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Ms. Duyen Kauffman
Dr. Jianwen She, Chief, Biochemistry Section, Environmental Health Laboratory
Ms. Alanna Viegas

DEPARTMENT OF TOXIC SUBSTANCES CONTROL:
Dr. Myrto Petreas, Chief, Environmental Chemistry Branch

ALSO PRESENT:
Mr. Davis Baltz, Commonweal
Ms. Nicole Quinonez, International Fragrance Association of North America
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DIRECTOR ALEXEEFF: Good morning, everyone. I'm George Alexeeff, Director of the Office of Environmental Health Hazard Assessment. Welcome you all here to the Scientific Guidance Panel for the California Environmental Contaminant Biomonitoring Program, affectionately known as Biomonitoring California.

Now, I want to thank the Panel and the public for their participation in this important meeting. And I just want to remind everybody that the meeting is being transcribed, as well as being broadcast via webinar. And so that if you are going to speak, please speak clearly into the microphone, so that our transcriber can hear you and so that those out in webinarland can also hear you.

I wanted to make -- well, first of all, I should probably mention about the basic details about exits and fires. So my first job, after I got my graduate degree, was working in a fire technology program at Weyerhaeuser Company. So every time I went to the -- a hotel with my boss, we had to check all the fire escapes.

Anyway, so I happened to check the fire escape today by accident, but it is functional over here, the fire escape. And then there are two escapes in the back over there, out the door to the left. And the restroom is out the door and to the left.
So I wanted to announce that Dr. Michael Lipsett is retiring at the end of 2013. And I wanted to acknowledge him for his years of service. And, you know, in fact, it's probably -- maybe has been mentioned that he hired me into State service, so I have a lot to thank him for. But especially for his more recent work and guidance over Biomonitoring California, which I know he was very passionate about.

One of the two things he was passionate about is Biomonitoring California and air pollution studies. I think those were his two great passions. Anyway he was planning on making it here today, but he was unable to. So we're hopping at a future meeting, probably in March, to, you know, thank him personally and acknowledge, you know, the specific contributions that he's made to this program and to State service. So I think I'll wait until that meeting to do that.

So at our last Scientific Guidance Panel meeting, it was held in Oakland in August 14th. And that was at a fantastic location. It was a wonderful location. And we reviewed the status of the current Biomonitoring California projects. We discussed the Program's new direction of screening for unknowns and provided initial input on strategy. We'll talk more in detail about that at this meeting.
Four pesticides were screened as possible candidates for biomonitoring in California in the future. And the Panel recommended that the Program prepare documents to support the consideration of all four pesticides as potential designated chemicals in the following order: Imidacloprid, glyphosate, glufosinate ammonium, and propanil.

We heard about something dear and close to my heart, CalEnviroScreen. It's a tool that was developed by OEHHA to evaluate environmental exposures in California communities and provided suggestions for future versions. And the Panel also discussed ways that CalEnviroScreen could inform future Biomonitoring California studies.

It's an interesting thing, in that both of those projects, Biomonitoring California and CalEnviroScreen, were sort of started around the same time without exact knowledge of where they would go. And so they've both made great strides.

For more information on the August meeting, you can -- including the transcript and a full summary for that meeting, you can visit the biomonitoring website.

So I think I'd like to now turn the meeting over to Dr. Luderer.

CHAIRPERSON LUDERER: Thank you, George. I'd also like to welcome everyone, members of the public,
Program staff and the Panel members. I wanted to let the remote participants, people who are interested in participating remotely, know that there are actually two systems that can be used today. So there's video webcast, which is -- there's a link to how to do that on the website for the Program. And there's also a webinar option, where you see the slides just with audio via Live Meeting. And you can also find a link to how to access that on the Program website.

So I'd like to just briefly go over the Panel goals for the meeting and talk about how we're going to handle public comment. So our goals for the meeting are to receive Program and laboratory updates and to provide input, to consider two structurally related classes of aroma chemicals as potentially designated chemicals, and those two are synthetic polycyclic musks and tetramethyl acetyloctahydronaphthalenes.

And we'll hear from Panel Member Oliver Fiehn about identifying novel compounds in untargeted metabolomic screens as well this afternoon.

For each of the agenda topics, we'll -- there will be time for Panel questions, public comment, as well as Panel discussion and recommendations. And I just wanted to let everyone know how we'll be handling the public comment. If a member of the public would like to
make a comment, he or she should fill out a comment card. And that can be obtained from Duyen Kauffman, who's holding up those blue cards there. And members of the public who are not at the meeting in-person are invited to provide comments via email. Biomonitoring California staff will provide those emailed comments to me, so that I can read them aloud during the meeting.

We will again divide up the public comment time by the number of individuals who wish to speak, so that we can make sure that we proceed on schedule and that all the commenters have the opportunity to speak.

So please also keep your comments focused on the agenda items that are being presented, and we'll have an open public comment period at the end of the day at which members of the public can bring up any topic related to biomonitoring.

