MEETING

STATE OF CALIFORNIA
ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT
ENVIRONMENTAL CONTAMINANT BIOMONITORING PROGRAM
SCIENTIFIC GUIDANCE PANEL

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH
AUDITORIUM
850 MARINA BAY PARKWAY
RICHMOND, CALIFORNIA

THURSDAY, JULY 20, 2017
10:01 A.M.

JAMES F. PETERS, CSR
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APPEARANCES

PANEL MEMBERS:

Megan R. Schwarzman, M.D., M.P.H., Chairperson

Carl Cranor, Ph.D., M.S.L.

Marion Kavanaugh-Lynch, M.D., M.P.H.

Ulrike Luderer, M.D., Ph.D.

Penelope (Jenny) Quintana, Ph.D., M.P.H.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY:

Gina Solomon, M.D., M.P.H., Deputy Secretary for Science and Health

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT:

Lauren Zeise, Ph.D., Director

Allan Hirsch, Chief Deputy Director

Amy Dunn, M.P.H., Research Scientist III, Safer Alternatives Assessment and Biomonitoring Section

Carl DeNigris, Staff Counsel

Alexander Hoepker,

Shoba Iyer, Ph.D., Safer Alternatives Assessment and Biomonitoring Section

DEPARTMENT OF PUBLIC HEALTH:

Robin Christensen, M.S., Biomonitoring California Grant Coordinator, Sequoia Foundation

Jianwen She, Ph.D., Chief, Biochemistry Section, Environmental Health Laboratory

APPEARANCES CONTINUED

DEPARTMENT OF PUBLIC HEALTH:

Nerissa Wu, M.P.H., Ph.D., Chief, Chemical Exposure Investigations Unit, Environmental Health Investigations Branch

DEPARTMENT OF TOXIC SUBSTANCES CONTROL:

Myrto Petreas, Ph.D., M.P.H., Chief, Environmental Chemistry Branch

GUEST SPEAKERS:

Axel Adams, M.S., University of California, San Francisco

Roy Gerona, Ph.D., University of California, San Francisco

Jon Sobus, Ph.D., National Exposure Research Laboratory, US Environmental Protection Agency

ALSO PRESENT:

Susan JunFish, M.P.H., Parents for a Safer Environment(PASE)

Aolin Wang, Ph.D., University of California, San Francisco

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P R O C E E D I N G S

MS. KAUFFMAN: Good morning. I'm Duyen Kauffman from the Office of Environmental Health Hazard Assessment. And we are going to start the meeting. So today's meeting is available via webinar. And for the speakers we do ask that you please speak directly into the microphone and introduce yourself before speaking. This is for the benefit of the people participating via the webinar and for the transcriber.

The materials for the meeting were provided to SGP members and posted on the Biomonitoring California website. A small number of copies of the meeting materials are available at the table near the entrance of the auditorium. A sample SGP packet is also available for viewing at this table.

We will break at 12:45 p.m. for lunch and take another short break at 3:00 p.m.

The location of the restrooms are at the opposite end of the hallway where you enter the auditorium. And emergency exits are here at the front of the room, on either side, and also as you exit the auditorium on either side. And with that, I'd like to introduce Dr. Lauren Zeise, Director of the Office of Environmental Health Hazard Assessment.

DIRECTOR ZEISE: Good morning, everyone. I'd like to welcome you to this meeting of the Scientific Guidance Panel for the California Environmental Contaminant Biomonitoring Program, also known as Biomonitoring California. So welcome to the Panel and to the audience in the room, and those attending on the web. And an early thank you for your participation in sharing your expertise with us.

So just to give a bit of a recap, SGP met on March 8th in Sacramento. And the Panel meeting was followed by a special event cele -- to celebrate the program's 10-year anniversary. Just to briefly recap the March meeting itself, the panel heard an update about our current program activities, and provided extensive input on the design of the multi-regional study. And that's now in our planning phases -- in the planning phase.

The Panel also heard a presentation from CDC, Dr. Breysse, the Director of the National Center for Environmental Health at CDC. And I heard about biomonitoring at a national level, and had a very good discussion with him about synergies between State and national efforts.

And then finally, we had three distinguished speakers kick off the discussion about the future program activities. That was Dr. Irva Hertz of UC Davis, Tom Webster of Brown University and Julia Brody of Silent

Spring, and then Dr. Gina Solomon, the CalEPA Deputy Secretary for Science and Health led the discussion.

Some of the key recommendations included that the Program should strategize on resource-efficient ways to choose studies that advance multiple program goals; expand efforts to generate data to evaluate regulatory actions and other interventions to reduce chemical-specific exposures; maintain the Program's strong efforts communicating study results to participants; and expand efforts to reach out to policymakers; and to continue to develop capacity for non-targeted analyses for identifying emerging chemicals of concern, and guiding targeted biomonitoring studies. And we're going to hear more about that topic later today.

So if you'd like more details, you can refer to the summary of the March meeting on our website, but we also have transcripts available on our website, Biomonitoring California website.

So just to conclude the recap of the March activities, it was a very nice event we had after the meeting to celebrate the Program's 10 years. It was a perfect way to celebrate the milestones, and we really appreciate your participation in that.

Now, looking forward to today's activities, we're going to start with some Panel business. As some of you may know, Dr. Asa Bradman has resigned as the SGP Chair and Panel member. And so just before lunch, we're going to have a tribute to Dr. Bradman. He's going to come in and join us, and we're going to thank you -- thank him for his service.

Second, I have the pleasure of announcing the appointment to the Panel by Governor Brown of Dr. José Suárez. He's an Assistant Professor in the Department of Family Medicine in Public Health at UC San Diego. Due to a prior commitment, he's not going to be able to attend today's meeting, but he'll be formally sworn in and participate in our next meeting, which will be November 9th here in Richmond.

So finally, it's my pleasure to introduce to you our new SGP Chair, Dr. Meg Schwarzman. Dr. Schwarzman has been a member of the Panel since 2014, and we're grateful she's agreed to be -- take on this additional responsibility. And so for those who -- of you who don't already know her, Dr. Schwarzman is an environmental health scientist at the UC Berkeley Center for Occupational and Environmental Health, and the Associate Director of Health and Environment at the Berkeley Center for Green Chemistry.

This past spring she designed and taught a new graduate class in environmental health policy at UC

Berkeley. And she recently returned from Alaska where she gave four talks, two radio interviews, and a webinar on environmental contributions to breast cancer.

So just this week Dr. Schwarzman advised the Department of Toxic Substances Control on the implementation of the Safer Consumer Products Program in her role as member of the Green Ribbon Science Panel.

Finally, Dr. Schwarzman received her medical degree from the University of Massachusetts Medical School and completed specialty training in family medicine at UCSF General Hospital, and received her MPH from UC Berkeley.

So please join me in welcoming Dr. Schwarzman to her new role as your Panel Chair.

(Applause.)

DIRECTOR ZEISE: So now I'll turn the meeting over for facilitation by Dr. Schwarzman.

PANEL MEMBER SCHWARZMAN: Thank you. And welcome, everybody. And I'm honored to follow in the footsteps of several Panel members who have preceded me as Chair. And I'll do my best to conduct the meetings as well as they have in the past.

So I want to start with the goals for the meeting. The first is our Program updates from Dr. Nerissa Wu, and then we'll go on to welcome our guest speaker Dr. Jon Sobus of US EPA, who is here to discuss advances in non-targeted analyses.

We're going to -- there's a quick switch in the agenda. So after lunch, we're going to consider the class organophosphorus pesticides as potential designated chemicals. And then after the break, we'll then hear from Dr. Roy Gerona of UC San Francisco about his work developing and applying an analytical method to measure glyphosate in urine.

We just made that switch this morning -- I'm sorry. We'll wait to hear whether that switch is finalized or not. Okay. It's back and forth this morning.

Okay. So for each agenda topic, we do, as usual, provide time for both Panel questions, public comment, and Panel discussion and input. And so the way public comment works, for those who haven't been there before, if you would like to comment on an agenda item, you can fill out a comment card. And Amy -- someone usually has those.

Oh, they're on the table near the -- at the entrance of the auditorium. You can turn the cards into Duyen Kauffman, and if you're joining the meeting via webinar, and you want to provide comments, you can do so by email, and the email comments relevant to the topic under discussion will be read aloud during the meeting.

We do subject public comments to time limits, if needed, depending on how many there are, and we'll divide the time allotted equally among the individuals who are wishing to talk about that agenda item. So please keep comments focused on the agenda topics that are being presented. And we also include an open public comment session at the end of the day for more general comments.

So with that, I'd like to introduce Dr. Nerissa Wu, who is the Acting Chief of the Exposure Assessment Section at the California Department of Public Health, where we are. And is also the acting lead for Biomonitoring California.

So Dr. Wu is going to provide an update on Program activities.

(Thereupon an overhead presentation was presented as follows.)

DR. WU: Well, welcome, everybody, panelists, and guests and Dr. Schwarzman in your new role. It's great to have you up there.

How is that?

Okay. Great. Im going to be giving the customary overview of the Program updates, and including some administrative news and project updates. And then as always -- I can just speak into -- or I can hold this

So I am giving the customary Program update today, administrative news, project updates, and then as we have in the last couple of Panel meetings talking more about the protocol we're developing for the multi-regional sampling plan, which is now going by its name of the California Regional Exposure, or CARE, Study.

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DR. WU: But first I want to talk a little bit about staff changes. We have had a number of staff transitions in the past few months. We've lost some staff. You might not know their names. They may not have presented to the SGP, but they're all part of the biomonitoring fabric and have all contributed in their own way to the Program: Susannah McKay, Suwati Anand, ShiZhong Wang, Sissy Petropoulou, Julie Frankenfield, Astrid Zamora, Joginder Dahliwal. And I just want to say thank you to those staff who have moved on and wish them well in their new positions.

We do have some new staff and they're highlighted on this slide in red. Lauren Baehner, Elizabeth Hall, Juliet Kinyua, Let Zhang, Robin Christensen, who of course is not new. You're familiar with Robin. She's been here many years, as our CDC grant coordinator, but she has joined State staff. I'm very happy to say she's part of the CDPH management team for biomonitoring, which is awesome, and Suzanne Wittwer who is our new CDC grant administrator, taking Robin's place.

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DR. WU: You've seen this slide before. This is our Program budget. With the end of fiscal year 2016-17, we have come to the end of our supplemental environmental justice funding. We've also come to the end of some limited term positions, which were established as part of a budget change proposal, or BCP. These are limited term positions that ended in June 2017, which is why we see this precipitous drop in our budget between '16-'17 and the current fiscal year.

We do have additional limited term positions, also created by a budget change proposal, which are scheduled to expire at the end of this fiscal year, June 2018. At this point, we don't seem to have a mechanism for extending those positions or making them permanent.

So this downward slope is projected to continue for the following fiscal year. And then in 2019 when our cooperative agreement with CDC comes to an end, we see that our budget will be less than half of what it -- of what we've had over the past few years.

We've talked about our budget issues in the past, and there's not a whole lot to say about it. Our staff continues to work very hard to be as effective and efficient as possible and to accomplish what we can with the budget resources that we have. You'll hear throughout my description of different projects that there are choices that have to be made as a program. We just need to -- there are things that we can and can't do given the resources that we have.

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DR. WU: Now, I want to turn to some of our project updates, starting with project BEST. And this is the Biomonitoring Exposures Study in the Central Valley in collaboration with Kaiser. We have finished analysis for the panels that were part of the original project plan. The perchlorate data is now ready to be returned to participants.

Those packets are actually in -- they're being created right now, and they should go out in the next week or two. Earlier this year we finished arsenic re-testing for participants who were found to have elevated arsenic levels. And those results were returned. And we reported out on that a couple sessions ago. We do have 56 remaining urine samples, which were never tested for metals. And because about 13 percent of our participants were found to have inorganic arsenic levels at the level of concern, we would like to go back to those untested urine samples and test them for arsenic, identify what their exposure source might be, and get that information to those participants. It seems like the right thing to do. So we will keep you updated as we proceed with that.

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DR. WU: For the Foam Replacement and Environmental Exposure, or FREES, Study. This is the study in which we're looking at a time point before foam and furniture replacement, and then at 6, 12, and 18 months after the foam or furniture is replaced from the home. We did recruit over several months, and then participants took varying amounts of time to replace their furniture or the foam in their homes. And so the time point of replacement, which spanned from October 2015 to November 2016, is quite spread out, which means their sampling at 6, 12, and 18 months, is also very spread out.

We have gotten all of our initial samples and returned those results to participants. We have completed the six-month sampling and are about to return about 15 of those results to participants. And we're about halfway through the 12-month sampling, and have just started the 18-month sampling point for some of our participants. And we anticipate that samples will be completely collected by the spring of 2018.

We're looking at the change in flame retardants in blood and urine following foam replacement or furniture replacement. We know that foam -- that flame retardant levels are decreasing in the environment, and that they're fluctuating over time and people as well. And so we wanted to have a control group to compare to this FREES cohort.

So we have recruited non-FREES participants, people who are not changing out the foam -- the furniture or foam in their homes. And their blood and urine samples were also collected in August 2016, and we're about to go back to those participants. So that will have a one year control group to compare to our FREES cohort.

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DR. WU: In the Asian/Pacific Islander Community Exposures, or ACE, Project, this is our Chinese population in San Francisco, for which we've recruited and collected samples from 100 participants. And we're looking at lead, cadmium, arsenic and mercury, as well as the per and polyfluorinated -- fluoroalkyl substances, the PFAS compounds.

We are actually in the results return phase of this project. We're calling notif -- we're calling participants with notifications of elevated metal levels. We have had one participant found with an elevated blood mercury level. And of our 100 participants, 54 of them had urinary arsenic levels at the level of concern, which triggers our speciation arsenic protocol, where we speciate into inorganic and organic arsenic, and take a look at the profile of the arsenic species to determine where that arsenic might be coming from.

Twenty-six of the participants actually had inorganic arsenic levels at the level of concern of 20 micrograms per liter. And those results will be followed up with notification to the participants, and collection of some additional exposure information, so we can work with participants to really identify their exposure source.

We're about to complete those calls. All results will be going to all of our 100 participants the next month, August 2017. And then following that, we're having an open community meeting for the general public in September in conjunction with APA Family Services, our community partner, to talk about the significance of the findings to the community as a whole.

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DR. WU: Now, I want to talk about some of the activities we've been able to accomplish under our environmental justice funding for this last year. The funding enabled us to extend our ACE Project, that I've just described, into a second community. And we've worked with the Vietnamese Voluntary Foundation, or VIVO, to recruit participants from the Vietnamese community in San

Jose.

So again, it's 100 participants, looking at those four select metals and PFAS compounds. And with our community partner and our recruitment team, we were able to recruit 100 participants in about two months. And so those samples are already at the lab, and we should have some results to report out next -- by the end of this year.

We also have the East Bay Diesel Exposure Project, which is being led by OEHHA and UC Berkeley, that's scheduled to start up this fall. And this study it will look at toddler and parent pairs over two different seasons in different neighborhoods of the East Bay. It will analyze urine for 1-NP, the diesel marker which we've discussed here with Dr. Chris Simpson.

And those results are going to be combined with exposure survey data, activity trackers, environmental samples, and a really cool mapping app, which will help under -- improve our understanding of diesel exposure. And we'll be hearing more about that at our November SGP meeting, which will focus on environmental justice.

We've also been able to do some community outreach and engage with community partners across the state. We've collected information from 84 different organizations around the state, including at least one from each of our eight California regions. And we've talked to community groups about what are your concerns, what are your priorities, and how can Biomonitoring work with you to move your agenda forward?

We've heard a lot about air quality, concerns about diesel exhaust in particular, pesticides, arsenic in water, gas and oil extraction. We've actually just gotten the report back from that team. And so we'll be digesting it and hopefully reporting back a little more in November.

We've also been able to initiate the Biomonitoring Matters Newsletter, which I think I highlighted at our last meeting. Our first issue has gone out. It's been distributed at different community events. It will be available in Spanish in the next month. And we are coming out with our second issue this fall. Both the newsletter and the community listening sessions I think are projects that help move our agenda forward, help us learn about what can be useful about biomonitoring to the community, and help bring information about the program to a wider audience, and we really hope to be able to continue these efforts despite the fact that this resource has come to an end.

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DR. WU: And now I'd like to turn our attention to the California Regional Exposure Study, formerly known as the Multi-Regional Sampling Plan. And just a brief reminder of what the CARE Study is. We are required, by our founding legislation, to conduct statewide sampling. And this was initially conceived as CalHANES with a budget of approximately $12 million annually. The Program was never funded at that level.

So we've decided to approach the statewide sampling on a regional basis, dividing the state into these eight regions that you see here, and conducting the sampling on a region-by-region approach.

And this modular approach gives us more flexibility to adjust to our budget fluctuations, if we have a year where we can accomplish more than one region; great. But at our -- under our current budget scenario, we're looking at one region per year, collecting 300 to 500 samples per region.

We are going to biomonitor for certain metals: mercury, arsenic, lead, and cadmium, but also molybdenum, antimony, cobalt, manganese, and uranium and for the PFAS compounds across the State. But this modular approach will also allow us to expand the parameters of the study. If there is perhaps an analytical panel of particular interest in some region, and we have the resources to do so, we can expand the study to accommodate additional panels or additional studies, intervention studies or nested studies, within the CARE Study construct.

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DR. WU: So one of the most important tasks of the study, and one of the first things we did was to try to develop a message and a study name that would adequately convey what we're trying to do, and the importance of the Biomonitoring Program, but also be accessible and understandable to the public, and be interesting enough so that it motivates them to pick up our recruitment materials and take a second look, maybe sign up for the study.

So we worked internally between our departments to come up with our message, what is it we're trying to say, what can Biomonitoring do for you, what it can't do. We want to be careful not to overpromise or overstate what biomonitoring can do.

And then we worked with communication experts to try to hone this into a message into something that would be -- that would attract attention, that would pique people's interest. And we conducted a series of focus groups, both in English and Spanish, in L.A. County to talk to people about, you know, what do you think of this image, or this name, or this motivational message? Do you like it? Do you hate it? Does it remind you of anything? What does it make you think of?

And our focus group participants overwhelmingly indicated to us that they would be most motivated to join a study to find out what chemicals are in their body, but also in learning ways to reduce their exposure. So we took those -- those motivational statements and rephrased it as a question, kind of to draw people into being interested in our study, but also because there is uncertainty about what a biomonitoring study will find.

We also wanted to link this to health. Again, not overstating what we might find in the study. And we've come up with the tag line of, "Are there chemicals in your body that could harm your health? Join the CARE Study to learn about lead and other chemicals in your body, and actions you and your family can take to help reduce your contact with these chemicals".

We have a longer message that's included on some of our fliers and other promotional materials. "Biomonitoring California is doing this study to measure chemicals in people across the state. This information will support efforts to reduce chemical exposure in Californians and improve public health". We do have a shorter version of this message.

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DR. WU: And I should point out, as I show some of our -- some of our design work, that nothing is finalized. We're still tweaking it with graphic artists, but this is just a mock-up of what our materials may look like. So this message of are there chemicals in your body that could harm your health, learn more by joining the CARE study, will appear on their postcard.

There's very little text, because again the point is to really just get somebody's interest, get them to look at the postcard and want to find out more. We do have this short statement translated into 10 different languages. We've looked at the most common languages spoken in households across L.A. County. So we've included English, Spanish, Chinese, Tagalog, Korean, Armenian, Vietnamese, Persian, Japanese, and Russian.

And the point of the postcard, regardless of how somebody gets the postcard, whether it's handed to them at a community meeting or they get it in the mail, is to get people to go to the pre-screening survey, which will be available online. We'll also have a phone number on here, so if somebody doesn't want to enter data online, they can call us and we'll help them fill out that pre-screening survey.

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DR. WU: This is a mock-up of the website where you would go to get more information about the study. There will be frequently asked questions and answers. And you see on the right, this is -- if you're interested in joining the CARE Study, the pre-screening survey is available in those ten languages that I've just described. You can click on that and go to a pre-screening survey, which will collect basic demographic information, a little bit more about the participants interested in joining up.

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DR. WU: We will still have our Biomonitoring California website, with which you're familiar, so that if somebody is looking for biomonitoring information and learns about the CARE Study, they can click over easily to our project page. And conversely, if a participant is reading about the study and wants to learn more about biomonitoring, they can easily go to the Biomonitoring webpage and learn more about the Program as a whole.

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DR. WU: So from this pre-screening pool, this pool of people who have filled out that pre-screening survey, we'll be selecting 500 participants, matching L.A. demographics as best we can, particularly in terms of race. L.A. County is already divided up into eight service planning areas by the L.A. County Department of Public Health. And we'll try to include participants from across all of these SPAs.

As people are invited to enroll, they'll receive a packet of information that will include a letter of introduction, the informed consent, the exposure survey, and a calendar, which will show them where and when we'll be collecting samples, so that they can make an appointment.

We are going to be working with a Salesforce platform, which is a very sophisticated cloud participant, or data tracking, software. So we'll be able to run reports in real time and look at metrics like what percentage of people have responded to the postcard, and what are the demographics in the prescreening pool, and where do we need to be doing extra recruitment?

And so we'll be using that to select our participants and figure out what the demographics are as people go through the study of how -- are people dropping out at a different points, and do we need to add more people?

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DR. WU: We have three parallel pathways of participation. We've got our intermet -- internet participants. And we hope that most people will be participating via the internet. Internet participants will be getting their packet via email. And they'll have a log in, a secure log in, to the Salesforce platform, where they can complete their informed consent, complete the survey, and make an appointment for sample collection all online.

We also have paper participants, so people who fill out the pre-screening and say, "I don't want to do this online. I'd like a paper version of the materials." We'll send them the informed consent and the completed survey to them in paper through the mail. They will fill it out on paper and mail it back to us. And then we'll make a phone call to make that appointment with them.

For people who indicate to us that they'd like to complete the study in a language other than English or Spanish, either by filling out the pre-screen in a different language or by indicating on the English or Spanish pre-screening that they would like language support, we will send them the packet, but then call with the help of an interpreter to make an appointment. Then we will complete the informed consent and the survey and complete sample collection all at that one appointment with the assistance of the interpreter. Again, this is all going to be tracked through Salesforce, so that we can keep good tabs on our data.

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DR. WU: Salesforce will also enable us to send out automated reminders, so don't forget to fill out your informed consent, don't forget to make your appointment, you have an appointment in the next couple days. We're hoping to minimize our losses of participants by having this reminder protocol. And for non-internet participants, the system will ping the staff and we'll be able to make those reminders manually.

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DR. WU: Once participants get to the point of booking their appointment, this is either on the internet or on the phone, they'll make an appointment for sample collection. We will have sample collection events across L.A. County to make it as convenient as possible for our participants. Each appointment includes a brief survey, so activities and diet over the last three to five days, a quick blood draw, and a urine sample collection.

Field staff are going to be in L.A. County for 12 to 15 weeks, during which time they'll be holding three to four events per week at these -- at these centralized sites. And then for participants who cannot make it to a site, either they're not convenient, the participant isn't mobile, the field staff will be reaching out to them to make home visits or to arrange a more convenient time for sample collection.

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DR. WU: So looking at our proposed timeline, we have submitted our IRB per protocol, and we are scheduled to appear before our IRB on August 5th. We will be conducting outreach to community partners and refining the materials and tools that we have, and we hope to be in the field starting our recruitment in January 2018.

This 12 to 15 weeks will go by. We'll collect our samples. And we want to be returning those results by January, as in '19. We also want to be in the field for region 2 starting our recruitment in January 2019, which means that we pretty soon need to be reaching out to our community partners and developing those relationships in region 2. We're very busy. We have a lot to do over the next six months.

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DR. WU: Finally, I just want to say that the comments from the Panel have been really helpful. You'll notice that we've incorporated a number of the comments. Dr. McKone is not here today, but he had some great suggestions about external reviewers for our surveys, and questionnaire materials, which we've done and that was useful. So we do continue to tweak the protocol, and I'm happy to hear your suggestions and continue honing our protocol.

CHAIRPERSON SCHWARZMAN: Thank you.

Okay. Is that better?

Thank you so much for that presentation. We have a few minutes for just clarifying questions from the Panel before we go on to public comment, and then a full Panel discussion period about this update.

So any clarifying questions for Dr. Wu?

PANEL MEMBER QUINTANA: Is this on?

