MEETING

STATE OF CALIFORNIA

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

DEVELOPMENTAL AND REPRODUCTIVE TOXICANT

IDENTIFICATION COMMITTEE

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Dr. Allegra Kim, Reproductive Toxicology and Epidemiology Section

Ms. Michelle Ramirez, Environmental Scientist, Proposition 65 Implementation Program

A P P E A R A N C E S C O N T I N U E D

STAFF:

Dr. Martha Sandy, Chief, Reproductive and Cancer Hazard Assessment Section

Dr. Lily Wu, Reproductive Toxicology and Epidemiology Section

ALSO PRESENT:

Mr. John Hewitt, Grocery Manufacturers Association

Dr. Robyn Prueitt, Gradient

Dr. Michael Taylor, NiPERA

Mr. Cosan Joshan Unuvar, Meyer Corporation

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P R O C E E D I N G S

DIRECTOR ZEISE: Okay. I think we'll get started. I'd like to welcome every one to this meeting of the Developmental and Reproductive Toxicant Identification Committee. I'm Lauren Zeise. I'm director of the Office Environmental Health Hazard Assessment. And today, we have one main item on the agenda, and that's the consideration of the developmental toxicity of nickel and nickel compounds, based on -- Jim, if you --

DR. DONALD: I'm sorry. We'll actually be presenting data not just on developmental toxicity, but also on male and female reproductive toxicity.

DIRECTOR ZEISE: Yes, I was getting to that. I'm sorry. So, yes, we'll be consider -- the Panel will be considering the reproductive toxicity of nickel based on three endpoints, developmental toxicity, male reproductive toxicity and female reproductive toxicity.

So I guess before we jump into the business of the meeting, I'd like to go through some logistics. First, the meeting is being transcribed and also webcast. So if you could please speak directly into your microphones. And then in terms of the -- just logistics, in the event of an emergency, we walk -- just walk out the exit doors, down the stairs to your right, and through the lobby, and we'll meet across the street.

Drinking fountains and restrooms, just walk out the back door there, and turn to your left, and go to the end of the hall. And then, of course, we'll be taking breaks during the meeting for the court reporter.

Now, I'd like to introduce our Committee, the Developmental and Reproductive Toxicant Identification Committee. To your left, far left is Dr. Suzan Carmichael, Stanford University School of Medicine; next to her Dr. Laurence Baskin, University of California, San Francisco School of Medicine; then Dr. Patrick Allard, University of California at Los Angeles, Institute for Society and Genetics; next to me is our Chair, Dr. Ellen Gold, University of California at Davis School of Medicine; next to her Dr. Ulrike Luderer, University of California at Irvine School of Medicine; then Dr. Isaac Pessah, University of California at Davis School of Veterinarian Medicine; then Dr. Aydin Nazmi, California Polytechnic State University, Department of Food Science and Nutrition at San Luis Obispo; and then Dr. Charles Plopper, University of California at Davis School of Medicine. So welcome to the Committee.

Now, I'd like to introduce OEHHA staff. So starting at the far end of the table facing the Committee is Allan Hirsch, our Chief Deputy Director; next to him is Carol Monahan Cummings, OEHHA Chief Counsel; next to her

is Dr. Martha Sandy, Chief of the Reproductive and Cancer Hazard Assessment Branch; then Dr. Allegra Kim, who sits within the section of the Reproductive Toxicology and Epidemiology Section; then Dr. Marlissa Campbell, who's a staff toxicologist in that same section; then Dr. Jim Donald, who's the Chief of the that section; then Dr. Poorni Iyer, who's a staff toxicologist in the section; and then Lily Wu, a staff toxicologist in the section.

And then I'd also like to introduce our implementation staff. Esther Barajas-Ochoa, and Michelle Ramirez, and Julian Leichty. So welcome to the staff.

Now Carol Monahan-Cummings will be making some introductory comments before I turn the meeting over to Dr. Gold.

CHIEF COUNSEL MONAHAN CUMMINGS: All right. Good morning. Before I start, I just wanted to introduce Ryan Mahoney, who's a staff counsel with our office. And he's one of our newer attorneys. But in the event I have to leave for some reason, he'll pop up here and be your attorney until I get back. So this is Ryan.

So every meeting I just go through a few reminders for you, since you only meet once a year and you have a lot of other stuff that you do.

So, first I'd like to remind you that in your materials, if you have hard-copy materials, there's a tab

for the criteria. And that is the listing criteria that was adopted by an earlier version of this Committee, that can help guide you in terms of questions you may have about the criteria you should apply to determining whether a chemical is known to cause reproductive toxicity.

So your listing decisions should be based on that criteria, and not considering the future impact of a listing, for example, whether or not warnings would be required. That's handled at a separate part of the process. Your duty is to determine whether a chemical or groups chemicals has been clearly shown through scientifically valid testing, according to generally accepted principles to cause reproductive toxicity in any of the three endpoints.

This standard is a scientific judgment call. It's not a legal standard of proof. This Committee can decide to list chemicals based on only animal evidence. There's no requirement that there be -- that a chemical be shown to cause reproductive toxicity in humans, and there's no requirement to determine whether or not the current human exposures to the chemicals are sufficiently enough to cause reproductive toxicity.

The members of this Committee were appointed by the Governor, because of your scientific expertise as the State's qualified experts on reproductive toxicity.

There's no need to feel that you need to be compelled to go outside that charge. You are a scientific committee.

In the event that you feel you have insufficient information or need more time to discuss the issues that are in front of you today, there is no requirement that you make a decision today.

You may also decide to list one or more chemicals in this category, but perhaps not the entire group. You may also defer a decision on some or all of the chemicals in the group to a subsequent meeting.

So the process is very flexible. It depends on your comfort level, in terms of the evidence that you have before you, and your discussions. Feel free to ask clarifying questions of me, or the other OEHHA staff during the meeting. If we do not know the answer to your question, we'll do our best to find it, and report back to you. That may require that we report back after a break, but we'll do our best to do that.

In the event that you have questions we can't answer today, and it affects the -- your decision process, that may be one of the reasons to defer a decision to a later meeting.

Do you have any questions?

CHIEF COUNSEL MONAHAN CUMMINGS: Okay. Back to you.

DIRECTOR ZEISE: Thank you, Carol. And now I'll turn the meeting over to Dr. Gold.

CHAIRPERSON GOLD: Thank you, and good morning. So I believe we're going to start with staff presentations first. And, Dr. Donald, I believe you're first on the agenda.

(Thereupon an overhead presentation was presented as follows.)

DR. DONALD: Sorry. I was told --

CHIEF COUNSEL MONAHAN CUMMINGS: Excuse me, before --

DR. DONALD: -- I had to turn this on and I forgot.

CHIEF COUNSEL MONAHAN CUMMINGS: Excuse me, Jim. Dr. Gold, I think that you were going to make a comment about public comments, about the timing. It's just on the agenda, so I don't know if you want to address it now?

CHAIRPERSON GOLD: Okay. So, excuse me. So pursuant to our usual process, each speaker from the public has five minutes. The public comments will follow the staff presentations and then any Committee questions. Then we'll have public comments, and each person has five minutes, except for those that made requests before or by September 11th, and received approval for longer comments. And I believe we have two of those. So blue cards are

available in the back table. So if you wish to speak, please fill out one and give it to either Esther or Michelle.

Now, Dr. Donald.

DR. DONALD: Good morning.

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DR. DONALD: So the Committee today is going to consider nickel and nickel compounds. And these chemicals were selected as potential candidates for consideration under OEHHA's process for prioritizing chemicals for consideration under Proposition 65.

Materials that were prepared consistent with that process were presented to the Committee in November of 2015. And after deliberation, the Committee recommended these chemicals for consideration for listing. So -- excuse me -- I'm beginning with just some general very brief overview points. And then the technical data will be summarized by staff from my section. We'll present in the order of developmental, female reproductive, and male reproductive toxicity.

Dr. Allegra Kim will present the epidemiologic data on each of those endpoints. And Drs. Marlissa Campbell, Lily Wu, and Poorni Iyer respectively will present on developmental, female reproductive, and male reproductive toxicity.

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DR. DONALD: Nickel -- excuse me, metallic nickel and various nickel compounds are used in many industrial and commercial applications. Those include stainless steel and other nickel alloys, in catalysts, batteries, pigments, and ceramics.

Obviously, nickel compounds encompasses a very wide range of chemical structures. Here are just a few of the more common ones, some of which you have data on reproductive toxicity available to you.

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DR. DONALD: The question of solubility of nickel compounds and its relationship to bioavailability was raised in comments that you received. So this table just posts together a little bit of information on the relative solubility of some of these compounds. The ones that are highlighted in red are those for which data on reproductive and developmental toxicity are included in the hazard identification materials you received.

Most of the data, in cases where we know the form of nickel that was used, were for compounds that are found at the top of the table, those that have high solubility in water. But as you can see, there are some compounds which have low or perhaps no solubility in water, for which we do still have reproductive toxicity data, though

relatively sparse data.

I would also remind, as I'm sure you're aware, that the solubility in water of some of these compounds may differ from its solubility in biological media, such as stomach acid.

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DR. DONALD: The compound at the bottom of the table, nickel carbonyl, one of the insoluble compounds is already listed under Proposition 65 as know to cause reproductive toxicity.

That listing is based on the developmental endpoint, and resulted -- or the listing was through the authoritative bodies mechanism and resulted from a formal identification of nickel carbonyl as causing development toxicity by the U.S. Environmental Protection Agency.

So at this point, unless the Committee has any questions for me, I will turn this over to Dr. Kim.

CHAIRPERSON GOLD: Thank you.

Any questions for Dr. Donald by the Committee?

Dr. Kim.

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DR. KIM: Epidemiologic studies that examined nickel as causing development toxicity included a broad array of outcomes, including spontaneous abortion, fetal growth parameters such as birth weight, congenital

malformations, autism spectrum disorders, or ASD, and transplacental carcinogenicity.

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DR. KIM: Most of the occupational studies included in the hazard identification materials were conducted in the same area, the Kola Peninsula of northwestern Russia, which is in the center of this map.

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DR. KIM: There were nine -- excuse me. There were two occupational studies of spontaneous abortion among workers in nickel refineries on the Kola Peninsula

The 1994 study by Chashschin et al. is the earliest epidemiologic study of nickel and reproductive toxicity that OEHHA identified. Chashschin reported that women working in nickel hydrometallurgy were 1.8 times as likely to report a spontaneous abortion than women working in construction. No statistical tests were reported.

The Vaktskjold et al. 2008a paper reported a case-control study focused on spontaneous abortion among workers at a nickel, cobalt, and copper refinery complex. The exposure assessment for this study was also used in several other studies in the hazard identification document, and this presentation, so I'll describe it briefly.

Measurements of about 500 individual nickel

refinery workers' urinary nickel concentrations and the water soluble subfraction of the inhalable nickel aerosol fraction in their work environments were taken in 1995 to 2001. Using these measurements and knowledge of refining processes and occupations, each refinery job was categorized as having background, low, or high exposure to nickel.

The 2008 study by Vaktskjold et al. included two distinct analyses. In the questionnaire analysis, the adjusted odds ratio for nickel exposure -- excuse me, the adjusted odds ratios for nickel exposure were 1.39 for low exposure compared to background and 1.27 for high exposure, and were not statistically significant.

In the birth registry analysis, the odds ratio for spontaneous abortion in nickel exposure adjusted for maternal smoking was 1.1 and was not significant.

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DR. KIM: Ten studies examined nickel -- exposure to nickel as a risk factor for fetal growth restriction, indicated by the following parameters:

Birth weight, a continuous variable; low birth weight defined as birth weight less than 2,500 grams, small for gestational age, or SGA, defined as weight below the 10th percentile for gestational age; body mass index of the child, or BMIC, and; head circumference.

Nickel exposures were assessed by measuring nickel in maternal and cord blood, urine, placenta, air pollution, and soil, and by refinery occupation category.

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DR. KIM: Three studies of fetal growth included measurement of nickel in blood, urine, or placenta. Odland and co-authors measured maternal blood in urine, infant urine, and in the 2004 study placenta. None of these studies reported statistically significant effects of nickel exposure on birth weight. There was a non-significant association between nickel in placenta and infant weight adjusted for gestational age and country. The association was smaller in multivariate analyses.

Hu et al. reported that nickel in maternal or umbilical cord -- in maternal or umbilical cord blood was not statistically significantly associated with birth weight.

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DR. KIM: This forest plot shows low birth weight results from five cohort studies. Each of these studies examined nickel as PM2.5 or particulate matter less than 2.5 microns in diameter. Nickel PM0.1 and Nickel PM10 were also examined in one study each. The studies were large and reported small associations between nickel particulates and fetal growth parameters, though they did

not separate the potential effects of nickel from those of all co-pollutants.

The first study on this chart by Bell et al. examined nickel PM2.5 and found an interquartile range, or IQR, increase in nickel PM2.5 was associated with an 11 percent increase in risk of low birth weight as shown.

Bell et al. also reported a significant seven gram reduction in mean birth weight associated with nickel, which is not shown in this chart.

Ebisu and Bell also examined nickel PM2.5 and found the adjusted odds of low birth was 5.7 percent higher per IQR increase in PM2.5 nickel, adjusted for confounders but not co-pollutants. The association between low birth weight and nickel was robust to adjustment for single co -- excuse me, co-pollutants with correlations less than 0.5 in two-pollutant models.

Basu and colleagues found no significant changes in odds of low birth weight associated with nickel, as shown here. They did report a statistically significant one gram decrease in birth weight with an IQR increase in nickel exposure.

Laurent et al. found that nickel exposure was associated with statistically significant one percent increase in odds of low birth weight; for nickel PM2.5, the first row for Laurent in this -- on the chart, and

nickel PM0.1, the second row on the chart, in each trimester and over the entire pregnancy.

Pedersen and co-authors examined effects of particulate matter, including nickel PM2.5 and nickel PM10 on infant size in European -- several European cohorts. Odds ratios for low birth weight were 1.14 for nickel PM2.5, and 1.29 for nickel PM10 in single-pollutant models, adjusted for potential confounders, but not co-pollutants, and they were not significant.

Pedersen also reported that nickel PM2.5 and nickel PM10 were statistically significantly associated with reduced head circumference, which is not on the chart. Adjusted betas for head circumference were at negative 0.6 for nickel PM2.5, and negative 0.46 for nickel PM10.

Adjustments for sulfur particles and particle mass concentration attenuated these associations, but they remain significant, except for the beta for nickel PM10 adjusted for sulfur PM10.

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DR. KIM: Vaktskjold and colleagues' 2007 cohort study used nickel refinery occupational exposure categories described earlier, and reported an adjusted odds ratio for small for gestational age, or SGA births, of 0.84, which is a statistically significant protective

association.

Another retrospective cohort stud by McDermott et al. examined low birth weight in relation to geospatially modeled concentrations of nickel, and seven other metals in soil, and reported no association between nickel and soil and risk of low birth weight.

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DR. KIM: Seven studies examined associations between nickel and congenital malformations including: Any birth defects, neural tube defects, genital malformations, musculoskeletal defects, and cardiovascular defects.

Exposures were assessed by occupation and measuring nickel in soil, fetal tissues, and newborns' hair.

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DR. KIM: There were three occupational studies of nickel and malformations among nickel refinery workers on the Kola Peninsula. Chashschin et al.'s 1994 cross-sectional study, mentioned earlier, reported elevated risks of malformations associated with nickel work and hydro -- with work in nickel hydrometallurgy compared to non-nickel exposed work. Relative risks were 2.9 for all structural malformations, 6.1 for cardiovascular defects, and 1.9 for musculoskeletal

defects. All were reported to be statistically significant.

Vaktskjold and colleagues 2006 and 2008 case control studies used the Kola Birth Registry and the occupational exposure assessment described earlier. The adjusted odds ratio for nick -- for nickel exposure above background and any genital malformations, undescended testes, or musculoskeletal defects were less than one and not statistically significant.

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DR. KIM: Two studies, both in Shanxi Province in China, where the prevalence of malformations is extremely high examined associations with nickel and soil. Exposures were assessed by village and not for individuals.

Mean soil nickel concentrations in these studies were almost identical: 41.3 micrograms per gram in the study by Huang et al. and 41.7 in Zheng.

Huang and colleagues focused on neural tube defects, or NTDs, and concluded that nickel had, what the authors called, layered level effects on prevalence of NTDs, with the lowest prevalence in areas with intermediate soil nickel concentration, while highest soil nickel was associated with medium NTD prevalence, and the lower soil nickel concentration was associated with high

NTD prevalence. They did not report statistical significance. Other metals also showed layered level effects.

Zheng et al. considered all birth defects and found a statistically significant dose-dependent decrease in risk of birth defects associated with higher concentrations of nickel in soil.

However, Zheng et al.'s lowest nickel category was less than 37.5 micrograms per gram, which is higher than the threshold for the high nickel concentration that Huang et al. used.

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DR. KIM: Friel and colleagues compared nickel concentrations in tissues of anencephalic and control fetuses, and found no differences in nickel concentrations between the two groups.

Manduca et al. measured metals in hair of newborns and found no differences in nickel concentrations between infants with and without birth defects.

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DR. KIM: Seven studies examined effects of pre- and perinatal nickel exposure on risk of autism spectrum disorder or ASD. The first three studies on this table were included in the hazard identification document published on July 27. The other four were provided in

September.

Most were case control studies and used air monitoring data to estimate exposure to nickel in ambient air. This table shows the ambient air nickel concentrations reported in these studies.

Windham and colleagues studied air pollution and ASD in the San Francisco Bay Area. The odds ratio for nickel and ASD was 1.46 and was statistically significant.

Kalkbrenner et al. conducted their study in West Virginia and North Carolina. And much of their study area was very rural. Nickel concentrations were much lower than in the Windham study. The authors selected children with speech and language impairment as a control group. Nickel was not associated with ASD in this study.

Roberts et al. conducted a case control study nested within a national cohort. The adjusted odds ratio for the fifth quintile of nickel exposure compared to the first quintile was 1.65, and was statistically significant as shown. This study also showed a dose-dependent effect among boys.

The McCanlies study was a small pilot study. Occupational nickel exposure was not significantly associated with ASD.

The studies by von Ehrenstein et al., Talbott et al., and Kalkbrenner in 2018 reported no statistically

significant associations for nickel and ASD.

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DR. KIM: Three registry-case -- registry-based case control studies examined nickel exposure as a risk factor for transplacental carcinogenicity, that is, cancer resulting from prenatal exposure.

Heck and colleagues used the California Cancer Registry to ascertain cases of neuroblastoma and retinoblastoma and air toxics data to assess exposure. The adjusted odds ratio for neuroblastoma and an IQR increase in average nickel exposure over the pregnancy was 1.08 for births within five kilometers and 0.67 for births within 2.5 kilometers.

The adjusted odds ratio for retinoblastoma and IQR increase in nickel exposure was 1.48, and was statistically significant.

Togawa et al. used data from Scandinavian registries to study occupational exposure to heavy metals and welding fumes and risk of testicular germ cell tumors in offspring. There were two types of exposure assessment.

The odds ratio for any paternal nickel exposure with or without exposure to other metals and welding fumes was 1.07, and for any maternal exposure the odds ratio was 1.07. Both of these were not significant.

The second type of exposure assessment used exposure indices, which were calculated as the product of the proportion exposed and the mean level of nickel exposure. The odds ratios for paternal and maternal nickel exposure index with testicular germ cell tumors were not significantly different from one.

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DR. KIM: Sorry.

Ni et al. conducted this cross-sectional study in an electronic waste recycling town to examine co-exposure to heavy metals as risk factors for oxidative damage to DNA. The beta for nickel in umbilical cord blood 8-OHdG, a marker of oxidative damage to DNA, was 0.215, adjusted for other metals, which were lead, cadmium, chromium, and potential confounders, and was statistically significant. 8-OHdG was more strongly associated with nickel than with other metals.

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CHAIRPERSON GOLD: Dr. Campbell, go ahead. Just -- your turn.

DR. CAMPBELL: Yeah. Eight studies of the developmental toxicity of nickel were conducted in rats by the oral route. Seven studies were conducted in mice by the oral route. Only one study was identified that exposed pregnant rats to nickel by inhalation. And

additional studies were conducted in rats and mice, and one in hamsters by various types of injection protocols.

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DR. CAMPBELL: These are the studies conducted in rats by the oral route. And rather than walk through each individual study in detail, I just want to comment on the overall data set. With the exception of the two Siglin studies, which used nickel sulfate as the test compound, all of these studies used nickel chloride for the oral studies.

It's worth noting that most of these, while providing information relevant to developmental toxicity, were actually reproductive toxicity studies, and therefore didn't necessarily and consistently perform detailed morphological examinations for external, internal, and skeletal abnormalities.

Therefore, while the discussion of the data is going to emphasize considerations of viability and growth, it shouldn't be assumed that nickel lacks the potential to cause morphological effects.

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DR. CAMPBELL: This graph shows mean live litter size in rats for those oral studies that reported it. The data are normalized as a percent of controls. Just to facilitate visual comparison of results across multiple

studies. Some of the studies noted on the previous slide were excluded from this graph, either because OEHHA did not have access to the original study report or because a data on live litter size wasn't reported in that study.

Doses of the various nickel compounds have -- well, there's only two, but have been converted to milligrams nickel per kilogram body weight, and grouped into ranges just to show the relationship between dose of nickel and magnitude of effect. Each color represents a different study or a separate generation or litter cohort within that generation -- within a generation.

The "a" tag indicates a statistically significant change at the P less than 0.01 level as was reported by the specific study paper.

Statistically significant decreases in litter size were seen only at the highest dose levels for two of the different studies in rats.

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DR. CAMPBELL: This graph is similar to the previous slide, but shows mean fetal weight normalized as percent of controls.

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DR. CAMPBELL: Moving on to oral studies conducted in mice. All of these -- all of these studies use nickel chloride as the test compound. On the

left-hand column, those three studies are the most recent whole animal developmental toxicity studies we identified, and all of them were conducted in the same lab.

Nickel chloride was given at different stages of prenatal development in order to evaluate the effects of exposure timing as well as dose. And the right-hand column shows three additional studies, all of which involved treatment given either just restricted to organogenesis or throughout most of gestation.

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DR. CAMPBELL: And this is just -- before we look more closely at the mouse developmental toxicity data, I wanted to point out that it -- it might superficially seem as if mice are more sensitive than rats to the effects of nickel on development. But what happened was, in fact, the test doses used in mice were much higher in general than those used in rats. And on this graphic, the doses given to mice are represented in red, while the doses given to rats are in blue.

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DR. CAMPBELL: This slide looks specifically at birth weight data from the Saini 2014b study. And they actually, within one study, gave nickel at different stages of gestation. And those stages are represented in this graph by the different colored bars, blue bars for

treatment during pre-implantation, red bars for treatment during organogenesis, and green bars for treatment during the fetal stage.