I also wanted to remind again, everyone please speak into the microphones and introduce yourself before speaking. This is for the benefit of people participating via the webcast as well as for our transcriber.

The materials for this meeting were provided to Scientific Guidance Panel members and posted on the Biomonitoring California website prior to the meeting today. There are also a small number of copies on the table in the back of the room, and one sample scientific
guidance folder for viewing also on that table.

We're going to take two breaks today, one around noon time for lunch, and another around 2:45 in the afternoon.

So there's been a slight change to the agenda this morning. Dr. DiBartolomeis is running into traffic, and so we're going to have the laboratory updates first, and then the Program update. So that's a little bit different than the agenda that was on the website.

So I'd like to start out by introducing the two speakers for -- who are going to be giving us laboratory updates, Dr. Jianwen She, who's the Chief of the Biochemistry Section in the Environmental Health Laboratory Branch at CDPH, and Dr. Myrto Petreas, the Chief of the Environmental Chemistry Branch in the Environmental Chemistry Laboratory in Department of Toxic Substances Control.

So Dr. She.

(Thereupon an overhead presentation was presented as follows.)

DR. SHE: Good morning and welcome, members of the Panel and audience. First, I also want to thank Mike Lipsett -- Dr. Mike Lipsett for his leadership. And as George said, coincidentally he also is the one who hired me into this position. He's not one the who hired me into
the State service, but he hired me into the position with Dr. Peter Flessel and Dr. Jed Waldman. So I personally and I also want to thank him for his leadership. I did learn a lot from him. I hope I can follow his examples to make good contribution to the Program.

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DR. SHE: Today, I will provide updates for Environmental Health Laboratory. This includes recent phthalate metabolite method update, project sample analyses status, preliminary FOX study results, BPA analog method development, a few recent publications, and finally our future work.

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DR. SHE: In the past, I have talked about automation of sample preparation for certain methods to increase sample throughput. Most recently, we have upgraded our phthalate metabolite method to an on-line or automated sample preparation system. There are many differences between the manual and automated sample preparation. Our goal was to increase sample throughput and the overall efficiencies of the method.

To accomplish this, we modified our existing HPLC-MS/MS system with additional equipment. There are many positive aspects to an on-line system. For example, this has increased sample throughput, no more time
consuming and labor intensive sample processing. This is all done automatically in a closed system, thus reducing the risk of potential contamination.

The amount of sample required for analysis has also been reduced from one milliliter to 300 microliters. A lot of benefit to the on-line system is that it's cost effective. Lastly, the on-line system has increased sensitivity and has lowered the detection limit.

In the future, we hope to validate on-line SPE sample preparation for other existing methods, like environmental phenol or specific OP metabolite method.

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DR. SHE: Another recent update to this method is the analyte panel has been expanded. In the past, as you can see, we can measure these six phthalate metabolites. But with this on-line system, we added four more metabolites. And the last column of the table shows the parent compound of metabolites. So currently, we can measure 10 metabolites for the phthalate.

The four new analytes are MEHHP, MIPB, MEOHP, MEHP. All 10 analytes EHL can measure are on the designated list. As you will notice, the new analytes are mostly coming from the parent compound DEHP. DEHP is a plastic-softening phthalate, and is highly lipophilic. DEHP is used in products like food packaging, toys,
medical equipment, PVC piping. And animal studies show that high level exposure to DEHP can damage the liver, kidney, and the reproductive system. All of the four analytes to measure the exposure of DEHP is important. We are looking forward to updating the Panel in the future with study results from all 10 analytes.

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DR. SHE: Since last SGP meeting, we have submitted the majority of the Pilot BEST results to the -- to EHIB for the result and release it to the participants. So we -- as a reminder, we only needed to focus on the last column, that's the Pilot BEST. We already talked about MIEEP and FOX in the previous meetings.

The box shaded in green indicate that analysis is complete, and that the data results have been submitted to EHIB. The box shaded in yellow indicate that either samples are currently being analyzed or the data is under review. Creatinine, phthalates, OP specific metabolites, hydroxy-PAHs, and metals in urine have all been released since last meeting.

Please note that the samples are only analyzed for arsenic species if total arsenic level were found to be above 20 ppb. Pilot BEST analysis of the 29 samples for speciation is complete and the data is currently under review. We hope to release this data along with the
environmental phenol and the perchlorate data to EHIB by the end of the year.

We encountered some small problems with environmental phenol, so we rerun most of samples. That's why we need extra time.

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DR. SHE: This slide graphically shows the geometric mean comparison of the metals in urine between the FOX project compared to NHANES data from 2009 to 2010 for adult men over the age of 20. For the FOX project, we analyzed 101 urine samples for four metals. Only three of the four metals we measured are shown here because the detection frequency for manganese was roughly nine percent in FOX cohort.

Both total arsenic and mercury were detected in 100 percent of the participants, while cadmium was detected in about 70 percent of the participants. FOX mercury data could not be compared to NHANES because of the detection frequency for that population was too low for the NHANES. These results are based on preliminary analysis, but we do not notice a significant difference between the two studies.