Okay. Thank you for that presentation. I just had a quick question. I'm sure this is being thought of, but if you could just comment on the extent to which the website for the participants is mobile friendly, since that will be a major mechanism by which many of the participants will access the materials.

DR. WU: That's a good question. I don't know the answer to that. We have just started our -- so there are two parts to that. One is our website, where you go for the general information. And our website at CDPH is kind of new, so I'm not sure how it translates to a mobile device or, you know, whether it has to be -- whether that has to be developed separately.

For Salesforce, that is also a new relationship. We're just working with them on our tools. So for participants who sign up, we would like those materials to be mobile accessible. It would obviously be very small. The questionnaires will be difficult to go through. You'd have to do a lot of scrolling, but we are exploring that as an option.

CHAIRPERSON SCHWARZMAN: Did you have a question?

PANEL MEMBER LUDERER: Yeah. Is this on?

It sounds like it is.

Okay. Thanks for that presentation. It's so exciting that this is starting. I'm really looking forward to seeing the results soon.

I just had a question. I know that you have a limited number of analytes that are going to be analyzed initially, and whether there are plans to, you know, collect additional specimens that will be archived and, you know, just tell us a little bit more about that?

DR. WU: Sure. We will have plenty of sample. We are taking blood, serum, and urine. And I can't remember the volumes off the top of my head of what we're collecting. But there will be plenty left over to archive for subsequent analysis, if that -- you know, partly if we want to add on analytical panels, but also, in the future, if we wanted to go back and do some longitudinal moni -- longitudinal monitoring over a region, we would have the availability of samples to do that.

CHAIRPERSON SCHWARZMAN: Other questions from the Panel?

Yes, Dr. Cranor?

PANEL MEMBER CRANOR: This is an easy question. Are you pretty happy with the postcards that you send out and the response people will give to them? I see you've consulted with postcard specialists, as well as communication specialists. I ask that, because I'm aware of other organizations that design terrible cards for trying to urge people to do things. And I just wondered what you feel about these?

DR. WU: Well, this is not a final design. We are still working with the graphic artist. So I think they will improve. This was sort of our mock-up of let's get the information we want onto the postcards.

Postcards I don't expect to be hugely successful. And we want to try it as a randomized sampling technique. But postcards, in general, I think don't have a huge response. I think they will have a much better response when we're giving them out, when there's sort of a community organization or a face associated with them. So the postcards have dual use.

We also have a flier with a little more information on it, which I think will be effective. We've seen bad postcards and good postcards. We've spent a fair amount of time trying to figure out what do we like or not like about postcards, what would make me pick this up and toss it in the garbage or pick it up and take a second look. And that's why we did the focus group.

So having -- having pictures that are appealing to people, having a language that somebody recognizes and says, oh, I'm going to take another look at this. We've done a lot of work to figure out what those things are that will be pique somebody's interest, and we're continuing to tweak that design.

PANEL MEMBER CRANOR: Thank you.

CHAIRPERSON SCHWARZMAN: Dr. Quintana, did you have a question?

PANEL MEMBER QUINTANA: I might discuss this further during the other session. But in terms of the postcards recruiting technique, I think that if you, of course, only rely on that, you will miss a lot of the population, especially lower income or not as competent English speaking.

So I'm wondering how -- if you've -- how much emphasis you plan to having community groups for everyone, kind of signs up right there, you know, kind of groups -- group information sessions through environmental justice groups, for example, that could really augment that sampling.

DR. WU: I would anticipate that the majority of our pre-screening people will come from community groups. As I said, I don't think the postcards will be -- I think we're hoping for a one percent response rate maybe. We're aiming pretty low.

Our community partnerships, which we are in development -- which are in development right now are mostly with environmental justice groups. And we're hoping to really draw from the population through our community meetings, being present at meetings, having our partners do a lot of recruiting for us, but also being at these events like you mentioned, and having somebody helping somebody with a laptop right there to fill out the pre-screening. So I think we are counting on the community outreach that we're doing to do most -- to contribute most to our recruitment.

PANEL MEMBER QUINTANA: Just a really quick follow up question.

CHAIRPERSON SCHWARZMAN: Yeah.

PANEL MEMBER QUINTANA: Some of the issues I've come across in community groups are this system that you have requires them to kind of sign up to be part of a pool and then maybe or maybe not get picked. And so I think that messaging has to be very careful, so that people aren't upset when they sign up and they aren't picked, you know, because they get all excited about this, what a great idea, and then my friend got picked and I didn't. We have that even for our small-scale studies. How come they get to participate and we don't, even if we -- they just hear about their friend participating and they want to sign up, but we don't have funding for additional participants, for example. And so just some thought maybe to that messaging to make sure it's not upsetting people, I guess.

DR. WU: We have talked about this quite a bit. There are communities who have been very enthusiastic about partnering with us on the recruitment phase. And their constituents have been like, great, we get to be biomonitored. And I think we do run the risk of over-promising.

So we are trying to craft language, which is clear, that this is a regional study. That we're really trying to represent across a county. It's very difficult. We want people to be excited enough to sign up, but not count on this as a community. This is not a community study. So your point is well taken, and it's something we're thinking a lot about.

CHAIRPERSON SCHWARZMAN: If other Panelists don't have questions, I have one question.

Anyone else?

Okay. I think -- you hinted at this a little bit, but I'm wondering if you could say a little more about your goals for recruitment in terms of representation of the community. I mean you hinted that you wanted to be representational. And it's obviously part of -- a big part of how you're targeting your outreach and recruitment, and the multiple languages that you're presenting the materials in.

But can you say a little bit more about the goal for representation? And are you over-sampling or are you just trying to get a representative population for the county or the region?

DR. WU: We looked at -- when we consider the parameters that we would like to cover in our representation, of course, race, socioeconomic status, geography across the region, age, sex, community, we realize with 500 samples we are not going to be able to do that. And we are also very much subject to who signs up through the pre-screening.

So we are -- we're putting a lot of emphasis on racial representation, just trying to match L.A. County demographics. And I think to whatever extent we can, our -- for example, our postcard distribution will be based on some socioeconomic status indicators, so that we're trying to get the materials out to a wide range of platform, a wide range of demographics.

But I think the reality is that we won't be able to really stratify across many of those parameters. We just don't have enough samples in the county.

CHAIRPERSON SCHWARZMAN: And then within the --

you said prioritizing racial representation, and then within that, you'll do a bit about age and gender or --

DR. WU: I think --

CHAIRPERSON SCHWARZMAN: -- how do this -- how do the categories follow kind of?

DR. WU: You know, we're still working that out how we set our goals, and then how we turn that into an algorithm for selection of the participants. It would be great if we could get some distribution across age and sex. But again, we -- we're sort of balancing the need to be representative with the need to just get 500 participants. And we're still working that out.

CHAIRPERSON SCHWARZMAN: Okay. Great.

One other question on that is are you thinking about using occupation and geographic location also as ways of sort of broadening the diversity of the people that you're sampling or not?

DR. WU: Geographic location certainly. By recruiting in each of the service planning areas, we're hoping to get interested participants from across the county. And we would want to -- we will want to select to make sure that we're getting people from different parts of the county. I think occupation is harder, because we are collecting occupational information, but it would be harder to stratify based on that.

CHAIRPERSON SCHWARZMAN: Right. So, but there's a difference -- I mean, I guess there is a question of stratifying -- your ability to stratify is related to your desire to recruit maybe. So I was going to say that, you know, the ability to stratify isn't quite as -- it's a -- it's a -- it's a longer-term or more ambitious goal than recruitment. But maybe I am thinking now that it is right to think about that first, because if you have representation, but it's not sufficient to stratify on, it's not that meaningful to have that representation.

Yeah. Okay. That's interesting. Thank you.

Any other Panel questions before we move on to public comment?

Okay. Duyen, do we have public comment from within the room?

MS. KAUFFMAN: No.

CHAIRPERSON SCHWARZMAN: Is there any public comment from online or from the webinar?

MS. DUNN: No.

CHAIRPERSON SCHWARZMAN: Okay. Then in that case, we can move on to the Panel discussion. And we're a little bit ahead of time, so we have 15 minutes.

Is this on?

So anyway, we have plenty of time for Panel discussion.

Dr. Quintana.

PANEL MEMBER QUINTANA: Hi. I have another question, I guess, about the study design. You said that you've been going around the state collecting information for community groups and environmental justice groups about their priorities. And I'm wondering to the extent to which those priorities have fed into what's being measured in this study.

And I'm thinking specifically, you know, I think the metals are a great thing to measure, because partly there's known health effects and you can interpret results to the community quite well. And you've seen, you know, elevated arsenic in preliminary data from different population groups, mercury. But for example if the community groups have been saying diesel exposure is a huge concern, have you thought about, you know, why PFASs versus 1-nitropyrene, for example.

I know the resources are -- and this question is being asked in the context of very limited resources, and I understand that.

DR. WU: We actually are considering adding 1-NP to the L.A. County. It's obviously a huge issue in L.A. County, or at least to a subset for our participants. Some of it is a logistics issue collecting enough urine samples, and then trying to figure out how to select participants for that particular analysis. But it is something -- diesel is actually the analyte we're considering adding at this point.

PFAS, I mean, the recent work has shown that there is a geographic contribution, because of your drinking water source. And we're very interested to see how PFASs vary across the state, and whether we can continue the work that DTSC has done to look at the connection between drinking water source and PFASs. So you're right, it has not risen to the top of our community discussions, but I think it's an important one for us to look at for public health issues.

PANEL MEMBER QUINTANA: So, yeah, I was not questioning the interest of that. I think it is extremely interesting. I guess my question was more your vision of how the community concerns kind of fed into the study.

Thank you.

DR. WU: Well these two things were developed somewhat concurrently, so we had selected metals, as you said, for public health reasons, and also because there is so much interest. We were also looking for analytes that would be sort of unifying to the State, things that are interesting across the different regions. But I think as we have heard more from the EJ communities, it will -- it's more of what we can add on to those statewide analytes. And there will be regions where -- who are very interested in pesticides. And that's something we want to drop into those regions, as opposed to diesel, which are L.A. County and other parts of Southern California.

Go ahead.

PANEL MEMBER QUINTANA: Just one last follow-up comment. I'm sorry, Panel. I guess the point of the study is to be statewide, it seems if you're going to measure 1-nitropyrene in L.A., you'd also want to measure it in Kern, if nothing else to show the huge difference that might exist or not, you know, tractors kick out diesel too, if you're a farmer, but -- so I think I'm not sure you should be -- have regional specific additions, because you do need a comparison. NHANES, one of the major wonderful uses of that is to be a comparison.

You know, so I just want to throw that out there that I would encourage if you do it one place, to do it all places.

DR. WU: That's a really good point about having a comparison group, but I wouldn't want to limit us to only doing statewide just because that will probably keep us from adding any additional panels just because of the resources issue. I mean, I wouldn't want -- I mean, there's so much uncertainty about our budget, so if we had money this year and the ability to add something on in this region, I feel like we should grab that opportunity.

CHAIRPERSON SCHWARZMAN: Are you done with that or should --

PANEL MEMBER QUINTANA: I'll stop now.

(Laughter.)

CHAIRPERSON SCHWARZMAN: Okay.

Dr. Cranor.

PANEL MEMBER CRANOR: This is a difficult question I realize, but hang on. California is not a poor state. The budget for this program has been going down. There are obviously other needs to protect the public health, so I suppose I have two questions.

To what extent -- I mean, let me -- one more background piece of information. I did have some conversations with people at the last meeting where there were lots of public folks. And I thought maybe they should speak to the Legislature. And I don't know, probably not much came of that.

But what are the prospects for better funding for the program, and what would you do with it? I guess those are the two -- the two questions.

In Calif -- I've talked to school teachers and they say, well, there's no money. Well, that's -- that's false. There may be no tax money, but California is not a poor state. So there could be funding there if it were needed, and what would you do with it?

I said it was hard.

(Laughter.)

DR. WU: I think this is a much longer discussion than we have time for. Everyone here works in public health and environmental health. And you all know the context within which we're all working. It's a difficult time. There's a lot of uncertainty about what future budgets may hold.

I think part of our challenge is to help define biomonitoring and environmental health as a wider discipline as important parts of public health, because the things that I think the public recognizes -- public and legislators recognize are more traditional public health and more traditional medical models of health.

And so my hope is that by starting our statewide program, we'll develop data and people will be like, well, when is our region going to get done? Why don't we have the statewide sampling plan that can be done in a one- to two-year cycle. And it's creating the awareness that this really is a critical part of public health. This is a critical part of why people get sick that will create that demand. You know, who knows if that's going to happen.

But in terms of what we would do, you've seen the study here. There are a number of things we have had to reduce in the study, in terms of numbers of participants, number of analytical panels, our ability to sample the state in a one- to two-year cycle. These are all -- these are all compromises. And I think the study is still valuable and will produce very interesting and valuable data, but it is not what we originally envisioned as a statewide sampling plan.

If we had the resources, we would cover the regions more quickly. We would have the ability to do 1-NP, and the phenols, and pesticides, and all of the analytical panels that -- which we have determined here have been -- are so important to public health.

What we're doing, it's interesting and valuable, but it -- it is not really statewide sampling as initially envisioned.

PANEL MEMBER CRANOR: Thank you.

CHAIRPERSON SCHWARZMAN: Panel questions?

Yeah.

PANEL MEMBER LUDERER: I have kind of a more specific question regarding kind of going back to your ACE Study, and -- you know, I under -- you found the -- quite a large number of high inorganic arsenic levels. And I'm wondering if you could talk a little bit about how kind of those findings have informed, you know, the kinds of questions you're going to be asking people about exposure in the CARE Study, you know, given that you've, you know, found these levels, and also if you have any information about what you found the source might be. You might not have that yet.

DR. WU: I think from our conversations with the participants, it's mostly a rice diet that's contributing to the arsenic levels. It's an Asian population. We eat lots of rice and fish.

So that has informed how we ask the questions in the CARE Study, but it's a very different population. So whereas our ACE questionnaire was many questions about species of fish, and how you eat your rice, and really on a micro-level focus on your rice diet and rice noodles and all this other rice product.

The CARE Study, partly because it's a different population, but also because we're trying to do these surveys online instead of with an interviewer, we've had to shorten that. And so there is -- there are fewer questions focused on specific analytes.

We do have the ability to do follow up though. If somebody is in the high-exposure group, we will do our -- have our usual follow-up protocol and then we'll be able to get into some more detail about what your potential exposures might be.

CHAIRPERSON SCHWARZMAN: Other Panel questions?

If not, I have one about the FREES Study. And I know you've presented this information to us before, but I was hoping you could just say again something about which flame retardants you're looking at. And obviously, in the older samples, the pre-samples, we would be looking at some of the chlorinated and brominated flame retardants primarily. But I'm wondering if you're also looking at some of the replacement flame retardants, so that we can see if those were lower before and higher after, even if they're not related to the foam replacement, but if like they were lower before, and then they predominate later unrelated to the furniture replacement.

DR. WU: So we are looking at PBDEs in blood and serum. And then we'll be looking -- there are three metabolites of organophosphate flame retardants that we're looking at in the urine samples. When people replace their foam or furniture, they were purchasing things that were labeled. Because of the new labeling law, they were able to purchase furniture, which should not have had any flame retardants in it. So our hope is that we would see a decrease in both.

CHAIRPERSON SCHWARZMAN: So -- and there's not actual testing of the foam, right, because the TB117-2013 label means it doesn't -- like it means that different flammability standard, so it presumably doesn't have to have the added chemicals, but it doesn't like exactly guarantee that there aren't compounds in it.

DR. WU: We were able to get foam samples -- the old foam samples from all of our participants. And actually, DTSC -- Myrto's lab is analyzing those foams. We don't have all foam from all of the new foam samples. Not everyone wanted us to cut into their new sofas.

(Laughter.)

DR. WU: So -- but we do have a number of the samples, and we'll be able to report out on that.

CHAIRPERSON SCHWARZMAN: That's great. Thank you.

Other questions?

Yeah, Dr. Quintana.

PANEL MEMBER QUINTANA: Do we have time?

CHAIRPERSON SCHWARZMAN: Yes, we do. We have plenty of time.

PANEL MEMBER QUINTANA: Hi. This is going back to the CARE Study. I'm curious about your -- I know you've submitted the materials to the IRB already, so I'm not saying you should go back and change things. But I'm just curious the language that you put in terms of permitting further analyses on these samples, and especially non-targeted analysis on the samples.

And as we discussed in previous meetings, there is an ethical issue, because you can pick up drugs of abuse, for example, when you run a non-targeted screen. Even if you don't want to see them, they're in the database for that sample, and they could be picked out by somebody. So I'm just curious of your comments about that.

DR. WU: Well, it's never too late to edit materials, even if an IRB reviews them. And they will probably have comments for us as well, but we do -- as we do in all of our informed consents, we have an option for people to donate their samples for storage after our initial samples are completed.

So they can archive. They can volunteer to archive them for additional sampling or analyses, or they can opt out of that, and then we destroy their samples right after we're completed with our initial analyses.

We do -- we describe them as additional environmental chemicals. And we state that we will not measure for any pharmaceutical, or recreational, or illegal drug. I can't remember exactly what the wording is, but we're clear to specify that we are not going to be looking for particular classes of drugs. We're only looking for environmental contaminants.

CHAIRPERSON SCHWARZMAN: Did that answer --

DR. WU: I realize that doesn't preclude that, like if it's in the data that comes out with non-targeted screening. I realize that is still an issue. We would not be finalizing that data. So even if the lab has it, it would not be reported to us as a -- to EHIB as a program. And so that information would not exist, for example, in the results return packets.

PANEL MEMBER QUINTANA: No, I agree. It wouldn't exist -- be reported. But it's just something to think about in terms of keeping data secure -- and, you know, it is -- it is an ethical issue. We came across it with house dust. We're finding all kinds of interesting stuff in house dust that we didn't expect. That's why it really came up to me, you know, so...

CHAIRPERSON SCHWARZMAN: Maybe, if I can add on to that question. Maybe the sort of bottom line is do you still have the opportunity to do non-targeted screening, if budget and interest kind of permits and suggests that, based on your IRB approval? Do you feel like that's consistent?

DR. WU: Yeah, I think we could do that. And I think we'll hear more about the non-targeted screening in our labs. Somebody may ask for that in the afternoon session.

But, yeah, I think our IRB does allow for these additional analyses, and we're not very specific in that, except to say that it is these environmental contaminants. We haven't gotten into the details of -- and I'm -- I'm not the right person to answer the question about at what point the lab could say, okay, this data we're not going to continue to identify or we're not going to report it out. Maybe actually Jianwen or June-Soo could report out and give you a little more information on that.

CHAIRPERSON SCHWARZMAN: Other Panel questions?

Oh, yes. Please.

PANEL MEMBER KAVANAUGH-LYNCH: Hi. Mel Kavanaugh-Lynch. I'm chewing on this idea that we'd like to analyze more things and sample more people, and not turn potential participants away. And I'm thinking about ways to leverage the public's interest in both other analytes and in bigger samples and particular populations.

Some of the things that are coming to my mind is offering the opportunity for crowdsourcing for saying to groups that say we want -- why don't you -- why aren't you sampling more of our people, or why aren't you sampling more of the things we're interested in? Saying we would be happy to do that, if you can help us find the funding for that, and even have participants pay for adding to their samples. On a -- and moving that into advocacy.

So in community meetings, where the community is bringing up wanting more people or wanting more analytes, encouraging them and even giving them sample letters to send to local, regional, and State bodies that have the opportunity to add funding, to advocate for more funding to the Biomonitoring Program. So kind of creating -- leveraging that -- those requests into actual advocacy.

DR. WU: I think it is important that we get creative about how we fund the Program. There is not really a State mechanism for us to function in that way, to bring in -- to like charge money for samples basically. And we're certainly not able to lobby or be part of a lobbying effort to encourage people to send letters to their legislators. That's something a community partner or an advocate would be able to do. And for those of you listening, that would be awesome.

(Laughter.)

DR. WU: But as the State, there are restrictions on what we can do.

CHAIRPERSON SCHWARZMAN: Other Panel questions or comments?

Anything else?

So you looked like you were thinking about something.

PANEL MEMBER KAVANAUGH-LYNCH: No.

CHAIRPERSON SCHWARZMAN: Okay. In that case, we are going to go on to our next presentation, which is by

Dr. Jon Sobus.

I'm pleased to welcome him here from US EPA. He'll talk about advancing and integrating non-targeted analysis research at US EPA. Dr. Sobus is a physical scientist at US EPA's Office of Research and Development, which is in the National Exposure Research Library -- Laboratory located in Research Triangle Park, North Carolina. He currently serves as task lead for the Rapid Exposure and Dosimetry Project within the Chemical Safety for Sustainability National Research Program. And we further serves as principal investigator and team lead for the Non-Targeted Analysis Research Project.

(Thereupon an overhead presentation was Presented as follows.)

CHAIRPERSON SCHWARZMAN: Thank you for being here.

DR. SOBUS: Thank you so much.

Does that work?

Okay. Is that better?

Thank you so much for the invitation to be here, for that nice introduction. I was actually here three years ago talking about a completely unrelated topic, but it's great to be back. I'm very excited to hear some really encouraging discussions from this first session. I think it's a really excellent transition into what I'll be talking about today.

I had the opportunity yesterday to actually speak with some of the staff scientists about common interests, common work. So that will be echoed in the presentation today. This is a fairly technical talk. I hope that's okay, but I really wanted to get into the specifics of what we're doing in ORD, and to clearly communicate that the purpose of building some of these tools, and databases, and dashboards is to make those resources available to the public, including State programs.

So keep in mind, everything I present today are tools that we're building to make available to you all. So again, I'm from US EPA Office of Research and Development.

Today, I'll be talking about tools that we're building to advance and integrate non-targeted analysis research.

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DR. SOBUS: So we are building our portfolio in non-targeted analysis for two key reasons. One, we need to have a better understanding of the drivers Of disease. We now believe that the majority of chronic disease risk is caused by differences in environments, rather than differences in genetics. But we don't have a great handle on what are the environmental components that drive disease.

From an agency standpoint, we have an interest in thinking about this problem prospectively. We need to be ensuring chemical safety, as well as human and ecological health. So we know that there are tens of thousands of chemicals registered for use in tens of thousands of products. And these chemicals, and derivatives of these chemicals, wind up in environmental systems. They wind up in people. They wind up in animals. So we need to ensure that these chemicals and their derivatives don't pose adverse health risks.

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DR. SOBUS: So I'm not sure how familiar everyone is with lexicon of non-targeted analysis. This is a quote that's often used to kind of frame the NTA issue, but this is a quote from Donald Rumsfeld that kind of presented a way of thinking about three different things. And I think it's very, very Relevant to the field of non-targeted analysis. So he said, "There are known knowns. These are things we that we know we know. There are known unknowns. These are the things we know we don't know. And then there the unknown unknowns. These are the things we don't know we don't know".

So how does that relate to exposure and analytical chemistry?

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DR. SOBUS: My understanding of much of what is discussed amongst the Scientific Guidance Panel and much of Biomonitoring California is reflecting targeted analysis. So this is looking at the known knowns. These are the compounds that everyone is very familiar with, and compounds for which targeted analytical chemistry methods have been developed.

In my experience, these compounds, the known knowns, represent less than one percent of what's typically in any given sample, environmental or biological. So by extension, we're looking at far less than one percent of the exposome.

So based on that, I would make the argument that we can't solve 21st century public health problems if we're kind of blinded to 99 plus percent of exposure. So we are very much looking at the tip of iceberg, and I think we need to dig a little bit deeper.

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DR. SOBUS: So moving on to the known unknowns. These are things that we know exist, but for which there aren't currently target methods, and there isn't currently exposure data. To give you some example, there are large public inventories of chemicals, millions of chemicals, that are known to exist that are manufactured.

We can develop what are called suspect screening analysis methods. So this is kind of a subsection of non-targeted analysis research. So a lot of what I'll talk about today focuses on these high throughput suspect screening analysis methods that try and take data that we generate on high-resolution mass spectrometry platforms and kind of cross-reference that to these large databases of known chemicals to try and find hits. So are any of these chemicals on this list present in any of these samples that we're examining?

Base on my experience, that gets us about five to ten percent of what's in a given sample, or five to ten percent of the exposome. So we made a lot of headway in developing efficient methods to do suspect screening analysis.

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DR. SOBUS: The final chunk is the unknown unknowns. So this is about 90 to 95 percent of what's in a given sample. This is often your biological metabolites, your environmental transformation products, things that we don't yet know exist.