The horizontal axis here shows live litter size as percent of controls, while the vertical axis shows a dose of nickel, and then the bars are grouped together by dose.

What you can see is the high dose of 185 milligrams nickel per kilogram per day was associated with reduced pup viability following treatment at any of the time points during gestation.

The mid-dose -- at the mid-dose of 92 milligrams per kilogram per day, a significant decrease in viability was seen only with the exposure on gestation day 0 through 5, the pre-implantation period. No effects on live litter size were seen at the low -- lowest dose used of 46 milligrams nickel per kilogram per day no matter what stage of gestation the treatment was given.

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DR. CAMPBELL: This graph was constructed, like the previous 3D graphs I showed you, for the rat studies of normalized data. In this case, live litter size in mouse studies is -- is represented as a percent of control. But the doses -- most of the studies use the same set of doses, so it wasn't necessary to group them up

into ranges in order to create the horizontal axis.

Many points show statistically significant decrements in viability of exposed mouse offspring, particularly at the dose of 185 milligrams per nickel per kilogram per day. Although it should be noted that all of those studies were from the Saini lab, while similar and higher doses from other labs didn't show significant effects on viability.

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DR. CAMPBELL: Turning from viability to weight data, this slide shows the birth weight data from the Saini et al. 2014b study. The birth weights normalized as percent of control. And again, the different bars represent the different exposure periods. The clumps of bars represent a different dose, and with the lowest dose on the bottom.

And what you can see here is a significant increase in weight decrements with increasing dose, as well as an effect of the time of exposure. Nickel exposure at the high dose was associated with decreased birth weights whenever it was given.

And treatment during our organogenesis represented -- during organogenesis at gestation 6 through 13 represented by the red bars produced a significant adverse effect at all of the test doses given.

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DR. CAMPBELL: Now, we turn to something a little bit different. The Saini 2014 study not only compared the effects of prenatal nickel exposure at birth, but the exposed offspring were then followed through the first six weeks of postnatal life with no additional administration.

And the next three slides just show the effects of both dose and days of exposure on postnatal pup growth starting here with nickel exposure during the pre-implantation period, or gestation days 0 through 5. The vertical axis on this slide is actual mean weight in grams and the data are not normalized.

Here, the different colored bars represent progressive weeks of postnatal age and then the clumps represent each dose. At the highest dose, you can see reductions in pup weight for each of the first six postnatal weeks following this very early gestational exposure. Significant effects were also seen at the middle dose used of 92 milligrams nickel per kilogram per day at postnatal weeks 1 and 6.

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DR. CAMPBELL: Now, this next slide in the set shows the effects on postnatal growth of nickel exposure restricted to organogenesis, gestation days 6 through 13. And what you see is there were significant decreases in

pup body weights at all doses and in each postnatal week from birth through postnatal week 6.

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DR. CAMPBELL: And the last one in this set shows the effects on postnatal growth of exposure to nickel limited to gestation days 14 through 18, the fetal period.

With this late gestation exposure no effects on post-natal weights were seen at the lowest dose of 46 milligrams per kilogram per day, but significant effects were seen each week with the two higher doses of 92 and 185 milligrams per kilogram per day.

What we don't know is what mechanism might occur, so that these gestation nickel exposures continue to affect post-natal growth or at what point, if ever, in their lifespan that adverse influence might stop.

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DR. CAMPBELL: Woops.

Okay. And this one just again shows the normalized data across the mouse oral studies for fetal or birth weights following prenatal exposure to nickel. Findings across studies showed somewhat similar pattern to the earlier slide comparing live litter size across studies. Again, significant decreases were seen in all the studies by Saini et al. with doses as low as 467 milligrams per kilogram per day when nickel was given

during organogenesis.

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DR. CAMPBELL: A question.

Sure. Yeah.

CHAIRPERSON GOLD: Dr. Allard.

COMMITTEE MEMBER ALLARD: Yeah. I just have a question with regards to Saini studies. Do you guys look into whether those were indeed separate studies, because it seemed to me a little bit surprising that one study would look at all three different developmental periods, and then they publish another paper with just one development period, then another paper with another developmental period. And then again, a paper with all three different development periods all in one paper. And I was just wondering whether that was just one data set perhaps that was divided into different papers?

DR. CAMPBELL: I have -- I'd have to look again to be 100 percent certain, but I think they were separate, because in the other ones they did sacrifice before birth and not follow them through post-natally.

COMMITTEE MEMBER ALLARD: Okay.

DR. CAMPBELL: But we can -- you know we can double check that. Sometimes they kind of fool you, you know.

COMMITTEE MEMBER ALLARD: Okay.

DR. CAMPBELL: Okay. Is that --

COMMITTEE MEMBER ALLARD: Yeah.

DR. CAMPBELL: Alrighty.

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DR. CAMPBELL: Okay. This is slide shows the details of the only available inhalation developmental toxicity study in lab animals. In this case, pregnant Wistar rats were exposed to nickel oxide aerosols at concentrations of 0, 0.8, 1.6, or 3.2 milligrams per cubic meter. Exposure was continuous from gestation day 0 throughout gestation, until sacrificed for evaluation on gestation day 21.

Maternal weight was significantly decreased at all three exposure levels. Fetal weights were significantly decreased in two -- at the two higher concentrations of nickel oxide. They did not provide data on live litter size or any other objective measures of fetal viability, but the numbers of fetuses were stated to have been unaffected by treatment.

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DR. CAMPBELL: There were an additional eight publications on experiments conducted by various injection methods in rats, mice, or hamsters. While injection is generally not a preferred route for animal studies as it's less relevant than oral or inhalation to human exposures,

these are included for completeness and also give a bit of a look at some of the other nickel compounds.

Although these -- this slide just shows four studies that were done by intraperitoneal injection. And they're all some form of nickel chloride. There's one in rats and three in mice. And just to make some general comments, each of these studies involve dosing on a single gestational day. In some cases, different days and/or different doses were compared. As was the case for oral exposures, both dose and day of exposure influenced outcome.

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DR. CAMPBELL: And this slide just shows the remaining injection studies. And I'm not going to go through these in detail. But I did want to comment on the one at the bottom, the Sunderman et al. 1983. It gave nickel subsulfide to female rats by intrarenal injection at a dose of 30 milligrams nickel per kilogram body weight at one week prior to breeding, so before the animals were pregnant.

The treatment was said to have resulted in intense erythrocytosis in the adult animals. Their pups were found to have had reduced hematocrits at two weeks postnatal age, though those values normalized as the pups matured and began to eat rat chow.

The offspring of the treated dams also had significantly reduced body weights at two and four weeks postnatal age. These findings could indicate a postnatal developmental effect on offspring following pre-mating maternal exposure to a nickel compound. Although, of course, it's totally unclear if the effect was specific to the subsulfide form and/or to the unusual intrarenal route that they used.

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DR. CAMPBELL: Just to conclude with a brief summary on the overall human and animal data pertaining to developmental toxicity. The human studies included five cohort studies of air pollution, which all reported small, but statistically significant, associations between exposure to nickel particles in ambient air, and adverse effects on measures considered to represent fetal growth.

Results from studies of effects of nickel on autism spectrum disorder, ASD, spontaneous abortion, congenital defects, and pre-term birth were inconsistent. Among the animal studies, regardless of species or route, the most sensitive and commonly reported adverse effects of prenatal exposure to nickel were reductions in viability and reductions in body weights of surviving offspring. Both the dose of nickel and the timing of exposure were observed to impact the frequency and

severity of effects.

And, at this point, we'll just pause if there's any questions from the Committee on either the human or animal developmental toxicity presentations.

CHAIRPERSON GOLD: Any questions from Committee members for the staff so far?

Okay. I think not, so we can move on with Dr. Wu.

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DR. KIM: Three epidemiologic studies examined effects of nickel on the female reproductive system. Outcomes were fecundity, clinical characteristics of polycystic ovary Syndrome, or PCOS, and pre-eclampsia.

Bloom et al. measured nickel and other metals in blood in a cohort of anglers, and reported that nickel was not associated with time to pregnancy, an indicator of fecundity.

Zheng et al. examined metals in relation to clinical characteristics related to PCOS in a case control study. A one microgram per liter increase in serum nickel was associated with a 12.6 percent reduction in sex hormone binding globulin adjusted for age, BMI, and waist-to-hip ratio.

Maduray et al. Reported that mean serum nickel was lower in pre-eclamptic women than in normotensive

women though the difference was not statistically significant.

And to Dr. Wu.

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DR. WU: The literature identified on animal studies of female reproductive toxicity of nickel encompassed several endpoints. The uterus was not a well studied endpoint. There were a few studies on the ovary and a multi-generation reproductive toxicity study that reported on estrous cyclicity. Numerous studies discussed reproductive index endpoints. Finally, three studies on milk composition were identified and the secretion of prolactin was also identified as an endpoint of nickel exposure.

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DR. WU: In an in vitro study by Rubanyi and Balogh examined the effect of nickel chloride using uterine tissue from Wistar rats. Nickel had dual action on uterine spontaneous contractions. In low concentrations, ten to the minus seven to 10 to the minus fifth molar, nickel chloride increased basal tone significantly, but had no effect on the amplitude or frequency of development or isometric force.

High concentrations of nickel chloride ten to the minus fourth to ten to the minus third molar inhibited

spontaneous contractile activity and decreased basal tone, which was antagonized by elevation of the extra cellular concentration of calcium. Exposure to nickel also caused mitochondrial structural damage and accumulation of glycogen.

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DR. WU: The effects of nickel vary from histological changes to functional alterations in the ovary. Nickel has been reported to disturb regular ovarian cycles, induce a dose-dependent anovulation, alter the secretion of several hormones, notably progesterone and cause histological alterations. Changes in weight and signs of oxidative stress were also noted.

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DR. WU: In a multi-generation reproductive toxicity study by Siglin et al. from Springborn Laboratories, different incidences of natural -- naturally occurring estrous cycles were reported for F0 and F1 females as shown in the first two bullets. The mean cycle lengths of F0 and F1 females were between four and six days long which is nearly a textbook definition of normal cycling in rats.

Oral administration of nickel sulfate hexahydrate over the course of two generations at dosage levels of up to ten milligrams per kilogram per day had no

toxicologically meaningful differences reported among the groups with respect to estrous cycling fertility indices, gestation lengths or the onset of sexual maturation in the F1 rats.

Albeit, the mean estrous cycle length appeared longer in the F1 generation as a result of treatment with nickel sulfate hexahydrate. OEHHA ran the Fisher's test for statistical significance comparing the controls to the treated groups and found the cycle lengths greater than ten days in the F1 generation approached statistical significance for the ten milligram per kilogram per day group. The exact trend test had a p-value of 0.053.

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DR. WU: Six studies were identified that examined endpoints which were encompassed by reproductive index. Nickel and nickel compounds might have effects on measures of reproductive index. However, it is difficult to pinpoint as the study designs evaluated are complicated by variables, including mating parameters, maternal fetal interaction, and period of evaluation.

Measures of reproductive index are more clear when evaluated as a whole with consideration of developmental toxicity endpoints. After oral administration or injection of nickel chloride during pregnancy in mice and rats, an increase in fetal death and

reductions in body weight of fetuses and offspring were reported.

Intraperitoneal injections of nickel chloride in mice on the first day of gestation were reported to have a higher frequency of both early and late resorptions, and a higher frequency of stillborn and abnormal fetuses.

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DR. WU: Maternal fetal transfer of nickel occurs in mammals via the placenta, and nickel has been detected in amniotic fluid and fetal blood. Nickel has also been detected in milk.

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DR. WU: Three studies of the effects of nickel on milk composition were identified. In cows, adding nickel to their diet produced no significant effect on milk production, milk composition, animal health or feed consumption. The solids, lipids, lactose, and fatty acids that compose milk were altered as a result of nickel exposure in rats. In rodent studies, changes in milk quality and production were shown after exposure to nickel chloride.

Reductions in liver weight in suckling pups were observed. And pups consuming milk from nickel-exposed mothers were observed to gain less weight.

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DR. WU: Prolactin secretion is reduced by exposure to nickel chloride in the dam. Secretion of prolactin is a normal pituitary function. Abnormal female prolactin patterns are known to alter the onset of maternal behavior needed for successful nurturing of young. It is possible that this action contributes to the changes in milk production and milk quality observed in nickel exposed rodents, as well as to negative consequences on the cycling offspring as suggested by perinatal mortalities reported in some studies.

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DR. WU: The endpoints examined in epidemiology studies and animal studies were widely varied. Human studies of female reproductive effects of nickel examined fecundity, hormonal effects, and pre-eclampsia. One study reported serum nickel was associated with a reduction in sex hormone binding globulin.

Studies in the animals reported adverse effects of nickel exposure on estrous cyclicity, release of some hormones associated with reproductive function, and alterations to the uterus and ovary. There were also studies on the effects of nickel -- there were also studies on the effects of nickel on the neuroendocrine control of prolactin in rodents and negative effects in offspring following changes in milk composition, after the

dams exposure to nickel and nickel compounds.

This concludes the presentation of female reproductive toxicity. If there's any questions, I'd be happy to answer them.

CHAIRPERSON GOLD: Thank you.

Any questions from the Committee for Dr. Wu or Dr. Kim?

Okay. So we'll proceed to male reproductive.

Dr. Kim, are you starting.

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DR. KIM: Yeah.

Nine studies examined associations between nickel exposure and effects on the male reproductive system. Nickel exposures were measured by personal air monitoring and in urine, semen, and blood. Outcomes were hormone levels and sperm and semen parameters, including sperm morphology, concentration, volume, motility, vitality, DNA integrity, and apoptosis.

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DR. KIM: Danadevi et al. measured nickel and chromium in blood of welders and unexposed men. Nickel and chromium were each associated with tail defects, viability, greater percent of sperm with slow or non-linear motility, and smaller percent of rapidly linear progressive sperm.

Nickel was more strongly associated than chromium with the percent of sperm with slow or non-linear motility. Hexavalent chromium is on the Proposition 65 list as causing male and female reproductive and developmental toxicity. Observed associations for nickel may be attributable in part to chromium, and possibly to other exposures associated with welding.

The three studies that analyzed nickel in semen were conducted in infertility centers. Slivkova et al. and Skalnaya et al. both reported no differences in semen quality. In the study by Skalnaya et al. Nickel concentrations were much higher than in the Slivkova study. And nickel was associated with semen volume below the reference level, as were copper and manganese and molybdenum.

Zafar et al. -- Zafar reported that nickel in seminal plasma was associated with reduced sperm concentration, semen volume, and sperm motility. Results for cadmium were similar. Of the 17 trace metals the authors analyzed, nickel was highly correlated with cadmium, copper, tin, and vanadium.

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DR. KIM: This graph from the study by Zafar et al. shows that men with normal sperm counts tended to have a relatively moderate semen nickel concentration, while

those classified as oligospermic meaning there were sperm, but the concentration was less than 20 million sperm per milliliter, generally had the lowest semen nickel. Men classified as azoospermic, or having no sperm, had the highest semen nickel concentration. A similar pattern was seen for cadmium, which is also on the Proposition 65 list for male reproductive and developmental toxicity. Analyses for -- were for single metals in this study.

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DR. KIM: Both studies on this slide examined associations between urinary metal concentrations and semen quality in men attending infertility clinics.

Zeng et al. reported nickel was -- quote, "Nickel was significantly associated with an increasing trend for sperm abnormal head. The p for trend was 0.03. When multiple metals were included, nickel and chromium remained in the model and the trend test for the association between percent abnormal head and nickel concentration was significant with p equal to less -- equal to 0.01. Sorry.

Zhou et al. reported that nickel concentrations in the fourth quartile were associated with increased comet tail length, indicating increased damage to sperm DNA, adjusted for multiple metals and other confounders.

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DR. KIM: Zeng et al. studied men presenting at a reproductive center. Third quartile urinary nickel was associated with lower serum testosterone, but nickel was not retained in the model with other metals.

Sancini and colleagues conducted a cross-sectional study among police working outdoors to evaluate the correlation between occupational exposure to low levels of nickel in urban pollution and plasma testosterone values. The authors performed personal dosimetry to assess environmental air exposure for 12 subjects. Nickel in air was highly and significantly associated with urinary nickel. Log urinary nickel was negatively associated with log plasma testosterone in the entire sample.

Wang et al. selected men from a hospital reproductive center. The highest quartile of nickel exposure was associated with an adjusted 20 percent decrease in total testosterone to luteinizing hormone ratio compared to the lowest quartile. Adjustment for other metals attenuated the association to 14 percent, but it remained significant.

And to Dr. Iyer.

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DR. IYER: After exposure to nickel salts, such as nickel sulfate or nickel chloride, a number of effects

on the male reproductive system were examined in several animal studies. These include effects on sperm. Other endpoints investigated include histopathological effects and reproductive hormone changes. Biochemical effects, such as lipid peroxidation and oxidative stress were also examined.

While I'm presenting these effects as four distinct endpoints, there is an overlap of these findings in some studies with more than one endpoint being examined.

I will summarize the findings in the next few studies, the next few slides, starting first with the effects on sperm.

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DR. IYER: So examining the effects on sperm. These include effects such as morphology as in abnormalities, motility and effects on sperm count, which is indicative of mortality. Over ten study reports presented findings of dose-related increase in abnormal sperm in seven species. Abnormal -- abnormalities were in the head, neck an tail region of the sperm.

In rats and mice, sperm count, as a measure of mortality, and sperm motility was significantly reduced by nickel in 10 studies. And in some of these included the studies that also presented abnormalities. And this

reduction was in sperm motility and mortality that was noted, was noted in both rats, fed a protein-restricted diet and those fed a normal diet. However, the percentage decreases in sperm count and motility induced by nickel were greater under protein restricted dietary conditions compared to normal diet conditions.

And these findings are supported by the in vitro studies showing changes in sperm motility and alterations in spermatozoa membrane integrity by nickel in bovine sperm.

Also, the findings appear to align with the report of reduced fertility index after male-only exposure in rats.

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DR. IYER: Examining the histopathological effects in the tissues of the male reproductive system, which is a reliable method for detecting effects on sperm production, rats or mice were exposed to nickel sulfate or nickel chloride, via the oral or dermal route, or via intraperitoneal injection. And one study involved exposure to nickel nanoparticles and nickel microparticles.

The effects differed across studies and the changes observed were dose-dependent and ranged from congestion and necrosis, and in some cases an increase in

frequency of localized apoptosis in the interstitium. Effects such as tubular degeneration, edema of seminiferous tubules, localized shrinkage, empty spaces in the seminiferous epithelium, and cell death was -- were observed.

In some studies, these histopathological changes were noted, along with a decrease in absolute and relative weights of the testis. In one study, degeneration of the germinal epithelium was time dependent with more tubules being affected.

Effects noted in the epididymis included: Degeneration, regressed epithelium of the cauda epididymis and presence of vacuolated cells. The authors note that the action of the metal on the epididymis varies from that on testis, and the damage produced in the epididymis and ductuli efferentes shows less of a tendency to recover suggesting that the epididymis may be more sensitive to the effects nickel than the testis.

One mouse study showed no histopathological changes in the testis or epididymis, but lower secretory activity of the seminal vesicle epithelium compared to controls was observed with a change of the epithelium from high columnar indicative of secretory activity to low cuboidal.

Overall, for histopathology, while there were

some limitations in the methodology used in the studies, consistently changes in the epithelial tissue of the testis epididymis were reported.

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DR. IYER: In the study in rats examining histopathological changes after exposure to nickel nanoparticles and nickel microparticles, that was mentioned earlier, a decrease in testosterone level resulting from testicular damage appears to have affected testicular spermatogenesis, and was exacerbated by reduced FSH.

Also, studies report dose-related decreases in testosterone production noted in the absence of cytotoxic effects in the mouse.

In another study, reduced testosterone production was noted in cultured rat, Leydig cells, via reactive oxygen species generation. These in vitro studies show consistent changes, such as decreases in testosterone levels, and oxidative stress as estimated by reactive oxygen species generation, apoptosis, and these support the in vivo findings that were described.

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DR. IYER: As mentioned earlier in some of the studies, nickel was used to exert toxic effects on male reproduction in order to investigate the mechanisms

underlying that toxicity. Biochemical assays were conducted to determine the levels of various biomarkers of lipid peroxidation and oxidative stress in the testes of rats and mice.

In addition to the empirical findings on sperm parameters and histopathology of the testes and epididymis, many studies have noted an increase in levels of testicular lipid peroxide. Along with the increase in level of lipid -- of testicular lipid peroxide, there was a decrease in antioxidant enzyme activities, including glutathione, and in some cases, the increased lipid peroxidation was accompanied with decreased testicular weight and a decrease in fertility rate.

Given that reduced serum and testicular ascorbic acid concentration and serum alpha-tocopherol levels were observed after nickel exposure. In some studies, simultaneous treatment with ascorbic acid was conducted and was found to significantly protect sperm from oxidative damage and improved sperm quality.

Also, nickel sulfate treatment caused testicular oxidative and nitrosative stress in rats, but simultaneous supplementation of alpha-tocopherol was found to be beneficial in combating against such stresses.

Multiple doses of nickel exposure produced moderate oxidative stress in the testis of mice, which was

associated with apoptotic cell death and DNA damage in the testis and in epididymal sperm, suggesting that nickel-induced testicular dysfunction at lower doses is either wholly or partially mediated through oxidative damage to macromolecules including damage to DNA.

Also, alterations in lactate dehydrogenase was -- were observed where increased levels were noted, along with membrane integrity being affected. And in some studies, decreased levels were observed along with decrease in testicular protein.

These biochemical alterations in the testes are markers of oxidative stress, and these findings led the authors to conclude that significant decline in sperm count may be due to membrane damage or to macromolecular degeneration by reactive oxygen species generated in the testes.

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DR. IYER: Human studies of nickel on male reproductive endpoints found that urinary nickel was associated with alterations in sperm morphology, lower plasma testosterone, lower testosterone to luteinizing hormone ratio, and DNA damage as indicated by the comet assay.

Observations from animal studies include effects of nickel on sperm morphology, motility and mortality as

well as histopathological effects and biochemical effects on the testis and epididymis. These effects may contribute to serum hormone decreases that are observed in animal studies, and are consistent with the findings noted in some studies in humans. And if you have any questions, I'd be glad to answer.

CHAIRPERSON GOLD: Any questions from the Committee for any of the staff?

Dr. Pessah.

COMMITTEE MEMBER PESSAH: I found it really helpful to have the charts in the first presentation, which actually gave you doses or concentrations. And I wonder how much of what was said subsequent to that, which didn't include concentrations or doses really should come into the discussion, because, you know, I think one has to look across what the levels are in serum or in urine, and really have a parallel in what the doses were or the concentrations were in the animal studies. So that would have been quite a bit more helpful.

