impact of that final stage of oogenesis, and again reported in multiple species using these kinds of assessment. And I think it's important to also put this back into context. This often comparison of BPS with -- it's perhaps more famous bisphenol BPA. And that similar to BPS, BPA has been described since 2003 in the work of (inaudible) as impairing the spindle morphology and meiotic maturation. So seeing that described in multiple species at really low concentration akin to what was observed with BPA was to me very significant.

We may not have a mechanism of action. That's obvious at least to me, but at least it's noteworthy that estradiol and synthetic estrogen like diethylstilbestrol also have been shown in a variety of species to also alter spindle formation and chromosomal alignment. So all these studies in my mind align.

So in terms of mechanism of action though, the part that is often mentioned the most is it's action on the nuclear hormone receptors, especially its potential

estrogenic modulation or just agonist activity on the estrogen receptors. You have alpha and you have beta. And many studies, BPS is often compared to BPA and other BPA analogs.

And I think the study that I relied the most on for this kind of investigation and comparison was the Kojima et al. 2019 paper, which was a very systemic comparison of BPA and eight of its analogs, including BPS of course, for both agonist/antagonist activities, because really depending on the concentration you can have it's modulation, right? It's not necessarily just agonist or antagonist. And they looked at the human estrogen receptors, so alpha and beta estrogen receptor, glucocorticoid, pregnane X receptor, and the constitutive androstane receptor. So it was -- it was a very comprehensive study testing a reasonable range of concentration.

And what was confirmed from this study and subsequent studies is that BPS shows a potent agonist activity towards both ER alpha and beta, although it is likely to be weaker than most of the other bisphenols that I used, and -- right and did not actually see a significant antagonist effect of BPS. So it seemed really focused on that agonist activity, you know, ER alpha and beta.

This was subsequently also confirmed in the NTP report and looking at the high throughput data that's available through the national toxicology program, the NTP, and that BPS was mainly active in the range of nuclear hormone receptor assays that they examined through its ER agonist activity.

But what -- I think what -- while this activity on nuclear hormone receptor is often mentioned, I think what we need to keep in mind is that ultimately it is not just a BPA-like chemical. And so that's also mentioned actually in the NTP report on BPAs and alternatives, that actually when you look at the collection of high throughput outcomes where BPS has been tested alongside BPA and many other analogs, actually BPS is quite dissimilar to BPA, one of the most dissimilar.

And so that brings me actually to two last studies. And this is my -- if you've counted, this is my fourth point by now, which is the fact that we tend to look at BPS in the way that -- being informed by what we've done with BPA what people have done with BPA, which is really focusing on this estrogenic activity or at least nuclear hormone modulation -- activity on modulation. And yet, they are quite different and they seem to be acting through pathways that we still don't really fully understand.

So I'm going to lean on two studies for that last point. One of them, as a disclaimer, is a study that came out of my laboratory, which is the C. elegans study, where we actually looked at the transcriptional outcome of BPA and BPS. And what we noticed is that while both BPA and BPS were reproductive toxicants, the -- and caused embryonic lethality and apoptosis in the germ line, what was really remarkable is that transcriptionally speaking BPA and BPS were remarkably different from each other. There was very little overlap in differentially expressed genes, DEGs.

And this has been repeated in other contexts including in a human study that looked at -- well in vitro study that looked at human primary preadipocyte differentiation, which is also a developmental endpoint. And they did similarly a RNA-Seq comparison of BPA and BPS and so really minor overlap I want to say between BPA and BPS. So I -- while we tend to focus on its hormonal activity as a mode or mechanism of action, I think it's clear that there's more to it than just that.

Okay. So in sum, looking at the mechanistic sets of evidence from my perspective, the part that we could align the best with the -- at least the animal studies that were mentioned was the impact on chromosome misalignment and alteration of meiotic spindle. This

again aligns with that we've known of other bisphenols, but also of estrogenic compounds such as DS and estradiol. And that therefore this, in my mind, from a -- from a mechanistic standpoint is a very strong set of evidence that links directly with reproductive performance and reproductive toxicity. And I'll end my comments there. Thank you.