To get at these compounds, since they're not in a database, you have to use true non-targeted analysis methods. These methods are very time and resource intensive. We definitely need ways to speed up that process. But certainly, we need to go down this path to really get at the bulk of the exposome. So I just want to make a final point that at a high level we use the term non-targeted analysis to really encompass both the suspect screening and the true non-targeted.

Most of what I'll talk about today is technically suspect screening analysis, but I'm just going to use the term generally "NTA".

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DR. SOBUS: So there are a variety of tools that are required when doing non-targeted analysis research. We can start on the left-hand side with analytical instrumentation. By and large, most groups that are doing NTA use mass spectrometry, and most use high-resolution Mass spectrometry. So this is what's used to generate information about unknown chemicals in any given sample.

Again, when we're doing suspect screening, we're going to use large chemical databases like PubChem or ChemSpider. Some of these databases have millions of compounds listed in them. But to kind of have that linkage between what's generated on a mass spectrometer and what's in these chemical databases, we need workflows to process that data to make some sense of that data. There are actually quite a few computational workflows that exist. Some are proprietary, some are open.

At EPA ORD, we are working very hard to make, what is called, our CompTox Chemistry Dashboard, both our screening database as well as a major component of our computational workflow. So I'll talk more about that in the upcoming slides.

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DR. SOBUS: So just to give you a glimpse into what the CompTox Chemistry Dashboard is all about. The image on the left-hand side here is the home page for the Chemistry Dashboard. You can see in the next at the bottom of that image that we currently have information for about 750,000 unique chemical substances in this inventory.

So this is a smaller number of compounds than what you might find in PubChem or ChemSpider. But what's very valuable about the Chemistry Dashboard is we have gone to great lengths to do a lot of curation of the chemicals.

So there is consistency between chemical names, synonyms, CAS numbers, and structures. We have programs and we had a lot of manual curation to make sure that there's very good quality data about these 750,000 chemicals within the dashboard.

So you can search individual chemicals by name, CAS number, InChIKey. You can also do advanced searches.

So for those in the room, or on the line, that are looking to do non-targeted analysis, we can do searches based on accurate mass, plus or minus some amount of error. You can do searches based on formula. And as you'll see in an upcoming slide, we can actually do batch searches on thousands of formula at once. And it will return all the structures that are consistent with those formulas. So it's a very powerful tool.

On the bottom we show a landing page for a given chemical. You can see it draws the structure. We have information on intrinsic properties, structural identifiers. And very importantly -- I'm not sure how many people are knowledgeable about our ExpoCast and ToxCast programs, but the dashboard also hosts exposure predictions for thousands of chemicals, as well as bioactivity data for thousands of chemicals from the ToxCast program and from Tox21. So all of these resources are available in the dashboard, which is why we're using this as a primary tool in our workflow.

For anyone that has interest in learning more about or using the dashboard, please feel free to contact Tony Williams, who's the project lead. He's a phenomenal guy and has built a really phenomenal product.

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DR. SOBUS: So I want to step back and talk about what we actually do when we do non-targeted analysis.

This field is borne out of metabolomics. And a lot of the workflow in metabolomics is consistent with NTA, but there are some inconsistencies, so I want to kind of go through just a very simple kind of five-step procedure that outlines how we go about doing NTA.

We can start with the image here, which is a total ion chromatogram that was analyzed on a mass spectrometer. This was an extract of one house dust sample. It was run in negative ionization mode. And there's about 300, what we call, molecular features shown here.

The molecular features are these peaks, which basically represent some unidentified chemical. They are defined at this time by an accurate mass, retention time and a mass spectrum. The goal is to look at these 300, and if we could, to try and figure out what they all are. That's very difficult to do, so we try and first prioritize them. We say which are of greatest interest to us?

By default, a lot of people look at the biggest ones first, which is a pretty good idea, but it's not always the best idea. So we've come up with a number of different procedures and algorithms for actually prioritizing these molecular features to figure out which ones do we look at first.

So once we prioritize them, the next step is to try and assign a chemical formula to each of these compounds. We also -- we often do that through a database match. So we're using the underlying database for the CompTox Chemistry Dashboard to do this matching.

So once we assign a formula to the priority molecular features, we then try and figure out what the correct structure is for that formula. This is probably the hardest part. As many of you know, there are lots of different structures that share a common formula.

So picking the correct structure, and you're often dealing with isomers, is very, very challenging, given that you even have correctly predicted the formula. So I'll spend a bit of time talking about how we go about picking the correct structure.

If we can get the correct structure, and even for compounds that are still undefined, we want to develop high throughput methods for predicting concentrations. This is a very valuable thing to do. And then ultimately, we want to understand where those chemicals came from. So we want to begin to implement some exposure forensics to figure out the sources of these chemicals and the samples that we are looking at.

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DR. SOBUS: So I showed just one example of one sample that was analyzed in one mode, and it had 300 features. Many of the samples that we look at have hundred, if not thousands, of features. And we look at tens, or hundreds, or thousands of samples across multiple modes. So there are thousands and thousands and thousands of data points to deal with. And this becomes very cumbersome in terms of data processing and data storage. This is one big challenge of NTA.

Another big challenge is that, again as I mentioned in the previous slide, there needs to be some prioritization. And historically, out of the metabolomics field, biases have been introduced to look at only high intensity compounds, and lots of experiments are done to compare groups.

So biological samples collected from a diseased population versus a control population, or upstream versus downstream of a water treatment plant. So these types of comparisons -- statistical comparisons are done to kind of identify features that are enriched. So this is very valuable to do. But again, you're biasing only to a very small group of compounds, and we need to step back and look at bit more holistically.

One of the biggest problems that the field faces at their -- there is no standardized methods. Every lab has their own way of doing this, which makes it extremely difficult to compare results from one lab to the next. That is potentially the biggest challenge that we face right now.

We need to come up with ways to overcome the lack of standardization. And then finally, most of the chemists in the room know very well from the targeted experiments that there needs to be some confirmation at the end of the day before some action can be taken. This is time-consuming. We're talking about thousands and thousands of chemicals, and we don't have standards for all these chemicals. How do we overcome that challenge?

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DR. SOBUS: So I'm going to talk about two different things we're doing to address these four primary challenges.

One, I'm going to go through as quickly as I can some of the workflows and tools that we're building at the agency, again with the intention of making available to the public. And then I want to finish by covering a collaborative trial that we've been leading now for about a year and a half that involves about 30 different institutions, including international partners.

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DR. SOBUS: So this is a broad workflow that is a little bit complicated so I'm going to step through it bit by bit hopefully without going into too much detail.

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DR. SOBUS: But as we begin this process for doing non-targeted analysis we always start with the raw samples. And I just want to make a point that these methods can be used basically for anything. All the images we show here are things that we are currently evaluating with our NTA methods. So a very, very versatile approach.

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DR. SOBUS: We take the raw samples, as we always do in analytical experiments, we do extractions, different clean-up steps, and we generate these total ion chromatograms, which represent, what we call, the raw features. These are just kind of unfiltered chemicals that are in these samples.

We need to do a bit of processing to kind of get down to again the things that are of interest, the things that are real, and the things that we need to take a closer look at.

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DR. SOBUS: So we have spent a good bit of time combining some vendor software applications, as well as writing our own programs that we intend to make open to the public to do processing of these raw features. So we're kind of getting rid of the stuff that isn't real, get rid of the stuff that isn't reproducible, and kind of hone in on the things that we really need to take a closer look at. So the output of these programs that we've written are basically data matrices that say here's a feature with an accurate mass, and a retention time. Here's where it shows up in all the different samples at all the different intensities.

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DR. SOBUS: So we can take that processed feature file, and then restrict it to things where we've been able to assign a formula to those features with some level of confidence. So now we've gone from potentially 50,000 things down to 20,000 things, maybe down to 5,000 things. So we are losing a little bit of the information. But again, we're focusing on the things that we have the most confidence in at this point.

So here we have a list of formulas This is just a snapshot. Typically, these lists of formulas are hundreds to thousands of formulas long. The next step is we use our Chemistry Dashboard. We have the batch search function that I mentioned before, and we will actually search these thousands of formulas and try and pull back all the candidate structures that are consistent with each formula.

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DR. SOBUS: Some of these formulas can have 10, 20, 30, 100 different structures. So this becomes the critical challenge of NTA, which chemical is right?

Assuming you got the formula right, which chemical is right? This is extremely difficult to do, and this is just one example where we pulled back nine structures for a given formula.

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DR. SOBUS: This gets complicated, so I won't really get into it, but this is where we spend a lot of our time. To date, we use something that's called a data source ranking procedure to nominate the most likely candidate structure.

Data sources is basically a popularity contest. So when you build a database with chemical structures, pulling information from different sources, from different lists, different inventories, the chemical is on every single inventory that you pull from. Those are all data sources. It means it's a very popular chemical, many people know about, probably high-production volume, and thus it's most likely to be the correct compound.

So we initially do a data source ranking, and we actually spit out on the dashboard the top ranked candidate structure based on data sources. So we get it right about 70 percent of the time when we do that, which is pretty darn good, but we need other ways of getting it right the additional 30 percent of the time.

So without getting into it, we have been actively building machine-learning models to predict retention time of candidate structure. Media occurrence of candidate structure. That's basically a probability -- so if you're looking at a dust sample, it's a probability for every candidate structure that that chemical could be found in dust, based on its structure.

And then we're also working on models to predict method compatibility. So if you used an LC/Q-TOF in positive mode, electrospray ionization what's the probability that you would have seen any of those candidate compounds.

So two of these models are complete, the papers are ready to go out. We're working on the third. But the point is in this graphic to kind of take all those pieces of information and to compile a composite score to then rank the candidate structures and try and get the right one 100 percent of the time, if you can. It's a lofty goal, but it's something that we're working towards.

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DR. SOBUS: So the next step -- now, we have a candidate structure, or perhaps a few candidate structures for each of the formulas that we've identified in the samples. Which ones do we care about?

We're still talking about thousands and thousands of features. Which ones do we care about trying to do additional experiments, confirmatory experiments on. This goes back a couple years where we had the idea of using ExpoCast data, and using ToxCast data about these chemicals that we're tentatively identifying to figure out which ones we care about.

So we took some information from our experiments, so the detection frequency of these compounds, the abundance of these compounds, how big is the signal in the sample, and we also pulled exposure information and bioactivity information about these compounds from the dashboard. And we build these nice little ToxPi's, which is basically a graphical with a numerical representation of how important the compounds are from a health risk standpoint.

So if they have high bioactivity and high exposure, and if they're in every sample at a really high concentration, that's a really important compound. That's something we really want to take a look at as soon as possible. If it has no evidence of bioactivity, limited exposure, if it's only in five percent of the samples at a really low concentration, probably something we can put on the back burner for awhile.

So we use the ToxPi approach again to prioritize the top ranked candidate structures, and then we do some additional experimentation. So this is just the MS/MS analysis, where we have prioritized parent ions that we select as a target list, do MS/MS experimentation, and to the extent that we can, we compare our observed MS/MS data to experimental data that exists in some database.

This works fantastically well when you have that experimental data. But guess what, you very rarely have that experimental data. There are now tools that will predict theoretical MS/MS spectra for many of these compounds. We are currently evaluating the performance of those tools.

I'm very optimistic that they're going to work well. But in the future, we will very much be relying on this theoretical predicted MS/MS spectra to do this type of matching.

Again, we would love to use standards where we can for confirmation, but you're just not going to have standards for a large majority of compounds that you've seen.

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DR. SOBUS: As a final step, to the extent possible, we want to use, what I would call, high throughput semi-quantitative methods to try and predict concentrations for these compounds. Whether we know what the structure is or not, we can predict the concentration. So this is a heat map that I made just using what I would call a global calibration curve.

So I took many, many, many chemicals and basically took the average linear trend across all those chemicals and then applied it across several thousand compounds. So this is just a heat map with some hierarchical clustering of samples -- or of chemicals measured in dust samples. You can see on this very simple example, we're still looking at six orders of magnitude range in estimated concentration.

The precision is terrible, but you're at least getting some number with which you can then do additional work.

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DR. SOBUS: So that is the basis for our workflow. Again, everything that we're building as part of that workflow, the goal is to make available to the public as open code, or as some function available in the dashboard. So hopefully, we can continue to work with the State scientists, other state scientists, local labs, et cetera in trying to implement these tools on a wide scale.

So I want to shift gears and talk about applications very quickly. I'll get moving.

There are three applications that I will cover very briefly. Exposure surveillance. So can we use these tools just to look for what chemicals are in different foods, products, et cetera. Again, chemical prioritization. Can we not only look at what's there, but figure out what's most important. And very importantly, look at what chemicals co-occur, where we might have these mixture effects from complicated exposures. And then I'll touch on exposure forensic. So can we identify chemical signatures of specific exposure sources?

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DR. SOBUS: So I'm very briefly going to cover applications of the workflow in three different instances. These are the results from a consumer product analysis pilot study that recently completed. We've submitted the article for publication. We worked with an analytical contractor using GC/GC low resolution time of flight mass spectrometry to evaluate 20 different product categories.

We have 12 different formulation categories, such as lipstick, tooth paste, sunscreen; seven different article categories, like carpets, cotton clothing, fabric upholstery; and then one food category.

Our contractor went out, they picked five random products from each of these categories. They did Soxhlet extraction and then again they analyzed these extractions using NTA.

Ten to hundreds of compounds were tentatively identified or confirmed in all of these products. If you look on the table on the left, you can see that the number of tentatively identified compounds was typically about an order of magnitude higher than those explicitly listed on ingredient list. So there's a lot more there than you may have thought.

When we took the tentative NTA hits and hit it against a consumer product database that we built internally, we found only about 20 percent of those chemicals show up on that inventory.

The take-home point here is there's a lot more stuff in products than we currently know about. And just very quickly on the right-hand side again, we can estimate concentrations for some of these chemicals and products, but there's a lot of work to be done considering things like extraction efficiency to improve the accuracy and precision of those predictions.

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DR. SOBUS: The second pilot study that I'll talk about was our seminal piece of work. This was published I think in 2015 or 2016. It was actually featured in a

National Academies Report.

This was a house dust pilot study where we looked at samples from 56 homes. We tentatively identified thousands and thousands of structures, and we used the ToxPi approach that I mentioned before to prioritize all of these candidate structures. We took, as a proof of concept, the top 100 candidates. We acquired standards for those, and we tried to confirm as many as we could.

We wound up confirming about 35 of the top 100 compounds. And through a lit search, we found 45 percent of those had never been reported. So as a proof of concept, many thousands of things in dust, many of the things that we find have never been looked for before.

And again, we're in the process now through some hierarchical clustering and some supervised and unsupervised techniques of trying to dig down into these samples using some meta-data about the homes, the age of the home, heating source, smoking, pesticide use, pets, et cetera, to try and see if we can have chemical fingerprints of some of these exposure sources. And if we're successful in that, that certainly applies to things like surface water, sediment samples, et cetera.

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DR. SOBUS: The last example that I'll talk about is a drinking water pilot study, where we actually implemented Brita filters. So these are filters that attach to a tap. We collected nine Brita samples over a period of about a month in the North Carolina triangle area, representing four municipalities and two private wells.

Again, we found thousands of features. We did our prioritization using the ToxPi approach here. Rather than showing the actual pi's themselves, we actually made a stacked bar plot.

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DR. SOBUS: But the same principles apply, we looked at the top 100 priority compounds. We only had 16 standards for the top 100 compounds, 15 of them were correct. So we can be very successful. But again, you're limited to the standards that you have when doing confirmation experiments.

Many of the compounds that we were able to confirm aren't on any current monitoring lists. So as a proof of concept, again, this is a very good approach for identifying emerging contaminants, in this case, in drinking water samples.

I'll point out that when we matched these compounds to some of our databases in-house, we found that the origin of these chemicals are from consumer product uses, as well as some industrial processes.

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DR. SOBUS: So I want to transition briefly, as briefly as I can, to kind of these lingering science questions. So we've come a long way, but there's much, much more to be done.

We know that methods are extremely variable from lab to lab. So what are the consequences of that variability. Are some of these methods better than others overall? Are some better than others in only specific instances for specific chemical classes, and how does sample complexity affect our ability to do NTA? We really don't have any handle on this right now.

How many methods would we really need to apply to comprehensively characterize any given sample? We have no idea. And then I made a point before that we don't have a lot of good experimental MS/MS spectra to help in candidate identification. And we certainly don't have standards for many of these compounds.

So how do -- how do we overcome that hurdle? And to go back to a point that I think was made earlier. Is there a way we can actually crowdsource exposure data, if we can make resources available to public laboratories.

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DR. SOBUS: So to address these lingering science questions, about a year and a half, two years ago, we established, what we call, ENTACT. So this EPA's Non-Targeted Analysis Collaborative Trial.

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DR. SOBUS: This is a visualization of kind of what it's about, who's involved, and what we expect it to produce. For those of you that are familiar with the ToxCast program, at EPA, this includes about 4,600 chemicals right now that are undergoing in vitro screening to get some type of bioactivity response data. We are taking advantage of the availability of these chemicals, and using it for this trial.

In addition to those chemicals, we have reference house dust from NIST, referenced human serum from NIST. We've actually had, through a contract, reference silicone wrist bands that were made for use in this study.

I have probably about 15 to 20 different institutions represented here. I think we're closer to about 30 now. We have many people that continue to keep joining to participate. And again, the goal of this is to kind of let everyone use their method to see what works best. What are the best tools in specific instances?

From all the data that's generated, can we have experimental data, these MS/MS reference libraries, and can that benefit the public? And then how can we support modeling for future applications where we're just never going to have data.

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DR. SOBUS: So part one is completely based on these synthetic mixtures. We have taken 1,200 of the 4,600 ToxCast chemicals. These are the highest quality chemicals that we have in terms of their stability, purity, et cetera. And we've included them in 10 different synthetic mixtures. The mixtures have anywhere from 100 to 400 individual chemicals, and they're actually shown here, and they're very lovely looking due to dyes, I imagine.

So we've given all these mixtures out to the collaborators. Everyone is currently doing a blinded analysis. They have no idea what's in them. They're applying their methods, and they're reporting to us what they think is in the mixture.

We then tell them here's what's actually in the mixture, and they go back and do an unblinded evaluation and say what did we get right, what did we get wrong, what did we miss completely? All that data comes back to me and I pull all my hair out.

(Laughter.)

DR. SOBUS: A subset of these groups have actually gotten multi-well plates that include all of the individual compounds that are in the mixtures. And this is just used to do additional confirmatory experiments and to generate reference data.

For part two, we're moving away from the synthetic mixtures, because they're not real samples - this is kind of best case scenario - and we're moving into these reference materials. What's interesting is we've extracted the reference materials and sent out the extracts, but then we've also spiked each of the materials with the mixture of ToxCast chemicals and then extracted and sent that out.

So each of the groups that's participating has the 10 mixtures as well as the six extracts of the reference material. All of this work is underway, data is starting to come back in. It's messy, but it's really, really, really interesting.

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DR. SOBUS: The final part is for, I think, we're at seven or eight vendors now. So basically, every company that makes high resolution mass spectrometers that's uses for NTA, they have received the full ToxCast library. We've given the 4600 chemicals on 13 384-well plates, and each of them are doing MS/MS or MS to the N, in some cases, experiments on these compounds. They're generating reference mass spectrometry files that they can then sell to their customers, but also will give to us to make public.

The point of this is, experimental data will exist for every high resolution mass spectrometry platform, and it will be available to anyone that buys that platform and uses it to screen for these 4600 ToxCast chemicals. So not far down the road, your programs -- your State-based programs will have access to this reference data, and can screen rapidly for the presence of these compounds in any medium. So it's a very, very powerful thing.

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DR. SOBUS: So that brings us to the take-home points. I personally think we're at a place now where we need to move forward with these methods, they need to become mainstream. I'm all about trying to build the tools so that the public can use them in facilitating the broad application of these methods.

I know, through painstaking processes, that there aren't standardized methods. Everyone has their own secret sauce, and we need to figure out ways to kind of bring everyone to the table and to come up with gold standards and benchmarks.

We're working very hard to do just that. So we're taking advantage of the fact that we have these ToxCast chemicals and leveraging that to enhance the NTA community. Tony Williams and his team have done a phenomenal job of building these massive rich chemistry databases, and we're sharing that internationally. We, internally, are developing these tools to allow rapid identification, characterization, and semi-quantitation, which I think is critically important.

We are working very hard integrating with lots of international partners that are very skilled in doing, particularly sediment and drinking water, which is really, really wonderful. And then finally, we're trying to apply this everywhere we can for discovery, surveillance, prioritization, and some of the things I didn't talk about, effect-directed analysis and biomonitoring.

So I think this is a very powerful approach that we're taking. We're very excited about having partners in the States, in academia and in local labs, and we're here to help anyway we can. And if there's any questions I can answer now or down the road, please done hesitate to contact me.

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DR. SOBUS: As I close, I just want to thank our research team in RTP. We have a terrific group of chemists, cheminformatists, and modelers. So I just want to acknowledge all of their great efforts and open it up to questions.

CHAIRPERSON SCHWARZMAN: Thank you so much for that presentation. It's the clearest explanation of NTA I've ever heard.

(Laughter.)

DR. SOBUS: Thank you.

CHAIRPERSON SCHWARZMAN: So I want to start with the opportunity for Panel members to ask clarifying questions, and then we'll have a chance for public comment.

It was so clear, we don't have any questions.

Oh, Dr. Cranor.

PANEL MEMBER CRANOR: A really naive question. So there are -- presumably, there are 84,000 substances registered for use in commerce. You claim there are tens and tens of thousands of them out there. What are they? Are they re-combined things? Are they rogue things? What are they?

DR. SOBUS: I think, A, there's million of them out there --

PANEL MEMBER CRANOR: Millions. Sorry.

DR. SOBUS: -- in my estimation. So tens of thousands registered for use. What's -- I think anyone that you talk to that's been doing this work for a period of time will tell you again that regardless of how big our screening database is -- ours is 750,000 substances, we're only seeing ten percent or less of what's in a sample. So what is that 90 percent?

PANEL MEMBER CRANOR: Yeah.

DR. SOBUS: Most people, A, are calling it exposure dark matter, which I think is pretty neat.

(Laughter.)

DR. SOBUS: So I've started to use that term too. But most people think that these are degradation products, transformation products, biological metabolites. So for the last two years --

PANEL MEMBER CRANOR: But if they're in -- if they're in commercial products, are they degraded or have they been modified to be in those products?

DR. SOBUS: That's a complicated question that I don't want to dig too deep in, but I'm -- I'm thinking more of when we have the inability to characterize a large percentage of the sample contents, I'm thinking more biologically and more environmentally and less based on the products.

PANEL MEMBER CRANOR: Yeah.

DR. SOBUS: I'm trying to remember back that -- the data that we got from consumer products came from contractors, so I don't remember what the break out in terms of percentages are. I would expect that there would be fewer degradation products, but certainly some, and certainly some interaction products as well.

So the percentage may be a little bit higher in products, but then you have issues -- issues related to packaging, and leaching, and migration, et cetera. So if we're ever going to get at that, I guess -- I guess the initial question is are those things important? And I guess the answer to that is you don't know until you have some idea of what they are.

So I'd certainly like to have an idea of what they are. So, you know, our thinking behind that, and I think some groups are starting to implement approaches to do this, are to figure out ways to use modeling systems to predict what likely degradation transformation metabolism products are.

And if you can generate them within reason without it exploding, you can then add them to a screening library and start to look for evidence of them being there. So if you take, you know, a list of 100 compounds and many of them you believe to be in a given sample, and many of them you believe under the conditions that they've experienced might lead to transformation products, you know, use some modeling tools to predict what those products might be, add them to the screening list, and then see if they're there.

So I think that's a first step, and that's something I've been trying to work towards for the last two years. Finding the appropriate models to do that has proven to be a challenge for a number of reasons. Again, we always want to use something that's open, so that if we can find that it works particularly well, we can then make it available to folks.

So finding appropriate tools that actually predict the correct compounds, and do it in a manner that it's consistent with our workflow is something that we're working towards. Hopefully, we'll have some success with that in the next year or so, but I think that's the path to doing it.

And even taking it a step further, I've seen some great presentations from some colleagues that are participating in our trial. If you can predict what those compounds might be, you then have a structure -- a theoretical structure of that predicted compound. Groups are beginning to predict the theoretical spectra of the predicted compounds from the known chemical products.