CHAIRPERSON GOLD: I would just add a comment that on -- not the last presentation, but the earlier ones on developmental defects, I think, and birth -- and the weights of the pups and so forth, it would be helpful if we had sample sizes, especially where we have non-significant findings, because that could very well

influence whether we -- it's statistically significant or not. So for future reference.

Any other comments or questions?

CHAIRPERSON GOLD: Does the recorder need a break?

THE COURT REPORTER: I'm fine.

CHAIRPERSON GOLD: You're good. Okay.

Then if there are no further comments by the Committee -- so a slight change of plans. I will go to the Committee presentations and discussions at this time. And there's been a little bit of a question as to the order. So for the benefit of the public as well as the Committee members, the planned order of presentations will be first human studies of developmental effects, then animal studies of developmental effects endpoints, then human female reproductive effects, then animal studies of female reproductive effects, then human studies of male reproductive effects, then animal studies of male reproductive effects, and then finally the mechanistic studies. Are there any comments or questions, or are people ready to go with that?

Yes. Yes. Good.

Okay. We'll get started then with human studies of developmental effects. And I've ask Dr. Carmichael to be the primary presenter and Dr. Nazmi, the secondary.

COMMITTEE MEMBER CARMICHAEL: Can you hear me?

Okay. Great.

Well, thank you OEHHA for all the wonderful preparation that you've done as usual to help us get through and summarize and review the literature, and for your summaries.

So I'm going to go through sort of in a similar order by sort of sets of outcomes at the epidemiologic studies. So I'll start with the spontaneous abortion. Just a couple of comments. There were only two studies. I felt like they had very -- some very substantial limitations in their design. For example, the first one, which Chashschin showed an odds ratio of 1.8, which was suggestive of an increased risk, but there was no statistical analysis, didn't know if it was significant, didn't really know where the -- how the study design was conducted.

And then the other study by Vaktskjold, both of these were in Russia, it said it was studying spontaneous abortion, but the outcome really seemed like a mixture of spontaneous abortion, stillbirth, and neonatal death, and that like it included, for example, live births that died within 168 hours that were less than 28 weeks. So it was just -- so basically, there were some strong limitations there. So I would say that we basically from the -- from

the human literature, we don't know whether nickel is associated with spontaneous abortion.

And next, I'll go to the birth defects studies. There were five studies. And just again, I feel like the studies were so limited that it's hard to even say whether there is -- you can reach a conclusion with the human studies. A really important principle of studying birth defects is that they -- typically, when we're studying them, it encompasses a wide variety of structural malformations that affect a wide variety of organ systems, and different severities and phenotypes and etiologies.

And therefore, when I see studies that lump them all together or don't tell me -- that don't say what was actually within the groupings, it's really, really difficult to make any conclusions.

And basically -- so basically the studies tended not to find associations. There were -- for example, the -- one of the studies of soil in China, reported a higher risk of birth defects. But again, it was -- they were -- that was the Zheng study in 2012. The birth defects were studied as one great big group. So it was uncertain what was included there. So basically, I'd say there's not sufficient evidence to really determine whether nickel is associate -- does or does not contribute to birth defects risk.

And the autism spectrum disorder studies. Basically, there were, as we've said, seven studies there. The study by Windham and others in 2006 was the first study that -- as far as I'm aware, looking at this outcome and nickel, and it was self-described as an exploratory semi-ecologic case control study. It had the advantage of being very large, and good diagnostic information.

And it did see an increased risk, and odds ratio -- adjusted odds ratio of 1.5 for the highest quartile of exposure. And this was based on -- the exposure was based on emission -- air emissions data from the National Air Toxics Assessment Program, or the NATA, data.

So they did see -- so this was the first finding -- first study of this association, and they did see some preliminary evidence. However, I'd say that there were -- there a number of limitations of this study. As they say themselves, it was difficult to tease out the effects of nickel from other higher -- highly correlated compounds. And they did not get into that analytically. And there are uncertainties about this -- the NATA data, and how that correlates with personal exposure levels.

But I think that the biggest strength here was that it did -- it did provide some justification for looking further. So then after that study, there were the

remaining ones that we've discussed. The one by Kalkbrenner had a similar design as the Windham study. And that was in North Carolina and West Virginia. This has some of the same strengths as well as limitations. And they did not see a significant association. And then

Roberts from 2013 used data from the Nurses Health Cohort study. So they actually had self-reported data on the outcome. And they did see a significant association with nickel among boys. However, after they adjusted for other metals that were correlated, the finding was no longer significant.

And for girls, which were only 15 -- around 15 percent of the cases, there was not a significant association observed in any of the models. And then there were the four studies -- additional studies that were published from 2012 to 2018. McCanlies, Talbott, Ehrenstein, and Kalkbrenner, and they did not find evidence for an increased association with autism spectrum disorder.

One, the study by Talbott, actually reported a reduced risk of ASD with higher levels of this -- of nickel in the air emissions data. So, in conclusion, there was one. The initial study was positive, but a limitation is in how the exposure was excess -- assessed, and it didn't adjust for other metals. So I feel like the

evidence is somewhat inconclusive at this point on that outcome as well.

And then I will go to the fetal growth studies. There were, I think, 10 of those. Several of them had more varied designs and stronger limitations that made it difficult, in my opinion, to really make any strong conclusions from them, and that was the Odland studies in Russia; and Vaktskjold, again in Russia; and McDermott, and Hu -- McDermott looking at soil samples and Hu looking at maternal and cord blood. And they didn't tend to find evidence for increased risk associated with the different ways that they assessed nickel exposure.

And then there were the set of five studies, which you also, in your presentation, grouped together. So there were several somewhat higher quality cohort studies conducted in the -- primarily in the U.S. and I do think -- Dr. Gold, I agree it's important to keep into context that these were rela -- they were -- some of them were very, very large cohorts, which kind of, I think, that plays into how we might interpret some of the findings, and their significance.

Excuse me.

The study by Bell was in Kentuck -- was in Connecticut and Massachusetts. And just to step back, all of these were based on air emissions data I believe. So

Connecticut and Massachusetts it included 77,000 infants, and they did report a significant decrease in birth weight. It was approximately an 11 percent reduction in birth weight, based on the interquartile range, which is basically looking at sort of the difference in risk between the people at the 25th and the 75th percentile of the exposure.

And I'd like to note that the maximum distance from the -- these were emissions data that have been sort of modeled or extrapolated to the census tract level, which is -- think of that as a neighborhood approximately.

And the maximum distance from the census tract to the nearest monitor was 45 kilometers. I think it's important also to think about how -- how all these studies had very different sort of criteria for which exposures they included relative to how far someone lived from an actual monitor.

Ebisu and Bell, in 2012, was a study of ten states on the east coast. So there was 1. -- let's see, about 1.5 million births in that study. And that represented about 17 percent of the original population. And that was partly based on restricting to people who lived within a certain distance of monitors. But the maximum distance that was from a monitor to a county border, because this was at the county level, was 76

kilometers.

And they found a significant association with low birth weights. They found a six percent increase, based on again an interquartile range. But again, the level of detail and the -- how specific the exposure was to where people lived was somewhat broad.

And then there were two studies in California. The Basu study found that nickel was associated with significantly lower birth weight. Again, this was A really large study of 6 -- almost 650,000 births. They had a more restrictive definition of where women lived relative to the monitors. It was that they lived in a zip code within 20 kilometers of a monitor.

And again -- so they found a significant association, but I'd like to note the that, for example, for the -- for birth weight it was only a one gram difference, which is pretty small in magnitude. So I think most findings were likely to be significant at that -- statistically significant with almost 650,000 births, so that's why I think it's really important to think about the magnitude there.

And then Laurent and others looked at births in L.A. county in particular. And they used, I think, it looks like a greater density of monitoring sites than perhaps any of the other studies overall, and used very

sophisticated pollutant modeling, especially relative to how some of the other studies went about it.

And they did find a significant association with low birth weight. Again though, it was a pretty large study. Let me see if I can find that in my notes. Oh, it's about a million births. And it was a one percent difference in the risk of low birth weight. So again, very large study, found a significant finding, but it was relatively small in magnitude.

And then we have Pedersen in 2016 combined data from eight European cohorts. They included 34,000 births, which is now starting to sound small, but it's not. And they had about, I think they said, 20 to 40 monitors per study city that was included from these eight cohorts. And they did not find a significant association with low birth weight with nickel, but they did see a significant association with head circumference. So they saw a significantly smaller head circumference. That's the only study that I'm aware of that looked at head circumference.

So in conclusion, based on all these studies, there were several that reported reduced birth weight with higher estimated exposure from nickel via air pollution. But I would interpret these with caution. The changes in risk tended to be quite small and the studies were large.

And in addition, in particular, the east coast

studies had a pretty high threshold for distance from monitors for assessment of nickel exposure. And other alternative explanations I think can still exist to explain some of these findings that weren't ruled out, such as nickel not -- nickel is -- could be a surrogate for other pollutants, which tended not to be adjusted, and other -- there may be other aspects of living in an area with higher nickel levels in the air or even living close to a monitor in particular.

So therefore, I think there's some suggestion that living in areas with higher nickel levels may be associated with small increases, but alternative explanations still exist for some of these findings.

And then we had a couple of studies -- there's quite a variety outcomes here. A couple of studies looking at transplacental carcinogenicity registry based case control studies. One, looked at neuroblastoma and retinoblastoma, which are cancers that primarily affect very young children. That's when most cases occur. And they had good -- they used cancer registry, had good diagnosis of the cases, which is always a big strength, but they were relatively small in the number of cases, because these are relatively rare outcomes with like 50 to 60 cases in each of these -- for each of these outcomes.

They did find and assoc -- an increased

association with retinoblastoma with an adjusted odds ratio of 1.5. The odds ratio for neuroblastoma was 1.1 and not significant. They did restrict their study to subjects that lived within a five-mile radius of a monitoring site. And they note that the studies -- it's interesting, because nickel has been found to be associated with lung and nasal cancers in exposed workers.

And there retin -- there's some animal evidence showing an association with nickel with retinoblastoma. However, I don't think they dealt with potential correlations across other pollutants. So I think that these findings are provocative. They have some biologic plausibility, but it is just one study on these outcomes. So it's hard to make firm conclusions at this point, based on one observational epidemiologic study.

And then Togawa from 2016, there is the only study of testicular germ cell tumors, which were diagnosed at age 14 to 49. And this was a multi-country study in several Scandinavian countries. It involved 8,000 cases. It had good diagnostic information. Study was population based, which improves its potential validity. They used -- they did not see a significant association. This study was based on parents' occupations. So that has its own limitations. So I basically say there's one study that wasn't significant. But I think it had some

limitations, so the jury is still out on whether -- so to speak, on whether there's an association -- evidence for an association in humans using human data.

And then there's the one study by Ni in 2014, that show -- found that there was a significant association between nickel and this marker of DNA damage, the 8-OHdG. And that was -- a strength of that study was that it was after adjustment for many other factors including other metals.

And there were two other studies that were -- that were available for review, one by Zheng looked at a composite measure of other adverse newborn outcomes. It grouped together things like fetal distress, pre-term birth and macrosomia. It did not find an association, but basically I think it's -- you can't really conclude from this study anything about those composite -- those outcomes that were -- are within a -- are important, but it's a composite, so I don't think we really have evidence regarding those outcomes that can be used to make any firm conclusions. And then there was one study on early childhood pneumonia that did not find an association.

And so in conclusion, I'd say there were some modest associations that were observed. Overwhelmingly, they were -- they were not significant. Among the ones that were positive, they tended to be for birth weight,

and they tended to be relatively small, based on the -- sort of the interquartile range that was observed, which is, you know, certainly doesn't represent the entire spectrum of what people might be actually exposed to.

And it also remains difficult to just -- to isolate the effects of nickel alone.

Those are my comments. Any questions?

CHAIRPERSON GOLD: Thank you.

Any questions for Dr. Carmichael from the Committee?

So, Dr. Pessah.

COMMITTEE MEMBER PESSAH: Just wondering, was there any analysis done for proximity to the actual monitors, and whether there was a relationship, because it seems like these monitors were quite a variable distance from --

COMMITTEE MEMBER CARMICHAEL: It was highly variable across studies from one that -- and, you know, they don't all report it either from, you know -- you know, up to average of 76 kilometers to really restricting to five kilometers. In a couple of the studies, I can't remember which ones exactly, looked at -- they would go from five kilometers to say 3. -- 2.5 or something more restrictive to see -- to ask that exact question to see whether the results held up. And I think that they --

they tended to in the studies that did it. But I'm sorry I don't remember which exact studies looked at that.

CHAIRPERSON GOLD: Any other questions for Dr. Carmichael?

Okay. Dr. Nazmi, anything you care to add?

COMMITTEE MEMBER NAZMI: So given Dr. Carmichael's pretty thorough review and OEHHA's phenomenal presentation - thanks for that - I'm just going to make three quick points summarizing some of the findings the way I see it. And I'm not going to get into individual studies. So my first point is that -- is a methodological one, that the methods of many of these studies might be considered weak, in terms of study design, analysis, and even sometimes reporting.

And even the larger studies, some of which Dr. Carmichael mentioned, and the higher quality studies tended to examine the impact of multiple chemicals. And it was impossible, or nearly impossible, to tease out the specific effects of nickel and nickel compounds on the outcomes that were being assessed.

My second point is that findings were largely not significant, and sometimes equivocal.

And my final point is that there were a few suggest -- studies that were suggestive of risk of nickel in compounds on some outcomes, and perhaps most notably

fetal growth and ASD that showed significant risk. Sometimes the risk was small and sometimes there were other factors to consider, as Dr. Carmichael suggested, in terms of study size on these developmental outcomes.

But that said, I think the weight of the evidence, the way I see it, is not -- is not very compelling.

CHAIRPERSON GOLD: Thank you.

Any questions for Dr. Nazmi?

Anybody?

Nobody.

Okay. Thank you.

So next we're going to discuss human studies of female reproductive effects. And I'll lead that discussion first, to be followed by Dr. Nazmi.

Okay. So as the staff indicated, we had three human studies that dealt specifically with female reproductive effects of nickel, and two additional studies that -- okay. I didn't receive the complete agenda, so I apologize if I'm a little bit confused, but I thank you for the correction.

Before we go to female, we will go to animal studies of developmental. Okay. And, Dr. Plopper, you are the primary discussant. I apologize for the confusion.

COMMITTEE MEMBER PLOPPER: Okay. Well, to begin with, I'd like to thank Dr. Campbell. You just saved us all about 30 minutes --

(Laughter.)

COMMITTEE MEMBER PLOPPER: -- because that was about half of what I had to say. And I want to also comment on OEHHA for their efforts to develop a consistent pattern of dose. I would say that the fairest way to look at this is that the literature on animal exposure is considerably uneven. And you did an excellent job of trying to translate that all into an exposure of nickel at a particular concentration and a time, and with a particular body weight, and per day, which makes the first graph you showed, overdoses, is a tremendous simplification and an accurate one, by the way, of exactly what the problem is here.

So I will -- I had a number of specific points to bring out that you've already covered. So what I would like to do is just spend a few minutes discussing how we could possibly look at this data. And the first of these is that there is a wide range of information that's available on what is essentially the viability of the conceptus.

And what -- that has been translated into the literature, and I made a list of about half of them. Part

of them are number of pups per implantation. Another is number of implantation sites per dam. Another is live pups per group or per litter. And in two cases, it's just the total number, which doesn't tell you if it was either the litters or the dams.

And another is, of course, the live pups and then the dead pups. And what's interesting in some of these studies where they report the dead pups, the live pups are the same. And this has been a tremendous -- it was tremendous problem for me, because I don't understand exactly how they got that way. And then the number of litters with live pups, and then some postnatal viability. And it depends. Some studies do it right at birth, and some do it days later.

So I don't mean to confuse the issue, but there are two ways to look at this. One is how many pups did they find that were dead one way or the other, and the other is how many were viable and how many survived parturition.

And I think the data that you have there, if you look at it from two points of view, one is how many survived, what was the litter size at the end, and then how many were found dead or didn't make it somewhere along the line? And the other confusion here is that some of the studies that assess pups prior to parturition do not

then necropsy the uterus of the dam to find out how many were implanted to begin with.

So we have that as another confounding issue here. And I think you did a nice job of pulling that all together. And when you see where the inconsistencies are in terms of either the live -- number of live pups in a litter or the number of dead pups, the inconsistency is based on when this measurement was made, and how thorough it was done.

And it drove me crazy. There's a couple studies where there's essentially no statistics. The statistics and they're lumping everybody together. Yeah, and it turned out the exposed animals had twice as many pups as the others, because there were twice as many. And so did they have more live litters? You can't really tell that from here. So I don't mean to be negative, but I think those are -- those needs to characterize what we do.

The other aspect of this is with this one -- and then the other part of this is how did -- what was fetal postnatal growth and where did they grow? And it's the same problem, because it's the same pups taken the same way. And in some cases, the -- its's prior to birth, some cases it's right immediately postnatal. Some cases it's some point during the postnatal period when lactation is going on. So you have that as an additional confusion.

And I think maybe I'm going -- I'll end up -- I don't want to get too negative on this. But when you go and look at the table, the graphs and the -- I went through every one and listed these out, the ones that don't show some sort of negative impact on either the dead pups or the litter size are the ones at either the lowest dose levels level.

And there is a clear dose response there no matter how you time it. And if you see from that graph you showed of the doses along there, well, you can tie those -- the dose is down to how much, but whether this had a negative impact on pup viability or pup death. It drives me crazy to have to use them both, but that's the way they're put out there.

And the -- so there's only -- there are two that show no -- no -- have a negative response, and didn't have anything at all. Those were ones that were -- the treatment was during pregnancy and the necropsy was assessed immediately after the last exposure, which very likely would reduce the chances. And the others were at the low end of that dose range, so which is you would expect. And there are two or -- there are two sets of very nice studies that do complete dose ranges, and one that looks at exposures at the early stage, the 0 to about 6 days pregnancy, another from 6 to 13, and then from 14

till whatever parturition is.

And when they assessed those on specific time frames, they do find that it does have a negative impact on the viability of the conceptus. Okay. The same thing is true for assessing the body weights. No weight gain for a large number of them. And it's due either to one -- the short term of the exposure or the short term after exposure, in which the -- in which the size or the fetus was assessed.

And it -- but it does -- and in some cases, where there were those detailed studies, like the ones in the mouse, which looked at specific time frames for exposure, it's obvious that the organotypic the middle time frame when all the organs and the embryo are organizing themselves, the tissue is organizing, it's establishing a relationship with the uterus. That is one of the most susceptible times for body weight gain, and it's also one of the susceptible times for death, as is the earlier pre-im -- pre-implantation one. And I will say that most of those studies that assessed implantation showed that that -- that the implantation was not affected by -- by the exposure, but the viability was.

So that meant that some of these conceptus initially established a relationship with the uterus, and then didn't -- didn't survive. And I think that was --

you covered about 90 percent of it. So I think I've identified it. And my take on -- from those two is that it's a dose -- both of those issues, how many pups there are and where they -- whether they survive is a dose-related and exposure-related problem, and as well as the ability of the fetus to then grow afterwards.

If you look at the papers that don't have that, there the ones that either have the very low concentrations or they have the exposures at specific times when the fetus would not be as susceptible. And I will point out you had a nice graph that showed the significant ones and the not significant ones. Well, if you -- actually, I redid a calculation on some of the ones that had different doses that were not significant, and they have a dose response. And if they had one higher concentration to expose, it would have been significant.

So it's -- it's there, but it's the low dose that did it. And I'm -- okay. I'm not going to take too much more time.

I want to say one other thing and that is that part of this issue of fetal growth needs to address exactly what systems are being targeted here. And there were four studies that actually analyzed the pups afterwards to find out what could be the problem. And the biggest problem, probably because it's the easiest one to

assess, was an impact on the musculoskeletal system.

And you can think of just about any bone in the pup's body, and it was either affected, because it wasn't there or there was very low calcification. And what that says is that there -- the two processes are -- at least two processes are susceptible there. One is the formation of the pre-bone. These are almost all cartilaginous bones in the cartilaginous. So that means that the cartilage didn't form to begin with, and then that it didn't ossify afterwards.

And rather than go into anymore details. If you want a list, I've got the whole list. And for me, it's very interesting, but I'm sure it's not what the public wants to hear.

So I don't have anything more to say. I thought it was reasonable consistent. The best studies showed the very clearest impact on time of exposure during gestation and the -- and the concentrations.

And I'll stop there.

CHAIRPERSON GOLD: Thank you, Dr. Plopper.

Any questions for Dr. Plopper by the Committee?

Dr. Pessah.

COMMITTEE MEMBER PESSAH: So I guess in summarizing what you just summarized, is there an effect of nickel or is it an effect of how the study was and the

outcomes were reported?

COMMITTEE MEMBER PLOPPER: No, there's actually -- that's a good point. I left that out, but she brought it up already. These are all soluble nickel compounds. And it is clearly the result of either -- they're either exposures to nickel sulfate or nickel chloride, and it very clearly has to do with the -- with the nickel that's there, I think. Sorry, if I didn't bring that out. That's all.

CHAIRPERSON GOLD: Any other questions for Dr. Plopper?

Okay. Dr. Allard, you're the secondary discussant.

COMMITTEE MEMBER ALLARD: Thank you.

Again, I think for the sake of time, because of the beautiful summaries that were performed, I will sort of skim through my notes here and just mention the most salient points.

I want to echo what was mentioned, which is the -- these two concepts of principles of inconsistencies that sort of emerged by reviewing the literature, as well as doses. And, of course, as Dr. Campbell really beautifully highlighted those two go hand-in-hand where the inconsistencies lessen as you increase the dose, right? That was highlighted in your graphs.

So the rat studies that tended to have lower doses were more inconsistent than the mouse studies that tended to be more consistent in reporting an effect from nickel exposure were perhaps more consistent because the doses tested tended to be higher. Although, I do want to highlight that even at high doses, there were still some studies showing no effect, while others did.

And at the end, skimming the entire body of literature presented in the document -- in the hazard identification document, as well as PubMed, at the end, it seemed unclear to me as to why some of the studies looking at the same -- around the same Exposure level would not -- would lead to inconsistent results.

So for the rat studies, I did tend to lend quite a -- lean, sorry, more, of course, towards the oral exposure, which I thought were more relevant. In terms much of the route of exposure. Although I did consider also the inhalation study that potentially could be relevant for in some occupational settings perhaps. I tend to lean more on the Siglin et al. study from 2000 -- so the 2000 Siglin A study, the one-generation study, where they did a beautiful dose response, and identified post-implantation losses and dead fetuses with a LOAEL of 10 milligram per kilogram per day, which -- and again, I want to highlight how useful that was converted in nickel

alone. So the compound was nickel sulfate, hexahydrate. And so nickel alone would be about 2.23 milligram per kilogram per day.