CHAIRPERSON LUDERER: Thank you, Dr. Allard.

We now have time for some full Committee discussion about any of the studies that were mentioned or other aspect -- other studies that were perhaps not mentioned, if anyone has any additional comments.

COMMITTEE MEMBER PESSAH: Did you want my two cents on mechanistic studies?

CHAIRPERSON LUDERER: Oh, I'm sorry.

COMMITTEE MEMBER PESSAH: No problem.

CHAIRPERSON LUDERER: Dr. Pessah. I guess I'm like running off to lunch here or something. Sorry about that.

(Laughter).

CHAIRPERSON LUDERER: My apologies. Yes, of course.

COMMITTEE MEMBER PESSAH: So Patrick brings up a very important point. It's clear from the mechanistic data available in the literature that BPS is active, but

it's also clear that it's not BPA. It's not acting in a sort of completely overlapping manner to the effects described in BPA. And there's some studies that have actually used informatics approaches to clearly define that they don't have identical mechanisms. I found that the chromosome misalignment and the MAP kinase signaling, the ERK1, ERK2 pathways were the most sensitive in the studies that I reviewed, but those are pleiotropic mechanisms. They -- the actual target engagement could occur at virtually dozens of possible biomolecules to produce the effects that were seen.

Having said that, they seem to be engaged in a relative concentration range that may be relevant to human adverse outcomes. I actually don't think estrogen receptor engagement is one of those mechanisms. There's really very little direct evidence of estrogen receptor engagement. More estrogen receptor signaling, and that would be downstream and would involve things like the MAP Kinase, Kinase, Kinase and so forth, ERK1, ERK2 pathways.

I really want to commend OEHHA at doing these cross-mechanism animal studies and to some extent epidemiological study comparisons of relevance, which got me to thinking about the relative concentrations. Clearly, one could calculate the median serum or plasma level of BPS in some of these studies that report levels.

I'm not sure how informative that would be since the half-life is somewhere below 15 hours. So, you know, you have to take it with a grain of salt, where concentrations in animals are shifting up and down all the time depending on relative time to dosing. Whereas, in the cultured dish, that's rarely done. It's just one steady state concentration, which may have huge effects in trying to interpret across study levels.

One of the things that I think really is important is that although the chromosome abnormality and alignment of chromosome alignment and spindle abnormalities, which is one of the most sensitive biomarkers of BPS, if you look at those studies, the dose response curves are convincing to some extent, but they're definitely not U-shaped. So I think in that respect, one has to say we may not be looking at common mechanisms or even phenomenon in those studies.

So I think that's all I really had to add.

CHAIRPERSON LUDERER: Thank you very much.

Now, we can move -- have some additional discussion if any panel members would like to add anything or comment on anything else.

Not seeing raised hands at the moment. And it is very close to noon.

Oh, Dr. Sandy and Dr. Moran. We'll start with

Dr. Sandy and then Dr. Moran.

DR. SANDY: Yes. I just -- we wanted to correct something we said earlier about the question on cancer. I'll turn it over to Dr. Moran.

DR. MORAN: Okay. Thank you very much. Yeah. Thanks for -- to the team that brought to my attention that in the study by Tucker et al., 2018, there were a couple of incidents -- actually a couple -- really two incidents of carcinoma in mammary gland at the median dose in animals observed up to 14 months, which is in this -- done less time than traditional for cancer development. You know, it's normal two years. So that for clarification. So some studies consider, you know, the cancer effect. It was not obvious. It was not statistically significant, and it -- given the, you know, the caveat of the time when the observation was made.

And in addition to that, I would just make a comment that the -- about the internal dose that was brought up here. There is one study at least that measure internal done in a traditional animal model for us. It's the Chen at al. 2016 study on C. elegans. So they took the time and really measured what is was inside the model.

You know, so I don't know, Dr. Sandy, if you want to add more about the cancer. That is not my specialty.

(Laughter).