So computationally, you can really take this all the way down the line. So I think a lot of groups right now are in the proof of concept stage to say can we even do it, can we make the prediction? And then once the prediction is made, how valuable is that prediction?

So we very much have that in mind to do, to explore, because it's the gaping hole in the work that we do right now. It's the 90 to 95 percent.

PANEL MEMBER CRANOR: That suggests one follow-up question, if I might. Do you have an example of something that you either have found or suspect that is pretty toxic that we didn't know about that wasn't registered, or it's one of these degradation products that turns out to be surprisingly toxic? Let's put it -- let me -- an extreme.

DR. SOBUS: Short answer, yes.

PANEL MEMBER CRANOR: Pardon?

DR. SOBUS: The short answer is yes, we do see that thing quite often. I really don't want to get into naming specific compounds. I have -- I will say generally I have colleagues at EPA RTP -- again, where I talked mostly about suspect screening, so is anything on the list that we know about present? I have colleagues that focus more on the true NTA.

So basically, if we match against a list, anything that doesn't match, that's what they want to look at. And it's a -- it's a laborious project, because you have to kind of take a very large number of compounds and kind of go through it manually and say what seems to be important?

In doing that, I know my colleague Mark Strynar, in particular, has found a number of perfluorinated compounds that aren't registered, that are potentially by-products of manufacturing processes. There is a decent number of these things at, what appears to be, decent concentrations in some water systems. So that would be one very good example. And I think there's been some media coverage of that of late, so there's a lot more follow-up work being done right now with several states and specifically with our lab as well.

So that's one great example of not necessarily knowing what you're looking for, but looking for the things that couldn't be matched that appear to be in high concentration, getting some idea that it could have a fluorinated signature, and then doing some additional experiments to kind of fragment that molecule and reconstruct it and figure out what the structure might be.

So that's a great chunk of work that's come out of our group in RTP over the last really five years or so. That's probably the best example I have.

PANEL MEMBER CRANOR: Thank you.

CHAIRPERSON SCHWARZMAN: Other questions from the Panel?

PANEL MEMBER LUDERER: Yeah. Thank you for that great presentation. And this dashboard just sort of makes me want to go out and try to use it.

(Laughter.)

DR. SOBUS: Oh, and there's a mobile app.

(Laughter.)

PANEL MEMBER LUDERER: But, you know, so -- so I have some more -- some questions. I mean, you were talking about all the many different platforms that there are, and everyone has their own way of doing these things. And so the -- you know, the study that you're doing where you're basically sending out the same mixtures to all these different people, I mean, I think that's going to go a long way towards start -- trying to start to resolve that and come up, as you said, with best practices.

My question is right now in the dashboard, is there a way, for example, for -- you know, if a user can, for example, I don't know, enter -- enter data about what was the platform they used, or how the samples were prepared to, you know, help narrow down what the unknowns are, or is that something that would be --

DR. SOBUS: No, that's the goal.

PANEL MEMBER LUDERER: -- in the future, but that's not there?

DR. SOBUS: That's the goal. So I guess the process that we're taking -- to let me step back and say the dashboard was meant to serve a different client. Tony -- so Tony was the original developer of ChemSpider, and we were Lucky enough to get him, I think, two years ago now. And he's been developing this dashboard for us, which is phenomenal.

So I'm very lucky to work with him and his team. And, you know, I kind of bring in the NTA users group internally, and say these are some things that we would love to see the dashboard. So we worked very closely to try and prioritize which of those things need to happen first.

So it's been about a year and a half now where we've been trying to integrate these batch searches based on formula. Another big thing is we want to do batch searches based on mass. As we're doing that, as we're building the functionality in the dashboard, in the background, we have a modeling team that are building the retention time prediction models, that are building the media occurrence prediction models.

So to the extent that it serves the public, we try and host those predictions on the dashboard as is, because we think it's particularly valuable. But even when we build -- let's say we build a retention time model, that model, at least initially, is benefiting only our group because it was built on the conditions of the analyses that we ran.

So kind of step two of that is how do you then figure out how to adapt that model to support all users?

And if you can build that model to support all users, then you need to have added functionality to allow people to upload the information that they uses. So I would say that we're -- that's down the road a little bit, and I'm probably speaking on Tony's behalf here. I really hope that we get there. I think he has the same vision for that, but a lot of things will happen between now and then. So we're -- we're very much in that proof of concept stage to say if we build this model, is it helpful? And if it's helpful, can we make it more versatile? And if it's more versatile, how do we build the tools in the dashboard to allow people to enter in their information. So that's where we're going. It's just a little bit down the road.

CHAIRPERSON SCHWARZMAN: Yeah. Dr. Cranor.

PANEL MEMBER CRANOR: One more question your comments just raised. Is there a way to shortcut some of this process? You mentioned in your answer to me that a number of these compounds were fluoride -- had fluorides in them.

Now, presumably lots of the fluoride compounds are very stable, and so is there a way to do a detection method for fluorides so you can pick them up or detection method for other chemical compounds that we know are toxic? I mean, there's a -- I'm not -- so I'm not a chemist -- neither chemist nor toxicologist, but I've read in toxicological books or talked to colleagues who have pictures of the molecules that have toxicological cancerous endpoints on them.

DR. SOBUS: Sure, sure.

PANEL MEMBER CRANOR: Are there ways to short circuit the process and pick out some of those things early?

DR. SOBUS: Yeah, so I have two answers to that.

PANEL MEMBER CRANOR: So a speculative question.

DR. SOBUS: And again, I'll address this from the suspect screening, things that hit against the database versus things that doesn't.

You need to do prioritizations in both. Okay. So I presented on that ToxPi approach. Okay. So these are for things that hit against the database. We've written code to automatically process this stuff. So you hit the database, you pull everything back. In pulling it back, you pull back the exposure data, you pull back the toxic data. It goes into a calculation in the program and spits out those things that are most interesting from a risk standpoint, taking into account both exposure and hazard. Okay. So that's -- that's done programmatically. That's easy.

Currently, I want to say we've got bioactivity predictions for fewer than 10,000 compounds. So 750,000 substances in the database. So it stands to reason to say that the Majority of things in the database don't currently have exposure and hazard data. So this is where you have to come up with computational approaches. So we are working with people within the National Center for Computational Toxicology - so those are Tony Williams colleagues and mine - that have built chemical read-across approaches and QSAR approaches for predicting bioactivity using models that are based on existing ToxCast data, as well as in vivo results.

So that is kind of something that we are actively working on now to use QSARs for bioactivity prediction, so at least we have some estimate of whether or not chemicals could be bioactive for things that hit against the database.

You know, we're still in proof of concept there as well. So where the data exists, programmatically we can pull it back and calculate it very quickly. Where the date doesn't exist, we're going to have to introduce the QSARs to make the predictions. So it will happen over the course of the next year. And then again, it will be done programmatically over that.

In terms of the true non-targeted work, things that don't hit the database, there aren't that many great approaches. Again, I'll mention two things that are commonly done, and then the third thing is something that we do. Number one is look for the biggest things. A lot of groups out there -- we -- we begin in what's called an MS-only analysis. So we're looking basically at parent ions. We try and collect as much information about those compounds as possible, and then we do our MS/MS experimentation where we generate fragment ions.

Many groups just kind of bypass the first step, and do an all ions, where they're basically fragmenting everything, and looking at all the fragments of all the compounds, which is an overwhelming amount of data, where they do what's called data-dependent acquisition, where they say whatever comes in as a parent ion, if it's above some intensity threshold, we're going to automatically do the fragmentation and then try and so the annotation downstream. So there, you're prioritizing based only on intensity. So that's approach one.

Approach two is, again, if you have groups of compounds, if you want to say -- and this is what's done in metabolomics, what are the molecules that are up-regulated in a diseased population versus a non-diseased population. Again, you're going to take everything that's here, everything that's here and make a statistical comparison, and what's left might be 50, 100, 200 things that are significantly different.

So now you're prioritized to look at those compounds. On the environmental end, you specifically mentioned fluorinated compounds. In terms of identifying halogenated compounds, which are always of great interest, we use something called a mass defect filter, which -- I'm not a physicist, I'm not a chemist, but my understanding is for compounds below a specific molecular weight, typically 600 or 700 daltons, if you -- if you round to an integer mass and then subtract from that the accurate mass, when you have a negative number, that's going to represent a negative mass defect.

And as it turns out, for halogenated compounds, you very often have this negative mass defect. So we've introduced into our programs a very quick calculation to calculate whether or not you have a positive mass defect, or a negative mass defect.

So a lot of my colleagues that studied the perfluorinated compounds implement the calculation and immediately look at the negative mass defect compounds and kind of sort it by intensity and say, here is the highest intensity negative mass defect compounds. These are halogenated compounds that are in the sample. Let's start there.

PANEL MEMBER CRANOR: Thank you.

CHAIRPERSON SCHWARZMAN: I think I'm going to save my question for the discussion, because we actually have a half hour of discussion allotted. And I want to go to public comment, and then we can get back to the discussion, because these are all really interesting questions and I think it's very applicable.

We have two comments, one of which is a question that -- to just be read.

So one is a question, which is what is bioactivity a parameter of the ToxPi key, and how is bioactivity measured. And the question is specifically in dust. And I think this is reference to slide 17 describing the workflow. So I think that's just a reference to bioactivity in the ToxCast data. Maybe you can just elaborate on that.

DR. SOBUS: Sure. I'll do the best I can at remembering this. So the ToxCast -- so there's a Tox21 effort that is a multi-federal institutional effort that has looked at a very, very large number of chemicals across a decent number of bioassays. The ToxCast program is specific to EPA. It's looked at a fewer number of chemicals. Again, I think it's about 4,000 right now across hundreds of bioassays.

So each one of these bioassays that I think were -- many of them were originally developed for pharma applications, basically look at the bioactivity of a given tested chemical in a concentration response format.

So you basically are testing a specific assay at increasing concentrations to try and determine a value called an AC-50. So that's an activation concentration, 50 percent. That gives you some information again kind of on this concentration response format. I'm not exactly sure how it's done, but it's determined from that concentration response information, whether it's a hit or not.

For our application, we are basically taking assay hits, so -- and different chemicals have been tested across different numbers of assays. So we basically take a percentage of active assays across all tested assays. So if something has been tested across five bioassays, and it was a hit in each assay, it's active 100 percent of the time. And that's going to be basically the number one bioactive compound according to our score.

So the dashboard, when you enter in your formulas as a batch search, there is a little button that let's you export it is a CSV file or in several other formats. You click on a variety of different data options that you can actually export. And one of those is on number of tested assays, and one of those is on number of active assays.

So you export that data, and then our programs automatically calculate percentage of active assays. And that gets rolled into our ToxPi calculation, which is basically bioactivity, exposure, detection frequency, and abundance all get normalized to a value of 0 to 1, and then you sum across all four things. So the most interesting compound would have a value of 4. It would be the number one compound bioactivity, number one exposure, et cetera, et cetera.

That's basically how it's done. I have very nice graphics that shows that in other presentations. So I'm sorry I don't have that with me today, but hopefully that's clearly enough.

CHAIRPERSON SCHWARZMAN: Also, for the public member who asked the question, but maybe if I could add just a quick follow-up question. It sounds like that's basically prioritizing, in terms of bioactivity, the higher number of assays on which it's positive, not degree of positivity. So -- or like lower AC. You know, lower AC would be more --

DR. SOBUS: That is correct.

CHAIRPERSON SCHWARZMAN: -- potent. And so if something is very potent at fewer say receptors, or in fewer assays, it wouldn't necessarily rise to the top as much as something that is active in many different assays and potentially different receptors.

DR. SOBUS: That is absolutely 100 percent correct. And I'll make the pitch out there for anyone who has interest in optimizing, you know, how we go about pulling, utilizing that type of information, every single component of this needs optimization. And at this point, it's kind of let's show that it works, lets try and apply it as broadly as we can to figure out where we are, and then we will work on the optimization of each area. That is absolutely an area that needs optimization.

CHAIRPERSON SCHWARZMAN: So the other written public comment that I should read is a question actually. Does current NTA at US EPA focus on parent compounds or metabolites as well?

DR. SOBUS: So we have our core group in RTP. There are other groups in Athens and Cincinnati and Las Vegas, and I believe other places that do -- that take similar approaches. Some of these are a bit more focused to metabolites. A lot of our ecological studies that may focus on fish species, looking at fish tissues and stuff like that, they're going to be a little bit more focused on the metabolite side of things.

Specifically, the work that we do, because I'm in the Chemical Safety for Sustainability Program, is very much focused on the parent compounds. Again, the goal is to try to bridge those two things to the best of our ability, which is going to involve some different modeling platforms to predict what the likely metabolites transformation products are going to be. So we're working towards it, but the work is that I've talked about today is more or less focused on the parent compounds for the time being.

CHAIRPERSON SCHWARZMAN: Okay. For the moment, maybe I'll have you surrender the microphone to our two public comment readers and then have you come back up for our discussion.

Oh, great. Never mind. Hang on to the mic.

DR. SOBUS: Good. I didn't want to surrender it yet.

(Laughter.)

CHAIRPERSON SCHWARZMAN: Sorry about that.

Okay. So we have -- Dr. Alex Hoepker from OEHHA and Aolin Wang from UCSF.

DR. WANG: Thank you so much for a very exciting and interesting talk. I just wonder, so before you mentioned that you cross reference a very huge database in the dashboard. Have you considered whether it would be helpful to cross-reference curated like library because some of the -- under some of the platforms like LC/Q-TOF? There are certain compounds that cannot be detected. So in order to like reduce false positive and also maybe to put -- to pick out candidates that are of interest to maybe scientists or of concern say the compounds that are used in baby products, particularly, would that be anymore helpful or...

DR. SOBUS: It's a great question. And I'll say this is a question that's debated a lot. A lot of our European colleagues are -- are pretty adamant that when you talk about suspect screening, they have to legitimately be suspects. You have to have some reason to believe that they might be there. You can't just take some massive list of things and say, is any of this there?

I disagree. I think if you have the means to screen against a large list, you should. I'm not seeing any negative consequence of doing that. You make a very good point that you could have some false assignments in doing that. We're evaluating to what extent that happens. But as a get around, Tony and his team have been working on basically anyone that contacts him that says, I have a listing of compounds that I have developed as a screening library.

He is accepting that information. He's developing linkages to our identifiers within the dashboard and then he's basically tagging that group of compounds as a specific list.

So I haven't spoken to him. We've been missing each other of late. But I think potential future functionality would be, rather than using everything to screen, to be able to select specific lists that you want to hit against as a subset of everything. So I think that functionality will be there, if people choose to use it.

Again, I'm of the mindset that I haven't really seen a lot of evidence that we're going to get a lot of mis-assignments or false positives by using a larger list. Again this is something I've gone back and forth on a lot of my colleagues about.

I think potentially, an important practical issue is, depending upon which piece of software you're using and which vendor instrument you're using, some physically can't handle a list of 750,000 substances. And this is something that we're dealing with right now. And this is -- it's really -- it's what's cool about the ENTACT Study is you're engaging everyone. And everyone is going to defend their approach for doing it, right?

But you have these discussions, and at the end of those discussions, you hopefully come to some consensus. Now, let me say with that, we're going to have a workshop in May of 2018 for all the participants and anyone else that wants to be there to talk about all of this, successes, hardships, path forward.

So we wound up in ENTACT, we had given the listing of 750,000 compounds to everyone. I had four people come back and say, I just can't do it. We physically -- our software will not allow us to do it. So we made a concession and gave them an abbreviated list of about 5,000 compounds.

Again, I had some people yell at me for doing that, because we're making it too easy. And I had other people praise me for saying thank you, now I can actually do this.

So it's yet to be determined what the best approach is, if there is a best approach. My mindset is I want to look for everything I can possibly look for and try and be very careful that I'm not getting a lot of these false assignments.

Good question.

CHAIRPERSON SCHWARZMAN: And our final public comment is from Alexander Hoepker from OEHHA.

DR. HOEPKER: Thank you very much for that presentation. That was really insightful.

I wanted to ask about there's really three steps issued that are cited, right, there's extraction, there's separation, and then there's detection with mass spectrometry, and sometimes the extraction and separation parts are not addressed as much.

DR. SOBUS: A little overlooked.

DR. HOEPKER: Yeah. And so I was wondering if EPA is currently developing orthogonal methods that are trying to fill some of these gaps in extracting and separating these chemicals. That was one sort of really big question.

But the other one -- the other question I had was GC seems to be overrepresented as a method of separation, I think, primarily because of the resolution and the large spectral databases. LC always seems to fall short. And I know we have a lot of capacity in LC of course at EPA. But I'm wondering for GC, because it is so over-emphasized right now, if there are thermal degradation issues, and if there are guidelines, red flags for certain chemical groups that exist that researchers need to be cautious of. I can think of peroxy acids, other organic acids that are prone to degradation. But I was wondering if there was any guidelines for other classes of compounds?

DR. SOBUS: There's a lot of good questions.

I don't know about any guidelines for thermal degradation. I would say to the extent that anyone doing this work has concerns and has an idea of something that we could look into, I think part of our job is to work with folks to try and look at what data we have in hand to see if we can do some experiments to perhaps model that type of activity.

It's interesting that you say that the GC is perhaps better represented than LC. My perspective is a little bit of the opposite, I think. We actually -- we're a fairly small group. We have an older straight TOF, and we have a newer QTOF and we're getting an Orbitrap.

We're trying to build our capacity. We currently -- really, we have -- we have a GC triple quad, and we're looking at getting a GC high resolution instrument, but that's potentially down the road a bit. So the bulk of the work that we're doing to date is LC based.

So -- and I think a lot of our collaborators particularly in Europe are primarily focused on LC. The nice thing is in ENTACT, we have a pretty good split. I'd say maybe 20 to 40 percent of the methods that will be employed are going to be GC based and the rest LC based. But we are working -- again, I think there's going to be three or four vendors that are applying GC approaches to characterizing these mixtures.

So again, there's going to be a lot of really interesting data that comes back from the analyses of these 4,600 samples. And again, someone like you that has a research hypothesis about thermal degradation, you know, to the extent that we can use the data that comes out of that trial to evaluate that hypothesis, that's something we want to do.

And we can even make those -- well, we have made those samples available to folks here for investigations. So that potentially is a good application. Help me with your first question again.

DR. HOEPKER: In some ways, you're addressing that already. I'm presuming there's going to be overlap between LC and GC, so there's ways of validating --

DR. SOBUS: Yes.

DR. HOEPKER: -- across methods too.

DR. SOBUS: Yes. There's -- and I will say, we're neck deep into our analyses of the SRM's, so that's the serum and the dust sample. And you've got -- we went and looked in the literature and tried to pull every compound that's been identified and quantified with targeted methods in the SRMs, and basically seeing how many of them can we see with this method, how many of them can we see with this method, how any can we see with both.

So that speaks to the kind of -- the reach of a given method for a particular chemical space, which gets to one of the questions I asked of we don't know how many different methods would be needed to kind of fully characterize a sample, if that's even necessary.

Did I miss anything as part of that first question? I feel like I did.

DR. HOEPKER: The extraction part.

DR. SOBUS: The extraction part. Good.

(Laughter.)

DR. SOBUS: No, that's a great question. And we went back and forth on this. The reality is you are one hundred percent correct. And I think more attention needs to be paid to that. Most people right now just try and use something that is going to give them, you know, kind of the biggest swath of information.

The biggest number of chemicals without biasing against any particular group. You're never going to get it all. Ideally, you would be doing multiple extraction procedures, multiple columns for chromatography, you know.

But it's all about bandwidth, right? If you do multiple extractions, multiple clean-ups, multiple columns for chromatography, multiple ionization sources, multiple platforms, LC and GC. You know, it gets multiple databases, big, small. You know, it just explodes.

So you've kind of got to do what you can to prove the methods using something that's a little bit more tangible. And then once you get a handle on that and have some type of benchmark for doing it, then I think you can extend. Like we want to get in to using HILIC columns, rather than C18 and C8 or in addition to. But it's just you've only got so many people, you've only got so much time. You can only do so much in a period of time, but that's absolutely something that I think has been not considered enough and something that needs to be considered more in future experiments.

Great questions.

CHAIRPERSON SCHWARZMAN: So we have time now for general Panel discussion, as well as audience discussion.

So, Dr. Cranor.

PANEL MEMBER CRANOR: Yes a follow-up question. You've given a very exciting and interesting presentation -- closer. Okay. At the same time, we know that there are a number of substances out there that are fairly toxic that we haven't done much with. Of the things that you've discovered, how do they compare in terms of toxicity, or do you even have any idea? This really goes to the question of the social benefits of the elaborate programs you're putting together, which are very exciting, I think, but in terms of the public health benefit.

DR. SOBUS: Um-hmm. So I guess I would potentially restate that as you asking me of all of the things we find, are the things that are currently looked for amongst the very top of that list? So if you find 5,000 things, and there's 300 things in NHANES, are those 300 things at the list of the -- top of the list of the

5,000 that we're finding?

PANEL MEMBER CRANOR: Yeah, and how they compare with the things we already know about?

DR. SOBUS: Right. So I'd say the things that we know to look for, that we've already determined we care about due tend to be at the top of the list, but they're amongst many other things that are also at the top of the list, and in many cases at much higher concentrations.

And --

PANEL MEMBER CRANOR: The things you found are at higher concentrations?

DR. SOBUS: The things that we're finding.

PANEL MEMBER CRANOR: Wow.

DR. SOBUS: And, you know, I'm not a chemist, but it's interesting when we -- when I work with the chemists, and, you know, my job is kind of to facilitate to write some of these programs, to kind of make everything happen quickly, when this stuff comes back, and I share a results file with a chemist and they look at what's at the top of the list, they're often blown away, because they -- you know, they may recognize a compound, but never thought that it might be present, and certainly not present at that concentration.

So it is really, really eye opening. And a lot of stuff makes sense, you know. I want to -- I always show this example of piperine. Piperine is what's in black pepper. It's basically in every home that we look at. That makes sense. You know, basically everyone uses pepper.

(Laughter.)

DR. SOBUS: Nobody ever really thinks about, you know, if you do this, basically anywhere in your home, you've got a bunch of piperine on your finger.

(Laughter.)

DR. SOBUS: So there's -- you know, there's a thousand chemicals just like that that are in every home. And when you sit back and when you look at the list of things that are on your finger, you're like, wow, that makes sense. So then you have to step back and say, what are the potential biological consequences of me being around that all the time.

So there are some really cool things that you find, but I think a lot of it makes a lot of sense. You know, the consumer product example that we gave I think is really cool of, you know, basically 80 percent of the things that came back aren't previously associated with consumer products. So what the heck are they and how are they getting there? You know, are -- you know for formulations are they coming from packaging?

So there's a lot of investigative work to be done

I think when you find some of these things. And that's why you want to be pretty careful about confirmation. You don't want to go, you know, chasing some compound that you think is there that you could be wrong on.

But I think there's -- there's a ton of work to be done as you generate these data sets to really dig in and say what does this mean? And that's what I would -- even if people don't have the capacity to make the numbers themselves, that's why we're trying to build the tools so that we can do the analyses or provide the tools to folks, so they can do the analyses, but also make the data available, so people can begin to mine it and say what does this really mean? Because there's just a ton of it there, and no one person or even small group of people can fully understand what's going on in any reasonable amount of time. So we need to kind of take it on as a big team effort, I think.

PANEL MEMBER CRANOR: Thank you.

CHAIRPERSON SCHWARZMAN: I'm going to ask -- take the Chair's prerogative and ask a question I didn't get to ask before. So one is just sort of a follow up on that, but then it kind of connects to my other main question, which -- so is it fair to say do you think that we don't have a very clear idea about toxicity for many of the compounds that you're detecting precisely, because they're -- they're the known unknowns, in a sense. Like, you find them, you can identify them, but they weren't the things that we thought to look for, because we have an understanding about how they're used and whether they're particularly toxic.

So would you say that you're generating sort of a list of commonly found compounds for which we don't have much toxicity data.

DR. SOBUS: You nailed it. And I think I view that as a big part of my job. Okay. So the ToxCast program, you know, you can only procure and evaluate so many chemicals, right? And they're up to, like I said, close to 5,000. So that's a huge undertaking. It involves tons of resources and a lot of time.

So given that there are 4,600 ToxCast chemicals, and 750,000 substances in DSSTox, what's the next list of things you want to screen in ToxCast?

So again, I view that as part of my job is to say here's the things that we routinely find. Let me work with some QSAR modelers and say does this have any potential to be bioactive? So that is a very important approach for kind of nominating things that should go in the next cycle of the program.