And I tend to lean more on that one, because I thought the outcomes reported there sort of aligned with some of the outcomes mentioned in human studies. Although -- so for me you know in the weight of evidence, that's sort of going the same direction that give me more confidence perhaps on the outcome of that study. And I'm particularly mentioning here the Chashschin, I guess, et al. Study from '94 in nickel refinery workers on page 27 that mentions a high incidence of spontaneous abortion and abnormal pregnancies overall.

Skipping, skipping.

The Saini studies, I really appreciated the fact that they try to identify the developmental window of increased sensitivity. Although, I tend to look at them from a more cautious perspective, perhaps one study instead of three studies. So I do not necessarily consider that there was perhaps repetition or confirmation of outcomes between the different studies.

But again, the -- the doses tested in all this these studies in mice were much higher than the ones done in rats. So at the end, for me, it came down to the dose. And the -- the lowest LOAEL, if that makes sense, reported

was for the -- the one-generation study in rats at 10 milligram per kilogram day. And this is -- you know, even with an uncertainty factor, this is much higher than the -- at least the average exposure level in human from all sources of -- which is estimated from -- in various documents, public documents at about 2.4 micrograms per kilogram per day.

So I -- at the end, I did not necessarily felt that there was a compelling picture here to -- for nickel, in terms of developmental toxicity, at least in general settings. Although some of the outcomes in animal studies seemed to align perhaps in with some of the outcomes in occupational settings, as I mentioned.

And I'll end my comments here.

CHAIRPERSON GOLD: Thank you.

Any questions for Dr. Allard by the Committee?

And how is the record doing? Are we ready for a break?

Ready for a break. Okay. So five minutes. Five minutes good?

Okay. So let's resume in five minutes.

(Off record: 11:48 a.m.)

(Thereupon a recess was taken.)

(On record: 11:55 a.m.)

CHAIRPERSON GOLD: So can we please try to

reconvene. Okay. First, I'll ask if the Committee has any questions on developmental studies, either in humans or animals at this time?

And then I'll apologize for misreading the agenda and jumping ahead. Too eager to jump the gun. But we will now start talking about female reproductive effects. And I will lead the discussion on humans, followed by Dr. Nazmi's comments. And then we'll go to animal studies.

Oh, and the plan is -- I think we can get -- I don't want to foreclose any discussion, but I think we can get through female reproductive effects and then take a lunch break. So that's the current plan. Okay.

All right. So as I started to say before, we had three human studies that dealt specifically with female reproductive effects of nickel and two additional studies that examined spontaneous abortions among nickel exposed workers.

The three studies of specific female reproductive effects included a small prospective longitudinal study, cohort study by Bloom of 80 non-pregnant women who participated in a survey. And the investigators examined blood nickel concentrations and those of other metals at baseline in relationship to the outcome time to pregnancy, which is a measure of fecundity or subfertility, which was detected using pregnancy kits.

So the statistical analyses in this paper considered a number of relevant potential confounding variables using appropriate statistical procedures. They found a small but non-significant increase in time to pregnancy in relation to blood nickel concentrations, and characterized their findings as detecting no association of nickel with the probability of a positive pregnancy test.

While the study design, exposure, and outcome assessment and analytic approach are strengths of this study, the small sample size makes the results largely non-conclusive. Additionally, we had a recent small cross-sectional study conducted in South Africa by Maduray of 43 women with eclampsia -- excuse me, pre-eclampsia, in 23 normotensive pregnant women, which showed -- showed concentrations of nickel and serum in hair samples to be non-significantly lower in women with pre-eclampsia and to be negatively correlated with diastolic blood pressure.

However, this study incorporated no multivariate modeling to control for confounding, even though their data showed significant differences in some important variables between the groups. So given the small sample size, which may have been inadequate to provide adequate statistical power to detect modest, but meaningful, potentially meaningful differences as significant, and the

lack of control of confounding these results, also can't be regarded as conclusive.

Then the third study by Zheng et al., was a case control design to compare serum nickel concentrations in 96 women with polycystic ovarian syndrome to those in 105 controls selected from the same medical center who did not meet the criteria for having symptoms of PCOS.

A blood sample for assaying nickel concentrations was obtained from each participant on days two or three of menstrual cycle. Mean serum nickel levels were significantly higher in PCOS cases than controls. And nickel levels were associated with a significant decrease in sex binding -- sex hormone binding globulin, and small increases in dehydroepiandrosterone and fasting insulin levels, controlling only for body mass index and age and waist to hip ratios.

The analyses focused on the relation of 11 different metals in relationship to 15 outcomes that included nine different reproductive measures. Thus while this study used appropriate statistical analyses, the relatively modest sample size, and many statistical tests conducted without adjustment for other potentially confounding variables and for the multiple testing, as well as the case control design did not provide a convincing or conclusive results regarding the relation of

nickel to reproductive analytes.

So the two additional studies which the staff mentioned and have already been mentioned in terms of the developmental context examined the relation of nickel exposure to spontaneous abortions and premature births, which could also be considered as female reproductive effects, as well as developmental.

So one of these was case control study of women employed in 14 nickel exposed work areas in Russia. A total of 474 cases and 4,571 controls from the birth register and 184 cases and 1,691 controls who completed questionnaires at these workplaces were included.

Assessment of nickel exposure was based on prior monitoring of work areas or measured urinary nickel concentrations. So the unadjusted odds ratio for nickel exposure in relation to spontaneous abortion from the questionnaire portion of the study was significant. But once adjustment was made for confounding variables, it was no longer significant, and was attenuated and there was no evidence of a dose response.

The results from the birth registry data showed no association of nickel in relationship to spontaneous abortion. And then there was one earlier cross-sectional study in Russia comparing 290 pregnant workers in a nickel hydrometallurgy refining plant to 336 working in

construction. Nickel concentrations were measured in departments at the plants and urinary nickel was also measured in workers.

And the study revealed spontaneous abortion rates in nickel-exposed workers of 16 percent and nine percent in the comparison group of construction workers for an odds ratio of 1.8, along with a nearly three-fold increase in structural malformations in the offspring.

And an increased rate of premature births. However, statistical testing was not clearly presented, nor was adjustment made for potentially confounding variables. And no comparison was made of nickel concentrations among pregnant workers in the refining plant who had spontaneous abortions to those who had term pregnancies. So all of these limitations tend to make the results of this study inconclusive.

So in conclusion, we have -- largely have modestly sized studies of varying designs and quality generally with only a single assessment of nickel exposure. Albeit, it -- they tended to be individual assessments as opposed to sort of ecological assessments. And they examine different female reproductive outcomes.

So the observed relations with nickel exposure have ranged from none to modest, and due to the designs, differences in the designs, analytic methods used,

limitations and exposure assessment, and the modest findings, it seems that none of them are sufficient or, compelling, or convincing in indicating a causal relation of nickel exposure to female reproductive effects.

I'll entertain any questions?

Okay. Seeing none.

Dr. Nazmi, I will ask you if you have any additional comments.

COMMITTEE MEMBER NAZMI: I have nothing further to add.

CHAIRPERSON GOLD: Okay.

That was quicker than I expected.

(Laughter.)

CHAIRPERSON GOLD: Okay. So any comments from the panel or questions?

Do we want to try and move on to animal studies of female reproductive and get that in before lunch? I think we can probably do that. Is that okay?

Okay. So, Dr. Luderer, you're going to start us off.

COMMITTEE MEMBER LUDERER: So I was going to start with talking about the evidence from the large number of studies that we've already heard discussed quite a bit today that dealt with effects of nickel exposure on prenatal mortality and growth as well as neonatal and

postnatal mortality, and growth.

And so I'll just, you know, summarize kind of my perspective on those studies briefly, because I think that is where the largest database is with -- for female reproductive toxicity with also the caveat that was brought up in the OEHHA document that it's difficult to sort out whether this is toxicity due to direct effects on the developing embryo or fetus versus the mother and we -- so -- but we will -- I think one can say that some of the effect is via the mother, and so that's why it's included under female reproductive toxicity.

So basically, I agree with what's been said that there's evidence that at the higher doses, so the studies that used higher doses tended to find effects both on prenatal, embryonic mortality, and -- you know, resorptions, various ways that that was measured in addition to finding dead fetuses, as well as fetal weight restriction, and postnatal, and neonatal mortality, as well as decreased weight gain postnatally.

So that was more likely to be found in the studies that used higher doses and at the higher doses in studies that used a wider range of doses.

Also, I think it is notable that effects on these endpoints were found in two different species, rats and mice, and via different routes of exposure that have

already been talked about, as well as during multiple different dosing windows. So then I think that the database is quite -- is moderately strong for an effect on the neonatal/postnatal mortality and growth being decreased with higher doses of nickel, as well as pre and -- prenatal embryonic death and growth restriction.

Going then to effects that are more specific for female reproductive toxicity, I'm going to talk next a bit about the effects on prolactin secretion. So there were four papers that reported on effects of nickel chloride on prolactin secretion by the anterior pituitary. And so two of those -- two of three in vitro experiments used male pituitaries and one used bovine pituitaries of unknown sex. Only one in vivo study used females and their weaning aged pups. And one reported on an in vivo study in males.

Now, all four papers did show that nickel chloride decreased prolactin secretion. Just to go into a little bit more detail. In the Carlson et al. study, they used male rat pituitary fragments in both static and perfusion cultures and found that one hour approximately long exposures to nickel chloride depressed the basal and stimulated prolactin secretion.

Similar results were found in cultured bovine pituitary fragments of unspecified sex. And in that same

paper, they also did an in vivo study with nickel chloride infusions in chlorpromazine anesthetized rats. And they again found does and time-dependently decreased serum prolactin concentrations.

Finally, there was an in vivo study that utilized subcutaneous injections and found decreased prolactin, but again that was male rats.

I think -- the reason that I think that those were in the document, and that I think they're relevant, is that we -- there is -- I think that the -- the effect of nickel chloride on prolactin secretion is not likely to differ between the males and females. We do have one female paper where we see that Smith et al. in their study in rats found that female rats that had been treated with the nickel chloride during -- in the drinking water, and that were euthanized after the pups -- their offspring had been weaned, that study did adjust for estrous cycle stage when the prolactin levels were measured. And they found a decreased -- significantly decreased prolactin in the highest dose dams.

They did not observe statistically significant effects on the pups. And they looked at two different -- the -- a first wave Of litters and a second wave of litters. However, looking at the first wave of litters, there was a large amount of variability in the prolactin

concentrations in that study. And the prolactin concentrations were very high, which to me raised the question of perhaps there was some stressor that occurred when those litters were euthanized.

The second litter, the -- there was actually a difference in prolactin concentrations in the highest dose group, which is the only dose group in the offspring for which prolactin was measured, but it wasn't statistically significant.

So overall, there appears to be more of an acute effect of prolactin -- on prolactin secretion with nickel chloride. Most of these studies just looked shortly after administration of prolactin. One study did look at one two, and seven days after a single dose and found that then actually prolactin increased. That was in males.

So I think the prolactin studies are relevant in light of another study, which I'm going to talk about next, which is the effects of nickel on mammary glands -- on the mammary gland and on milk composition. So there were two studies investigating the effects nickel on milk composition, but one of those -- only one of those also investigated effects on the mammary gland in the lactating female and on endpoints related to offspring development and -- and in that study was Dostal et al. study.

Unfortunately, the route of exposure here was

subcutaneous injections, which I don't think, you know, as we've already talked about, are not relevant to humans. Nonetheless, I think it is an important study, because it is the only study that really looked at mammary gland effects, and also measured nickel concentrations in the milk.

And they found that repeated daily exposures to nickel chloride raised the nickel levels in milk significantly from less than two micrograms per liter that was four daily doses to 513 in the 50 micromolar per kilogram group and over 1,000 micrograms per liter in the 100 micromolar group.

And concomitant with these increases in the milk nickel concentrations, they also reported decreased mammary gland weight in the lactating females, as well as decreased total RNA per gland, while the DNA per gland was not altered and this resulted in decreased RNA to DNA ratios.

And also, this then was associated with alterations in the composition of the milk, so that there were increases in the milk solids and lipids, while the protein and lactose concentrations in the milk were dose-dependently decreased. This study importantly also -- which some of the studies that we had talked about earlier noted that there was decreased water consumption

at high doses of nickel. This study actually did separate experiments where they pair watered the lactating females, and showed that not all the effects were due to decreased water consumption in the females, and I thought that was quite important.

So I think that looking at the prolactin effects, together with these effects on the mammary gland and milk composition that the effects on prolactin in lactating dams of nickel may play a role in this altered nickel composition, since we know that prolactin is very important in that -- in lactation.

And this -- the Dostal et al. study also noted decreased postnatal pup weight again, which we also observed in many of the studies that we talked about earlier. And the study suggests that the altered milk composition may play a role in that, which was observed in quite a lot of studies, but this is the only study that looked at milk composition together with that.

Getting into some of the female, the next set of studies I'm going to talk about is for the ovarian toxicity of nickel in animals. This database is not as deep as the database of the development database that we talked about. It's, I would say, somewhat supportive of nickel being an ovarian toxicant.

There -- and again, we have one study that used

subcutaneous injections, that's the study by Forgacs et al. The -- this study provided details on group sizes and statistics, which some of the other studies that fall into this group of the ovarian studies did not do.

So I think it was -- it showed an effect with subcutaneous injections that were begun on the day of estrous. So they performed vaginal cytology on the rats. And then on the day of estrous they began subcutaneous injections of nickel sulfate in this case for -- and they continued the injections for every four days for 21 days.

So there -- they were analyzing estrous cycles throughout this entire 21 days. And they showed alterations in the estrous cycles. Importantly, in this study, they showed individual animals and their estrous cycling. Although, they didn't present a statistically -- a statistical analysis, they showed that one out of 13 in the control group, five out of 14 in the 10 milligram per kilogram group, five out of 14 in the 20 milligram per kilogram group and 11 out of 13 in the 40 milligram per kilogram groups had two or fewer cycles during those 21 days.

Considering that a normal cycle is four to five days in length, that's a dramatic decrease in the normal number of cycles. And if you look at that with Fisher's exact test, there's a very significant effect with a p

value of 0.001, which they did not do, but they do provide the data where you can do that.

They also then collected over -- from the eggs from the oviduct at the time of euthanasia, and they reported that there was a decrease in ovulation. However, again, if you go to their estrous cycling date, you can see that the rats that had a vaginal cytology that was consistent with the day of estrous when you would expect to find the ova in the oviduct, those rats all ovulated. And they had the similar numbers of ova ovulated compared to the control rats.

So it looks like their decrease in ovulation was really having to do with the effect on estrous cycling and whether or not they attempted to collect the ova on the day when you would actually expect to find ova in the oviduct.

So -- and consistent with that, they didn't notice any differences in the number of corpora lutea when they did histology.

The other thing that his paper looked at was progesterone measurements collected from the ovarian vein in anesthetized animals prior to euthanasia. And here they -- only the highest dose group had a decreased progesterone response to HCG. But again, given that this was dose -- the dosing occurred during different points in

the estrous cycle of the animals, it's a little difficult to -- to interpret.

The second study I'll talk about briefly was the Rao et al. study, where this was a rats and mouse study where they dosed the mice for 30 days, and they observed a decreased ovarian weight that was dose-dependently decreased, as well as protein content of the ovaries. And in this study, similar to some of the -- the male reproductive studies that were so -- that were summarized earlier, there was evidence of oxidative stress in the ovaries with decreased ovarian glutathione -- the antioxidant glutathione and ascorbic acid, or vitamin C, as well as decreased activity of several antioxidant enzymes, superoxide dismutase, and catalase, and increased lipid peroxidation.

A caveat with this study is that they were sacrificed on random estrous cycle stages. They didn't evaluate estrous cycling. However, you would expect that this would decrease the ability to detect differences. In the -- I'll briefly talk about the two-generation studies really looked at ovarian weights and histology.

The Price et al. all study looked at those -- both studies looked at those endpoints, Siglin et al. also report on estrous cycling. There were no effects on ovarian weights or histology noted in either of those

studies, in -- to -- in the F1 generation in the Price study, and in the F1 and F2 offspring in the Siglin et al. study.

The estrous cycling was not affected in that study, the Siglin et al. study. But as was noted by the presentation, there were -- there were quite significant differences between the F1 and F2 generation in terms of their estrous cycles. In particular, the F2 generation had -- appeared to have had large percentages in every group, including the controls that were not cycling.

But there was a near significant increase in the number -- the percentages of the offspring that had cycles longer than 10 days, as was presented earlier, but that was only in the F2 generation.

So I would say overall for the ovarian data, they're suggestive, but -- and information to conclude that nickel chloride may be an ovarian toxicant. But again, as I noted, there are some inconsistencies among the studies, largely having to do with the -- the different endpoints being examined in different studies.

The evidence is strongest for the oxidative stress-related endpoints and for the disruption of cycling by the parental exposure.

For the -- there were also a few studies database in the database looking at uterine toxicity. Two studies

that focused on uterine toxicity, and I think they both suffer from weaknesses. One was an in vitro study, one was an in vivo study that lacked sufficient experimental details. The two-generation studies both reported no effects on uterine weights or histology, but it didn't appear that animals were euthanized on the same estrous cycle stage.

And I should also say about the two-generation studies regarding the ovarian histology that no follicle counts were done. And there was not a lot of detail in -- just given about how many sections per -- at least that I found were analyzed.

So overall, I would conclude that for the animal database regarding female reproductive toxicity, the largest database is for the effects -- is from the developmental studies looking at prenatal and postnatal mortality and growth. And there -- I think as we talked about earlier, there is sufficient evidence to support that there is an effective nickel at the higher doses on those endpoints.

There's some evidence for ovarian toxicity of nickel, but the quality of the study and the lack of similar endpoints being observed in different studies limits the strength of the conclusion that one can draw from those studies.

CHAIRPERSON GOLD: Okay. Thank you.

Any questions for Dr. Luderer?

Dr. Allard.

COMMITTEE MEMBER ALLARD: Yeah. Just a question. We'll talk about it when we talk about mechanisms. But several studies have suggested that perhaps that decrease in milk production, or differences in milk composition could be responsible for the difference in weight, or perhaps even death. And I didn't find in the document that was provided to us any studies doing cross-fostering.

COMMITTEE MEMBER LUDERER: No, there weren't.

COMMITTEE MEMBER ALLARD: Did you find some elsewhere --

COMMITTEE MEMBER LUDERER: No.

COMMITTEE MEMBER ALLARD: -- that would be able to parse that out?

COMMITTEE MEMBER LUDERER: No.

COMMITTEE MEMBER ALLARD: Okay.

CHAIRPERSON GOLD: Any other questions for Dr. Luderer?

Okay. And the secondary discussant then is Dr. Plopper.

COMMITTEE MEMBER PLOPPER: Well, I'll keep this short. I agree with what she said. And it's not a solid literature. The in vitro studies suggest that their

uterus and the ovary may be targets, but you already pointed out those limitations. And I will say that to try and understand what the impact of this would be on female reproduction, I guess most of the studies don't -- as you pointed out, they do their exposures if they want to check something. They don't necessarily follow an estrous cycle.

And what disappointed me was that in some of the necropsy, the live studies where they tried to find it out afterwards, they didn't go back and count corpora lutea, so they don't know what we're seeing here. So I would agree with everything she said. Just my concern.

Thank you.

CHAIRPERSON GOLD: Okay. Thank you.

Any other questions or comments?

Okay. I suggest that we take a lunch break, and we'll come back to male reproductive studies when we return after lunch.

Forty-five minutes maybe? So 10 after 1:00 we'll reconvene, if that's -- Carol you have something first.

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah. I just wanted to remind the members as usual not to talk among yourselves about the issues that you're considering today. Maybe just talk about the weather at lunch. And this is the same for the members of the public. If you -- if you

talk to someone from the public about this, then you should disclose that when you come back from your lunch.

CHAIRPERSON GOLD: Okay. Thank you. If there's nothing else, let's break now for lunch and return at 1:10.

(Off record: 12:22 p.m.)

(Thereupon a lunch break was taken.)

A F T E R N O O N S E S S I O N

(On record: 1:10 p.m.)

CHAIRPERSON GOLD: Okay. Can we try to reconvene, please?

Okay. Having discussed developmental effects and female reproductive effects, we're now going to talk -- move on to talk about male reproductive effects first in humans, then in animals. And then we'll have a final presentation about mechanistic studies. So, Dr. Baskin, you're going to lead us on human male reproductive effects.

COMMITTEE MEMBER BASKIN: Thank you. And thanks to the scientific panel for already presenting a really fantastic synopsis of the scientific papers that we were able to review. So I'm going to discuss the human male reproductive studies. I guess backing up a second, nickel is important for something in our body and nobody really knows what that is, but It relates to I think iron transfer. And like all of the rare elements, we need them, but we don't really understand them quite well.

So there's nine human studies. And the methodologic -- there's a lot of methodology issues here, in that there's not fantastic controls, a number of the patients recruited were from subfertile populations. And most importantly, there were a huge number of

co-variables. In other words, in the majority of studies, they were testing for other heavy metals. And they also didn't take into account environmental issues, such as smoking, alcohol, or potentially other exposures, which are consistent with the problems of epidemiologic studies in general.

Breaking it down, there were four cross-sectional studies in humans that examined the effect of nickel using reproductive endpoints, which indirectly were essentially semen parameters or plasma testosterone. These were performed basically all over the world. And as mentioned, there's a number of compounding factors, which make it really difficult to interpret whether nickel is the critical issue or causative agent.

There were five other cross-sectional studies, which looked specifically at sperm parameters. A majority of these were done in China. And during the same time period of these studies, data really from the World Health Organization shows that semen and sperm parameters have really dropped across the world, but they not are necessarily related to fertility. So again, some issues with how this data should be interpreted.

The Zeng, Zhou, Sancini, and Wang study were the most significant looking at specifically the levels of nickel in the urine. And they were able to associate that

again with subpar sperm quality, but there's really no causation that can be proved from this epidemiologic evidence.

The final study, the Zafar study looked at the toxics metals, not just zinc, but did look at zinc plus a number of other ones in human seminal plasma. This was a study done in Pakistan. And again, they showed some decreased parameters. But this study, as well as the others, was really -- there are multiple limitations, mostly due to sample size, and again the co-variables.

So summarizing the nine human studies. They're just not of high enough quality to really definitively show that nickel was a causative factor. Although, there's clearly an association.

I'm going to move on to the animal studies, unless there's questions.

CHAIRPERSON GOLD: No, I think what we'll do is have the secondary discussant for human and then we'll move to animal. So first, are there any questions or comments for Dr. Baskin?

Okay. Dr. Carmichael, I believe you're the secondary discussant.

COMMITTEE MEMBER CARMICHAEL: Yes. And I have no further comments. Basically, Dr. Baskin made all the points that I would have.

CHAIRPERSON GOLD: Very good.

Dr. Baskin, I guess you can go on with animal male reproductive

COMMITTEE MEMBER BASKIN: The animal studies show a little bit of a different outcome. And I think there were 24 -- well, 24 plus maybe three, depending on how you interpret that.