DR. SANDY: No. Thank you very much.

DR. MORAN: Yes.

CHAIRPERSON LUDERER: Yeah. Thank you for that clarification. And I'm looking. I don't see any additional raised hands. So as I was saying, since it is noon now or almost noon, we can go ahead to take a lunch break. And before we go on the break, I'd like to ask the OEHHA Chief Counsel Carolyn Rowan to give the Bagley-Keene Open Meeting Law reminder for us.

Thank you, Carolyn.

CHIEF COUNSEL NELSON ROWAN: Thanks. I'd just like to quickly remind the members that during breaks you aren't allowed to talk amongst yourselves about the subject matter of the meeting, and that includes phone calls, text messages, and chats.

My recommendation would be that you also not talk to third parties about the items being discussed at the meeting. If you do, then you'll need to disclose that fact that you had the discussion with someone and give the general content of that discussion when we return.

And that's it for me.

Thank you.

CHAIRPERSON LUDERER: All right. Thank you, Carolyn. So we will take a 45-minute lunch break. So we'll reconvene at 12:45. So everyone have a good lunch

and we'll see you all when we return.

(Off record: 12:00 p.m.)

(Thereupon a lunch break was taken.)

(On record: 12:46 p.m.)

CHAIRPERSON LUDERER: All right. Hello again, everyone. Welcome back. It's time now for public comment on the agenda item -- on this agenda item that we've been discussion all morning on bisphenol S. And so the public should feel free to comment on any aspect of the presentation or discussion.

And Amy is going to share some slides with us on how to provide public comment. So as a reminder, you must be in the Zoom meeting to provide oral comment. And instructions for how to the join the meeting are available on OEHHA's webpage for this meeting and are also shown on this slide. If you would like to make a public comment, you can click on the Zoom webinar raise hand icon to indicate that you would like to speak. And when your name is called, you will be prompted to unmute yourself. Please then unmute yourself and provide your comment. You my also state your name and affiliation. And just another reminder that public comment is limited to five minutes per speaker.

So I'd like to ask Kiana, if there are any raised hands? And is there anyone else wishing to provide public

comment?

MS. VAGHEFI: I'm looking through the list and I do not see any raised hands.

CHAIRPERSON LUDERER: All right. So then seeing none, I'll bring the conversation back to the Committee for any further discussion on the matter before the vote.

So any Committee members who have additional comments or discussion they would like to make, please raise your hands and I'll call on you.

I'm see not seeing raised hands.

Then we will -- I'm going to ask everyone if you are ready to vote. Is there anyone who's not ready to vote? That might be the simpler question. Please raise your hands.

Okay. All right. It looks like everyone is ready to vote. So then I'm going to read the question before the Committee, which is what we will be voting on. And that is, has bisphenol S been clearly shown through scientifically valid testing, according to generally accepted principles, to cause reproductive toxicity based on female reproductive toxicity?

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

Dr. Allard.

COMMITTEE MEMBER ALLARD: Yes.

CHAIRPERSON LUDERER: All right. Dr. Auyeung-Kim.

COMMITTEE MEMBER AUYEUNG-KIM: Yes.

CHAIRPERSON LUDERER: Dr. Baskin.

COMMITTEE MEMBER BASKIN: Yes.

CHAIRPERSON LUDERER: Dr. Breton.

COMMITTEE MEMBER BRETON: Yes.

CHAIRPERSON LUDERER: Dr. Carmichael.

COMMITTEE MEMBER CARMICHAEL: Yes.

CHAIRPERSON LUDERER: I will vote yes also. And Dr. Nazmi.

COMMITTEE MEMBER NAZMI: Yes.

CHAIRPERSON LUDERER: Dr. Pessah.

COMMITTEE MEMBER PESSAH: Yes.

CHAIRPERSON LUDERER: And Dr. Plopper.

COMMITTEE MEMBER PLOPPER: Yes.

CHAIRPERSON LUDERER: All right. That is everyone. So by my count that is unanimous, all nine members voted yes. And six are required to add a chemical to the list.