I didn't mention, but I think some of the criticisms of existing in vitro high throughput screening programs is the lack of capability for testing metabolic capacity. We're constantly testing the parent compounds, and the limited testing on mixtures.

So this is something that again we really hope to contribute to these high throughput screening programs is to say here are our things that we're finding over and over again. We believe this to be the structure. This is not a parent chemical. This is not registered for use, but it's something that's out there everywhere at very high concentrations.

Perhaps, we should test the activity of that compound in ToxCast. At the same time, you know, if you look at every possible combination of every possible chemical, you have an untestable number of mixtures, right? So how do we use our empirical data to say here's a cluster of compounds that always co-occurs and it always co-occurs when you have this exposure source. If this is relevant say to children's health, maybe this is a mixture of seven things that we really need to test the activity of to see if it's additive or if there's a synergy, something like that. So that's something through our work we hope to contribute back to the ToxCast program.

CHAIRPERSON SCHWARZMAN: So that's really helpful, and it kind of connects to my earlier question, which is you mentioned that you didn't talk a lot about the application of these methods in biomonitoring studies. And it's really exciting to hear about, you know, environmental applications, and consumer product, and household dust and all of that sort of thing. It's lots of interesting stuff.

But I wonder if you could elaborate a little bit about some of the challenges and opportunities in applying NTA in biomonitoring samples. And it occurs to me just from a not very expert perspective in this that there's a lot of -- but as a physician, I have some sense of the number of biological compounds that are in our bodies and what is -- is there -- is it a ton of work to distinguish among or between sort of big classes of chemicals to which we're exposed from the environment, and our own endogenous chemistry?

DR. SOBUS: Great question. So I actually had dinner with I Steve Rappaport last night, and we talked about that for about two hours. The -- you know, the definition of the exposome, as representing the totality of exposures is -- is conceived, construed differently by groups of people. And it's done in such a way that it's kind of fit for purposes for their application.

So whereas, you know, my mandate -- agency's mandate, my activities to support that mandate are to evaluate a lot of the environmental component of the exposome. Certainly, there's that endogenous component that's critically important, and often the focus of many exposomics, and certainly metabolomic studies.

So I think -- I haven't heard any naysayers yet, but my thinking is a lot of the effort to date done in biological samples, particularly serum, has focused on the higher concentration compounds, more often the endogenous molecules, because they're easier to detect, because they're at much higher concentrations.

Steve has pointed out through publications, through presentations that it is harder to get at the environmental chemicals because they are at lower levels, and you have some signal suppression. You've got all of this other biological stuff that's really highly concentrated, and then the environmental stuff is lower level.

So that's why we're looking at serum right now. And I will say it is more of a challenge to characterize the environmental chemicals in biological samples, particularly serum, because of some of those signal suppression issues. We do tend to see lots more stuff and see stuff more successfully in the environmental samples. But there's no reason that we can't do xenobiotic, a component of biological sample at the same time that we do the endogenous component.

So, you know, the path forward -- and we're working with some partners in the field like Scripps that manage XCMS and METLIN. You know, we've incorporated into METLIN, the DSSTox compounds. So now folks that do metabolomics and that look routinely at the endogenous compounds for some type of enhancement that could be mapped to a biological pathway, and to an apical endpoint, you know, we're providing them with the ability to also look for the environmental chemicals.

So I think in the near future, we're going to be in a paradigm where you either have individual groups that look at both sides of it, and then can draw relationships between the xenobiotic, exogenous, as well as the endogenous. But even if you have, you know, distinct communities, they're at least aware of what's going on on the other side, and then folks that can bridge the data across, that's where we need to be.

But I don't think we're too off -- too far off from doing it. So again, you can -- you can do what you can do. And from my standpoint, you know, there needs to be more work done on the environmental end, just because it's lagging behind the metabolomics community, but I think we're really close to having kind of a bridging of those efforts. And I really don't see a whole lot standing in the way of it.

CHAIRPERSON SCHWARZMAN: Other Panel comments.

Dr. Quintana.

PANEL MEMBER QUINTAN: Do we have time?

CHAIRPERSON SCHWARZMAN: Yes, we have time.

PANEL MEMBER QUINTANA: Hi. When you were talking, I was thinking about the old days. I think I'm old enough to talk about the old days in, for example, genotoxicity testing. In a way, we used to do the opposite of what you're doing. So instead of looking for everything in a sample, we might take say urine from a group of nurses that handle cancer drugs, and compare it to urine of nurses who didn't, and see whether the urine was more mutagenic, for example.

DR. SOBUS: Right.

PANEL MEMBER QUINTANA: So not even looking for what was in there.

DR. SOBUS: Right.

PANEL MEMBER QUINTANA: Or the same thing for house dust. Is an extract of house dust more mutagenic than this house dust? I'm just wondering, if you take, for example, your house dust samples, 56 samples, and you tested the extracts say in ToxCast or something, and you looked at the 10 most mutagenic -- just taking that as an endpoint. There's many other endpoints, reproductive, whatever. Like the 10 most mutagenic compared to the 10 least mutagenic, could you subtract out a bunch of compounds and see what was different or how much have you explored that kind of approach to kind of use a downstream gatekeeper on environmental samples, like house dust, like urine, like other things.

DR. SOBUS: Sure. Sure. It's a great idea. It's something that I'll say we're actively pursuing. We're not far enough along on any particular study where we have results to report, but this is something we're working towards.

I had one other slide on NTA applications, and the bottom two bullets were grayed out. The first of those two bullets is called the effect-directed analysis. So that's exactly what you were describing, where you effectively take an extract of a sample of interest, introduce it to some type of test system, look for a response in that test system, and then you fractionate at different stages the mixture itself. So making it into two fractions and testing the two fractions. And then if one of the two fractions is bioactive break that active fraction into two, and you just successively break it into smaller fractions, so you figure out what are the primary components of that mixture that are driving the bioactivity.

So this is something that's done a fair bit. We have a lot of European colleagues that are absolutely experts in this field. It's something we want to work towards. I believe there are actually platforms now where this can be done in an automated fashion. I think that's -- we talked about this when we had a workshop. I think our workshop was two or three years ago now in RTP. And we had a whole discussion about the specific issue. And again, the field was kind of split between some people being adamant that this is the correct approach, and other people saying it does work very well, but it's extremely time-consuming.

I think there was a comment that said, one effect-directed analysis, one Ph D. This is --

(Laughter.)

DR. SOBUS: It takes that long to -- to do a successful experiment. Now, people really pushed back on that and said that, you know, the tools have been developed in an automated way, so that this can be done fairly quickly. So I think you're absolutely on point. I think now that we have the means -- again, you can't really do effect-directed analysis without having the underlying non-targeted analysis tools. You can identify the active fraction, but if you can't figure out what the chemicals are in that fraction, you know, have you really succeeded.

So I think now that we have the underlying tools to do the NTA, we can start to introduce it for different effect-directed analysis applications.

PANEL MEMBER QUINTANA: Just a very quick follow up that I guess the care to which the samples were collected, if you're using real-world samples, that would be to me proof of something we should be worried about. If it's in real people's homes, you know, like you said, or in urine for kids, I'm just wondering if you've thought about using some of the National Children's Study samples that were collected with such care from Irvine and other places, because they do have the air that -- the water, the -- all the components that might feed into that exposure. So I'm just curious if you've explored that.

DR. SOBUS: We absolutely have. Not necessarily National Children's Study, but certainly other well-known programs where samples have been collected and archived with care. We've had some -- some conversations with folks across the pond. So EPA and NIEHS share a campus. So we've been having some conversations lately about opportunities for some existing studies and for some upcoming studies, where we can start to kind of go down that path.

I think one of the important things about me being here is, you know, we're not CDC. We have a fairly small group of people. We have really two instruments right now that we're using. We're not in a position to be looking at thousands of samples right now. Our role is really to help build the methods to do proof of concept, to build the computational tools, and to make that available to the public.

And again, you know, what we've found is be it through, you know, data coming back from contractors or from collaborators, the real challenge to the community right now is folks saying, yeah, I can do that. We can do NTA. And then you have no expectation of what's going to come back.

And then sometimes, money is spent, time goes by, data comes back, and you say this wasn't what I was expecting, because there's no way of really evaluating when you say you can do NTA, what is it that you can really do. And that's why there's -- it's critical to have again some standards to which we hold laboratories to say if you can do it, you need to be able to at least do this.

And at the same time, there really needs to be a network of laboratories, so that when we have big studies with thousands of samples, maybe millions of samples, we have the capacity to take that on, because this isn't something -- you know, it's neat when we make these presentations and people get excited and, oh, you can do this work. And, you know, our initial inclination is, yeah, let's do it. And then my managers always look at me and they're just like what are you talking about? You can't that do that. You don't have the capacity to do that.

(Laughter.)

DR. SOBUS: So you -- you need to pick and choose, but I think the path forward for that is building a network of practitioners so that you can spread samples around, and then adhere to some agreed-upon standards for performing the work. And I think that's how -- the only way we're going to handle doing rigorous analyses on some of these precious samples for which great care has been taken to collect and store.

CHAIRPERSON SCHWARZMAN: Dr. Cranor, and then Dr. Luderer.

PANEL MEMBER CRANOR: One more hard question.

(Laughter.)

PANEL MEMBER CRANOR: I think many of us thinking about toxic substances grew up on carcinogens, and mutagens, and such things as that. On the -- of current concern, are xenohormones, you might say. And they typically operate at much lower con -- much lower concentrations, and they can disturb things. Can your system accommodate those, incorporate them, and identify them? I mean, this is a really speculative question, but --

DR. SOBUS: When you -- so when you say identify them, is this under the assumption that they've been identified as being bioactive for endocrine effects, or something like that, at low concentrations, are you asking if we can identify the effects or are you asking --

PANEL MEMBER CRANOR: Can we identify other ones --

DR. SOBUS: -- can we identify the chemicals, given that --

PANEL MEMBER CRANOR: -- that will have those effects?

DR. SOBUS: Broadly I think, yeah. I think to the extent that -- I mean, you say the chemicals are very low level. Obviously, we're not going to have the sensitivity with a broad non-targeted method on a QTOF that you would on a dedicated triple quad where you're focusing on 10 compounds. You're just not going to get it, but you're not too far off.

You know, we're -- in these SRM samples, we're picking up on pretty much everything that people have seen with targeted, trace level, triple quad analyses. So I think, you know, the vendors are really pushing the sensitivity on these instruments to where we can see them.

Whether or not we would anticipate if they would have effects at low concentration, like similar compounds, truthfully, we have a staff of computational modelers whose job it is to figure that out. So that's why I kind of turned to them to say, what do you have available to you to predict bioactivity, so that if I find this compound, I can come to you and say is this something I need to be worried about? Do you anticipate this thing could be bioactive in any way? Does it resemble anything else you've looked at before that was bioactive with a low concentration? So that is the workflow.

PANEL MEMBER CRANOR: Thank you.

CHAIRPERSON SCHWARZMAN: Dr. Luderer.

PANEL MEMBER LUDERER: Yeah, I wanted to just kind of get back to this -- the question that we were talking about with the metabolomics versus the, you know, exposome, and that, you know, a lot of -- there's maybe more work that already that has been done on the metabolomics side. And so one question I had in terms of -- I mean, obviously, if you're biomonitoring, it is really important to, I think, integrate those two things, and whether in the dashboard, in your 750,000 compounds, does it include any endogenous compounds or is it only, you know, xenobiotics --

DR. SOBUS: I'm pretty sure it has some, but I could -- I could not even take a guess at how many.

The reality is Tony and his team have so many connections in the field, and it's kind of -- and I believe -- I forget who the person was at CalEPA. He was just in contact with someone last week about an OEHHA chemical list, that was integrated in the dashboard over night. So it's -- his job is kind of networking and identifying people with rich databases that he can map to through the dashboard.

So when we started this work, we were looking at a list of, I want to say, like 7,000 compounds. And then version 2 was 32,000 compounds, and then it was 300,000 compounds, and now it's 750,000 compounds. This has happened over the course of two years.

So -- and again, this isn't just random pull stuff in and it's there. They've built automated programs and protocols for doing curation of that chemical information. And with that, they put flags on it. So if you go out to a massive public database with millions of compounds, there are often inconsistencies in the drawn structure, in the CAS number, which may no longer be registered in synonyms, in SMILES notation, and InChIKey.

So the programs effectively look across all of those components to make sure that they are consistent.

And if they're not consistent, they flag them. And once they're flagged, there's at least some attempt for at least a subset of the compounds to fix the errors. So those data quality flags exist in the database.

So to the extent that Tony and the team can kind of map out or link in and at least run the automated programs to kind of check all of these different pieces of information, that's how stuff gets pulled in. So how much of that that's got pulled in over the last two years that represents endogenous, I really don't know. I would say, by no means, is it anywhere near the majority, but some of it's probably in there.

Again, I think the most explicit action to kind of bridge those two things was Tony sharing the DSSTox content with those that manage METLIN which is a repository for mostly endogenous compounds.

CHAIRPERSON SCHWARZMAN: This may be an overly simplistic question, but I'm wondering how -- like for the purposes of this Program, of the Biomonitoring Program, something that sounds interesting to me to think about as a Panel would be to start from -- get our hands on a list of compounds that is like -- this one that I was referring to kind of the known unknowns of it's not the ones that are looked at in NHANES.

DR. SOBUS: Sure.

CHAIRPERSON SCHWARZMAN: It's like what is the kind of next set of compounds that we don't know a lot about that's showing up in environmental samples, or consumer product samples, dust samples, or biological samples that we should be thinking about is this something we should be looking at in a biomonitoring study in California

DR. SOBUS: That's a fantastic question. And I mean it's a very logical and important question, and perhaps surprisingly something that we haven't really talked about doing. I think everyone is so kind of focused and nested in their individual application that no one has really stepped back to say, okay, broaden it to what should we be looking for on a wide scale. I mean, we certainly from -- you know, you could do that a number of ways. You could say, do you want to focus that on a medium or do you want just want to -- to just broadly say give me a list of a hundred chemicals that you think are the next biggest deal?

That's something that I think any one group could kind of nominate their list. But what potentially would be more powerful is to look to the community, the people that are doing this, and say what is it that you think for any reason is the most important thing? And then, you know, it's kind of -- it's kind of a voting and a tally.

And we could put together that list.

And I think the network of participants would be a fantastic group to kind of query and say what would you nominate? From that, we could absolutely put together a really interesting list.

CHAIRPERSON SCHWARZMAN: That's my wish list.

(Laughter.)

DR. SOBUS: That's a -- that's a fantastic question.

CHAIRPERSON SCHWARZMAN: That's what I would like to suggest we do, because it's like using non-targeted analysis just direct targeted analysis to inform targeted analysis.

DR. SOBUS: That's absolutely the idea.

CHAIRPERSON SCHWARZMAN: And that's what, I think, would be a really exciting kind of next step.

DR. SHE: Jianwen She. I like to also help answer this question that the California Biomonitoring Program would use low targeted approach to select the chemical groups for us to work on.

So Dr. Sobus presented a very excellent talks. For the Biomonitoring Program, we need to look at what's practical for us, what the EPA can do, what can we do? So I think for us generally, we will focus on application of the tools that are already built in.

And incidentally, like some of the -- for me, some of the unknown unknown problems that's like artifical intelligence. We can build off a goal, narrow down on the board game, or we can broaden the search, like an exhaustive search that you can find all of the unknowns or you can do your ...... search, so you focus on specific areas. As artificial, that's how human solves unknown problems.

So for -- so I use this energy to compare mass spectrometry based non-targeted search for us. Instead of the broadest first search, we need a depths-first search. What I mean is depth-first search. Depth first search first, we focus on class of chemicals. That many class of chemicals you can pick up. For mass spectrometry, like Dr. Sobus said, all halogenated chemical compound that have specific mass spectrometer features, mass deficiencies, isotope profiles, mix and match easier to search.

But as poly-halogenated compound the ECL is working on, they may consider this group of chemicals who find a chemical maybe unique, new there. Like, we found poly-halogenated dioxins that's not registered. Aside far dioxins not registered, mainly by-product from the chemical or industrial process can be a group for us to look at.

For us, we -- in the past, we proposed using BP-3 analogs. So we still working on this group of chemicals. So that's my general comment.

And anything regarding specific progress, like Dr. Yu-Chen Chang can comment, if you are here. Do you want to make any comment on this part of the work?

DR. CHANG: So I'm Yu-Chen. In response to one of the SGP meeting that California EPA presented, there was a concern for UV filters. So at EHLB, our primary focus is to do untargeted screening primarily focused on the class with a structurally-related compound, which is benzophenone class.

So right now, since we have a very limited man power, so there are very limited compound we can screen. And database is right now limited, and we have a whole list of structural library available screen for this class of compounds. And we are in the process to expand our class to environmental -- to phthalates, which also listed as designated chemical for the list as a whole class, which we don't have the capability to screen for the whole class.

So right now, that's what our focus is on, the UVA filter, benzophenone structurally-related class.

Thank you.

CHAIRPERSON SCHWARZMAN: Any further comment from the Panel or from the audience that we should include before we move on?

I would just -- oh, there's one more.

Martha.

DR. SANDY: Hi. I'm Martha Sandy from OEHHA.

I just wanted to follow up on what -- one of the points that Alex had made about the extraction method for non-targeted analysis. And you have also talked about, well, what is the chemical space we're covering?

I would like to suggest, if possible, you could come up with a couple different ways of extraction to try to cover as much of the chemical space as you can and just remind folks of an example, where looking for food mutagens for years and years and years, researchers did extractions and looked at one type of extract, and they found a lot of things but they missed acrylamide.

(Laughter.)

DR. SOBUS: It's a point well taken. And, you know, I don't know the best approach, if, you know, there can be composite extracts. But we were -- we're very conscious of this. And again, we could have bit off a bit more and shared the raw samples with participants in our trial and asked them to kind of apply their own extraction procedures.

If it turns out that we should have done that, I will take all the blame for missing an opportunity. And perhaps we should have, but there's so much variability downstream on processing that we thought if we had too many undefined variables - this is a classic statistics problem, right - that if you've got 10 variables and 30 methods, it's going to be very difficult to pinpoint which of those variables is more largely responsible for affecting the outcome than something else.

There's got to be a reasonable relationship between the number of variables and the number of -- in this case, of laboratories. So I think -- we collectively made the decision, but I can take the blame for it if it was a bad decision, to kind of just make one extraction and to send that out.

I think in follow-up efforts that could be done individually by any laboratory, but certainly by a consortium as well, that is something we need to look into more, because obviously there's going to be some issues with being too narrow in the wet chemistry procedures.

CHAIRPERSON SCHWARZMAN: All right. Thank you very much. I'm very intrigued by this notion of both how we can expand our non-targeted analysis studies, and also how we might inform some subsequent decisions about more targeted screening based on this future collaborate on getting this wish list -- my wish list of up and coming compounds.

So I want to trans -- thank you so much for your presentation --

DR. SOBUS: Thank you all very much.

(Applause.)

CHAIRPERSON SCHWARZMAN: -- and I want to transition now to turn the mic back over to Dr. Lauren Zeise.

So this is the part where we're going to honor Dr. Asa Bradman for his service to the Committee.

DIRECTOR ZEISE: Yes. We wanted to take a few moments to thank Asa. Asa, would you like to stand up?

Great. Okay.

(Thereupon an overhead presentation was presented as follows.)

DIRECTOR ZEISE: So on behalf of the Biomonitoring California program, I'd like to thank you for your outstanding service as a member of the Scientific Guidance Panel.

Asa was on the original Panel appointed in 2007 by Governor Schwarzenegger. And he assumed the Chair position in 2015. So in this program's 10th year -- it's Asa's 5th -- 10th year as serving on the Panel. And we really do have deep appreciation for all of your outstanding guidance, hearing your wide-ranging knowledge of assessing exposures to chemical hazards, long experience garnered as a researcher at UC Berkeley, and a lot of groundbreaking studies, particularly in children, including the CHAMACOS Study.

So we really did benefit so much from -- the Program did benefit so much from your unique insights and advice as an SGP member, and then your enthusiastic member -- your enthusiastic leadership as Chair.

So as I think we all know, you've long been an advocate of biomonitoring of children. And again, your many thoughtful contributions during SGP meetings have helped us shape key aspects of the Program's work over the past 10 years, and put special attention on exposures affecting disadvantaged groups and children.

So I know I speak for all Program staff in saying we'll miss working with Dr. Bradman as Panel members and SGP Chair. And we wish him the very best as he takes on fresh challenges, including some collaborations with State researchers. And we look forward from hearing about your future contributions.

Thank you, Asa.

(Applause.)

DR. BRADMAN: I just wanted to thank you, Lauren, and everyone on the Panel, and the Program, and the community that's been so supportive of this Program. And again, you know, I just want to emphasize how important this effort really is. And I have a feeling -- I'm here today. I have a feeling I'll be back at future meetings, or at least participating online, because these issues are so important to me, and also we'll have ongoing collaboration.

Thank you.

(Applause.)

CHAIRPERSON SCHWARZMAN: So that concludes the morning portion of the meeting. And I think we're ending about 15 minutes before the Program says so, but I have permission from the powers that be to do so with hope that we can actually move the afternoon session a little bit earlier, just because some Panel members have to leave a little bit early and we want to get to the discussion that has to do with a Panel vote in time to make sure we have all the Panel members here.

So we have an hour for lunch, which means we'll resume at 1:30. And that's -- just to highlight for people, that's a little bit shorter lunch than we've had in the past. So it's recommended that folks eat in the cafeteria that's right here and be back to resume the meeting right at 1:30 for our afternoon session.

And before we break for lunch, I just want to invite Fran Kammerer to give us our -- oh, it's Carl today. Sorry. Changed staff -- to remind us about the Bagley-Keene.

STAFF COUNSEL DeNIGRIS: Carl DeNigris, staff counsel for OEHHA. Just a reminder that the Panel is subject to Bagley-Keene, and refrain from discussing any matters that are before the Panel outside of this forum.

Thanks. Have a good lunch.

CHAIRPERSON SCHWARZMAN: Okay. So we'll conclude the meeting for this morning and resume at 1:30.

Thank you.

(Off record: 12:32 p.m.)

(Thereupon a lunch break was taken.)

AFTERNOON SESSION.

(On record: 1:34 p.m.)

CHAIRPERSON SCHWARZMAN: Okay. We're going to start. I'm going to bring the afternoon session to being. Thank you for coming back from lunch.

Before we start, I just wanted to mention apparently we're out of the packets of materials at the table when you walk in. But just so that everybody knows, all of the materials are posted online. So if you're wanting access to anything and it's not available in physical form here, you can get it on the Internet at the Biomonitoring California website.

So I'm -- I'd like to welcome Dr. Roy Gerona. He is here from UCSF. He runs the Clinical Toxicology and Environmental Biomonitoring Lab at UCSF, and has collaborated with the Program on Reproductive Health and the Environment at UCSF on suspect screening for endocrine-disrupting chemicals in biological samples. And most recently, Dr. Gerona's lab has been developing biomonitoring methods for pesticides including glyphosate, glufosinate and other organophosphate metabolites. And that's bio -- in biological samples obviously.

So he'll be presenting today his work on glyphosate. And thank you so much for coming to be with us.

(Thereupon an overhead presentation was presented as follows.)

DR. GERONA: Okay. Am I on?

All right. I'm on.

Okay. First of all, I'd like to thank Sara and the Advisory Panel for actually inviting us here to present the work that we're doing on glyphosate. I will not be the only one presenting this afternoon. There has been changes in the schedule of the graduate student who has done a lot of work in the method development for this work. So I'm going to be introducing him first, let him talk, and then I will follow it up with the applications that we've been working on.

Axel Adams graduated from the University of Wisconsin - Madison in 2014, which is where I came from as well. I did grad school there. And immediately after being admitted to the Joint Medical Program between UC Berkeley and UCSF, approached me and has in mind that what we wanted to do for his Master's thesis is really to develop methods for biomonitoring.

So the work that he will be presenting here is his Master's thesis. I am lucky to have him in the lab. His is a very good analytical chemist. And what he'll present -- or what you'll -- what you'll hear from him this afternoon are the challenges part, because often the paradigm here in America is you give the challenges to your graduate students --

(Laughter.)

DR. GERONA: -- and then you seek out the opportunities later once the challenges have been resolved. So she will be -- he will be presenting the challenges part in developing the method for glyphosate, and I will be following it up with opportunities where we think we would be able to contribute in epidemiological studies concerning glyphosate.