In animal studies, of course, we can directly test the effects of zinc. So splitting up these studies, four of the studies had an end number of less than five, so I just kind of discarded that based on the fact that that was insufficient statistically. And one was looking at nanoparticles, which didn't seem 100 percent germane to what we were looking at.

Six of the studies, and these are -- backing up a sec, the majority of studies are done in rats with a number of them done in mice, and then a few other asterisks, which I'll get to. Six of the studies showed no effect, and seven of the studies had pretty decent solid scientific evidence that nickel caused gonad toxicity.

And what I mean by gonad toxicity is they either looked histologically and there were beautiful sections of the testicles showing abnormalities in respect to nickel in a number of these studies. They looked at enzymatic or

biochemical defects in the testes. And then ultimately they looked at sperm morphology. So I think the science was reasonable solid there.

The studies that I would cite as the Das study from 1997, the Das study from 2000, the Doreswamy study from 2004, the Zara study in 2012, and the Murosky study of 2012. So these really spanned, you know, close to at least two decades.

Again, the majority of studies were done in rats. One of the questions I had in relation to these were -- the effects were clearly there, but were they reversible. That issue wasn't really addressed. In general, it seemed to be dose related, and the studies were done in multiple different fashions, but some were done by gavage, which simulate, I'm assuming, you know, ingesting nickel. Others were done intraperitoneal, which obviously would not simulate, you know, drinking water or ingesting it.

And then finally, I wanted to highlight four studies and these were somewhat indirect studies. And what I mean by that is the authors assumed, based on their review of literature, that nickel was toxic to the gonads. And so they weren't trying to prove that nickel was -- they are already basically made the scientific assumption that nickel would cause toxicity. And these studies were really focused on seeing if they could prevent the

toxicity.

So right there it's reasonably incriminating that they felt that nickel was essentially a bad actor to the gonads, and again based on enzymatic biochemical or histologic, an analysis of the sperm. And they used various different methods to try to prevent nickel from being a problem. And these studies specifically are the Das study in 1997, the Kakela study in 1999, the Jargar study in 2012, and the Xie study in 1995, if I'm pronouncing that correctly.

So, in summary, there is some concerning animal data that based, I think, on our standards of scientific valid testing that shows that nickel in animals, or soluble nickel I think specifically in an animals can have deleterious effect on male reproduction.

CHAIRPERSON GOLD: Okay. Thank you.

Any questions or Dr. Baskin?

Okay. Dr. Allard, you're the secondary discussant.

COMMITTEE MEMBER ALLARD: Yes, I actually agree with Dr. Baskin. And I came to the same conclusion that the data that exists actually supports an effect on male reproductive systems. So I do also want to basically highlight the fact that a lot of the studies were done by injection, which I did not actually give too much weight

to, because I did not believe that this was a significant route of exposure. And also, I think it's important to highlight that the distribution of nickel, depending on the route of exposure is actually quite different. So I really considered the oral route and mainly considered those studies.

Although, of course, I try to see whether the outcomes align between injection and oral studies. So I guess I'm not going to necessarily mention all the studies that were done. I particularly leaned on Kakela et al. myself from 1999, where male rats were exposed to nickel chloride hexahydrate in drinking water. I was sort of surprised actually at the size of the effect where there's a very strong reduction in the size, especially at 28 days of exposure and the viability of the pups.

Other studies also mentioned a dominant lethal effect, which we can perhaps talk a little bit more when we talk about mechanisms next.

So to make a long story short, I think altogether the studies, as I mentioned, clearly highlight effect on spermatogenesis in particular. There's definitely, across the many studies, perhaps there were -- in the comments that were mentioned that the fixation protocols do not seem to be consistent. But looking across all these studies, the effect on the seminiferous tubule was

actually quite consistent, even if different procedures were actually -- were actually used. So, to me, that actually gives strength to it.

The other component that made me decide that this effect is likely real is the fact that the doses administered to get this effect is actually much lower than the other endpoints that we've talked about so far.

So, for example, the apoptosis LOAEL in the seminiferous tubule was obtained at 2.5 milligrams per kilogram per day by gavage. And actually there was still a trend at 1.25 milligrams per kilogram per day. So not significant, but it's still trending. So, right, altogether, I'm supportive of a male reproductive toxicity for nickel.

CHAIRPERSON GOLD: Okay. Any questions by the Committee about male reproductive?

Okay. Seeing none. The last section we're going to deal with is mechanistic studies. And again, Dr. Allard is on. You're going to be the first discussant about mechanistic studies.

COMMITTEE MEMBER ALLARD: Okay. So moving on to mechanistic studies. It was clear from the document that there are actually not that many mechanistic studies. Although, different kinds of mechanisms were mentioned as potential ways that the nickel could actually act.

So the way -- the way that I've organized things myself in my mind is actually followed an adverse outcome pathway paradigm. So with adverse outcome pathway with, if you're not familiar with it, this is just a way to organize different data sets and components of data sets where you try to organize things from a very molecular level molecular level on the left end and try to link that at the different level of organization from cellular to organ to organism to population response.

So I -- what I tried to do is actually link the different elements that we've talked about so far today from human population, and then actually backtrack to try to find the molecular events that could be explanatory of the various outcomes that have been discussed.

So I'm -- normally, you would have one AOP per sort of endpoint. And I'm -- that would be a lot of AOPs, considering everything that was mentioned today. So I'm not necessarily going to do that. I'm going to highlight the ones that I thought were perhaps the most convincing ones.

So the -- some of the ones that I considered, were issues with pregnancy, neonatal/perinatal deaths, and especially male fertility issues, because of the -- what I believe to be the strength of the -- of the rodent data in that -- in that sense.

So looking at all this, the sort of four different mechanisms that I gleaned from the literature from this document, as well as the larger body of literature, were the fulling oxidative stress and DNA damage is something that comes back quite often. Actually, in the hazard identification document itself. So it's a commonly cited cause for the impact of nickel on a variety actually of biological systems.

So, for example, here in the testis, the study from Doreswamy et al. from 2004, while performed by ip injection showed an increased level of signs of oxidative stress such as, for example, lipid peroxidation. Something that was actually observed in other tissues as well in other studies.

The -- but to go back to the Doreswamy et al. study, they also, together with signs of oxidative stress, detected DNA damage in testis and epididymis in a concentration dependent manner, which they basically surmised is linked to the generation of oxidative damage, which would make sense, but is not necessarily directly shown here.

Again, signs of oxidative stress have been identified in other tissues, such as in the ovary, the Rao et al. study from 2009 points out again lipid peroxidation in the ovary following nickel chloride exposure in mice at

all stages.

And I want to link that back to the signs of oxidative stress in human studies, although again of varying qualities. But the Ni et al. study from 2014 highlighted oxidative damage as measured by a 8-hydroxydeoxyguanosine levels in umbilical cord blood. And the larger body of literature has also indicated that oxidative stress can be detected in the blood correlating with nickel exposure.

And I think while I build my own AOP, I was comforted in routing some of the outcomes of the -- the outcome that I think would derive from this is definitely the decrease in male fertility. And other AOPs have actually been done, albeit for aquatic species, with nickel. So Briggs et al. in 2016 published an AOP for nickel, again in aquatic species. And they do mention our -- the generation of reactive oxygen stress and oxidative stress as being perhaps one of the main causes of the different endpoints that have been observed in aquatic species.

Although they also mention disruption of calcium and magnesium as being potentially also involved in -- in different endpoints.

So the -- again, looking at the literature presented here, and the wider body of literature, the

generation of oxygen species, reactive oxygen species and DNA damage could be the cause, especially of the increased apoptosis that was mentioned several times across various studies, but especially in the testes in males.

We've also talked about the alteration of hormonal production and levels. This could be linked again to the male decrease in sperm production observed in animal studies. So, for example, the -- I hope I don't mispronounce. I probably will -- the Forgacs et al. study from 1998 looked at the production of testosterone by Leydig cells in vitro in response to gonadotropins and so that nickel sulfate exposure decreased the production of testosterone. This was also observed in other studies that I'm not necessarily going to mention here.

Another study by the same author in 1997 looked at granulosa cells in response to HCG and saw that the progesterone production was also dramatically decreased by nickel sulfate. So the production of hormones and the ability of nickel to alter the production of hormones could potentially be responsible for, at least again in males, the decrease in sperm production that's observed.

There was some mentions of alteration of enzymatic level which is the third mechanism. Although, I think the evidence was very thin on that one. And the last one is the milk production/composition change. And

at least I felt on this end that the -- the data was a little bit stronger, again to explain why there's elevated perinatal death. Perhaps milk is not sufficient to provide nutrition at these stages, and the reduction in prolactin observed after nickel exposure could be explanatory in that sense. Although, we don't really understand why prolactin could -- would be decreased after nickel exposure.

If indeed, as has been observed in other systems, calcium and magnesium and homeostasis is altered, perhaps that could explain why prolactin production would be decreases.

So I want to step a little bit -- to conclude my part here, I wanted to step a little bit away from just the studies I mentioned in the hazard identification document. Just like I did last time, I do tend to look at the ToxCast data of in vitro data sets to look at what, you know, the collection of the hundreds and thousands of assays that have been performed what nickel seems to show activity towards.

And it seems that at an extremely low level, nickel sulfate in the nanomolar range has a very strong ability to activate P53, which I thought was very consistent -- oh, and sorry, this was corroborated in other studies and reviews of toxicogenomics data that

showed the P53 pathways, dependent pathways, were strongly altered following nickel exposure.

So I thought the apoptosis -- the apoptotic findings that I often mentioned in the testis were really congruent with the in vitro ToxCast data generated in a completely different cell type. We're talking about colorectal cell HCT116, I believe, cell line. But this very strong ability to activate P53 and lead to Apoptosis was consistent with many of the findings that pertain to the male reproductive toxicity.

I -- one thing that I was surprised not to see anywhere was the mention of a epigenetics. And yet, there's quite a bit of data out there about epigenetics and nickel exposure. DNA methylation seems to be strongly altered by nickel. Actually, DNA methyltransferase activity seems to be lowered by nickel exposure, at least in Chinese hamster sells. That is the Lelal study from 1998. So we've known for a while now that DNA methylation is altered by nickel. And yet, this has not been necessarily explored further in any of the studies that we've looked at so far today.

And histone modifications also seem to be altered at least H3K trimethyl and H3K9 dimethyl show from the Brocheto & Costa 2015 study in PBMCs in peripheral blood mononuclear cells show the global levels of these marks

seems to be decreased. And again there's a lot of developmental effects that could be, of course, caused by DNA methylation. This is extremely sensitive and I'm not saying that this is necessarily to be part of an AOP, but this is something to think about when we think about nickel, and something to explore -- to explore further.

So, basically all of this together in terms of the mechanisms, it's clear that we are only at the beginning of being able to build an AOP based on the data that exists -- the mechanistic data that exists, but that there's definitely convergence of studies that show that nickel exposure can create a reactive oxygen species and an oxidative stress response that this is likely causative, or perhaps in parallel, causes DNA damage, which eventually increase apoptosis. And again this could be explanatory towards the male reproductive toxicity findings.

I'll end my comments there.

CHAIRPERSON GOLD: Thank you.

Any questions for Dr. Allard?

Okay. Seeing none. Dr. Pessah, you're our secondary discussant.

COMMITTEE MEMBER PESSAH: Sure. So I think the potential mechanisms were covered in quite some detail. Thank you. There is some data that hasn't been included

in this report, which really addresses some of the known influences of nickel, maybe not vis-à-vis with regard to developmental or reproductive toxicity, but it's just in general about the toxicity of nickel, which involves shifts in metabolism in a number of in vitro systems, in particular the hypoxia inducible factor alpha if -- one alpha, which seems to be quite sensitive and seems to impact mitochondrial function. And so that probably should be taken into account, given that it's one of the more potent effects of nickel.

Again, its relationship to everything we've heard is perhaps less clear, because those outcomes haven't been measured in a -- in a reproductive study.

Other than that, I really don't have much to add.

CHAIRPERSON GOLD: Okay. Thank you.

Any comments or questions by the Committee at this point?

Dr. Plopper.

COMMITTEE MEMBER PLOPPER: Well, one of the things I'd like to hear addressed is the fact that if this -- the mechanisms that have been proposed, if they're in other systems with other toxicants that continued exposure alters the biology of the targets. And they actually become resistant, particularly for oxidant stress. And I'm just wondering how that would fit into

this picture, because some of the studies we talked about are long-term exposures in humans and in animals, and there's less response. And I'm just wondering if part of -- if that is -- if oxidant stress is a mechanism -- I know there's probably no literature on it, but it's just been striking me from what you've been saying that these are things that would normally -- would very often -- not normally, but would very often alter with time.

So when we have some studies that are three-generational animal studies, the survivors of the survivors of the survivors are the ones that are tested in the third generation. So are those the ones that most easily develop a resistance, because they become adapted?

That's all I -- just -- I don't know if that helps or hinders, but it's something I think we need to think about, because the human studies those are long exposures. And that's usually long enough to cause a resistance, a change if it's an oxidant stress type of mechanism.

CHAIRPERSON GOLD: Dr. Allard, do you want to comment or...

COMMITTEE MEMBER ALLARD: Yeah, I -- I would actually need to go back to look exactly at the studies and make correlations, but I thought -- I think that question of timing, at least in my mind, might explain

why, in some studies, they actually see a decrease in antioxidant response, and in some studies they actually see an increased response to -- to potential oxidative stress, so -- but in -- but in both cases, they took that as showing that there was an altered way of dealing with oxidative stress, whether they saw a decrease in SOD or an increase in SOD, for example.

So perhaps the timing would actually explain the discrepancies between the studies in seeing, you know, either a lowered response or a heightened response.

CHAIRPERSON GOLD: Dr. Pessah, did you want to add anything to that?

COMMITTEE MEMBER PESSAH: No, I just want to caution that an outcome of oxidative stress is totally pleiotropic and non-specific, and virtually any foreign substance can be associated with a change in the level of oxidative stress markers.

And so whether this is really an AOP or a just a consequence of the exposure that would have occurred whether they used any metal for that matter really is unclear at this point. So that's why I thought maybe if you look into the mechanisms of shifts in metabolism, which are really important in metabolically active tissues, such as testes and sperm, that might give you some insight into an AOP.

But I'm less -- less convinced that just showing that you have oxidative stress biomarkers are at all causative for some of these other effects.

COMMITTEE MEMBER ALLARD: I absolutely agree. I think -- as I mentioned, I think we are really at the early stages of trying to thread some of those findings together. What I did appreciate though in the other AOP for aquatic species that was published is that they -- they made similar, I would call them, jumps at this stage, talking about how nickel can affect iron homeostasis and iron itself, but being important for exploration could explain some of oxidative -- oxidative stress findings that were found across multiple species at this stage.

But right, it's a very sort of broad, you know, oxidative stress and then what? You know, how do you move on from that? I lean much more on the apoptotic pathway especially because this was identified in vitro at extremely low levels and in the normal range.

CHAIRPERSON GOLD: Okay. Any other comments or questions at this point?

Okay. Can we take one minute to organize the public comments. I have three requests. And I just want to make sure there are no more.

Two of them we know about, right. Yeah. So we have -- we gave 15 minutes to the folks from Gradient, and

15 minutes -- excuse me -- to the folks from NiPERA, because they requested that by the deadline.

And then we have two others now for five-minute presentations. If there's anyone else, this is your last chance.

Okay. So we have Robyn Prueitt from Gradient.

(Thereupon an overhead presentation was Presented as follows.)

DR. PRUEITT: Thank you. Thank you very much for the opportunity to speak here today. I'm Robyn Prueitt, a toxicologist at Gradient, an environmental and risk sciences consulting firm. And I'll be providing comments on the epidemiology evidence regarding the potential reproductive and developmental toxicity of nickel and nickel compounds. And while some of this will reiterate what was presented by both the staff and the Committee here today, I will also discuss how we evaluated study quality in a fairly systematic way, and how we considered the form of nickel evaluated in the studies.

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DR. PRUEITT: So we evaluated the 40 epidemiology studies reviewed in the hazard identification materials as well as the four additional studies of autism that were sent to the Committee members after the original hazard identification materials were completed.

On our initial review, we found that one developmental study and one male reproductive study were -- did not evaluate statistical associations between nickel exposure and any health outcome. So we found that these studies are not informative for evaluating potential health hazards of nickel.

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DR. PRUEITT: So for our evaluation, we first assessed the quality of the studies by conducting a standardized risk of bias analysis, which I will explain further on the next slide. We then evaluated the study results with consideration of how the factors that affect the risk of bias impact the interpretation of the results, and also with consideration of the form of nickel evaluated.

Then we integrated the evidence across the studies that evaluated the same type of outcome considering the consistency of the results, and placing more weight on studies with lower risks of bias when possible.

When integrating the evi -- when -- and we did this because when integrating evidence, it's important to give appropriate weight to results based on study quality rather than just whether the findings are positive or null.

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DR. PRUEITT: For the risk of bias analysis, we used the risk of bias rating tool that was developed by the National Toxicology Program's Office of Health Assessment and Translation. And this tool is used to assess the Study quality characteristics that may impact the validity of a study's results. We sign risk of bias ratings to each study across nine different domains including the three key domains of exposure assessment, outcome assessment and confounding, as well as the domains of selection bias, attrition bias, statistical methods, exposure levels, temporality, and the form of nickel.

And so using this tool, we found that all of the epidemiology studies have a moderate risk of bias, and thus are generally a low quality, which decreases the reliability of their results.

In general, most of the studies did not employ appropriate statistical approaches to assess potential confounding. They used area level exposure measurements or assessed exposure using indirect measures that were not validated. And they were not able to assess the temporal relationship between nickel exposure and the outcome of interest. And this indicates a high risk of bias in these domains.

Some studies had a higher or lower risk of bias

across more domains than others. And the results of studies with a lower risk of bias across the key domains are likely more reliable than studies with a higher risk of bias across these domains.

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DR. PRUEITT: For the three studies of female reproductive outcomes, they evaluated non-non-occupational exposures, which are primarily to soluble nickel sulfates and nick oxides. And each study evaluated different outcomes. As discussed earlier, the studies with time to pregnancy and pre-eclampsia is the outcome reported no association. And then a third study reported higher nickel concentrations in the serum of polycystic ovary syndrome cases, as well as decreased levels of sex hormone binding globulin with in -- with increasing serum nickel concentrations.

But there were no changes in other clinical chemistry parameters that would be expected to change in relation to sex hormone binding globulin levels.

And as discussed earlier by the Committee, this study they looked at many different hormones and did not adjust for multiple comparisons. So this decreased sex hormone binding globulin levels in the absence of any other changes -- related changes, you know, could be a spurious results.

So given the factors that contributed to the moderate risk of bias for this study, as well as the other studies, particularly a lack of accounting for important confounders, likely selection bias and an inability to assess the temporal relationship between exposure and outcomes, these reported associations need to be confirmed in other studies before they can be used to support a hazard listing for nickel as reproductive toxicant. So overall these three studies did not provide evidence for a causal association between exposure to nickel and female reproductive outcomes.

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DR. PRUEITT: So we evaluated eight cross-sectional studies that evaluated male reproductive outcomes. And as with the studies of female reproductive outcomes, all these studies evaluated exposures to primarily soluble nickel sulfates and nickel oxides. And studies evaluated associations between nickel exposure and hormone levels, sperm DNA damage, and sperm function parameters.

And the table on the slide shows the results for each specific endpoint with a zero indicating no association, and up or down arrows indicating an increase or decrease in -- of the endpoint respectively.

And as you can see from the table, the results

are inconsistent across studies. It appears that a lot of the null results were not presented by the staff earlier, but I think we have a complete listing here in this table. It's notable that in five of the eight studies, the participants were infertile or were male partners in couples undergoing infertility assessment in China or Pakistan. So the results of these studies are not generalizable to the general U.S. population.

Because all of the studies were found to have moderate risk of bias, the validity of their results is questionable, and they do not support a hazard listing for nickel or nickel compounds as male reproductive toxicants.

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DR. PRUEITT: Of the developmental studies, seven evaluated birth defects with five reporting null or statistically significant negative associations with nickel. And three of those studies had a low risk of bias across many of the domains evaluated, which may increase the reliability of their results.

And two of those studies evaluated associations with high occupational exposures to soluble nickel aerosols in a nickel refinery, where high exposures to insoluble forms of nickel such as sulfidic, oxidic, and metallic nickel also occur.

And of the two studies reporting statistically

significant positive associations, one was an occupational study that was criticized by the editors of the journal it was published in for being incompletely documented, and was deemed inconclusive. And so that's the Chashschin study. Also, its results were not reproduced in a more thorough study of the same cohort of workers that reported no results, which is one of the studies by Vaktskjold. I'm not sure I'm pronouncing these names correctly.

And so because the majority of studies reported null or negative results, including those with more reliable results, they do not support a causal association between exposure to nickel or nickel compounds and birth defects.

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DR. PRUEITT: So of the 10 studies that evaluated low birth weight, five reported null or negative associations with nickel exposure, four reported statistically significant positive associations, and one reported a borderline significant positive association.

One study reporting a negative association, and two of the studies reporting positive associations have a lower risk of bias across more domains than the other studies, so their results are likely more reliable. And the two studies reporting positive associations were multi-pollutant studies that used univariate analyses to

evaluate associations, that's the Ebisu and Bell and the Bell study.

And then the study reporting a negative association, another study by Vaktskjold is an occupational study with high exposures to multiple forms of nickel. And as Dr. Mike Taylor will be noting in his presentation that will come up after mine, an independent analysis by a biostatistician, Steve Seilkop, indicates that this study had adequate statistical power to detect the effects on low birth weight reported in the Ebisu and Bell study, if they are indeed causal, at nickel concentrations that are much lower than those estimated for the workers in this study.

This demonstrates the importance of testing hypotheses generated by individual pollutant analyses in multi-pollutant studies. So given this analysis, as well as the inconsistency of the results for low birth weight across studies, even those of similar reliability, the studies evaluating nickel associations with low birth weight do not provide evidence to support a causal association.

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DR. PRUEITT: Seven studies evaluated autism spectrum disorder, including the four studies sent to the Committee members after the original hazard identification

materials were completed. Five of these studies reported no association with autism, including one study that evaluated occupational exposures.

Of the two studies reporting statistically significant positive associations, these are limited by a lack of confidence in the exposure assessment, a lack of accounting for important confounders, and inappropriate statistics. So it's unclear if the positive results are attributable to bias or confounding.

Overall, the majority of studies reported no results. And together, they do not support a causal association between nickel exposure and the development of autism.

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DR. PRUEITT: A few additional studies assessed associations between nickel exposure and adverse pregnancy outcomes, DNA oxidative damage, and the development of early life cancers. And each outcome was evaluated in only one or two studies, and none of the studies accounted for potential confounders.

And in addition, the results across these studies were largely null. So overall, they do not provide evidence to support a causal association with nickel exposure.