So let's see, yes, Amy then -- well, I guess I've already -- I've tallied the vote and you have also tallied the vote. And then -- so Lauren then, I will turn it over to you for summary of the Committee action.

DIRECTOR ZEISE: Okay. Certainly there were a unanimous vote of the attending members, nine yeses. And so the chemical will be added to the Proposition -- bisphenol S will be added to the Proposition 65 list for reproductive toxicity for the female reproductive endpoint. Back to you.

CHAIRPERSON LUDERER: All right. Thank you. So there's a 15-minute break scheduled, but I don't think we need that --

DIRECTOR ZEISE: I don't think we need it.

CHAIRPERSON LUDERER: -- since we just came back.

DIRECTOR ZEISE: Yeah.

CHAIRPERSON LUDERER: All right. So then we'll move right on to the consent item, which is an update of the California Code of Regulations, Title 27, Section 27000, list of chemicals which have not been adequately tested as required. And we're now ready to take up this item, so we're being asked to affirm changes in response to submissions from the U.S. Environmental Protection Agency's Office of Pollution Prevention and Toxics, the California Department of Pesticide Regulation, and U.S. EPA's Office of Pesticide Programs have indicated that there are no changes.

This is a ministerial duty of the Committee in that we rely on information provided to OEHHA by the

Department of Pesticide Regulation and U.S. EPA to identify the chemicals that need to be added or removed from the Section 27000 list. So I'd like to invite Environmental Scientist in the Proposition 65 Implementation Program Kiana Vaghefi to give the staff presentation on this item.

Kiana

MS. VAGHEFI: Thank you, Dr. Luderer. Let me share my screen.

(Thereupon a slide presentation).

MS. VAGHEFI: All right. Proposition 65 requires the State to publish and update annually a list of chemicals that are required to be tested under State or federal law for carcinogenicity or reproductive toxicity and that have not yet been adequately tested as required. This list can be found in Title 27, Section 27000 of the California Code of Regulations and is commonly referred to as the Section 27000 list. It's a separate and distinct -- it's separate and distinct from the Proposition 65 list of chemicals known to cause cancer or reproductive toxicity. The Section 27000 list has no regulatory impact. It does not require that any testing be done. Rather, it is a source of information concerning chemicals that need further testing pursuant to State or federal law.

To update the list, OEHHA requests information from the California Department of Pesticide Regulation and the U.S. Environmental Protection Agency's Office of Pollution Prevention and Toxics and Office of Pesticide Programs each year.

This year, OEHHA staff reviewed these responses and identified one recommended change to the Section 27000 list. Addition of 2,2,3-trifluoro-3-(trifluoromethyl)oxirane, also known as hexafluoropropylene oxide, or HFPO. Based on information received from U.S. EPA's OPPT, further carcinogenicity, reproductive toxicity, and developmental toxicity testing are required.

The letter from OPPT, along with additional background, response letters from DPR and OPP, and a mock-up of the proposed change are available in the staff report provided to the Committee and posted online on November 23rd. The proposed change is also shown on the slide.

As Dr. Luderer mentioned, this is a consent item and a ministerial duty of the Committee, in that the DARTIC and CIC committees use the information provided by DPR and U.S. EPA to identify the chemicals that need to be added to or removed from the Section 27000 list. We ask the Committee members to vote in favor of the proposed

change, so OEHHA can update the list.

I'll now turn it back over to Dr. Luderer and I'm happy to take any questions.

CHAIRPERSON LUDERER: Thank you, Kiana.

Are there any questions from Committee members? And as a reminder, this is a consent item.

Any questions, let's see, from Committee members?

I'm looking and I do not see any raised hands.

So in that case, then I will read the question that we would be voting on, 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 will now call your names and ask you to vote yes, no, or abstain on this question in alphabetical order. So Dr. Allard.

COMMITTEE MEMBER ALLARD: Yes.

CHAIRPERSON LUDERER: Dr. Auyeung-Kim.

COMMITTEE MEMBER AUYEUNG-KIM: Yes.