Axel.

MR. ADAMS: Yeah. Thank you so much for having me here today.

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MR. ADAMS: So to begin with the analyte in question, N-(Phosphonomethyl)glycine, I'll be referring to it as PMG, it's IUPAC name, also generically known by the trade name glyphosate.

It's a broad spectrum herbicide. It was originally discovered by a Swiss chemist, and was initially patented as a chelating agent. And it actually works as a chelating agent to induce its herbicidal action by chelating a manganese co-factor for one of the enzymes involved in aromatic amino acid production in plants.

It's the most widely used pesticide in the world with 113.4 million kilograms used in the United States in 2014 alone. That represents about a 30,000 percent increase from its original usage in 1974. And it's the most widely used domestic pesticide. It's also -- which means that in terms of like home usage, applying on lawns, applying in home gardens, things of that nature.

So it's used in a couple different ways. The primary way, and probably the way most people are familiar, is in conjugation with PMG-tolerant crops. So things like Roundup Ready soybeans, Roundup Ready corn that are developed to have enzymes that are resistant to the herbicidal action of PMG.

But it's also widely used with non-PMG tolerant crops. For instance, it's used as a preceding application to kill down weeds in fields. It's also used as a desiccation agent. So they'll apply it to, for instance, grain to hasten drying prior to harvest. And it's also used as a spot application in different agricultural settings like orchards, and also municipally, so like along railroad tracks, and parks, things like that.

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MR. ADAMS: So lately, the safety status of this pesticide has been contested. It was classified as not likely to be carcinogenic to humans by the US EPA in 2015. In 2014, the International Agency for Research on Cancer found an association between PMG exposure and non-Hodgkin's lymphoma in a meta-analysis, that looked at a number of different studies. It was slightly problematic because some of the studies were rather old at that point, and did not really assess exposure at moderate levels. And then there were issues with kind of the distribution of these settings and where they took place. So it may not have been representative of exposure in the general sense.

But nevertheless, in 2015, IARC performed its first review of PMG and classified it as Group 2A probable carcinogen. And there have been a number of different in vitro, in vivo studies kind of looking at different ways in which it might be carcinogenic. And there are a number of different proposed mechanisms, which is kind of beyond the scope of this talk, as we are analytical chemists.

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MR. ADAMS: Most methods for measuring PMG have been indirect methods. So what that means is you have the analyte in question, you conjugate a different moiety to it, so you're able to measure it better, and then you're able to do, for instance, gas chromatography-mass spectrometry. And there have been very few direct methods.

Direct methods are advantageous because they require less processing, which makes it easier to do large -- a large number of -- or very high throughput analysis, which is important for biomonitoring. They also generate fewer kind of toxic waste products. And green chemistry is kind of the goal in this day and age.

Of the direct methods, even fewer are applied in human matrices. So 2008, there's Wang et al. that looked at serum. It had a limit of quantitation of 5 nanograms per ml with ion-pair chromatography. You had Yoshioka et al. who used Obelisc N column, which is also the column we used. They had a limit of detection of 20 nanograms per ml, limit of quantitation of 90 nanogram per ml.

And then you had Jensen et al. 2016 that were looking at urine and also milk matrix. And their LOD and LOQ were reported based on the individual transitions. So I'll talk about this a little bit more. That's kind of problematic, and isn't very common in the literature. And there are several other problems with that paper that kind of call into question whether or not it's a good method.

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MR. ADAMS: So PMG itself is a very difficult analyte. It's kind of like -- being able to measure it effectively is sort of like the holy grail of small molecule mass spectrometry. And the reasons for that is that it's very low molecular weight. It has basic and acidic features. It doesn't have an analytically useful group like a fluorophore or chromophore. It has several different pKa's, and it's also a chelating agent, as I mentioned previously, for both bivalent and trivalent cations.

But fortunately, it's a very stable molecule. So the half-life in water is about 33 days. In soil, it's half-life has been reported up to about 250 days. And there's not very much data in half-life in other, you know, human or biological matrices.

Also, it is very stable in terms of in the soil. So it's a little bit unique among pesticides as it adsorbs very strongly to soil particles. And they can stay, you know, for great periods of time in soil. They can also be degraded on the soil absorption surface, so AMPA, which is the most commonly reported breakdown product, but also to sarcosine and formaldehyde that are subsequently broken down just to CO2 and water.

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MR. ADAMS: So what we did is we used liquid chromatography tandem-mass spectrometry, a very high fidelity method for analyzing small molecules. And the basic principle is it's a series of a different separation techniques. You separate first based on the overarching chemical features of the molecule, so its polarity. And then you separate based on the mass-to-charge ratio of the parent ion. You fragment that and then you separate it, based on the mass-to-charge ratio of the daughter ions. So it's kind of like a -- like a Russian nesting doll of separation science.

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MR. ADAMS: So when we were developing the method, first we looked at tap water, because that would be a very simple method to do. We were able to do that with external calibration, kind of a standard method. We just acidified the water, add our C-13 internal standard, and run a calibration curve, kind of very bread and butter analytical toxicology.

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MR. ADAMS: Add then what we found is in terms of precision accuracy studies, it's very straightforward, as you would imagine, not a complex matrix.

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MR. ADAMS: Now, the issue was in terms of transferring it to urine and breast milk, those are much more complex matrices than tap water, as you'd imagine. And there were several different problems with both matrix. So usually what we want to do is we want to, if we're doing external calibration, run a calibration curve and a matrix blank. So in terms of urine, we would get drug-free urine or urine that's free of the analyte.

But it had been reported in the literature actually by Jensen et al, that drug-free urine that was commercially available had trace -- a trace signal of PMG in it. So that's problematic, because then where are you getting your drug-free urine?

We also reproduced that and found that in the drug-free urine we were obtaining, we could also detect a signal. You can use synthetic urine which is not quite as good as human urine, because you're not capturing all the different aspects of the metabolome.

Similarly with milk, there's no commercially available like breast milk or even bovine milk reference standard. So really what that leaves you is internal standard addition. So that's internal calibration.

So basically what you're doing is you're spiking the analyte at different levels into the individual sample that you have. So if you have it for Mr. A, you're going to have four aliquots of Mr. A's urine spiked at different concentrations. It's really a robust method. It's a little bit annoying because you have to do more aliquots and submit it for analysis, but it is very robust.

The other problems with PMG is it elutes very near the solvent front. So in the liquid chromatography, you basically have a dumping of a bunch of different analytes at the same time, as soon as you open the column into the mass spectrometer. And that develops a bunch of background noise and interferes with your signal-to-noise ratio.

So looking at urine, we were able to do that relatively straightforward. We looked at drug-free human urine and we also collected urine from lab members, which is not usually what we do.

(Laughter.)

MR. ADAMS: You know, anyway, but -- so we diluted it 10 times, and acidulated it with formic acid. We spiked in our internal standard at very high concentrations, so there wouldn't be any interference with naturally occurring C-13 PMG, which occurs as part of the synthesis process. And then we ran our standard addition curve in each individual sample.

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MR. ADAMS: What we found was we were able to get very good precision and accuracy, very linear within the standard calibration. What this graph is showing is that there's something known as rotational matrix effects. And that means that the matrix effects vary among individuals in a concentration-dependent manner.

So that's ideal for dealing with -- that's an ideal form of matrix effects to deal with with standard addition. There's also translational matrix effects, which you can't deal with standard addition. So we were able to demonstrate that it was a very valid way of dealing with matrix effects to use standard addition.

And we were able to achieve a limit of detection of 0.1 nanograms of PMG per ml, so 0.1 parts per billion.

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MR. ADAMS: Moving on to milk, which is a very, very difficult matrix. It's far more difficult -- a far more difficult matrix than, for instance, blood. And the reason for that is it's a complex mixture. So in milk you have a bunch of different fractions. You have a solution of globular whey proteins, you have colloidal casein suspension, and you have lipid emulsion. The casein suspension is very interesting, because in the casein micelle, which is a protein micelle, you have a lot of different cations.

And as you recall, PMG is a chelating agent. So there's -- it's thought that it could be interacting with these casein micelle. So you have to drop out the casein micelles, drop out the whey proteins, deal with the lipids, because the lipids are a very problematic source of matrix effect, in analytical chemistry, and then finally, you know, get as much of your analyte as you can in the aqueous solution.

And there is a number of different ways you can deal with each one of these different sources of obstacles separately. But what we did is we diluted it to deal with -- basically to separate out the mixture, acidifying it, basically dropping out the casein micelles, going past isoelectric points so they drop out solution. You can then centrifuge it to bring those to the top of the solution, because they're a very low density protein.

You can then denature the globular proteins with dichloromethane, centrifuge it, drop that to the bottom, and then dilute the aqueous solution again. The DCM also acts as a liquid-liquid extraction, so you're able to remove a lot of the phospholipids, and then just have the PMG in your aqueous fraction.

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MR. ADAMS: So what we were able to find is that when we looked at precision and accuracy studies in milk, we were able to get good precision, good accuracy, very good linear range across a pretty wide range -- dynamic range of 1 to 40 parts per billion. We were also getting relatively good recoveries, as low as 1 part per billion, and a limit of detection of slightly under 0.2 parts per billion for milk and a limit of quantitation of 1 part per billion for milk.

There are two statistics that are important for standard addition, the cap on Q statistics, which are calculated based on the level that you're expecting to see in your sample. And there haven't been good studies on actual levels that are observable in milk, so these are uncalculated. We can kind of estimate what they would be.

And the reason you would do that is you would adjust the fortifications for the standard addition in order to ensure that you have like the maximally valid internal calibration curve.

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MR. ADAMS: But in summary, it looks like it -- this way of dealing with the complexities of the milk matrix, in conjunction with the complexities of PMG as an analyte, is a relatively reliable way of doing it -- doing so.

So problems with our method, there are several. One is that the Obelisc N column, which is a HILIC column that we used, and I mentioned previously is also used by Yoshioka et el. for dealing with blood as a matrix, degrades relatively rapidly. And it's an expensive column. We've been able to do about 1,000 injections in milk before it degraded.

So it is kind of an expensive method in that regard. And that was also reported by Yoshioka that they had problems with degradation just from how dirty the matrix is. And HILIC columns tend to be a little bit more fragile. The standard addition also necessitates 4 aliquots per individual sample. So if you're analyzing Mr. A's urine, you're going to have 4 aliquots of his urine that you have to run.

So if this is -- our method is 6 minutes, so you have 4 by 6 minutes of time on the machine, and as you multiply that out for large and larger data sets, that's a substantial increase in time, and also time in preparation for the lab technician who's preparing the samples.

However, it's slightly counterbalanced, because you don't have the external calibration curve to create. And we figured that, you know, the increase in validity of the method counteracted the extra time it took. Also, with the current method, AMPA was not detectable due to the short retention time. I mentioned previously that it's eluting very close to the elution front. So you're basically having a dumping of all these different analytes at the same time, and AMPA's was in there.

And as result, you have a higher background noise that makes it -- your limit of detection and your limit of quantitation rise higher. Also, whether or not AMPA is a good analyte to look at is up for debate. It had initially been thought that AMPA was the degradation product of PMG. However, there's more and more evidence suggesting that in, you know, for instance, soils or environments that are naive to PMG, it's more commonly broken down by soil bacteria sarcosine and formaldehyde, and subsequently to CO2 and water.

In which case, you don't have a very good stable degradation product to analyze. And so it may be in terms if you're actually looking at accessing exposure, it may just be worthwhile to focus on PMG itself, as opposed to this other degradation product, which may not represent the major pathway of degradation.

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DR. GERONA: Thanks for that clear presentation.

I think that it's the most exciting part of the presentation that we have this afternoon.

(Laughter.)

DR. GERONA: So now that the challenge has been, you know, answered, or responded to, our task in the lab is to find ways by which we can apply this method. And then fortunately, there are a lot of studies. One thing to use the method.

I would preface this -- this set of slides by saying that we are analytical chemists. And so what we intend to do is to present you the data and have other experts start the dialogue on how that data should be interpreted. We will not comment on the implications of a lot of this data, because we think that it is outside our area of expertise. So we're presenting you the data. So let's start the dialogue and let's plan on what to do with the data.

Obviously, some of this data needs to be verified. That's why we're also looking for partner laboratories that can verify the results that we will be presenting to you this afternoon.

So immediately, or even before the method development started, we already had partners who are actually require -- who require that the data be developed, so that we can provide precise and accurate data on glyphosate in biological samples.

We have five cohorts that we're currently working on. Some of them have been completed. Some are ongoing, and some are about to start. So for the first cohort, the Detox Project, it actually started as the Feed the World Study. And this is a study that is sponsored by the Organic Consumers Association.

These are crowdsourced urine and tap water samples collected across the United States. At the end of the -- at the end of the project, we have about 252 urine samples collected. This study has been completed. And so we'll present to you the data that we've obtained from this work.

Now, one of the lessons that we learned from the Feed the World or the Detox Project is that the midwest is, as expected I guess, has -- basically, the cohort from the midwest from The Detox Project has the highest levels of glyphosate that we're detecting. And so we were very interested on finding specific cohorts in the midwest to see what would be the levels of glyphosate that we will be measuring.

So there are two cohorts that we were able to find. The group of Paul Winchester in Indiana has provided us with urine samples from pregnant women. The total number of samples is about 283. We will be presenting the results of the first 83 samples this afternoon. We have run all 283. The remaining 200 needs to be analyzed.

The third cohort is very interesting. This cohort was not really meant for studying glyphosate. This was actually a cohort from Gail McCarver at the Medical College of Wisconsin. And this was a cohort where Gail was looking at BPA levels the first year of life.

So Gail has time points from day 0 to day 365 urine samples from these infants, where we previously measured BPA. But when the -- when the project was over, we have a lot of left-over urine samples. And so when this opportunity came in, we thought this is an excellent set of samples to look at whether -- especially the day 0 urine, day 0, day 2 urine.

And so we collected and gathered leftover urine samples. We were able to actually collect 36 samples -- my timer is not flashing yet, so that's good -- 36 infants. And we have complete samples for day 0, day 2 as the first time point; the first week, so that's about day 7, day 10; days 180 to 185, that's six months; and then day 360 to 365, that's about one year.

It's ongoing. We have done our initial studies, and I would just say that we have some interesting results, in the sense that we're finding glyphosate even in some day 0 urine samples. So we know that this is controversial. It's going to be controversial. I guess if we were to report it. And that is the reason why it's very important that our study is verified by an independent lab, that the blind samples can be forwarded to the independent lab to see whether they can reproduce the results that we're getting in the laboratory.

I'm not going to be discussing more of cohort 3, except saying that, you know, that's our initial finding that we're getting.

Cohort 4 -- and this is again -- you know, we're very lucky to be able to collaborate with the MARBLES Study. Cheryl Walker from UC Davis will be able to provide us with 60 breast milk samples, 30 from children with -- that were diagnosed with autism, and 30 that are age-matched control. So we're getting the breast milk of obviously the mom.

We're also going to be getting the urine samples collected when these children are being conceived, first trimester, second trimester, and third trimester urine samples from this specific cohort. We haven't received the samples. We're very excited to receive them and start the analysis.

And ultimately, our group wanted to actually demonstrate that -- or, not demonstrate, but ask the question would the mom's exposure, is that actually being delivered to the baby in the first year of life. If the only source of food of the baby is mom's breast milk, are we going to be able to find glyphosate in the infant's urine.

So it's a study where we're collecting matched serum, urine, breast milk, infant urine. And we're recruiting for this particular study.

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DR. GERONA: So let me -- let me present the first cohort. So as I've said, the Detox Project is a crowdsourced project. What we did here is the Organic Consumers Association partnered with us. We were the lab testing the urine samples for the individuals who wanted to actually have their glyphosate level in their urine tested by a laboratory.

So in this particular study, we were limited, of course, with data that we can get from individuals. So unfortunately, there's no questionnaire that is associated with this study. We were given limited demographic information like the gender, the age, where the samples came from, and some times we're given tap water as well.

So most of the samples came from the United States.

Is this also a pointer?

Oh. Okay. So we were able -- so the collection or the analysis of sample -- Five minutes. I can go over by another five, right, so I have 10 minutes?

(Laughter.)

DR. GERONA: So there are 252 urine samples. We actually collected samples between April of 2015 to February of 2016. As you can see, the number of samples, a bit lopsided with the west coast. There's about 124 samples. It's very interesting that when we did this work, what we were expecting is that most of these people who are sending us samples are very much aware of the debate that is going on on glyphosate.

So we were expecting that these people are probably people who are very much concerned about the food that they eat. And we were thinking, oh, gosh, probably what we will see is nothing that we can detect.

So it was actually a surprise to us to get an 86.1 percent detection frequency overall for the United States. The midwest, as I've said, it's slightly higher than all the other sources.

As you can see here, the median that we're getting is about -- sorry the geometric mean that we're getting is about 2.5, slightly higher for the midwest. There's no statistical significance, or they're not statistically -- they're not significantly different, even though you have this levels of 2.3 and 2.8.

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DR. GERONA: So when you have a crowdsourced sample, you have to report back. So one thing that I need to disclose for -- four minutes or seven minutes.

(Laughter.)

DR. GERONA: -- to disclose is that these people paid for the analysis, right, so -- because we don't have any funding to actually support the analysis. So they paid for the analysis a very minimal cost. They really don't know who's analyzing their sample, because we did not announce this -- that UCSF has announced -- that they're blind to whoever.

So one of the things that you worry about, especially when you report back to the lay public is if you tell them, oh, your glyphosate is 3.1 ppb, how will that person interpret it, right? It's always the interpretation of that data that is an issue.

So when we give them the report backs, we give them some reference points on how they could possibly interpret the data. This is not the only -- the only information that we provide. There are -- they get a sheet where they have the averages for all the different parts of the United States, the averages for the months that we were collecting, but they also get this table where we have their values in green, the running average at the time that we actually gave the results to them. And these all other dots is in that batch where is there -- where is their value with reference to the other people that we measured for that particular batch.

We also compare what we're getting to other studies that have already been reported in the literature --

Seven minutes now.

-- a European study, which is by Hoppe et al., and then the averages of the farmer study, where they -- these are farmers from Minnesota and North Carolina, where the method, I think, is HPLC, where they looked at the levels of glyphosate on the application day of glyphosate, post-application day 1, post-application day 2, and post-application day 3. Now, there was also a study where they compared non-farm versus farm households.

And so what's presented here are the averages for the adult male, and the adult female, and then the child. And as you can see, it's clear here that there's really not much difference whether you're in a non-farm or the farm setting. And most -- in both cases, it's the child that has the higher -- higher level of glyphosate that was measured.

Now, this is an additional graph, and what I want you to specifically look at here are the last two spreads or scatter data for this specific set of data. What we did here was we collected all the samples that were collected from California between April 19th to June 28th. And so we asked the question, are we repeating this observation from the Curwin et al. Study?

And the answer is yes. If you look at the same household, the average that you'll get actually for the child is higher than for the adults.

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DR. GERONA: Then -- six minutes -- again of pregnant women. So I told you we will present the first 83 urine samples that we have studied. And as you can see, this is a heat map of the agricultural use of glyphosate in the United States. There is a particular interest in the midwest, because that is where most of the agricultural use is happening. So we were thinking, okay, so it would be very interesting to see the exposure levels of people in the midwest.

And that's what we did here. So we have, in this particular table, we're comparing it with the Detox Project, where we have level -- the statistics for the whole U.S., the whole midwest, and what we saw in Indiana. You have 83 samples from Indiana. The detection frequency is about similar to the detection frequency of -- for the midwest, which is slightly higher than the average for the whole United States. And the geometric mean is slightly higher also for Indiana.

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DR. GERONA: I have three slides, so you will let me finish, okay?

(Laughter.)

DR. GERONA: So this study we were able to contextualize the results a little bit more, primarily because when they collected the urine samples, they were already looking at potential association between pesticide use -- and not necessarily just glyphosate -- of pesticide use and potential sources of exposure. So they have a questionnaire associated with this study.

This -- I think the -- this is the same cohort where they look at association between atrazine levels and potential exposure sources. So in terms of demographics, as you can see here, what's so interesting and what's probably expected, we looked at those that are living in the rural area, the suburban and the urban area, and you see a statistical significant -- significantly different levels between those living in the rural and the suburban area.

We don't quite know how to explain this. So -- and the other demographics that we got is the BMI, prenatal. So this is prenatal BMI of the women, where we got the urine samples from. And as you can see here, the healthy ones are the ones that have the higher level, and the obese ones are the ones with the lower level.

As I've said, you know, we don't now how to interpret -- well, our collaborator is still studying this data set.

I would like to preface though that these are only 83 samples. And that's the reason why they adding the 200 samples that we're still analyzing would be very, very good to see whether this will actually be still the same.

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DR. GERONA: And so the other thing that we did is we divided the levels into terciles. And as you can see here, what's interesting is when we did that particular division of the data, we found out that -- we looked at full-term births only -- that there is a trend in this particular data. I won't comment in the statistical significance yet, because we're still adding on data on this particular cohort.

But you can already see a trend that the ones with low values have longer pregnancy length than the ones with the higher values. We actually did a different type of analysis -- or they did a different type of analysis which I will show in the next slide.

For the birth weight percentile, how did we get the birth weight percentile? So we looked at published curves of birth weight at specific gestation age, and looked at the birth weight percentile of each of the individual babies that are part of this particular study.

And again, so what you're seeing here is that the ones -- the ones that have high levels of -- the high tercile has the lowest birth weight percentile average.

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DR. GERONA: And then finally the last slide that I'm presenting, so I told you that, you know, we did the opposite. We looked at -- instead of dividing the values, let's look at term births again, let's look at their gestation at birth, and see when we group those at 37, 38, 39, 40, 41 what will we see in terms of the levels of glyphosate that we were measuring.

As you can see here, so those between 40 and 41 -- statistically significant with P-value of 0.02. Those with obviously stars, they're statistically significant also. This one is not statistically significant. You'd probably wonder why. What we're not showing here is the spread of the data, and that's why it's not statistically significant.

Now, this is -- this is an interesting coincidence. We -- so in this particular cohort, they also asked the stress level of the subject at the time of the collection of the urine sample. And so they asked them to judge it between 0 to 5. And as you can see here, the week of gestation correlated with the stress level, the ones with the highest gestation length has the lowest average score.

And the trend that you're seeing here, quite similar to the trend that you're seeing with the glyphosate level. It's a coincidence. We're -- you know, I -- we would want to know whether there is really an association between this.

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DR. GERONA: And so what are the future directions? We would want to revalidate the milk method with actual milk samples from UC Davis. And Axel is still not off the hook. He will still come back to the lab and do this.

(Laughter.)

DR. GERONA: We will finish the current collaborative epidemiological studies that we're doing. We want to assist another lab with methods run transfer. This is key because we think we think -- we really wanted another lab to independently validate the results that we're getting.

There have been several groups that have already approached us to do work. Aside from those five, there are three more groups that we're probably working with in terms of urine, and breast milk, and tap water levels. And we would incorporate this method in a multi-analyte method for polar pesticides. We're also -- these are some of the analytes that we are working on as well. These are only some. There are several we have about, I think, 25 now, organophosphate metabolites, glufosinate, and 2,4-D.

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DR. GERONA: And with that, I would like to thank all our funders and collaborators. This was Axel when he's much younger.

(Laughter.)

DR. GERONA: Some people who have done a lot of work on this also. It's not -- Matt Friesen is my RA who started the lab with me. So he initially was developing the method for tap water. And Ann Gordon is the QA manager of the lab. Anita Wen is our expert in method development and validation, and acts as a consultant in the lab.

These are the collaborators that we have in those cohorts, which I think has already been mentioned. And special thanks to Asa, because we have been consulting him also in this study.

And with that, we will -- Axel will take all the questions.

(Laughter.)

(Applause.)

CHAIRPERSON SCHWARZMAN: Thank you very much. We'll start with clarifying questions from the Panel.

PANEL MEMBER LUDERER: I have one.

Can you hear me now?

Okay. I thought -- and I may have just misunderstood this, but when you were talking about the milk analyses -- by the way this is really exciting work and thank you very much for this -- the great presentation.

I thought you said something about that PMG is interacting with the casein and the micelles. So I -- what -- and maybe I didn't understand what you meant by interacting. But then if you're centrifuging to remove the micelles, aren't you going to be removing some of the PMG in the sample?