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DR. PRUEITT: So, in conclusion, we found that all of the reviewed epidemiology studies have a moderate risk of bias, indicating that they're not valid according to generally accepted principles of study quality.

Most studies evaluated associations with exposures to soluble and oxidic nickel. And the results were largely null or inconsistent across studies evaluating the same outcome. The studies of nickel refinery workers had additional exposures to sulfidic and metallic nickel and their results were largely null, or with any positive results not being reproducible in more reliable studies.

Overall, the epidemiology studies do not provide clear evidence for associations between exposure to any form of nickel and reproductive or developmental outcomes. Therefore, they do not provide sufficient or even limited evidence for recommending nickel and nickel compounds for listing as reproductive toxicants.

Thank you.

CHAIRPERSON GOLD: Thank you. Are there any questions from the Committee for Dr. Prueitt?

Thank you.

DR. PRUEITT: Thanks.

CHAIRPERSON GOLD: Next, we have Michael Taylor from NiPERA and he was given 15 minutes as well.

(Thereupon an overhead presentation was Presented as follows.)

DR. TAYLOR: Very good. Thank you. Thank you for the opportunity to address the Committee today.

I'm Mike Taylor. I'm a toxicologist NiPERA. NiPERA is the science branch --

Microphone on. Yes. I'm just not close enough.

NiPERA is the science branch of the Nickel Institute, which is a global association of leading nickel producers. And I'm here today obviously to tell you our view of the data available for the proposed listing of nickel and nickel compounds for reproductive and developmental toxicity.

We solicited advice from experts in reproductive and developmental toxicity, both for animal evidence that experts at Exponent reviewed Dr. John DeSesso and Dr. Amy Williams, and -- as far as the human evidence, the experts at Gradient, Dr. Julie Goodman and you've heard from Dr. Robyn Prueitt, and also Steve Seilkop who's an independent biostatistician.

And they've all submitted written comments. And this presentation will summarize those comments and expand on them a little bit. I hope you've got the opportunity to -- to review those receive -- you've both received and got the opportunity to review those written comments.

--o0o--

DR. TAYLOR: So while we know exposure is not a criterion for listing under Proposition 65. For reproductive effects though, it's the consideration of internal doses, systemic exposure in bioavailability that are important, when evaluating the consistency of study outcomes, both across different population studies, workers and general public, and, of course, between humans and animals as well.

As far as the general population the -- by far the greatest route of -- the greatest source of exposure -- internal exposure is via the diet, because nickel is naturally contained in our diet. It's essential to plants and found in plants in this -- in our diet.

It's also found in the earth's crust, so it's a natural component of water. And found in drinking water, the absorption of nickel is highest in drinking water in the fasting state up to 27 percent absorption. But absorption is much lower from food, typically one to three percent, and even lower than from insoluble compounds, which might be found in soil or nickel metal or nickel alloys.

And I think this is a point I want to make about the relevance of different routes of administration. And what can happen between an oral route of administration

say, and then perhaps an injection or an I.P. route, where during the oral route when the absorption is lower and controlled, nickel is bound in the serum to proteins and, of course, absorption is regulated.

During injection, or I.P., studies that -- those mechanisms were overwhelmed. There's a lot more free nickel available in the serum, and then a lot more systemic availability that is artificially high that you couldn't get through a relevant route of exposure.

But regarding inhalation, the public is exposed to soluble and oxidic nickel compounds in ambient air at very low levels. And, in fact, the blue doesn't even show up I think on that chart as far as the contribution at normal levels to systemic exposure in the public.

Workers can be exposed to soluble, insoluble compounds or metallic nickel, depending on what their processes are that they're working on. And welders can be exposed if there's nickel in their materials to complex oxides call spinels.

And at very high levels of inhalation exposure, this route can dominate systemic delivery, as you can see. So again, it's important to consider these different exposures when thinking about different studies, and done by different routes.

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DR. TAYLOR: So as I've alluded to, and we've talked about already some today, that nickel can be found in different chemical forms. They have different physical chemical properties, and thus different toxicological properties, the water soluble nickel compounds, and that includes nickel chloride, nickel sulfate. They're both very soluble. And that's what the majority of studies have been done on.

And insoluble -- water Insoluble nickel compounds, and then metallic nickel, that's the form of, you know, obviously, nickel metal and in the form of nickel in alloys.

And the bioavailability and the systemic toxicity of the soluble nickel compounds are much greater than that, of the insoluble compounds and of nickel metal. And very early on, we mentioned nickel carbonyl. It's already on the list. And it's a -- while it's relatively insoluble in water, it's an organometallic and is actually pretty highly bioavailable. And it's a -- it's a different animal. We're not really discussing it here in -- as far as these compounds go.

And speaking of compound, nickel metal obviously is not a nickel compound. It's elemental nickel and it has a different mechanism of nickel ion release. It requires corrosion on the surface to release nickel ions,

rather than the dissociation that's involved with the dissolution of nickel from nickel compounds.

And obviously it's in a zero valence state. It's grouped with nickel compounds for -- I think, for this evaluation because it's grouped with nickel compounds for the Proposition 65 listing for carcinogenicity. And it's important to remember that the carcinogenicity effect is by inhalation -- by inhalation only is a local respiratory tract effect, and not a systemic effect.

And actually, nickel metal typically has a lower -- or a carcinogenicity classification as far as not being a known human carcinogen. And all this goes together just to talk about the different properties that these different compounds and nickel metal can have, and why they should be considered separately for Proposition 65 listing for reproductive toxicology.

--o0o--

DR. TAYLOR: And if that's the case then, there would be a separate consideration done perhaps for each group of compounds, and then for nickel metal for each classification -- classification of effect that's been discussed here today.

And I think in that case, the strength and consistency of the evidence available by each of those -- for each of those groups could be considered. And also

bioavailability and toxicokinetic data could be considered as well in those decisions.

That's what we've done in our written comments, and our outside experts have done that as well. And I'll go through these. But as you can see, in our view, we view that listing is warranted, based on the soluble nickel effects on developmental toxicity, just based on animal studies only.

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DR. TAYLOR: So about those developmental effects, we -- the high dose oral exposure of soluble nickel compounds, both in rats and mice, increased developmental effects consistently, and increased -- dr. Plopper, we just call them perinatal mortality, because it's -- it's -- it could be several things, but it tends to cause the death of pups around the time of parturition.

And so the perinatal mortality effect is -- is what we've seen and what's seen consistently. There can be other effects at higher doses, but the perinatal mortality effects in rats is the most sensitive effect in the most sensitive species. The lowest low effect level.

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DR. TAYLOR: And I do -- I do think the evidence shows that rats are more sensitive than mice, just because the low effect levels in rats are much lower than the no

elect levels that are seen in mice.

The human evidence has been -- has been well discussed here, but there's been suggestions of effects in some multi-pollutant studies that are -- that are done in the -- on the public at nanogram per cubic meter air exposure levels. And then those effects weren't seen in a refinery case control study with highly exposed female workers in microgram per cubic meter exposure levels. And as explained in comments by Dr. Steve Seilkop that those -- those studies did have the power to detect those effects if they're real.

So we feel that just based on the animal evidence only, the Proposition 65 listing of soluble nickel compounds for developmental toxicity is appropriate.

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DR. TAYLOR: So this graph compares the internal exposures of -- in the form of urinary nickel levels across different studies, and where effects have seen and have been -- have and haven't been reported. And in this case, the worker studies here are the urinary levels in the Vaktskjold studies. And the Vaktskjold studies were a continuation, I'd say, of the Chashschin studies, where Chashschin was -- is actually a co-author on the Vaktskjold studies.

The Chashschin studies started the -- started the

interest in the effect. And therefore there was -- what -- pretty much the definitive study design with the Vaktskjold studies and the birth registry -- the Kola birth registry established, and the Vaktskjold studies were then performed, and at much higher urinary nickel levels than is seen in the general public, where effects are sometimes reported in multi-pollutant studies for things like small gestational age. Those effects were not repeated or not realized in the -- in the worker studies.

And then this also compares to the levels where the rats have seen and not seen effects as far as the internal exposures there go.

And this just would say either -- either the mechanism of action for these effects in rodents either aren't relevant for humans or that humans just aren't achieving -- aren't able to achieve those levels of internal exposures even at very high levels of workplace exposure that aren't realized anymore in the workplace in modern times.

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DR. TAYLOR: As far as the insoluble compounds go in the nickel metal as far as developmental toxicity, we felt that there's no reliable animal studies that properly assess these effects for insoluble nickel compounds in nickel metal, no definitive study that can be used to make

a determination of causality. But insoluble nick compounds and nickel metal are unlikely to cause these effects just due to their much lower bioavailability than the soluble compounds.

For instance, they have 100-fold or greater oral absorption of nickel from nickel oxide and nickel metal shown in toxicokinetics study. And also, there's much lower acute toxicity values for these -- for insoluble compounds in nickel metal than soluble compounds just showing the systemic toxicity is limited.

So we feel that in these compounds -- in soluble compounds and nickel metal have not been clearly shown to cause developmental toxicity.

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DR. TAYLOR: As far as female reproductive effects go, Exponent did a review of those studies and used only the ones that are done by relevant routes of administration, in this case just compared the oral studies. And those found that only -- they only thought that the paranoid or mortality effects of the soluble compounds warranted hazard listing. There weren't clear effects that the maternal effects were involved. It wasn't clear that maternal effects were involved. And they just -- they concluded that the listing should be for developmental toxicity only for the soluble compounds.

I'll move on as my colleague has already covered the human studies. But as far as female reproductive toxicity, neither nickel compounds, soluble compounds insoluble compounds or metal, have met the listing criteria.

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DR. TAYLOR: The male reproductive toxicity, again when only considering the relevant routes of exposure, Exponent looked at those studies that looked at different aspects of male reproductive toxicity, and found them -- the results to be highly contradictory across the studies. In those cases, there were some problems with testicular histopathology, fixation. It takes special fixative to rapidly penetrate the membranes or the covering of the testes and fix those tissues before they break down.

And it's important to remember that the large and robust multi-generational rate studies of solu -- of soluble nickel compounds have not shown any effects on male fertility. Soluble compounds though, we wouldn't expect either to have effects for insoluble compounds or nickel metal either.

So in that case, our view is that no compounds, either soluble or insoluble compounds, nor nickel metal have met the criteria for listing.

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DR. TAYLOR: The last thing I'll show you is that the European Commission in 2008 evaluated the same set of data that -- at least up to what was available to them in 2008 to decide on their classification in Europe for different -- for nickel compounds and for nickel metal, and at that point, decided that only the soluble toxicity -- soluble compounds met the criteria for developmental toxicity based on the animal evidence. The insoluble compounds metallic metal did not, specifically because of a lack of bioavailability. So the harmonized classifications are just for developmental toxicity for soluble compounds, not for reproduction infertility or for effects versus via lactation in Europe.

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DR. TAYLOR: So our request is that you consider this consistent messaging from both our experts and from Europe and list for developmental toxicity. However, the main request is that you consider all these compounds separately, because they have different physical chemical and toxicological properties.

Thank you.

CHAIRPERSON GOLD: Thank you very much.

Any questions from the panel?

Thank you.

We also have John Hewitt from Grocery Manufacturers Association.

MR. HEWITT: If I could just ask, Chair Gold, for the timer to stop just long enough for the comments to get handed down, so that you'll have them in front of you, because I'll reference those in my oral comments to try and be succinct and quick as possible. Thank you.

CHIEF COUNSEL MONAHAN CUMMINGS: John, do you have a copy for the court reporter.

MR. HEWITT: Carol, they are making their way.

CHIEF COUNSEL MONAHAN CUMMINGS: Oh, there they are.

MR. HEWITT: And...

Thank you Chair Gold for your indulgence in letting me pause long enough to hand those out. And for the record, John Hewitt on behalf of the Grocery Manufacturers Association. Today, I'm here also on behalf of the Council for Responsible Nutrition and the American Herbal Products Association. My apologies that we did not meet the timeline for submitting comments in a timely fashion. What you have in front of you are our written comments, and I'll try to summarize those as quickly as possible.

The -- I'll five you the desert first, the conclusion first. It's our position that the umbrella

term nickel and nickel compounds encompasses dozens of distinct chemicals, each with its own unique properties and toxicity level. We believe that listing all of these chemicals under a single Proposition 65 listing would violate Prop 65 and the implementing regulations.

CRN, GMA, and AHPA would urge this committee to reject this proposed listing and instead to consider only whether individual compounds of the nickel and nickel compounds category can clearly be shown through scientifically valid testing to cause reproductive toxicity with the meaning of Prop 65.

To support that, I would just -- I would ask that the Committee focus its attention to the bottom of page two and the top of page three of our written comments. In that section, and I'll grab some of the -- just a few of the key sentences there for the benefit of the audience as well. You know, we believe the proposed listing seeks to label dozens of chemicals, metallic nickel and every nickel compound, both soluble and insoluble, as reproductive toxins with a single stroke.

We believe that this Committee should endeavor to be far more specific than the proposed listings to apply specifically the scientific evidence before it.

I would say that in looking at the second paragraph in paragraph -- on page three, OEHHA itself

acknowledged in its scientific document in support of the proposed listing that quote, "The various nickel compounds differ in toxicity", end quote. And that is from page nine.

Despite this, of the dozens of unique nickel chemicals in existence, OEHHA noted that only six forms of nickel are typically used in the reproductive toxicity studies, three forms of nickel salts, and three elemental forms. As a result, from its very outset, we believe the information provided to this committee for its consideration concerned only a fraction of the known nickel compounds.

Furthermore, studies have indicated that the absorption not only differs due to the solubility of nickel compounds, but also in the context of the route of exposure. OEHHA acknowledged this in its scientific document in support of the proposed listing. The conclusion -- and the conclusion by Sunderman et al., which states that the dietary constituents significantly reduce the bioavailability of nickel.

OEHHA also notes in this conclusion was further supported by the findings of Solomons et al. and Nielsen et al. and those are on page 12 of OEHHA's documents.

Again, we believe these findings suggest that the very -- that there exists varying levels of toxicity, even

among the different routes of exposure to nickel compounds. And seeing that I have about a minute left, I will do my best to summarize, Chair.

So we would reiterate our previous point that each form of nickel must be evaluated for toxicity before determining its potential for listing. We would respectfully direct this Committee's attention to page nine of the Nickel Producers Environmental Research Association's comments, which points out that IARC, NTP, and the European Union all make categorical distinctions between metallic nickel and nickel compounds, and have classified them differently based on the varying levels of carcin -- carcinogenicity.

We would also direct this Committee's attention to the 2008 European Commission report cited on page 11 of the aforementioned comments, which conclude, the insoluble nickel compounds such as nickel oxide and sulfides and also metal -- metallic nickel did not meet the criteria to be classified as reproductive toxicants.

Seeing that I'm out of time, I will summarize Chair Gold and say that, in summary, scientifically valid testing does not exist to show the entire category of metal -- metallic nickel and nickel compounds in this case would cause reproductive toxicity.

Thank you, Chair.

CHAIRPERSON GOLD: Yeah. Thank you.

Are there any questions?

Okay.

The last person that we have is Joshan Unuvar. I apologize if I mispronounced.

MR. UNUVAR: Thank you very much for the opportunity. And I agree with the previous public comments that nickel metal and soluble nickel compounds have to be evaluated separately to prevent needlessly alarming the public.

As mentioned, nickel exists in food and water. And I have -- I gave some examples in my written comments. For example cocoa powder is 12,000 micrograms per kilogram. So it would be pretty hard to believe that, you know, going to the grocery store and buying something, you would be exposed to potential toxicants, and there is no direct link to it.

Also, I want to comment on the fact that if anything in general is being considered to be listed, it should come with the safe harbor level or some minimum level, because we have the most experience here in this room. But once -- if something is listed without the safe harbor level, then it goes -- it could potentially go to the court system and the courts decide what that level is, or some settlement between two companies or two entities

decide that. And it's not the right way to go, I believe.

And I want to -- so if -- if we don't go specific in the listing, like some of the commenters said or don't use safe harbor levels, you get companies making some unnecessary warning to the public. I have an example here. This is from a local glassware store basically saying the eyewear products in the store can expose you to the chemical nickel metallic which is known to the State of California to cause cancer. So as far as I know, metallic nickel is exempted from cancer listing.

So basically, what they're saying is if you wear eyeglasses you could get cancer. It's simply not true. But this is what the public is being exposed to.

And I'll have another example. I recently purchased -- leased an electric vehicle and had a -- had this warning on the side window. It says, "Operating, servicing, and maintaining a passenger vehicle or offhighway motor vehicle can expose you to chemicals including engine exhaust, carbon monoxide...", and so on. So this was a pure electric vehicle. Doesn't have engine or exhaust. But here we go, I am being warned against cancer, breathing exhaust, or engine -- idling the engine and stuff like that.

So a warning might -- a listing might come easy or might seem like the right thing to do at some point.

But the implication of that -- of those listings could be very different what the -- what the Board or the Committee intends to do.

That's it.

CHAIRPERSON GOLD: Thank you.

Dr. Sandy, did you want to comment?

DR. SANDY: Thank you. I'll just clarify the listing, because it's been talked about a few times, for these compounds as causing cancer under Prop 65, we have separate listings we. Have one for nickel in parentheses metallic, so metallic nickel is listed, and then a separate listing for nickel compounds as causing cancer.

And those listings are based on findings from the report on carcinogens of the NTP, which has classified nickel compounds as known to be human carcinogens, and metallic nickel as reasonably anticipated to be a human carcinogen, as well as IARC, which is classified metallic nickel in Group 2B as a carcinogen, but nickel compounds as Group 1, known human carcinogens.

CHAIRPERSON GOLD: Thank you.

Does the Committee have any questions for this presenter?

Thank you very much.

So I'm going to ask the recorder if you'd like a break?

THE COURT REPORTER: No.

CHAIRPERSON GOLD: No. Would the Committee like a break?

Keep going. Keep going.

Okay. So that now that we're done with the public comments, and the discussion by the staff and the Committee, we can have further discussion as the Committee wishes before we take a vote.

So if there's anything further anyone wants to say or ask the staff, now would be the time?

Dr. Luderer.

COMMITTEE MEMBER LUDERER: Just something that was raised I think in the presentations and also kind of mentioned during the panel presentations earlier today, and as well as the OEHHA staff presentation is that the majority of the studies that we reviewed for the experimental animal studies really were for soluble nickel compounds. And I think when there were very few -- there was one study -- actually, I think two studies by the same group of the nickel nanoparticles and microparticles. So it may be worth the panel kind of revisiting what we -- the studies that were positive were, for the most part, I believe the soluble nickel compounds.

CHAIRPERSON GOLD: Thank you. So did you want to make a comment, Dr. -- so I was going to raise this as a

question, so thank you for making the comment. The question is whether the Committee wants -- I have actually two questions. One is whether we want to consider separate soluble insoluble in metals? I'd be interested in hearing from the Committee on that question?

COMMITTEE MEMBER ALLARD: The -- if you move on to the question of solubility, then what would be the cutoff? I guess that's my question. We need to determine as a cutoff for solubility to call something soluble versus insoluble. And, of course, opens a gray area that most of the data was with sulfate and chloride versions. And I personally feel more comfortable making a decision about those rather than extrapolate to any other forms, especially because you would assume that the distribution could be completely different, and therefore the effect would be different.

CHAIRPERSON GOLD: So you brought up my second question, but you also have a subset of the first questions, which is if we specify solubility, then we have to the amount of solubility, or to consider that.

The second question is whether we are talking about all nickel or nickel -- and nickel compounds, or if we want to restrict it to certain types of nickel.

So again, I open the floor to comments?

I encourage you to speak now, because I want to

hear you before we vote.

CHIEF COUNSEL MONAHAN CUMMINGS: Could -- sorry, could I make a comment?

CHAIRPERSON GOLD: Yes.

CHIEF COUNSEL MONAHAN CUMMINGS: I would remind you that when we did the opening comments that if you're uncomfortable and you want more information, or you're -- you know, you need more time to think about something, it's entirely okay for you to do that. If you want us to follow up on something, you know, for whatever reason, you want a definition of soluble - I don't know if there is one - you don't have to feel compelled to vote is all I'm saying.

CHAIRPERSON GOLD: Dr. Luderer.

COMMITTEE MEMBER LUDERER: Okay. I'll just say that I agree with Dr. Allard that it would be difficult to define a cutoff for solubility. And that the vast majority of the studies were the nickel chloride and sulfate that we reviewed. And so if we were -- if the panel thought that restricting the -- that the conclusions are really limited to those compounds, then that might be a better way to go than solubility.

CHAIRPERSON GOLD: So just to clarify, so you want to specify the compounds, is that -- is that what you're suggesting?

COMMITTEE MEMBER LUDERER: I'm agreeing that that would be something that I would like to hear what the other panel members have to say as well.

CHAIRPERSON GOLD: As would I. So again, I open it up to the Panel.

Dr. Pessah.

COMMITTEE MEMBER PESSAH: Thank you. I think you've opened up a can of worms, because I totally agree that the form is going to be very important in determining the weight of evidence. But also, I think the literature suggests that whether you're fasting or have food in the gut, can have a 40-fold difference in bioavailability. And if dietary intake is the primary form, why would we choose solubility over whether the studies were fed or fasted, right? I mean, we didn't account for that in our discussion.

CHAIRPERSON GOLD: Anyone else have a comment?

The only clarification I would make about the nickel chloride is that's true of the animal studies. I think in many of the human studies we don't know --

COMMITTEE MEMBER LUDERER: Right.

CHAIRPERSON GOLD: -- what the source of exposure is.

Other comments?

Dr. Luderer.

COMMITTEE MEMBER LUDERER: Although, just to respond, I mean, I think the issue of fed or fasted, I mean, in humans obviously we can't -- it could be either.

COMMITTEE MEMBER PESSAH: Right, but if it falls on the animal studies to really make an informed decision, then you can't ignore it, right, because then we didn't present the data in terms of what the evidence was for fasted versus fed studies, did we? I don't remember did we do that?

COMMITTEE MEMBER ALLARD: Sorry. We did not, but ultimately, the compounds tested in animals, except for a few nanoparticles once, most of them were just chloride versus sulfate, or chloride and sulfate, sorry. So whether it was fasted or not, that -- most of the evidence was just about these two, and that's it.

CHAIRPERSON GOLD: Dr. Nazmi.

COMMITTEE MEMBER NAZMI: I might ask counsel to comment on the allowability of us voting on specific compounds versus what the original charge was. Are we changing something, if we --

CHIEF COUNSEL MONAHAN CUMMINGS: Well, you're -- as I understand it, I'm not a chemist or a toxicologist. But if you're talking about potentially listing a couple of the chemicals within the category of nickel and nickel compounds, you can do that. If you feel like the evidence

isn't enough to do the whole class, or group, or however you want to define it, you can do that. It's totally fine.

There is enough notice. I mean, if you went outside nickel and suddenly said we want to list chromium, which is already there, you wouldn't be able to do that.