CHAIRPERSON LUDERER: Dr. Baskin.

COMMITTEE MEMBER BASKIN: Yes.

CHAIRPERSON LUDERER: Dr. Breton.

COMMITTEE MEMBER BRETON: Yes.

CHAIRPERSON LUDERER: Dr. Carmichael.

COMMITTEE MEMBER CARMICHAEL: Yes.

CHAIRPERSON LUDERER: I will vote yes also.

Dr. Nazmi.

COMMITTEE MEMBER NAZMI: Yes.

CHAIRPERSON LUDERER: Dr. Pessah.

COMMITTEE MEMBER PESSAH: Yes.

CHAIRPERSON LUDERER: And Dr. Plopper.

COMMITTEE MEMBER PLOPPER: Yes.

CHAIRPERSON LUDERER: All right. Again, the vote is unanimous, nine yeses, which is the -- a unanimous vote. And so then Amy will tally the vote and provide it to Lauren.

DIRECTOR ZEISE: So I think we've just heard that we've got a unanimous vote --

CHAIRPERSON LUDERER: Yes.

DIRECTOR ZEISE: -- to make that change to the Section 27000. So thank you.

CHAIRPERSON LUDERER: Uh-huh. And then next we have some staff updates on Proposition 65 listings, regulations, and litigation that have taken place since our last meeting. So Kiana then, can you -- will you present the chemical listings and safe harbor levels?

Thank you.

(Thereupon a slide presentation).

MS. VAGHEFI: Yes. Thank you.

All right. The screen is up.

All right. Thank you, Dr. Luderer. I'll be providing you with an update on important Proposition 65

developments since the last DARTIC meeting. I'll start by going over the chemicals or endpoints added to the Proposition 65 list as well as data call-ins requesting information on chemical toxicity. Then I'll review adopted and proposed safe harbor levels. After that, I'll turn it over to our Chief Counsel Carolyn Rowan to provide an update on other regulatory actions and significant Proposition 65 litigation.

NEXT SLIDE

MS. VAGHEFI: Since the Committee's last meeting, 11 chemicals have been added today the Proposition 65 list: 1-bromo-3-chloropropane, 1-butyl glycidyl ether, glycidyl methacrylate, 1,1,1-trichloroethane, leucomalachite green, anthracene, 2-bromopropane, dimethyl hydrogen phosphite, coal-tar pitch, fluoro-edenite fibrous amphibole, and silicon carbide whiskers were all added as carcinogens.

NEXT SLIDE

MS. VAGHEFI: Today, the DARTIC considered listing BPS as causing female reproductive toxicity. BPS remains under consideration for listing as causing developmental and male reproductive toxicity. Information from the BPS data call-in will be used in preparation of a hazard identification document for a future DARTIC meeting on these endpoints.

Additionally, OEHHA issued a data call-in on vinyl acetate to solicit information related to its carcinogenicity. This information is being used in the preparation of a hazard identification document for future consideration by the Carcinogen Identification Committee.

NEXT SLIDE

MS. VAGHEFI: All right. Since the Committee's last meeting, a cancer no significant risk level was adopted for inhalation exposures to antimony trioxide, which will become effective January 1, 2024. We proposed an update to the no significant risk level for exposure to ethylene oxide from two micrograms per day to 0.058 micrograms per day.

And now I will turn things over to Carolyn.

NEXT SLIDE

CHIEF COUNSEL NELSON ROWAN: Thanks, Kiana. I have a couple updates on other regulatory actions that have taken place since the Committee last met.

First, OEHHA's regulation regarding exposures to acrylamide and cooked in heat processed foods became effective on April 1st, 2023. This regulation provides a limited exception to the Proposition 65 warning requirement when acrylamide levels are at the lowest level currently feasible. And it sets forth safe harbor levels for acrylamide and specific food categories.

Second, on October 27th, 2023 OEHHA noticed a proposed rulemaking that would amend and add new sections to the safe harbor warning regulations. OEHHA is proposing amendments to sections 25601, 25602, 25603, and 25670.2 and would add new sections 25607.50 through 25607.53 to Title 27 of the California Code of Regulations.