MR. ADAMS: Yeah. So that's a very good point. And so the reason -- the way that we deal with that is by adding the heavy isotope of the PMG itself. So basically you add that at the beginning, and then you're able to do recovery studies. And we were finding that we weren't losing that much during the analysis of that heavy isotope.

The other good thing about standard addition is it kind of takes into account the losses that you're going to have within the sample itself. So you might have -- you know, depending on the external matrix that you're using, you may have different casein concentrations, and then you'd expect a difference.So standard addition deals with that in and of itself.

The other thing is that the casein interaction is more of theoretical one. It hasn't been well demonstrated, and well -- because there isn't very much data on analytical chemistry in milk as a matrix. So what we did is we looked at analyses for other different smaller organic acids in milk.

And the thought is that -- so casein is a linear protein -- low density and linear protein that organizes itself in micelles in milk. And if you can bring it down -- bring down the pH of the solution past the isoelectric point, you basically disrupt the associations between the proteins. And since it's such a low density, it actually floats to the surface.

And so presumably you'd have freeing of the glyphosate within the aqueous layer, and then you're able to dip your pipette tip, sample the aqueous, and then do your subsequent liquid-liquid. But the internal standard is how we deal with losses.

PANEL MEMBER LUDERER: That was added before you did all the process?

MR. ADAMS: Yeah, exactly. That's how we calculated recovery.

DR. GERONA: It may -- I may just add to this. You know, when -- we started developing the method in 2014, because we actually have collaborators already in 2014. When we look at all the methods that are published in the literature, they're all using external calibration.

And so we dried our darndest to actually repeat or reproduce some of the -- some of the work that they were doing. But with tap water we're able to do that.

With urine and breast milk, we were not able to do that. And our findings consistently is that every urine sample is unique.

And so you would get very different matrix effects from one urine sample to the other. That's when we basically made the decision and say, okay, what other alternative methods can we use? If external calibration is not working, then what other -- what other armaments does analytical chemists have to approach this problem?

And that's when we shifted to standard addition. That's the reason why, you know, every time we were asked about the method -- and we have been requested by several different laboratories to basically just tell them what our method was. And we say -- we're telling them that, you know, we want our work published, because it's -- in the end, it's really a simple method that we're able to work on, at least for urine, not for milk. Milk, as you see, is quite complicated in that.

MR. ADAMS: And something, too, I failed to mention is another reason why internal calibration is kind of perfect for milk as a matrix is during the lactation course for an individual, the constitution of the milk varies. So like early on, you have the cholesterol. It's a more heavy lipid milk. And then it goes and you get increasing protein, and then you get increasing levels of lactose, all of which build in background signal.

And so if you're going to be using, you know, a commercially available drug-free human milk, where is that milk from? And also, there are variations in the composition of milk throughout the day. There are variations based on what the mother is eating. And so internal calibration is a way of dealing with all those different factors at the same time.

CHAIRPERSON SCHWARZMAN: Other Panel questions?

Okay. Then we have some time for public comment. Is there any public comment?

There's one in the room. Did you want to --

DR. GERONA: So you believed our presentation?

(Laughter.)

DR. SHE: Thank you very much for the excellent presentation regarding delineating a very polar compound in the very complex mixture like urine.

So as you're very well aware, the limitation of the standard addition method, which is throughput. But as far as you already know, the standard addition method solves the rotational matrix effect perfectly, which was traditionally used for the metals analysis, because they do not require longer analysis.

So basically, I think you find a very smart approach to solve the problem. And my only comment is how you resolve the throughput problem for the wide, large epidemiological research, because basically, every single sample you do a calibration on it. That's the first comment.

The second one is we did try using it because for the OPFR. And I discuss with Myrto, we use the standard addition, but we haven't summarized data, because OPFR tend to be very polar.

And another approach I think you are already searched. You referred as indirect analysis, which is more kind of -- I guess, you mean is derivatized, which after derivatized analytes, the polarity has changed. And then now you can extract the analyte and separate it from matrix, so -- which is indirect, but when the industry start with the PMG, that's EPA request that provide method on the derivatized method that did that. Our laboratory used derivatized approach, for example, to analyze the DAPs. That's the CDC's method.

And also, we use derivatized method to analyze the PAH. And so it seem to be okay after you derivatize. Now, suppose you eliminate the matrix effect. One is you compensate matrix effect by vertical approach. So you can -- can you give us some -- a little bit more details in review of this method?

And also, we -- I'd like to emphasize, we may be a candidate to get your method transferred, because we work on different disease, but if that's Nerissa and other program leaders' decision, but we are possibly a good candidate, because we work on the urine, and on the similar chemicals.

DR. GERONA: You know, thanks for the -- for your offer, because we're actually -- so to answer your first question about throughput. That is the reason why we need partners, and that is the reason why we're looking for labs where we can assess whether the method that they will be developing its -- if it's standard addition, we want to assess whether there's concordance in the data between our lab and your lab.

The high throughput problem with standard addition, I mean, that's one of the reasons why we -- originally, the Feed the World Project really wanted us to continue the work, but they were predicting thousands of samples every month. And I told them with -- with the approach that we have, it will not just be compatible with the number of -- that number of samples.

For one, this is just one part of several studies that we're doing in the lab. We have probably a dozen projects going on at the same time. And so you're right, you know, one of the disadvantages of the, you know, standard addition method is instead of analyzing one, for every sample we analyze four, and then we -- obviously, we run that in replicates also. I would let Axel comment on some of the -- some of the methodologies that were also in the literature, and I'll also supplement it if needs to be.

MR. ADAMS: Yeah. So thank you. In terms of the throughput, it's really not that bad for the urine preparation, because that's basically dilute and shoot just by four.

With milk, it's a pretty arduous method, because it's -- you know, it's -- as you precipitate out the casein and phospholipids, you have to make sure not to disrupt it back into the solution when you sample the aqueous layer from below. And it's a very -- it's kind of a technical method from start to finish, and it takes a lot of time.

That we don't have a good answer for. It's -- you know, the method is at it stands. And it's a little bit slower. But that being said, I think it's a pretty robust approach for a lot of different analytes in milk. And there are not very many methods published for different analytes in milk, because it's such a difficult matrix.

With regards to conjugation derivatization methods, when we approached this, we were initially thinking of throughput and we wanted a direct method for throughput reasons. And so from the very beginning from method development, we were reviewing direct methods and trying to figure out a direct method that didn't require a derivatization step and the additional time and cost involved with that.

And so there are -- there are a lot of derivatization methods out there that are very good. But from our end, it was more kind of like you had to pick a problem with throughput on one end or the other. And so we opted for something that was maybe a little bit more tedious in terms of time, but didn't require those other resources. And so it was something of an arbitrary decision, I guess.

DR. GERONA: Yeah. And in addition to that, you know, I know that throughput is always -- with epidemiological studies, it's always something that drives the study that, you know, you need to be able to analyze this number of samples in this given amount of time. But for us, when we were starting it, the question that we were asking is has somebody got it correctly already?

And so to us what is more important is if we're going to be developing the method, we don't care how slow it is. We would rather give you the correct answer in the accurate and precise method, then -- and then worry about the high throughput later.

There are now -- so now that we think that we actually have a method that is reliable, we can start thinking and we can start the dialogue with different laboratories to see whether are there ways by which we can actually shorten the method or maybe do it faster, if there is really a need to do that based on the interactions that we have with different groups?

CHAIRPERSON SCHWARZMAN: Moving on from clarifying questions, we have time now for discussion, and if you wouldn't mind staying up. It often turns into discussion with the presenters.

So I'll open it up to discussion from the Panel and also welcome discussion from the people in the room.

CHAIRPERSON SCHWARZMAN: Dr. Quintana.

PANEL MEMBER QUINTANA: Hi. Thank you for the presentation. And I know that your samples, especially the first ones you show, were not randomly selected or anything. But you were speaking about the study, and I was expecting more variability across the regions than I saw. And I'm wondering if you were surprised by that in general? Even the data you showed later, kind of a surprising amount in lots of people and quite similar.

DR. GERONA: Yeah. So -- and if I can comment about that. So there are a lot of surprises in that Detox

Project results. I mean, I've already pointed out one, right? Why are we detecting glyphosate in 86.1 percent of this population?

If the people that we're already -- we're already screening for are people who are already aware, and most likely are eating organic food, who are very much aware of their day-to-day exposure, we were thinking that we will get so much less.

But I guess if you look -- so the FDA started -- actually, I might have it here. So these are -- these are some of the other interesting data. If we were given one hour presentation --

(Laughter.)

DR. GERONA: -- we would actually be able to present all this data.

So if you look at -- so there has been effort in actually looking at what could products contain -- contain glyphosate, and how much levels are you actually getting from each one of these?

And so -- and you -- can

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DR. GERONA: You know, I'm presenting this to you -- so some of the product -- common products -- Cheerios is 1,125.3 ppb. Ritz crackers. These brownies from Kashi, who doesn't eat that, right?

(Laughter.)

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DR. GERONA: Some honey has 41 ppb glyphosate.

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DR. GERONA: Quaker Oats, Stacy's Organic -- this is organic, Stacy's Organic Simply Naked Pita Chips has one. California -- California wines have it.

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DR. GERONA: And all of this. I mean, who doesn't eat Oreo's, Lay's? I eat Lay's all the time, and so --

(Laughter.)

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DR. GERONA: So as you can see, we present you with all this data on the sources and the levels that we're measuring. I think it's easy to understand why we're getting what we're getting. That pretty much everything you eat -- almost everything you eat might have some -- the worst thing is this, there are food that we -- you know, you would think -- so, for example, protein bars, for example, right? It's like before I go to the gym, I'll eat my protein bar. That's healthy. Well, what kind of protein is a protein bar?

Soy. And how much of the soy in the United States is GMO soy?

MR. ADAMS: Ninety-six percent.

DR. GERONA: This is why he's my graduate student.

(Laughter.)

DR. GERONA: And so I think that in itself answers the question. We looked at tap water. Tap water, as -- I don't know if I mentioned this -- we analyzed 110 tap water samples from The Detox Project, only two actually have glyphosate, one from Simi Valley and one from Quincy, Washington. The levels is 0.02 ppb. And we were asking ourselves why is it that tap water won't have it?

Well, if you think about the treatment of water for your tap water, there is a step of phosphate -- phosphate precipitation. And glyphosate has phosphate. So we do think that, you know, well it's good that our tap water does not have glyphosate, but the sad -- the corollary to that is even if your tap water doesn't have it, you still have it. Where else is this coming from, right? I think it's clear that the answer is probably the food that we eat.

MR. ADAMS: Yeah. The other thing I'd like to add is that in addition to these reference values, the UN FAO basically set an ADI for PMG of 60 milligrams per person per day. And they estimated the average intake for this pesticide for 17 model diets. And those range from around 88 to 530 micrograms per person per day.

So that's a -- you know, we're reporting in parts per billion or nanograms per ml. So really it kind of makes sense, the levels we see based on, you know, residues that are already in different food products and even, you know, estimates of dietary intake.

DR. GERONA: Can you mention something about why -- where we're seeing it in organic food?

MR. ADAMS: Oh, yeah. So there was a study done by the USGS, in I believe 2014, looking at levels in about 4,000 hydrologic samples. And what they found is that they had the highest detection frequency as you might expect in drainage ponds, things like that where there's high levels of agricultural runoff.

But something that was quite interesting was that they were detecting it relatively -- at relatively high levels in terms of detection frequency. About 71 percent of precipitation samples had detectable levels of PMG. And the reason for that is pesticide drift.

And so even if, you know, say there is an organic production field where they're producing organic soy for Kashi Brownies or whatever, you're still going to be exposed to pesticide drift. There's also -- there's also an issue where, as I mentioned previously this is a very stable pesticide in terms of soil adsorption. It's rather unique in that aspect. The phosphate forms associations with different aspects of the basic crystal matrix of the soil and can stay there for very long periods of time. So even if it was a conventional field in the past, there may still be residues that are being incorporated.

There was also a study in Norway, in I believe 2015 or 2016, that looked at levels in different soybeans. There was -- there was organic soybeans, conventional, and Roundup Ready. And they were still detecting residues in organic soy. So there's still, you know, exposure from pesticide drift, runoff, and also just residues in soil.

DR. GERONA: So -- and that is probably the reason why, you know, in these 83 urine samples, we actually also surveyed organic food consumption of these individuals. And so even though this obviously relied on whether participants are telling the truth or not, you have the always/frequently/rarely/never, there was no significant difference in terms of the levels that we're finding versus those that are frequently organic -- consuming organic food and those that are not consuming organic food.

CHAIRPERSON SCHWARZMAN: May I ask just a quick follow-up question on that? You mentioned this study of the different soys. And there was glyphosate residues in the organic soy, but was there -- were there differences in the Roundup Ready versus the --

MR. ADAMS: Yeah, that was the conclusion that they had as well, that in Roundup Ready, there are higher residues than conventional soy. So as I mentioned previously, you're not going to be using PMG as an herbicide in non-Roundup Ready soy, because you'll kill the soy, but it's still used as a desiccation agent, and a field pre-treatment agent. So there are still residues that are being -- there are still going to be residues on the crop itself.

CHAIRPERSON SCHWARZMAN: But there was a difference observed?

MR. ADAMS: Yes. Yep.

CHAIRPERSON SCHWARZMAN: Other comments or questions?

DR. PARK: DTSC, June-Soo Park. It was a great presentation. Thank you.

This might be a very naive question too. First, in the -- it's quite an acidic compound. I don't know what was the motivation you developed, the method to analyze breast milk? The urine sound good matrix for this kind of compound, Mr. Adams, as an alternative?

But you struggled and spend a lot of time and effort to develop this method due to the complication of matrix I believe. So that was the first question. But I -- my main question was the -- also you developed the method. You know, that you have -- you sound like you have a very robust one.

I wonder, you know, the non-persistent chemicals like glyphosate, I don't know if this -- have you tried some -- tried to measure some depuration effect or anybody done it? Like you kind of have a different period of, you know, the breast feeding and any decreasing over the time. So that was kind of main -- my major question.

DR. GERONA: So let me address the motivation first, why we developed a method for breast milk. When we were approached, there are two questions that our collaborators are asking. At the time that they approached us, there has been a lot of claims that because glyphosate is polar, that you will not find it in a matrix that is quite hydrophobic. And as you can -- as you know, breast milk has a lot of nonpolar compounds.

The second concern is when you talk about compounds that could potentially be an endocrine disruptor or toxic, the concern always is which is the most vulnerable part of the population, right? The vulnerable part of the population, of course, are infants. And then we were asking also the question, well, but if the infant -- so if the source is food, infants are not necessarily eating all this -- eating all this solid food, so the main food for the baby is either formula or breast milk.

And so what we wanted to -- the reason why we started developing the method actually is to ask that question, whether the exposure of the mom, which we were predicting to be a high detection frequency. This translates to an exposure of the infant.

And so as you can see in the slide that I presented, that particular study is still ongoing. And ultimately, that's really the study that we wanted to publish -- to actually say that in these matched samples from different biological matrices between the mother and the infant that we may or may not be seeing glyphosate.

MR. ADAMS: Yeah. The other aspect of that is that there was a unpublished study that was performed by an advocacy group, Moms Across America, that used immunoassay to detect PMG in breast milk. And they found, I think it was, 7 out of 11 samples. Immunoassay is not going to work very well for an analyte like this, because it's small. It's N-(Phosphonomethyl)glycine, so it's very similar to amino acids. It is a phosphate group. Everything in your body has a phosphate group.

And so we wanted a mass spec method that could basically double check those kind of studies. The other thing is it is very polar, and you wouldn't expect that to necessarily partition into milk. But there were a couple kind of biological reasons that we were wondering whether or not it was going into milk.

And one was whether or not milk was acting as an ion trap. So the idea that this compound might be going across into a new environment that has a different pH, changing its polarity by its protonation status and not diffusing back. So there are a couple different systems that that acts on, like the uterus can act like that, and you can have crop passage of drugs that accumulate in the amniotic fluid, because it's acting as an ion trap.

The other thing is that so there are all type of amino acid transporters, LAT1 and LAT2. And those have been shown to transport PMG across epithelial barriers. And those are present in mammary epithelium. So we know that there's a transporter by which it could possibly get into milk. We think that there may be this acid trap idea or acid trap mechanism happening as well. So we would -- just wanted to develop a method that could be able to answer that question, if anyone wanted to ask it.

Further more, dairy in Wisconsin -- we're both from Wisconsin, so, you know, dairy --

(Laughter.)

MR. ADAMS: -- dairy is big, and we wanted to develop a method that could be also applied in the dairy industry for quality assurance for, you know, milk samples.

DR. GERONA: We also have happy cows in Wisconsin, not only in California.

(Laughter.)

CHAIRPERSON SCHWARZMAN: Do you have a follow-up question?

DR. PARK: Yeah, just one more thing. I'm from Wisconsin too.

(Laughter.)

DR. GERONA: Oh, so maybe we should collaborate.

(Laughter.)

CHAIRPERSON SCHWARZMAN: Other questions or discussion points, comments?

Okay. One more.

PANEL MEMBER LUDERER: I do have one more quick one. In your comparison in the Detox Project graph, I noticed that the European study levels were really low.

DR. GERONA: 0.17.

PANEL MEMBER LUDERER: Yeah, and is that using the same method?

DR. GERONA: No.

PANEL MEMBER LUDERER: No. That was my question.

DR. GERONA: So -- so -- yeah, yeah, yeah.

PANEL MEMBER LUDERER: How comparable are the different studies?

DR. GERONA: So that's actually -- so when you compare -- especially when you compare between studies. The first thing that an analytical chemist could ask is what's the method, right? And what's the LOQ of the method?

Because I think what gets lost in the translation of a lot or interpretation of a lot of the studies, I mean there have been plenty of studies that have looked at biological samples. And the conclusion of the study is glyphosate is not detectable in serum. That glyphosate is not detectable in urine. Glyphosate is not detectable breast milk.

Well, the general public might be fooled to actually accept that. But if you're talking to an analytical chemist, the first question that an analytical chemist would ask is what's your limit of quantification?

Because a lot of the studies that are being published where the conclusion is that if you look at the limit of quantification, it's 20 nanogram per ml or 50 nanogram per ml. Of course, you know, you will not detect glyphosate, because the levels, as we looked at in urine, we -- the levels that we're seeing are below that.

Now, so the European study used -- so the report was, and we cannot verify it. The report was GC-MS/MS with an LOQ of 0.15 nanogram per ml. You are asking about -- you're asking about the difference, right? There -- so if you actually contextualize that result, and look at the values based on which country, because there are several countries, so that's -- I think, if I'm not mistaken, that's about 183 individuals from 18 different countries, right?

There is a correlation on how lax the regulation is for using GMO products containing glyphosate, and the levels that you're seeing. The more lax the country is, the higher is the level.

Well, we are very strict here in the United States, right? And so that's -- that's sarcasm.

(Laughter.)

DR. GERONA: And that's probably the reason why, you know, you're seeing a higher level here in the United States. That's potentially the reason why, you know, you're asking about what is the difference, right?

The levels that -- the geometric mean is about 0.70 nanogram per ml, but I've also seen other higher levels depending on the country that sent us the sample.

MR. ADAMS: And if I can add on, so with the IARC ruling that ruled it is a probable carcinogen. That meta-analysis looking at PMG exposure and non-Hodgkin's lymphoma -- I re-did the meta-analysis a couple years ago. And the issue with that meta-analysis is that there are relatively few studies but the distribution of the studies is -- you know, there's heavy weighting of studies in Sweden, and France, than there is Canada, and then there are two -- two or three studies from the United States.

The United States studies are pre-1995. The importance of that is that's the emergence of PMG-tolerant crops. And so it doesn't really capture modern exposure levels in the United States, and its heavily weighted towards countries that have more stringent regulation with regard to pesticide application.

The other problem with that meta-analysis and that collection of studies is most of it's questionnaire based. Well, we know that farmers are petty good at reporting questionnaires and exposures, but there's really no -- I guess no double checking in terms of actual levels of the analyte in their system.

And so, yeah, I guess that was another main -- major impetus for us was to study this very difficult analyte, and see what we can learn from developing methods about it for application in the other small molecules we look at. And then also develop, you know, methods and tools for people to kind of tease apart some of these issues in the epidemiology regarding this particular compound.

CHAIRPERSON SCHWARZMAN: Great. Well, thank you both very much for your interesting work and for coming here to talk with us about it.

(Applause.)

CHAIRPERSON SCHWARZMAN: Okay. So I think we're actually -- we have the go ahead to continue without a break, which we were -- the reason that we're trying to move this discussion up is just to make sure that we have a chance to get a vote among the Panel members on our next topic before some have to leave early.

So we're going to move right into the next agenda item, which is the potential designated chemicals: organophosphorus pesticides. And I want to introduce Dr. Shoba Iyer who is a staff toxicologist in the Safer Alternatives Assessment and Biomonitoring Section at OEHHA. And she will be present a brief summary of information on the class organophosphorus pesticides as potential designated chemicals.

(Thereupon an overhead presentation was presented as follows.)

DR. IYER: Thank you. Is this on?

Testing.

Sounds okay?

All right. So the purpose of this agenda item is for the Panel to consider the class of organophosphorus pesticides as potential designated chemicals.

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DR. IYER: As a brief reminder, designated chemicals can be considered for biomonitoring by the Program. Chemicals are designated based on inclusion in CDC's National Reports on Human Exposure to Environmental Chemicals program and on recommendations by the Scientific Guidance Panel, or SGP, for Biomonitoring California.

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DR. IYER: Some organophosphate insecticides are already on the list of designated chemicals based on their inclusion in CDC's National Reports on Human Exposure to Environmental Chemicals program. Some examples of already designated organophosphate insecticides that are still in use in California are acephate, chlorpyrifos, diazinon, dimethoate, malathion, and naled.

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DR. IYER: The class we are discussing today is organophosphorus pesticides for which we are using a structure-based definition, that is phosphorus-containing organic compounds used as pesticides. The class of organophosphorus pesticides does include all of the organophosphate insecticides already on the designated list, as well as other subclasses, such as organophosphinates and organophosphonates.

The class also includes any organophosphorus pesticide currently in use or that may be introduced in the future. Shifts in pesticides being used are likely to occur. For example, the emergence of herbicide-resistent weeds is a factor the can affect pesticide selection.

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DR. IYER: With regard to past SGP actions on this class of pesticides, in March 2009, the Panel recommended that all already designated organophosphate insecticides be added to the list of priority chemicals. And last July, the SGP reviewed our preliminary screening of three pesticide classes. The Panel recommended that OEHHA prepare a potential designated chemical document on organophosphorus pesticides. We followed this recommendation, and the document we prepared was posted two weeks ago on the SGP meeting page.

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DR. IYER: Here is a list of the criteria for recommending designated chemicals, which also applies for classes of designated chemicals. The criteria are: Exposure or potential exposure, known or suspected health effects, the need to assess the efficacy of public health actions, availability of a biomonitoring analytical method, availability of adequate biospecimen samples, and incremental analytical cost.

And please note that these criteria are not joined by the term "and".

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DR. IYER: We selected the seven organophosphorus pesticides listed on this slide to highlight in our designated chemical document. These are all in current use in California. In my talk today, I'll be giving an overview of information on these seven pesticides relevant to the criteria for designated chemicals.

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DR. IYER: On this slide and the next slide, I have the chemical structures of the seven highlighted organophosphorus pesticides. Here are the structures of bensulide, ethephon, ethoprop, and fosetyl-aluminum.

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DR. IYER: And here are the structures of glufosinate-ammonium, glyphosate and tribufos. And I want to show you just for reference the structure of an organophosphate pesticide, tetrachlorvinphos, which is already a designated chemical. The organophosphate component of the structure is outlined in the blue box.

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DR. IYER: This table shows you the ranks of the highlighted pesticides that were in the top 100 used agriculturally in California in 2015. These ranks were obtained from the California Department of Pesticide Regulation, or DPR, and they are based on the number of pesticide pounds applied for agricultural use. Ethoprop and tribufos did not rank in the top 100. And among organophosphorus pesticides only, glyphosate is the most highly used agriculturally in the State.

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DR. IYER: On this slide, time trends for six of the highlighted organophosphorus pesticides are displayed. I'll show glyphosate on the next slide. The graph here on the left plots agricultural use based on data from DPR. The time trend for glufosinate-ammonium here is particularly interesting. There were supply chain issues that resulted in significantly decreased agricultural use of this herbicide a few years ago with use increasing again in 2015.