CHAIRPERSON GOLD: So, in other words, we could make it more restrictive. It's fairly broad and general.

Dr. Plopper.

COMMITTEE MEMBER PLOPPER: So that's what happened with, was it, nickel carbonyl or something? You've already -- one of them has already been listed, correct?

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah, but I think that just depends on the listing authority.

COMMITTEE MEMBER PLOPPER: Or EPA, yeah.

CHIEF COUNSEL MONAHAN CUMMINGS: You know, it was identified specifically by that authority. I don't know.

DR. SANDY: EPA.

CHIEF COUNSEL MONAHAN CUMMINGS: EPA. So it -- they didn't do it as a group, they identified a single chemical.

COMMITTEE MEMBER PLOPPER: One other comment. I'd just like to follow up on this issue. I'd prefer to see it done by compounds and not by a solubility issue,

because I agree with Isaac, the solubility of these compounds and their ability to be taken up depends on so many biological factors that haven't been assessed here. I don't know that we would have time to go through and even discuss all the opportunities.

So if -- I would feel comfortable with the solubility as a soluble compound, but not an issue of setting a limit, because I don't know that we could really do that.

CHAIRPERSON GOLD: Can I just clarify? I thought I heard you say at the beginning that you wanted it by compound. But at the end it sounded like you said solubility versus not so, just for --

COMMITTEE MEMBER PLOPPER: No, I'm just saying soluble. If we could -- we have -- I think there's data that could -- a reasonable division could be set -- made one way or the other for soluble. Two of these soluble nickel compounds. That's all. Specific, yes, since it's already been done before.

CHAIRPERSON GOLD: Two specific compounds. So are we saying nickel chloride and nickel sulfate, is that what we're saying?

COMMITTEE MEMBER PLOPPER: That's what I'm saying.

CHAIRPERSON GOLD: I don't want to put -- I'm not

putting words. I'm asking the question.

COMMITTEE MEMBER CARMICHAEL: I have a question.

CHAIRPERSON GOLD: Dr. Carmichael.

COMMITTEE MEMBER CARMICHAEL: Is it -- that it's want to divide that further, and is that distinguished from metallic nickel?

CHAIRPERSON GOLD: Can you answer that question, Dr. Allard if metal is different from -- I don't mean to put you on the spot.

COMMITTEE MEMBER CARMICHAEL: I mean, I'm just not -- I'm not sure how many different -- what the -- what the most sensible groupings are, not trying to figure it out, but we haven't mentioned metallic nickel, I don't think, unless that's just in the insoluble group.

COMMITTEE MEMBER ALLARD: Right. I think at this stage we're only talking about the -- whether bolus of studies are really about, which are the nickel chloride and nickel sulfate. And where I think we've been mostly talking about non-grouping at this stage, right. Sort of ignoring metallic nickel.

CHAIRPERSON GOLD: So if I can get a sense of the Committee, I hear an argument for limiting this to nickel chloride and nickel sulfate, based mostly on the animal studies, I would state. Does anyone want to argue something in addition or different from that?

COMMITTEE MEMBER CARMICHAEL: I just wanted to clarify is that -- that's true for all of the different endpoints that we were talking about, that those two compounds are what they -- the evidence boils down to?

CHAIRPERSON GOLD: Since we're dealing it with animals, largely because in the humans it's different, I think, than the animal studies. So I'll put it to the -- maybe to Dr. Baskin about the male studies and then I'll do similarly with the female and developmental.

Not to put you on the spot.

COMMITTEE MEMBER BASKIN: No. No. I mean, we obviously haven't done our homework in relation to this specific question. But how hard is it to distinguish soluble from insoluble nickel compounds? That seems to be -- I mean, metallic nickel, like the nickel in my pocket, it seems like it's insoluble. If you ingest it, kind of a problem, or can be a problem from multiple different reasons, because the nickel can get absorbed evidently from your stomach acid.

So that's where I think the issue is. I mean, all the studies they looked at were really soluble nickel. They weren't testing whether the rat ate a nickel.

(Laughter.)

CHAIRPERSON GOLD: Dr. Luderer, do you want to comment from the perspective of the female reproductive in

the animal studies. I think you have, but just to be clear.

COMMITTEE MEMBER LUDERER: I mean, the Majority were nickel chloride and nickel sulfate. There was one group that looked at the nanoparticles and microparticles in male and female. But the female study I thought had a lot of -- it was not a very well done study. There were a lot of problems with that study. And since it was only one, I kind of hesitate to -- to base a decision about those particles entirely on the one study at least certainly regarding female reproductive toxicity. Whereas, there's a large database for nickel chloride and nickel sulfate.

CHAIRPERSON GOLD: Dr. Plopper, do you want to say anything about the animal studies for developmental effects in terms of solubility versus specific compounds?

COMMITTEE MEMBER PLOPPER: Well, almost all the very definitive studies in animals were with the soluble compounds, the chloride and the sulfate. They were dose responsive, two species.

CHAIRPERSON GOLD: So -- and, Dr. Donald, has put up the slide that shows that the compounds we're talking about are largely highly soluble, correct?

DR. DONALD: Yes.

CHAIRPERSON GOLD: So the question is again, do

we want to talk about solubility, in which case we probably should specify the amount of solubility, or do we want to talk about specific compounds, or do you need more information, which is yet another alternative? Do you not want to make a decision today?

Dr. Allard.

COMMITTEE MEMBER ALLARD: I think this table raises a lot of questions. Again, I personally only feel confident making a decision based on data. So we have mostly data about nickel chloride and nickel sulfate again. But if you assume that solubility is the main factor here -- although we have to take into account other things, as mentioned earlier, then we would be ignoring the nitrate version, which is to be sitting, you know, quite nicely in between the solubility between chloride sulfate.

The cutoff would be a discussion that could be extremely lengthy and would require a lot of further discussion. Ultimately, most of the studies are chloride and sulfate. And again, I'm going to repeat myself here, but if most of the data is on these two compounds, then we can only decide on these two compounds personally.

CHAIRPERSON GOLD: Anyone else have a comment?

So if we were to restrict it to nickel chloride and nickel sulfate, I want to hear if anyone on the

Committee would be uncomfortable with that or have a different preference?

So I take no comment as you prefer to restrict it to these two compounds?

Anyone disagree with that?

COMMITTEE MEMBER BASKIN: Yeah. When you look at the solubility in water, there's somewhat of a cutoff point between 93 and 1.13. Although, the compounds that are insoluble have also been listed as hat -- as hazardous. So let's say we -- we go with that plan, just the two compounds that have been looked at, do we relook at the other compounds another time?

CHAIRPERSON GOLD: Okay. We can request that of the staff if you want to do that, but I -- so I'll ask the question of staff. I presume we were given everything you could find on nickel and nickel compounds, is that correct?

COMMITTEE MEMBER BASKIN: So the other issue is is that we think nickel, not the chloride or not the sulfate, is the issue here. So I personally would lump them all together.

DR. DONALD: To answer your question, Dr. Gold, yes, we provided all the data we could identify relevant to the potential for nickel and nickel compounds to cause reproductive toxicity.

CHAIRPERSON GOLD: Okay. Anyone have any other comments, because now we have a couple of opinions?

Again, one option is to say you're not prepared to make a decision today. Does anyone feel that they're in that position?

Everyone is prepared to vote today.

COMMITTEE MEMBER BASKIN: I mean, I'm prepared to vote because there's not anymore data that we're going to get to vote on. Is the staff kind of in agreement?

DR. CAMPBELL: (Nods head.)

COMMITTEE MEMBER BASKIN: It looks like there was a very thorough analysis performed.

CHAIRPERSON GOLD: Dr. Sandy and then we'll go to --

DR. SANDY: So we've given -- as Dr. Donald said, we've given you everything on the developmental and reproductive toxicity of nickel. I'll offer to you the possibility of looking at other information if -- as Dr. Baskin said, if you think it's nickel, would it be helpful to you to consider how nickel compounds are listed and metallic nickel as causing cancer, or some other toxicity endpoint, the thought process of how to, and why to list that broad group, or if you feel that Dr. Baskin --

CHAIRPERSON GOLD: Would that be helpful is the question, right?

DR. SANDY: Exactly.

CHAIRPERSON GOLD: To show the way of thinking how it was thought about for a different endpoint. Would that be helpful?

Dr. Donald.

DR. DONALD: If I could also offer. If you believe today that any subset of the chemicals presented to you have been clearly shown to cause reproductive toxicity, you could choose to vote on those today and defer decisions on others to a future meeting if there's relevant information that you would -- further information you would like to consider for those.

CHAIRPERSON GOLD: So yet another possibility. Dr. Pessah, you had a comment or question or something.

COMMITTEE MEMBER PESSAH: No. I think I'll hold my tongue.

(Laughter.)

CHAIRPERSON GOLD: And Dr. Nazmi, you look like you had something to say or ask?

COMMITTEE MEMBER NAZMI: I think what I was going to say was going to lead to that option three.

DIRECTOR ZEISE: So just to clarify then, option three would be to look separately at those two compounds for which there's the most evidence. And then at a future meeting for OEHHA to make a presentation about

potential -- different kinds of nickel compounds and how they might form different kinds of ions and get into the chemistry a bit more, and also potentially present on some other findings for other endpoints is that what option three is? I just want to clarify what Dr. Nazmi and Dr. Donald suggest.

CHAIRPERSON GOLD: Dr. Nazmi.

COMMITTEE MEMBER NAZMI: I wasn't necessarily advocating for option three, but --

(Laughter.)

COMMITTEE MEMBER NAZMI: -- I left like that's where it was going.

CHAIRPERSON GOLD: So I think I might even hear four options. So let's see if I can state them and then see if we can come to a consensus. And the reason it matters is if we're going to vote, we need to know specifically what we're voting on.

Okay. So one possibility is to vote on nickel and nick compounds sort of the broad category. Another possibility is to restrict it to nickel chloride and nickel sulfate. Another possibility is to say we don't have enough information to vote at all today and seek more information. Oh, there's one more. Another one is to divide it on solubility. And the last one is to get further information on how nickel might get metabolized or

the toxicity of it in other contexts and get more information and defer the decision until we have that.

So I hear maybe five possibilities. And again, the reason this matters is because if we're going to vote, we have to vote on something specific. And, you know, we have to know exactly what we're voting on.

So I would appreciate a sense of the Committee in terms of -- well, let me ask the question. Do people want to vote today or do they want to defer? Let me ask that question.

How many -- this is just a straw poll. Can we do that?

CHIEF COUNSEL MONAHAN CUMMINGS: (Nods head.)

CHAIRPERSON GOLD: Okay.

COMMITTEE MEMBER ALLARD: So I'm -- personally, I'm ready to vote, but it depends what we've -- to vote on nickel chloride and sulfate. I'm not ready to vote on all nickel and nickel compounds.

CHAIRPERSON GOLD: Okay.

COMMITTEE MEMBER ALLARD: Because then that would just be mixing a lot of things that I would not feel comfortable voting on. So it depends what we're voting for

CHAIRPERSON GOLD: Yeah. Okay. So fair enough. So we could -- we could vote on that and still request

other information if we want it for future -- for the future.

So that -- Dr. Pessah.

COMMITTEE MEMBER PESSAH: So I was just wondering, I'm trying to draw a parallel with what we've done in the past on other things we've brought to the Committee. You know, I'm going to use this as an analogy. It may be an inappropriate analogy, but if you -- if you're trying to make a decision on an organophosphate, you don't make a decision on all organophosphates simply because they're not all the same. And when I look at this chart, at the physical chemical differences across nickel compounds and nickel metal, it's vastly different from the range of physical chemical properties of organophosphate.

And so what you're asking us to do is make a decision on whether it's just about nickel, regardless of the form or what we have evidence on, which are the two that were mentioned previously.

If I had to make a suggestion, I would be more comfortable with just going what we have data on.

CHAIRPERSON GOLD: Me too.

Counsel has a comment.

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah, I don't know if it adds anything to the conversation. But I did want to mention that under Prop 65 currently in terms of

metals, chromium, including hexavalent compounds, is listed for reproductive toxicity in cancer. Mercury and mercury compounds are listed for reproductive toxicity. Arsenic, including inorganic oxides are listed for reproductive toxicity. Arsenic inorganic arsenic compounds are listed for cancer. Nickel compounds are listed for cancer. We already knew that, including metallic nickel separately. Beryllium and beryllium compounds are listed for cancer. Cadmium and Cadmium compounds are listed.

So I'm not saying that the data was, you know, the same, and they only did -- tested two things. But just for your -- for context, it is -- it wouldn't be an unusual kind of approach.

COMMITTEE MEMBER BASKIN: Can I ask the staff a question? And this relates probably mostly to human studies. But when they measured, for example, nickel in the urine, were they doing mass spec just on nickel?

DR. KIM: That is my understanding.

COMMITTEE MEMBER BASKIN: That was kind of mine too. So all of these compounds that are soluble, it doesn't matter whether it's chloride, sulfide, or the ones that we didn't study, implies we're really looking at nickel. And to me, if nickel chloride and nickel sulfate are concerning animals, I would look at nickel acetate,

and nickel nitrate, and nickel subsulfide, and nickel carbonate and be concerned. So I personally would lump those together based on -- I get it that they're Different molecules. But we're looking at the nickel aspect. We're looking at the heavy metal aspect of this compound.

CHAIRPERSON GOLD: Before we make a decision, I would just again comment that the two compounds we're talking about were mostly in the animal studies. In the human study, some of them measured nickel in urine, some in blood. They had different findings. And some looked at airborne nickel. So I think we need to think about the totality. But if we're largely being driven by animal studies, then that's a consideration.

So, Dr. Pessah, you have something to say?

COMMITTEE MEMBER PESSAH: I doubt any of the human studies speciated. I think it all looked at total nickel.

THE COURT REPORTER: Your microphone.

COMMITTEE MEMBER PESSAH: Oh, Sorry.

The human studies, when they measured nickel, were total tickle. They didn't speciate.

CHAIRPERSON GOLD: Which was sort of -- well, sort of argue in favor of what Dr. Baskin is saying, in terms of considering all nickel. So it sounds to me, correct me if I'm wrong, that people want to vote today,

but we're -- right, unless somebody says we really need more information and want to defer the vote. But then it comes down to whether -- I hear both things. A couple of people that want to restrict it to two compounds and a couple of people that want to look at the more generic topic of nickel and nickel compounds.

Dr. Luderer.

COMMITTEE MEMBER LUDERER: Well, maybe one way to sort of think about it a little bit more broadly would be to go back and ask those of you who reviewed the epidemiological literature in detail. I mean, if the strength of the epidemiological database is -- as a whole, is strong enough to make us concerned about the developmental reproductive effects of nickel, then that would be an argument for broadening -- you know, for considering all nickel compounds, because as you say, we can't -- we really don't know what the -- what specific nickel compounds humans are exposed to.

CHAIRPERSON GOLD: Let me ask a ask question of counsel. Is it possible to have different categorizations for different outcomes? So for female, it might -- you know, it might be one -- it might be nickel and nick com -- I'm just giving you an example.

CHIEF COUNSEL MONAHAN CUMMINGS: Yes.

CHAIRPERSON GOLD: And I don't mean this. And

for something else restricted to just nickel chloride and nick sulfate. Can we do that?

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah. You can go endpoint by endpoint for whatever grouping you do. I was also going to mention you don't have to only have one question. You can -- you know, if some -- if you want to vote on whether you want to listen to all, you know, the soluble ones or all of them. And then depending on how that comes out, then -- if -- then you can look at the more individual ones. It's entirely up to you. You can have more than one question.

CHAIRPERSON GOLD: Dr. Carmichael.

COMMITTEE MEMBER CARMICHAEL: As far as the -- I would say the weight of the evidence is from the animal literature, rather than the epidemiologic literature.

CHAIRPERSON GOLD: I would agree. And so with the possible exception of male reproductive, do you feel like the human studies are contributory? No. Yes. No.

Please speak into the microphone.

COMMITTEE MEMBER BASKIN: The human studies, the epidemiologic studies often don't show causality. They show a concern. And that's why the animal studies were done. I mean, we've done this for years.

CHAIRPERSON GOLD: Okay. So here's -- if I can -- I don't want to put words in -- I'm asking a

question. So, Dr. Carmichael, you were the primary discussant on the human developmental studies. And you said just moment ago that you thought most of our -- most of this is being driven by animal studies. Would you say that for the developmental outcomes?

COMMITTEE MEMBER CARMICHAEL: Yes, the developmental outcome as well as the male reproductive outcome effects, which I was the secondary on, but didn't give extra comments on. So therefore, as I'm thinking about this, I'm going with what the specific types of compound were, however we define that.

CHAIRPERSON GOLD: From the animal studies.

COMMITTEE MEMBER CARMICHAEL: From the animal studies.

CHAIRPERSON GOLD: And, Dr. Baskin, you're nodding your head. Are you in agreement?

COMMITTEE MEMBER BASKIN: I'm in agreement.

CHAIRPERSON GOLD: And so -- and I would say from the female reproductive, I don't think the human studies are particularly contributory. So anyone else want to make a comment at this stage?

Do they disagree with what's been said?

Dr. Pessah.

COMMITTEE MEMBER PESSAH: So if the human studies are all based on total nickel, and they're not convincing

or compelling, and the animal studies only looked at two forms of nickel predominantly. I still raise the issue of how can you generalize in the animal studies when you've really only got two forms of nickel that have been studied.

CHAIRPERSON GOLD: So you're arguing for restricting it to those two compounds?

COMMITTEE MEMBER PESSAH: Based on that, yes.

CHAIRPERSON GOLD: Is that a yes.

COMMITTEE MEMBER PESSAH: That was a yes.

CHAIRPERSON GOLD: Okay. And so -- can I come back to you in second. Dr. Baskin, you were making the argument of more generic -- or general formulation rather than restricting it, so comment.

COMMITTEE MEMBER BASKIN: I mean, sodium chloride has chloride in it and it's not dangerous unless you take mega amounts. Okay. Nickel chloride is probably dangerous. Certainly dangerous in the animal studies. I think we're studying nickel here, so I'm okay incorporating -- I'm personally okay incorporating the other nickels that are -- that are on the list, soluble nickels. Nickel and you -- that's I guess tricky to define.

CHAIRPERSON GOLD: Right. So then we get into the issue of solubility.

COMMITTEE MEMBER BASKIN: Yeah.

CHAIRPERSON GOLD: So you're still arguing for a broader inclusion. Dr. Allard, you were wanting to make a comment.

COMMITTEE MEMBER ALLARD: Yeah, I mean for what it's worth, because it's not about developmental reproductive toxicity. It's again going back to the ToxCast data set. I was surprised looking at it last night again how different the profiles were for nickel sulfate and nickel chloride. There's no explanation behind it. It's just the -- you know, different parameters and different assays that were run, but not the same pathways came out between nickel chloride and nickel sulfate.

I'm not necessarily -- I love mining the data set. I don't know all the details of it, and I'd love to talk to someone about it, but I can just relay that the profiles are different between the two compounds.

Is it then just about nickel? And I'm not sure. The ToxCast data set to me makes me believe that maybe it's not just about nickel, that there's other parameters, physical characteristics that makes things different.

CHAIRPERSON GOLD: So are you arguing for --

COMMITTEE MEMBER ALLARD: I'm still arguing for the same thing to only -- personally, I would only feel

comfortable voting about sulfate an chloride, and with perhaps more discussions at a later date about the other compounds, but for today, just chloride and sulfate, personally

CHAIRPERSON GOLD: Dr. Nazmi.

COMMITTEE MEMBER NAZMI: I wrote down these options as we've been chatting. And I tend to agree with Dr. Baskin that we are examining nickel. So I guess I would, for the straw vote, favor lumping them together, as we're thinking about the votes. And I would feel comfortable voting on that.

CHAIRPERSON GOLD: Okay. I have another question for counsel.

(Laughter.)

CHAIRPERSON GOLD: So is it an option to have two sets of votes?

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah, that's totally fine. That's totally fine, yeah.

CHAIRPERSON GOLD: We could have one that's -- woops, sorry -- generic nickel --

CHIEF COUNSEL MONAHAN CUMMINGS: You can do the whole -- the whole group first and then --

CHAIRPERSON GOLD: -- All nickel. And we could have another one that's nickel chloride and nickel sulfate.

CHAIRPERSON GOLD: Right, or soluble, or whatever ones you want to vote on.

CHAIRPERSON GOLD: Soluble. Again, if we do soluble, we should come up with a level of solubility?

No, don't have to.

DIRECTOR ZEISE: Can you comment?

CHIEF COUNSEL MONAHAN CUMMINGS: You don't have to. Although I think giving us some advice in terms of what you are thinking of on solubility. I mean, I think Dr. Baskin was saying that there's a fairly simple cutoff point, at least on this chart. But, you know, whether or not that's the correct one, I don't know. But you don't have to actually set a number to -- we just need to kind of understand what you mean by soluble.

CHAIRPERSON GOLD: The only comment I would make is that nickel by itself would fall below the cutoff I think the way you defined it, and yet you're saying all nickel, so...

CHAIRPERSON GOLD: Please use the microphone.

COMMITTEE MEMBER BASKIN: We have to define it, so everybody knows that we're talking about. So even though I agree that insoluble nickel can be dangerous, we could -- we could simply, for the point of discussion, say insoluble versus soluble, so it would include all the compounds on the list there, except for the bottom three.

CHAIRPERSON GOLD: Okay. So any degree of solubility is what you're saying?

COMMITTEE MEMBER BASKIN: Or what we choose to vote on. So I'm not a chemistry expert. So my recommendation is that because there seems to be some concern here. And I'm also concerned too that we do a couple different votes. But I think we need to come back to the Committee and get a chemistry expert to tell us what -- you know, what this really means, if staff can provide that.

CHAIRPERSON GOLD: That suggests deferring the vote.

COMMITTEE MEMBER BASKIN: Well, I think we can -- I mean, you can vote for all of it, and you can vote for just the two compounds and see where the numbers fall.

CHAIRPERSON GOLD: Or you could vote for solubility versus non-solubility.

COMMITTEE MEMBER BASKIN: Correct.

CHAIRPERSON GOLD: We could have three votes.

COMMITTEE MEMBER BASKIN: Correct. Dr. Plopper, one second.

COMMITTEE MEMBER PLOPPER: Since we're talking about nickel, aren't we talking about nickel ions -- anions? It's the nickel anion that's the problem. And I was trying to look through this literature to find out if

somebody has actually characterized when they measure nickel, what is it? What is the nickel that's the problem here, and an ion -- and an anion has a different reactivity than a -- than the whole metal itself, and particularly the -- what are -- the ones that I see that are insoluble are also the ones that don't react well with just about anything. And so I -- but I'm not a chemist.