The proposal is a continuation of a similar rulemaking proposal that was initiated in 2021, which OEHHA voluntarily withdrew to take additional to incorporate public input. The proposal would provide information to consumers and disincentivize unnecessary prophylactic warnings by amending existing short-form safe harbor warnings to include the name of a carcinogen, or a reproductive toxicant, or both for which a business is warning.

The proposal includes a two-year period for businesses to gradually transition to the new warning. And the proposal also includes safe harbor status for short form-warning content on food products, clarifications to internet and catalogs safe harbor warning requirements, and warning options for off-highway and motor vehicle and recreational marine vessel parts.

A public hearing is scheduled for tomorrow, December 13th, and the public comment period is scheduled

to close on December 20th. OEHHA will have a year from the date of the notice to submit a final package to the Office of Administrative Law.

NEXT SLIDE

CHIEF COUNSEL NELSON ROWAN: Okay. On the litigation front, I'd like to update you on a few developments. On November 7th, 2023, the Ninth Circuit Court of Appeals issued a decision in the National Association of Wheat Growers versus Bonta case. The decision held that Proposition 65 warnings for exposures to glyphosate are not purely factual and uncontroversial and violate the First Amendment of the U.S. Constitution. The deadline to file a petition for rehearing is December 21st.

The California Chamber of Commerce versus Bonta case also involves a first amendment challenge. That one is to acrylamide warnings for food. And the case is pending in federal district court. I don't have any significant updates to report on that.

In September of 2023, the Personal Care Products Council filed a another First Amendment challenge to warnings for titanium dioxide, airborne unbound particles of respirable size, for cosmetic and personal care products. The plaintiff filed a preliminary junction motion and the court has yet to rule on that motion.

Finally, Physicians Committee for Responsible Medicine versus Newsom case, which is a challenge to the decision not to list processed meats as a carcinogen is still pending in Sacramento Superior Court. And I don't have any significant developments to report on that case.

NEXT SLIDE

CHIEF COUNSEL NELSON ROWAN: And that's it for my litigation update. Thank you.

CHAIRPERSON LUDERER: Thank you very much for the update. So I'm now going to ask OEHHA Director Lauren Zeise to summarize the Committee actions.

DIRECTOR ZEISE: Thank you, Ulrike -- Dr. Luderer.

So the Committee unanimously voted to add bisphenol S to the Proposition 65 list based on reproductive toxicity for the female reproductive toxicity endpoint.

The Committee also unanimously voted to add HFPO to the Section 27000 list based on a U.S. EPA requirement for carcinogenicity and reproductive and developmental toxicity testing. And this was a consent item.

So with that, I'd like to thank the Committee for all the effort that went into preparing for today's meeting and for participating in today's meeting. We really do appreciate all the work that goes into making

judgments about reproductive toxicity and covering our -- all the materials that are submitted to you to review. So very much appreciate that.

We also appreciate the participation of the audience and attending this meeting. And then finally, I'd also like to thank the OEHHA staff from the Reproductive and Cancer Hazard Assessment Branch, in particular the Reproductive Toxicology and Epidemiology Section. You can imagine the tremendous amount of effort that went into preparation of the hazard identification materials. So that was a big lift and appreciate all that effort.

I'd also like to thank the Proposition 65 implementation team and the legal team for getting this meeting organized and together, and for all of the legal and other ways in which those two teams support this effort.

So finally, I just want to wish everyone a Happy Holidays and again express my appreciation and turn it back to you Ulrike -- Dr. Luderer

CHAIRPERSON LUDERER: Thank you, Dr. Zeise.

And I would also like to thank everyone, all the staff who worked very hard on this and all the members of the DARTIC, and also wish everyone a very Happy Holidays.

And with that, I declare the meeting adjourned.

(Thereupon the Developmental and Reproductive Toxicant Identification Committee adjourned at 1:07 p.m.)

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 16th day of December, 2023.

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