And the graph on the right shows you the time trend of pesticide pounds sold for these same highlighted pesticides. The information in the graph on the right is compiled by DPR based on self-reported data from pesticide registrants, pest control dealers, and pesticide brokers.

Note that these data include pounds sold for any use in California, including agricultural, institutional and home use. So comparing the time trends for agricultural pesticide use with those for pesticides sold can provide a rough idea of the potential for non-agricultural use. Of note here, are the apparent increases in pounds of the glufosinate-ammonium and fosetyl-aluminum sold in 2015.

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DR. IYER: On this slide, I'm showing you the same type of time trend information for glyphosate. The isopropylamine and potassium salts of glyphosate are the primary forms used. The trends for each of these salts are shown in red and blue in the graphs, respectively. And the black triangles in each of these graphs show the sum of all forms of glyphosate used or sold, including the two primary salts as well as other salts.

The trend in the sales graph on the right indicates the magnitude of glyphosate use in non-agricultural applications in California.

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DR. IYER: We searched for information on retail availability of products containing the highlighted organophosphorus pesticides to determine the potential for home use. We located herbicide products containing glufosinate-ammonium and glyphosate for gardens and lawns; a turf herbicide with bensulide; a plant growth regulator containing ethephon, and a fosetyl-aluminum fungicide for plants and lawns. Some of these products are widely available and home use could represent a potentially significant exposure pathway.

And ethoprop and tribufos are restricted pesticides and are generally not available for purchase or use by the general public.

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DR. IYER: This slide summarizes detections of the highlighted organophosphorus pesticides in environmental samples collected in California. DPR's report on 2015 data collected as part of the Air Monitoring Network noted one detection of bensulide that year. The detection was in a Salinas air sample, but was below the limit of quantitation of 9.3 nanograms per meters cubed. The air samples collected that year were also tested for tribufos, but that pesticide was not detected.

We located two detections of glyphosate from tap water testing in the state from 2004 to 2009: one in the Imperial Irrigation District in 2005 at 16.5 micrograms per liter, and another in Bakersfield in 2006 at 32 micrograms per liter.

In reviewing groundwater data from the State Water Resources Control Board, we identified two detections of glyphosate in the last 10 years: one in Los

Angeles County in 2009 at 1.3 micrograms per liter, and another in Santa Barbara County in 2013 at 20 micrograms per liter.

We did not locate any California studies of the highlighted pesticides in dust. For more details on these studies in the slides, as well as for details from some non-California studies, you can refer to the document we posted online.

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DR. IYER: Potential toxicity concerns for the highlighted organophosphorus pesticides include neurotoxicity, carcinogenicity, developmental effects, endocrine effects, and respiratory effects. These effects are consistent with those associated with organophosphate pesticide exposure. We covered known or suspected health effects of the highlighted pesticides in more detail in our document, so you can find additional relevant details there.

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DR. IYER: This table summarizes the organophosphorus pesticides listed under Proposition 65 and classifications by the International Agency for Research on Cancer, or IARC. The highlighted pesticides are in the top rows of this table, and the pesticides already on the designated list are in these bottom rows.

All but one of the pesticides in this table have been identified as carcinogens. And oxydemeton-methyl is listed as a reproductive toxicant under Proposition 65.

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DR. IYER: The next few slides will cover information we located on the potential to biomonitor the highlighted organophosphorus pesticides.

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DR. IYER: I'll start by showing you the structures of metabolites and breakdown products for selected pesticides. The major human metabolite and breakdown product of glufosinate-ammonium is 3-methylphosphinicopropionic acid, or 3-MPPA.

For glyphosate, aminomethylphosphonic acid, or AMPA, is the primary metabolite and also a breakdown product.

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DR. IYER: The major human and rat metabolite of ethoprop is O-ethyl-S-propyl phosphorothioate. And bensulide oxon is a rat metabolite, and also a breakdown product of bensulide.

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DR. IYER: This table summarizes serum and urine detections of the highlighted organophosphorus pesticides in past biomonitoring studies for the parent compounds and some metabolites. The serum detections of bensulide, ethoprop, and tribufos were reported in a doctoral thesis we located. Ethoprop's metabolite, O-ethyl-S-propyl phosphorothioate, was detected in a different study in urine. Glufosinate-ammonium and its metabolite 3-MPPA have been detected in serum. Glyphosate has been detected in serum and in urine, and its metabolite AMPA has been detected in urine.

One study looked for AMPA in serum, but did not detect it. And, of course, Dr. Gerona and Axel Adams just presented their interesting findings from their recent work on glyphosate.

Back here.

The designated chemical document provides details on other studies summarized here, including detection frequencies and ranges of levels measured. We did not locate any California biomonitoring publications.

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DR. IYER: In considering the potential for bioaccumulation, we obtained log octanol-water partition coefficients, or log KOWs, based on experimental data that were tabulated by US EPA. OEHHA has identified a log KOW of greater than or equal to 4 as indicating potential concern for bioaccumulation. Bensulide and tribufos are the only highlighted pesticides that have log KOWs greater than 4.

With regard to potential for bioaccumulation in fish, US EPA concluded that this was not a concern for bensulide. US EPA determined that tribufos could accumulate in exposed aquatic or terrestrial organisms, but also noted that metabolism and rapid elimination are expected to mitigate the bioaccumulation concern.

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DR. IYER: In evaluating environmental persistence, we focused mostly on soil half-lives. Each bullet in this column of soil half-lives for the highlighted pesticides shows results from one study. We also looked up some persistence information from US EPA's ecological risk assessments.

US EPA's assessment of bensulide predicted it to be extremely persistent in terrestrial ecosystems. US EPA concluded, based on studies across various media, that ethoprop is moderately to strongly persistent in the environment, but they also noted that there is significant uncertainty in the estimates of ethoprop's environmental half-lives due to observed variability in persistence measured at different sites, and the potential impact of previous use history at the sites.

Glufosinate-ammonium degrades moderately rapidly in soil to 3-MPPA. US EPA concluded that glufosinate residues may persist in aquatic environments. And they also reported that glufosinate is expected to remain primarily in the water column rather than in sediment with concentrations decreasing over time via dilution and metabolic degradation.

US EPA reported that the persistence of glyphosate in soil appears to correlate with climate. A publication we located found that glyphosate persists longer in soil under cool and dry conditions, and that same study also found that AMPA persisted in soil longer than glyphosate did, even under warm and moist conditions.

And US EPA noted that tribufos appears to be more persistent than is typical for most chemicals in this class, which is illustrated by the very long soil half-life of 745 days reported in one study.

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DR. IYER: Moving along to analytical considerations; the California Department of Public Health's Environmental Health Laboratory currently measures two specific urinary metabolites of the organophosphate pesticides chlorpyrifos and diazinon, and they also measure four non-specific dialkyl phosphate metabolites.

Additional method development would be required for Biomonitoring California to measure any of the highlighted organophosphorus pesticides. But as you heard earlier, there are methods available for a number of these.

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DR. IYER: One of the criteria for recommending designated chemicals that I showed you in an earlier slide is assessing the efficacy of public health actions to reduce exposure. For the class of organophosphorus pesticides, adding them to the designated chemicals list would allow the Program to select any member of the class to be included in a future study, to have the flexibility to choose analytes appropriate to the particular scenario of interest, and to track the levels of exposure and how they change over time and by region. The results of biomonitoring studies can inform ongoing State efforts to reduce pesticide exposures of concern.

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DR. IYER: The slide shows the Panel's options for this agenda item. The Panel could recommend adding organophosphorus pesticides as a class to the list of designated chemicals; the Panel could defer, pending more information; or the Panel could recommend against adding organophosphorus pesticides as a class to the list of designated chemicals.

This concludes my talk, and I'm happy to take any

Panel questions.

CHAIRPERSON SCHWARZMAN: Yeah. We have a question.

Dr. Cranor.

PANEL MEMBER CRANOR: Let's see if I can turn this -- oh, is this on?

You mentioned that you have in here that some of the organophosphates linger in the soil much longer. I didn't see that apparently they can also be stored in human fat for a period of time, is that correct?

DR. IYER: I didn't look into that aspect of the pesticides. We were looking primarily at potential for biomonitoring, so the biomonitoring studies are what I focused on, aside from soil.

PANEL MEMBER CRANOR: I guess, was -- I'd looked at a toxicology book in looking at risk, and if they're stored and there is other exposures, they can interact, they can -- they're add -- they can be additive. And so it does seem important to know what's going on out there, which the Biomonitoring Program would do, given these -- the variety of facts about it.

DR. IYER: Yeah. My best guess would be that some of the information we located on log KOW might give you the best prediction of what might stick around in the fat. So bensulide and tribufos were the two that we've found had log KOWs greater than the cutoff of concern.

PANEL MEMBER CRANOR: I just saw a couple of them had long -- long half-lives.

DR. IYER: Yeah. Yeah. The others, aside from bensulide and tribufos, had very low log KOWs.

CHAIRPERSON SCHWARZMAN: Do you have a question?

PANEL MEMBER LUDERER: Well, yeah, I guess -- just kind of related to that too, I mean, looking at the data that we were just presented in the last presentation, I think it was from the agricultural study where they had the day of the application of the glyphosate, and then subsequent days. So that one doesn't, you know, seem to go down relatively quickly, at least in the applicators. But yeah, I agree that the log KOWs that are elevated for those do lead to the one to be concerned about accumulation in humans, as well as other organisms.

I just -- I guess -- are we supposed to start talking about whether we want to designate yet or are we still on questions?

CHAIRPERSON SCHWARZMAN: No, this is clarifying questions at this time.

(Laughter.)

CHAIRPERSON SCHWARZMAN: Any other clarifying questions?

We have time now for public comment, and then we'll have our conversation.

So are there any public comments from the room?

There will be one.

Is there anything on the web that we should be aware of?

MS. DUNN: There are none.

CHAIRPERSON SCHWARZMAN: Nothing. So we'll have one public comment.

Dr. Bradman.

DR. BRADMAN: Hi. I just want to comment a little bit on this issue, and also perhaps related to Roy's presentation too. I think one thing we know with pesticides that when they're used, we're exposed to them. I mean, in our studies, we found materials that had been used in the field that historically never -- no one would even think they would get into people's homes or get into people's bodies outside the fields, but they do. Whether those are of concern or not is, of course, another issue, when we look at risk and health effects.

But I think understanding exposures to these materials is really important. And like consumer products, like air pollutants, these are things that we're all exposed to. So I would encourage real -- the opportunity to really carefully look, particularly at the cholinesterase-inhibiting compounds, and there's several on here that are included. And then other heavy-use compounds. I think it's really important that they be looked at and at least the beginning stages of understanding of what the exposures are.

CHAIRPERSON SCHWARZMAN: Do we have any other public comments?

One more.

MS. JUNFISH: Hi. My name is Susan JunFish. And I'd like to express support for also monitoring organophosphates, particularly the ones that are used by the mosquito vector control districts throughout the State, such as malathion is listed on that list. And I believe another very commonly used organophosphate. I think it's of serious concern what's happening when there is evidence from a very important study that was done in I think it was 2006 at Harvard School of Public Health showing an efficacy of ULV spraying for West Nile Virus. They showed that reproductive rates in communities that did ULV spraying versus communities that did not spray, there was difference in the reproductive rate -- the number of eggs being laid by West Nile Virus-carrying mosquito species.

DR. IYER: I'll just add a --

CHAIRPERSON SCHWARZMAN: Yeah, I was going to say about the presence of malathion on the list.

Go ahead.

DR. IYER: Yeah. Yeah, just to clarify, malathion was included on the slide as an example of a chemical that is on our designated list, and it also falls under the broader class of organophosphorus pesticides. So just to clarify.

CHAIRPERSON SCHWARZMAN: It is already currently on the designated chemical list?

DR. IYER: That is correct.

CHAIRPERSON SCHWARZMAN: Okay. Any other public comment before we move on to general discussion?

Okay. So, why don't you go first, Dr. Luderer.

PANEL MEMBER LUDERER: I mean, I was just looking at the criteria list. So, you know, from what we've -- we've heard in the document that was given to us, as well as the earlier presentation, there's -- certainly the exposure to the public criteria I think is met by these compounds, given the wide detection of the ones that have been measured in biomonitoring specimens and the use in California in agriculture, and other uses, that there is -- I think we -- it meets the known or suspected health effects. You know, we've heard about several -- many of -- of the ones that we talked about today, how many of them are considered to be carcinogens, as well as, you know, other neurological endocrine disruption effects that have been reported for many of these compounds.

I think there's also the need -- it meets the criteria for the need to assess the efficacy of public health actions. You know, some of these organophosphates have -- are now not permitted to be used, you know, indoors in residential settings. And so will we see a decline in levels of those -- you know, that's something that we need to be able to assess.

And then we've also heard that the biomonitoring analytical methods, you know, exist to be able to analyze these specimens. So I think, based on all of these things, that we certainly meet multiple criteria for designating these compounds - the organophosphorus compounds.

CHAIRPERSON SCHWARZMAN: Thank you for that summary of the evidence and how it meets with our criteria.

Before -- I guess I'll jump in with a comment that goes off of one of the things that Asa just mentioned about the document that you prepared I noticed there was the mention of the -- looking at how many cholinesterase inhibitors were applied in proximity to schools. I wonder actually if you would speak about that data for a minute, because that was a really interesting sort of subset to me, of this class.

Do you mind? Sorry to put you on the spot.

DR. IYER: No problem. I'm -- we were trying to focus on a selection of information to present in the slides. And I didn't cover that in the slides, but yes -- so we took a look at the report that the California Environmental Health Tracking Program put together, where they examined the use of agricultural pesticides used near California public schools. The -- that work was done in 2010.

And so I tried to get a feel for what -- which of our highlighted pesticides were included or were used close to schools. So of those organo -- highlighted organophosphorus pesticides, bensulide, ethephon, and glufosinate-ammonium were among the top 10 pesticides applied within a quarter mile of schools in one or more of the counties they assessed in the State. And bensulide was additionally one of the top 10 pesticides classified as a cholinesterase inhibitor used within a quarter mile of schools in the counties they looked at.

I'm aware that there's ongoing work that I think will give us some more information on more recent expo -- potential for exposure near school sites. So that's what I'll add to that.

CHAIRPERSON SCHWARZMAN: Thank you.

I think one of the other things that was striking to me, in addition to your sort of rundown of how the evidence really meets a lot of the criteria - although, of course, we aren't required to meet all of them - is the getting back to this concept of designating a class as opposed to designating individual chemicals. And I think this is a conversation that we have each time we look at a class, but it seems as relevant for this class of chemicals as for every other class that we've looked at that there is the -- there are these dramatic shifts in the market that can happen relatively quickly, based on sort of external pressures or even like that supply chain issue that you highlighted that happened where one drops out and others presumably fill the place in the short-term, and that are dictated by factors that we wouldn't predict or know about in time to like designate a chemical.

And particularly, I'm sort of curious about what's going to happen with the increasing public attention on glyphosate, and Roundup Ready crops, and the -- with the listing under Prop 65 what's going to happen in terms of public pressure on the manufacturer.

And it just, you know, glyphosate is obviously of concern because of its shear volume of use, as we've heard about. And it's -- not persistence. That's using the wrong word, but it's presence in so many of the -- both environmental monitoring and biomonitoring samples.

But I'm -- I'm interested not just in -- it raises the point to me not just of pointing toward glyphosate, but thinking, well, these could shift pretty quickly, and the emphasis on what's being used could shift pretty quickly. And I -- for that reason I'm really, I guess, intrigued by and supportive of the Program's continued movement toward highlighting a whole class of chemicals, and sort of enabling the Program to look at those as the market dictates.

Any other comments or thoughts?

Yeah.

PANEL MEMBER QUINTANA: Hi. Is this on?

After all that drama, I was just going to say I agree with you.

(Laughter.)

PANEL MEMBER QUINTANA: No, just to second the idea of this -- that graph that you prepared. And I want to also congratulate you on, and your -- all the staff on another really excellent and thorough report. But I think that the graph on how the changes and use over time, you know, can be very dramatic, even year to year, I think that's such an important point that supports the idea of designating a class.

CHAIRPERSON SCHWARZMAN: Okay. Thoughts or points for discussion?

I'm getting the sense that the Panel is ready to make a motion. Would anybody like to make a motion?

PANEL MEMBER LUDERER: I move designate -- to designate the organophosphorus -- that the Panel vote to designate the organophosphorus pesticides as a class.

PANEL MEMBER CRANOR: I'll second.

CHAIRPERSON SCHWARZMAN: So individual member votes. Maybe we'll start at this end.

PANEL MEMBER QUINTANA: Yes.

PANEL MEMBER KAVANAUGH-LYNCH: Yes.

CHAIRPERSON SCHWARZMAN: I will vote for that also.

PANEL MEMBER CRANOR: Since I second, yes.

(Laughter.)

PANEL MEMBER LUDERER: Yes.

CHAIRPERSON SCHWARZMAN: So I think I'm supposed to say that the Panel has therefore moved to -- that the chemical class organophosphorus pesticides be included as designated chemicals as a class in the California Environmental Contaminant Biomonitoring Program. And so I think that actually concludes our -- this decision portion around this topic, and we can -- we're doing beautifully for time.

And we have some time designated now that's for open public comment that could be about the items we've discussed today, and specifically this afternoon, or other public comments related to the Biomonitoring Program. And we have time for that now. And because I think we're a little early, I'm -- I don't -- I don't want to -- we have one. There we go.

And if people can continue to suggest that they'd like to make a public comment because you didn't have much heads up about this.

MS. BUERMEYER: Hi. Nancy Buermeyer with the Breast Cancer Prevention Partners. I didn't have a comment before you voted, but now I do, which is to say I'm really glad you voted the way you did. We, at the Breast Cancer Prevention Partners, have concern about these pesticides, because there have been some indications of connection to breast cancer, which is obviously of concern to us. And I just -- I was prompted to say something by the reference to the Health Tracking Program's report on pesticide use within a quarter mile of schools.

And I think that report has generated interest in changing the regulations around pesticide use and reporting through the Department of Pesticide Regulation. I actually haven't tracked that as carefully as I would like to, but that report is suggesting there's going to be a change in public policy. And so it would be great if there could be a commensurate tracking of changes in exposures based on that change in policy.

I think the challenge, as we all know, is the funding to do that, right, because you'd want to get that baseline now, so you'd have a point of comparison, or at least be able to go back and find samples from now. And I think it just -- it raises the ongoing challenge of the ability of the Program to do all that it can and should without the resources to do that.

And I don't have an answer to that obviously, but it's a challenge that continues. But I -- but we also strongly support the designation of classes of chemicals, as you did here, and as you have for phthalates and some other things because of that volatility of how companies are using, changing, moving from chemical to chemical or even just tweaking a particular chemical to a different very similar chemical, and being able to try to keep up with that along the way. So the short version is thank you for voting for that.

CHAIRPERSON SCHWARZMAN: We have a public comment here.

CAL/EPA DEPUTY DIRECTOR SOLOMON: Hi. This is Gina Solomon of EPA and -- It's green. In response to the previous comment related to a pending regulatory action on pesticide use around schools, the California Department of Pesticide Regulation did propose to take several actions related to pesticide use around schools and day care centers, public schools and public -- publicly licensed day care facilities in California that would ban most types of applications of all pesticides within a quarter mile, or at least any dispersive applications.

Then there are some exemptions that allow smaller buffer zones for less dispersive type applications. And then there's also a warning or information -- informational component to the regulation.

The regulation underwent two public comment periods, and is expected to be finalized fairly shortly. So it would come into effect in the coming year. So if there were interest in looking at any potential changes over time, that would be something that could happen fairly soon.

CHAIRPERSON SCHWARZMAN: Great. Thank you.

Other comments?

DR. HOEPKER: Alex Hoepker, OEHHA. Just a clarifying question. Do the criteria of recommending designated chemicals equally apply to chemical classes? I think it does, but I just wanted to clarify that. And a second question related to that, how do we group chemicals? Obviously, this is an obvious example of organophosphate as a class of pesticides, but I'm wondering if there's space to classify chemicals also by receptor endpoints relating to cancer and reproductive toxins?

CHAIRPERSON SCHWARZMAN: Shoba, do you want to start with that?

DR. IYER: Yeah. So the answer to the first part of your question, yes, the same criteria applies to both individual chemicals and chemical classes, the criteria for recommending them as a designated chemical or class.

And my understanding is that thus far we have defined some chemical classes as structure-based definitions or chemical function-based definitions, but at this time no toxicity endpoint-based definitions.

CHAIRPERSON SCHWARZMAN: Can you say anything about whether that's possible, or is there anything that's preventing the program from doing that?

DR. IYER: I think it gets -- it gets to be more complicated doing that. You know, known or suspected health effects is one of the multiple criteria we have for recommending a chemical or chemical class. And that information might not exist for a chemical. So this is one of the challenges around considering that, that I'm aware of.

CHAIRPERSON SCHWARZMAN: I know this isn't exactly public comment, but I just remember something that I was going to say earlier and I forgot, but I think it's become salient. I'm thinking about, you know, on part of the -- one of the criteria for recommending a chemical as a designated chemical is the potential to monitor levels around an intervention study. And whenever I think of glyphosate, I think of the intriguing example of the City of San Francisco, and what -- the action they took under the -- their precautionary principle ordinance, in which they targeted the use of glyphosate in public areas in the city.

And since they weren't taking legal action on it, but they were implementing their precautionary principle ordinance about its use, what they did is gather the gardeners together who used glyphosate in the city and said where do you use it? And, you know, they came up with a whole list of areas like right of ways, and areas where there's invasive -- natural areas where there's invasive plants and airport, and median strips, and -- et cetera, et cetera, you know, public parks and things like that. And then they asked the gardeners what else could you use, like what are the alternatives?

And they came up with a long list of alternatives from like flaming weeds, to hand weeding, to just feeding them compost, and making the substrate strong, to putting goats out. And this whole creative range of possible alternatives.

And they ended up -- through looking at that, they ended up, you know, identifying other alternatives for most uses. And there were a few uses for which they retained glyphosate, for example, medians of busy roads, where it's too dangerous to put workers in the median and do hand weed, and also like SFO, where they can't shut down the runways and put goats out.

(Laughter.)

CHAIRPERSON SCHWARZMAN: And -- but as a result of that intervention, they reduced glyphosate use by 90 percent in the city. And that was a completely non-regulatory intervention. And it just -- I mention it here, because I think it's an intriguing model that imagine if Biomonitoring California could have gotten in on that, and looked at before and after and what we could learn by that kind of intervention.

So just as long as we're thinking of organophosphorus pesticides, it's a story that sticks with me, because of its creativity and its non-regulatory approach and how much we might be able to learn from looking at similar situations. So that's my addition. Does anyone else have any comments before we close the meeting?

And nothing from the web that we need to be aware of?

MS. DUNN: Nothing from the web.

CHAIRPERSON SCHWARZMAN: Sorry, one more in the room.

MS. JUNFISH: I appreciate your comment about San Francisco. I think they're a great role model for the rest of the cities in California, and the world over actually. This is not related to biomonitoring, but just as a quick addition to what you said, the County of Irvine has been testing steamers. And so hot steaming to kill weeds. And that has been a really great alternative to most emergent herbicides -- broad spectrum herbicides.

Susan JunFish.

CHAIRPERSON SCHWARZMAN: I think my favorite option in that was the flamethrower.

(Laughter.)

CHAIRPERSON SCHWARZMAN: That's what I would want to use.

(Laughter.)

CHAIRPERSON SCHWARZMAN: Okay. With that, thank you all for your contributions for today's meeting. I'm supposed to announce that the transcript of the meeting will be posted to the Biomonitoring California website when it's available. And that our next Scientific

Guidance Panel meeting will be on November 9th here in Richmond.

And with that, I think we'll adjourn the meeting. Thank you all.

(Thereupon the California Environmental Contaminant Biomonitoring Program, Scientific Guidance Panel meeting adjourned at 3:27 p.m.)

CERTIFICATE OF REPORTER

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

That I am a disinterested person herein; that the foregoing California Environmental Contamination Biomonitoring Program Scientific Guidance Panel meeting was reported in shorthand by me, James F. Peters, a Certified Shorthand Reporter of the State of California, and thereafter transcribed under my direction, by computer-assisted transcription.

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

IN WITNESS WHEREOF, I have hereunto set my hand this 28th day of July, 2017.

James F. Peters

JAMES F. PETERS, CSR
Certified Shorthand Reporter
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