So I -- I don't know. I think it would help to have some perspective on the chemistry that's floating in there. Is it anion when it's being measured as soluble. If it is, then that's a real concern, because it's -- it doesn't -- doesn't matter how many compounds you use. If it makes anions, then that's what the problem is. Yes, I think that's a concern.

CHAIRPERSON GOLD: Dr. Sandy.

DR. SANDY: Yeah. So my early suggestion to come back with some information. If I may, I'll just read some quotes from the report on carcinogens substance profile on nickel compounds. "Nickel compounds generate nickel ions in target cells at sites critical for carcinogenesis", and then it goes on. Another partial quote here is, "Metallic nickel can slowly dissolve in the human body and release ionic nickel". Another one is, "Both soluble and insoluble forms of nickel caused genetic damage".

So just to give you a flavor of its -- I don't --

it doesn't appear that the report on carcinogens used soluble versus insoluble, because they recognized that it's nickel ions for that endpoint, and I was suggesting you might want to think about how other people have looked at the toxicity. Is it nickel?

COMMITTEE MEMBER BASKIN: So what you're saying, in the right situation, you know, the nickel metal or nickel alloy, if it's under the right conditions -- so let's say a child swallows itself and there's acid in the stomach, the nickel ion could be released, even though it's deemed basically insoluble, and it could be dangerous, and it was deemed a carcinogen. Am I interpreting that correctly?

DR. SANDY: I -- the way you've put it, I cannot -- I don't want to say if that exact example was what was in the minds of the folks on the Report on Carcinogens Panel, but I was just giving you some quotes, they are saying that metallic nickel can slowly release ionic nickel into cells in the body.

COMMITTEE MEMBER BASKIN: So the implication sis that metallic nickel, which is considered insoluble, could potentially be dangerous, is that correct?

DR. SANDY: Yes, they're saying metallic nickel can slowly dissolve in the human body and release ionic nickel. And another sentence I read, it's nickel ions in

the target cells that they believe are critical for carcinogenesis. And the question for you would be do you think that's critical for developmental and reproductive toxicity?

COMMITTEE MEMBER BASKIN: Thank you.

CHAIRPERSON GOLD: Dr. Allard.

COMMITTEE MEMBER ALLARD: Just based on the amount of data that I saw on PubMed, I think it was easier for carcinogenicity to come to this conclusion, just because they were so many more studies performed with regards to nickel's carcinogenicity than what I've been able to find. And I saw with regard -- in the -- in the HID with regards to reproductive and developmental toxicity.

So, yeah, I think there was just a lot more confirmation of that possibility with regards to carcinogenicity in terms of data available.

CHAIRPERSON GOLD: Dr. Pessah.

COMMITTEE MEMBER PESSAH: I agree with what was just said. I was going to say the same thing.

CHAIRPERSON GOLD: Okay. We can have a one-minute comment from the audience, if you like.

Mr. -- could you identify yourself

MR. UNUVAR: Joshan Unuvar. I'm a material scientist, Ph.D. I agree with the ion versus metal

distinction. And we can see that nickel is dissolving, but very, very little. It's only 1 -- 1.13 milligrams per liter. But if you look at the nickel chloride, it's almost five and a half orders of magnitude larger. So we're -- we can't really put them in the same basket. And I want to ask for a little more clarification. The way I read it, nickel in alloys are exempt from the carcinogen listing. And you might have -- some other people here might have data on that. I don't have the printout, but it's specific excludes nickel in the metallic form and alloys.

CHAIRPERSON GOLD: Yeah. I'm not sure -- I don't -- I'm sure the cars -- what the CIC has done is relevant particularly to us, or -- and they took it from an authoritative body as I understood. So I think what this Committee has to decide, which chemical it's going to deal with it, right? We can be guided somewhat by what's been done, but it's for carcinogenicity, and we have to decide about developmental and reproductive.

MR. UNUVAR: Sure. Sure. And also just -- let's say you swallow nickel piece. It's a one-time exposure, and it's not -- not like being exposed to the air inhalation every day. So it's not -- doesn't maybe fit the profile of continuous exposure. And it's so slow that it's not going to affect it.

CHAIRPERSON GOLD: I would also point though, the solubility is in water. And the concern is the acid in the stomach, so -- all right.

One quick comment, Dr. Taylor.

DR. TAYLOR: Thank you very much. I appreciate it. I do this for a living, so I'm enjoying the discussion. And I would look at that chart and say that your dividing line, you know, for soluble I would call it right under nickel ammonium sulfate. Look at the orders of magnitude difference between that and nickel subsulfide. Nickel subsulfide certainly we consider it an insoluble nickel compound, or it's -- you know, as a sulfidic compound. So I would just say that the carso discussion is fair, because that's a local effect in the lungs. Nickel only causes inhalation respiratory tract carcinogenicity, a local -- requiring, you know, the particles to be there.

We're talking with repro a systemic effect, which requires dissolution and systemic delivery. And in that case, I would say that if you swallow a nickel, even in the stomach acid -- and you can do these kind of tests of metallic nickel and nickel compounds in the stomach acid and see how much is released.

You know that this is a threshold effect. We've looked at the thresholds and the blood levels it requires

to see them, that you don't reach those. You cannot reach those thresholds by swallowing an insoluble compound even in stomach acid.

CHAIRPERSON GOLD: All right. Thank you.

I'm going to suggest that we take a break.

(Laughter.)

CHAIRPERSON GOLD: And I'd like the Committee members to think, but not discuss the issues among themselves. And let's say 10 minutes, and we'll reconvene at 3:15.

(Off record: 3:04 p.m.)

(Thereupon a recess was taken.)

(On record: 3:15 p.m.)

CHAIRPERSON GOLD: Can we reconvene, please. I'm going to pose one question to the Committee and then I'm going to propose a way to vote. So my first question is, does anyone on the committee want to defer and not vote today?

So everyone is prepared to vote one way or another.

Okay. Then counsel has suggested an approach to the voting that I think would be very helpful. Are we ready to vote or is there more discussion?

Dr. Baskin.

COMMITTEE MEMBER BASKIN: So I appreciate we got

this lovely folder, which were the public comments and the comments from people who had interest here. And from the NiPERA -- if I'm pronouncing that right -- production, page 16, the conclusion in blue, "The only effects that have been clearly shown through scientifically valid testing, according to generally accepted principles are the developmental toxicity effects observed in animal studies with soluble nickel compounds.

So clearly NiPERA, who I'm only familiar with because of this book, has a definition of soluble versus insoluble. So I think we can come up with that definition too. And if it's simply the bottom three are insoluble and the top three are soluble, I would be -- I would propose we vote if we want to use soluble in a definition, based on that.

CHAIRPERSON GOLD: Okay. Just to clarify, what staff would like, if we decide to do that, is that we don't need to come up with a cutoff level or anything like that, but just give them a sense what they need to consider in determining solubility. Okay.

Okay. All right. So the proposal is that we'll vote three times, if necessary. But the first vote will be the most broad -- most broad generic category, so nickel and nickel compounds. And depending on how you feel, you may or may not be comfortable with that, you

vote the way you want.

Okay. If that settles it, then we don't need to vote further. But if it doesn't, then we'll go to the next sort of smaller -- slightly smaller category of soluble versus insoluble. And if that doesn't settle it, then we'll go to the narrowest, which would just be the two compounds.

Everybody in agreement with doing -- that approach. Any problems with that approach?

Okay. And we're going to do this for three outcomes. So three times three, if necessary. So nine votes, if necessary.

Yes.

MR. UNUVAR: My I read a section --

CHAIRPERSON GOLD: Carcinogenicity?

No, I think we're talk about -- I think we're, A, done with public comments, and B, dealing with reproductive developmental.

Okay. Are we ready to vote?

Okay. So bear with me. We're going to go broad and -- okay. Yes. All right. So first, we're going to -- we're going to do this in the order that we had the presentations in terms of the outcomes we're dealing with, so we're going to start with developmental, and we're going to start with the broadest category of nickel and

nickel compounds, okay, just to clarify what we're voting on.

So the question before you is has nickel and nickel compounds been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity? All those who believe yes -- all those voting yes, please raise your hand.

(No hands raised.)

CHAIRPERSON GOLD: All those voting no.

(Hands raised.)

CHAIRPERSON GOLD: Two, three, four and four. We've got eight.

Okay. All right. So that -- unless you want to do it that way.

DIRECTOR ZEISE: Oh, no, no.

CHAIRPERSON GOLD: No, that's fine. That's fine. That's what I was going to do, but.

Okay. So for developmental toxicity we'll consider now a soluble versus insoluble. Good. Okay.

COMMITTEE MEMBER ALLARD: I'm sorry. Can you tell us what the third kind of vote will be?

CHAIRPERSON GOLD: The third one will be the two compounds.

COMMITTEE MEMBER ALLARD: Okay. Making sure. Thank you.

(Laughter.)

CHAIRPERSON GOLD: Okay. Has soluble nickel been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity? All those voting yes, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Those voting no please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: So anyone abstaining?

(No hands raised.)

CHAIRPERSON GOLD: Well, I voted, but are we missing one vote. I'm missing one vote, so let's do it once more.

All those voting yes for soluble nickel compounds.

(Hands raised.)

CHAIRPERSON GOLD: Four -- five. I missed you sorry.

All those voting no, just to confirm.

(Hands raised.)

CHAIRPERSON GOLD: Three. Okay.

No abstentions.

We have one more. Okay -- oops, I'm sorry. All

right. Still dealing with developmental outcomes.

Has nickel chloride and nickel sulfate been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity? All those voting yes, please raise your hand.

COMMITTEE MEMBER PESSAH: Can you vote twice?

CHAIRPERSON GOLD: Yes. These are sort of exclusive.

(Laughter.)

(Hands raised.)

CHAIRPERSON GOLD: So that's four, five, six.

All right. Let me state it again, so we're clear. What we're voting on. We did soluble versus insoluble. Now, what we're doing is nickel chloride and nickel sulfate. Now, let me repeat. Has nickel chloride and nickel sulfate been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity? All those voting yes, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Three four -- we have eight.

So no noes and no abstentions.

CHAIRPERSON GOLD: Okay.

COMMITTEE MEMBER PESSAH: I'm sorry. I need to

change my vote on the last one to soluble. It was soluble to yes, because I think I voted no on that. Sorry. I apologize.

CHAIRPERSON GOLD: So you voted no, so we need to vote again.

DIRECTOR ZEISE: I think to clarify the record, it would be good just to ask the question again.

CHAIRPERSON GOLD: Okay. All right. So going back to the second vote. Okay. Has soluble nickel been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity? All those voting yes, pleas raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Six.

And those voting no.

(Hands raised.)

CHAIRPERSON GOLD: Two.

And no abstentions.

CHAIRPERSON GOLD: Okay. So that takes --

All right. So next we're going to do female reproductive toxicity in the same way in the same order. Are we clear?

Okay. All right. So the broadest category now. So has nickel and nickel compounds been clearly shown

through scientifically valid testing, according to generally accepted principles to cause -- no, that's the wrong one. Sorry -- yes, to cause female reproductive toxicity. All those voting yes, please raise your hand. This is for nickel and nickel compounds?

(No hands raised.)

CHAIRPERSON GOLD: Those voting no?

(Hands raised.)

CHAIRPERSON GOLD: Eight.

No abstentions.

Okay. Now, we'll vote on solubility and female reproductive toxicity.

So has soluble nickel been clearly shown through scientifically valid testing, according to generally accepted principles to cause female reproductive toxicity? All those voting yes, please raise your hand.

(No hands raised.)

CHAIRPERSON GOLD: All those voting no, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Eight.

And then finally for female reproductive toxicity, has nickel chloride and nickel sulfate been clearly shown through scientifically valid testing, according to generally accepted principles to cause female

reproductive toxicity? All those voting yes, please raise your hand?

(No hands raised.)

CHAIRPERSON GOLD: All those voting no, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Eight.

And no abstentions.

Very good. All right. Now, we're going to finally go to male reproductive toxicity with the broadest category, so nickel and nickel compounds. Has nickel and nickel compounds been clearly shown through scientifically valid testing, according to generally accepted principles to cause male reproductive toxicity? All those voting yes, please raise your hand.

(No hand raised.)

CHAIRPERSON GOLD: All those voting no, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Eight.

No abstentions.

Now, we're going to solubility. Has soluble nickel been clearly shown through scientifically valid testing, according to generally accepted principles to cause male reproductive toxicity? All those vote yes,

please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Six yes.

Those voting no, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Two.

No abstentions. So that solves this one.

So we're done.

DIRECTOR ZEISE: Yes.

CHAIRPERSON GOLD: Thank you. I think this is the most complicated vote I've been involved in. Well, we don't do the last two compounds, because they're covered by the solubility one.

CHIEF COUNSEL MONAHAN CUMMINGS: Right.

CHAIRPERSON GOLD: Correct?

That's the -- Okay. So I -- yes, we're clear now.

Okay. So the staff though has asked for just general guidance. We don't need cutoff levels, just sort of -- what sort of information or criteria would guide you -- would help to guide them in determining solubility? So anyone wishing to comment on that?

No guidance.

Okay. They're a bright group. We're going to leave it to you.

(Laughter.)

COMMITTEE MEMBER BASKIN: We can give you guidance, but I think that you're way smarter than us to sort this out. But if you were asking me and pin me to a wall, I would say above nickel.

CHAIRPERSON GOLD: Does that include nickel, nickel and above or nickel --

COMMITTEE MEMBER BASKIN: No, above nickel.

CHAIRPERSON GOLD: Above nickel.

Thank you.

Okay. So I believe we're on staff presentation at this point?

So, counsel, are you prepared to do the presentation?

CHIEF COUNSEL MONAHAN CUMMINGS: I think the -- Michelle is first.

DIRECTOR ZEISE: Carol, this is the consent item.

CHAIRPERSON GOLD: Item 3, the consent item.

CHIEF COUNSEL MONAHAN CUMMINGS: So sorry. Totally forgot about that one. Hopefully this will take just a second.

So our -- can you get the slide up?

MS. RAMIREZ: Yes.

CHAIRPERSON GOLD: We provided you with a staff report on the Section 2700 list of chemicals which need

testing under Prop 65. This is a separate list from the list of chemicals known to cause cancer or reproductive effects. If you look in your materials --

--o0o--

CHIEF COUNSEL MONAHAN CUMMINGS: -- hopefully, you can see something like this. And hopefully you looked at that. It's got several pages.

So our -- since this is a consent item, we're just asking you to say yes or no about whether or not we should make the following changes:

--o0o--

CHIEF COUNSEL MONAHAN CUMMINGS: So for -- we would like to know whether or not we should remove these chemicals. I'm not going to try and --

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: -- pronounce them. I can say Borax.

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: But these three chemicals that were recommended by the Department of Pesticide Regulation to remove because they say they've been fully tested.

So, yes.

CHAIRPERSON GOLD: Can we have a show of hands of everyone that approves of removing these from the list?

Oh, sorry. We do have a voting form.

So based on the recommendations in the OEHHA staff report, should Section 27000 of Title 27 of the California Code of Regulations be amended, and as indicated in Section 6 of the staff, report those: --

DIRECTOR ZEISE: Can you wait till the end?

CHIEF COUNSEL MONAHAN CUMMINGS: End of what?

Oh, I can just show you all -- I mean, we can show you all of these at once. Basically, they were covered in the report. These are the ones that DPR recommended that we. Remove

--o0o--

CHIEF COUNSEL MONAHAN CUMMINGS: These are the ones that you U.S. EPA recommended that we remove from that list.

--o0o--

CHIEF COUNSEL MONAHAN CUMMINGS: These are the -- the one chemical that they suggest that we add to the list from Department of Pesticide Regulation, and the types of testing that they're asking for are listed there.

--o0o--

CHIEF COUNSEL MONAHAN CUMMINGS: And the Department of Pesticide Regulation is recommending that we modify these two listings in the way it's marked here, so we're taking out the only one is required caveat, so it

sounds like they want all of those to be done.

And the petroleum oil and classified, they're making it more clear what they're applying it to. So hopefully that helps.

--o0o--

CHIEF COUNSEL MONAHAN CUMMINGS: And I think there's one more. And this one -- this chemical that is quite long would be added to the list for -- and asking for particular types of testing that are listed on here.

CHAIRPERSON GOLD: Okay. So can I get a vote from the Committee on making the amendments that are recommended in the report that you just heard?

All those voting yes, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Zero noes, and no abstentions.

Thank you very much.

Okay. Anything else from counsel?

Not until after Michelle, I think.

(Thereupon an overhead presentation was presented as follows.)

CHAIRPERSON GOLD: Okay. So we're going to staff updates now.

MS. RAMIREZ: Hello. Since your last meeting, we have administratively added two chemicals to the Proposition 65 list. The first slide here shows that

Vinylidene chloride (1,1-Dichloroethylene) and TRIM VX were added for cancer

--o0o--

MS. RAMIREZ: The next slide has the chemicals under consideration for administrative listing or modification of existing listing. A notice of intent to modify the listing of ethanol in alcoholic beverages was published on August 3rd, 2018. This is proposed under the Labor Code listing mechanism for the cancer endpoint. Bevacizumab is under consideration for administrative listing under the formally required listing mechanism for the female reproductive and developmental endpoints. The notice of intent to list was published on October 5th 2018.

--o0o--

MS. RAMIREZ: And since your last meeting, four safe harbor levels have been adopted in regulation. A no significant risk level of 108 micrograms per day has been adopted for Malathion effective April 1st, 2018; a no significant risk level of 1,100 micrograms per day has been adopted for glyphosate effective July 1st, 2018; a no significant risk level of 0.88 micrograms per day has been adopted for vinylidene chloride effective July 1st, 2018; and a maximum allowable dose level of 290 micrograms per day has been adopted for metham sodium effective October

1st 2018.

--o0o--

MS. RAMIREZ: On this last slide, as you can see, we've also proposed safe harbor levels for three chemicals. No significant risk levels have been proposed for bromochloroacetic acid, and bromodichloroacetic acid, and maximum allowable dose levels have been proposed for end N-Hexane by the oral and inhalation routes.

And now I'll turn things back over to Carol.

Thank you.

CHIEF COUNSEL MONAHAN CUMMINGS: Thank you. So this is just a quick update on litigation related to Prop 65. And I don't recall exactly what date it was that you all met last time. So this may or may not be new to you. But we have our first case in the federal court under Prop 65. We were part of litigation that was filed by the National Association of Wheat Growers among many others against our office and the Office of the Attorney General challenging the warning requirement for the chemical glyphosate under the first amendment.

So the companies and individuals are arguing that give a warning is contrary to the first amendment rights to not give false and misleading information.

So that case is pending currently. It's -- the court entered an order against the Attorney General's

office preventing any enforcement of the warning requirement pending the outcome of the case. The court did dismiss our office because the listing of glyphosate is not subject to the first amendment. It's government speech, so we're out of that case, but watching it closely.

Recently, the case was stayed because there's three cases pending in the Ninth Circuit Court of Appeal that are about warnings and compelled warnings, not Prop 65 ones. But given the decisions in those cases, we can find out what might happen in ours.

So we also have -- in the state courts on appeal, we still have the case that was brought by the American Chemistry Council challenging the listing of BPA. It's been sitting in the court of appeal for several years now.

And also, the American Chemistry Council case challenging the listing of DINP, which is a carcinogen listing, I believe. And then a case brought by Syngenta challenging the listing of the triazines the whole class of pesticides known as the triazines is pending in the trial -- in the court of appeal.

We were successful in defending all of those listings, but now they're -- those cases have been appealed.

We still have a couple older cases in the trial

courts. That two of them are just derivative of the triazine and BPA cases. They had to do with the Public Records Act. And a brand new case was just filed very recently in September 2018 against our office by a group that one of the plaintiffs groups that enforces Prop 65, the Center for Enviro -- let's see, Center for Environmental Research on Toxics, is that correct? I just called them CERT.

DR. SANDY: Education and Research on Toxics.

CHIEF COUNSEL MONAHAN CUMMINGS: Education and Research on Toxics. That's correct. Sorry. And that case is challenging our proposed regulation that would find that the exposures to listed chemicals in coffee -- carcinogens in coffee don't pose a significant risk of cancer, in that particular chemical mixture. So we're being challenged on that.

Oh, yes, just -- just because we -- we've got two different listings for BPA. The one that is in the court of appeal was for the developmental endpoint. BPA is also listed as a female reproductive toxicant. It's currently on the list.

And the other thing I don't usually bring up regulatory actions, unless we get sued over them, but I did want to mention that we finally have adopted -- fully adopted their regulation that we worked on for about five

years or so to update the -- the warnings for Prop 65. You're -- you'll -- if you haven't already, you're going to start seeing some new ones that actually include a name of a chemical, and a source of exposure, and a URL to our -- our website. We have a separate website that just has information on chemicals, and locations, so that Californians can actually find out what it is they're being exposed to, and maybe how to reduce their exposures.

And so that was a very long process. And that regulation finally went fully in effect at the end of August. And so hopefully that will resolve some of the issues where people just don't know. I mean, you can list the chemical, but if it's -- they don't know how they're being exposed, it's not very helpful.

So thank you.

CHAIRPERSON GOLD: Thank you.

So that I believe concludes the staff updates.

So now we'll have a summary of the Committee actions.

DIRECTOR ZEISE: Okay. So the Committee deliberated on nickel and nickel compounds. And by a vote of six to two identified soluble nickel for the male reproductive toxicity endpoint, as being clearly shown according to scientifically valid testing, according to generally accepted principles to cause male reproductive

toxicity.

Similarly for developmental toxicity, the Committee added soluble nickel -- the soluble nickel endpoint as being clearly shown through scientifically valid testing, according to generally accepted principles.

So those two chemicals will -- I'm sorry, so soluble nickel will be identified as known to cause reproductive toxicity for both the developmental toxicity and male reproductive toxicity endpoints. And the Committee declined to list the general class of nickel and nickel compounds for any endpoint.

Then with respect to identifying chemicals known requiring testing, the Committee affirmed the changes that OEHHA proposed to Section 2700.

And so that's the Committee actions taken today.

Then I guess I'd just like to end with thank yous to the Committee for taking time out of your busy schedules, and for all the deliberation today to get us to closure on what nickel -- the nickel and nickel compounds consideration.

And I'd like to thank the members of the public for providing public comments for coming to the meeting to comment, and for listening on the webcast, and, of course, thank the RCHAB staff, the legal staff, and the implementation staff for all their hard work to prepare

the materials for the meeting, and to make the presentations at the meeting.

So thank you all. And I'll just finally turn it back over to you, Ellen.

CHAIRPERSON GOLD. Anybody have any further comments or questions?

Okay. I would like to thank the staff and the Committee for their diligent and conscientious efforts on this particular issue. And for everyone who made presentations today.

So thank you.

And with that, I believe we can adjourn.

(Thereupon the Developmental and Reproductive Toxicant Identification Committee adjourned at 3:43 p.m.)

C E R T I F I C A T E O F R E P O R T E R

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 24th day of October, 2017.

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