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DR. SHE: The next few slides I'll talk a little bit about arsenic analysis and the speciation. As I
mentioned, total urinary arsenic was measured also in 101 FOX participants. The protocol for urinary arsenic speciation shown here and the follow-up survey for study participants with elevated inorganic arsenic levels were developed by -- mainly by OEHHA with input from EHIB and other parties like EHLB.

If the total urinary arsenic level was greater than or equal to 20 ppb, then urinary arsenic was speciated for six different species. In this case, the total number is equal to 29.

Of the 29 participant samples that were speciated, five of them were found to have elevated inorganic arsenic and related species.

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DR. SHE: Of the six -- you can see from column 2 we measured six species of this. Six analytes we measured they can be broken down into two different categories, one is inorganic arsenic and related species, one is organic groups that include two species.

Most of the species were detected in some, if not all of the 29 participants. Arsenic V was not detected in any of the participant samples. For the participant whose sum of four of the inorganic species above 20 ppb, we call them have elevated levels of inorganic arsenic. For this case -- this was the case for five of them, and we found
for this five DMA. So it was dominate species. Generally, DMA is the most frequently excreted urinary arsenic metabolite, and this species ends up being the greatest contributor to the inorganic urinary arsenic levels. This is consistent with CDC's finding.

A follow-up survey was offered to the five participants with urinary inorganic arsenic greater than 20 ppb. The survey results are currently being reviewed.

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DR. SHE: CDC has established the level of concern for total arsenic to be greater than 50 ppb. So to use this criteria, we found two participants the total arsenic level is greater than 50 ppb. And for this two participants, arsenobetaine and arslenocholine was a major contributor to the total level. And we think the recent fish or seafood consumption is likely source of this organic species. A notification letter informing them of their elevated levels and that is likely due to the consumption of recent seafood meal will be sent with their results to these two participants.

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DR. SHE: In last SGP meeting, we also talked about Environmental Health Laboratory is developing bisphenol A analog method. So here the slide shows four analogs include BPA substitute. So I like to give a
little bit more update about this method.

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DR. SHE: Currently, we validated this method. We only analyzed six sets of samples, as we talked before in the past, to test the measures of robustness and the precision, we need at least to run 20. So this is very early report.

We prepared three levels of the quality control samples at 1 ppb, 10 ppb, and 50 ppb. So the column 3 shows the precision for 10 ppb and 50 ppb. Usually, we require at least -- we can get a precision better than 20 percent. So you can notice for BPS, the last row at 20 ppb, we have 27, which is slightly higher than we like to see.

Also, for the second row for the chemical BPAF, for 50 ppb, somehow we get 25. So the precision is not that great yet. And also the -- but for the accuracy, this is relative recovery, by the way. General acceptance is 70 to 130, so we are over the accuracy, right. So for the QC sample at 1 ppb we have some contamination issues withstanding the result. Other challenging part, there are no commercial available isotopes labeled standard. We consider that maybe also a reason why sometime we do not get good the precision. We use a surrogate standard which is an isotope labeled BPA for all of the four other ones.
But this surrogate data may not be able to compensate the loss or contamination introduced through the process. So we tried to resolve this issue a little bit further.

We are looking forward to updating the Panel in the future, and -- but the method is hopeful after we have run 20. And at the same time, if we're able to procure the new standards, we have better precision for the real sample analysis.

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DR. SHE: In addition to our routine analysis, we also try to publish the method. Since 2013, we have four publications. Usually, we focus on analytical result, and -- sorry, analytical method itself. So we hope, in the future, we can work with our -- within the Program with the PIs to publish analytical results more, because analytical results required to give to participant and the many other issues, so laboratory cannot allow to go to publish them alone. So, however, the publication you can see is an analytic method.

For example, we also -- we did like a matrix effect analysis and published two. And today, we will hear Dr. Fiehn talk about unknown analysis. So we have published some papers also in the area. And we have one publication accepted for -- so, at this moment, it is in press. So I hope we can in the year -- in the future
years, we can produce more scientific journal publications.

You can visit Biomonitoring California website for a list of the biochemistry sections the publications, possible also the ECS publication.

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DR. SHE: For the future, we like to finish all of the data results for the Pilot BEST, even we have encountered some difficulty for certain method that we hope we can solve that quickly. Analyze all of the laboratory collaboration samples. Analyze -- something happening? It's okay.

Analyze Expanded BEST samples, and complete and improve BPA analog method and also develop OP flame retardant method in the urine, and possibly bundle it with current DAP or OP -- specific OP method, because they are structurally similar. They all show in the urine. So by bundle them, we can improve both the method, also not affect our current workload of -- so we can -- one side analyze the routine samples, on the other side develop a new method.

Thank you.

CHAIRPERSON LUDERER: Thank you very much, Dr. She. And congratulations on the recent publications and on the development of the new phthalate assay. That was
very interesting. Also, great to hear that the DEHP and DIBP have been added as well, and the new environmental phenols development as well.

Do we have any clarifying question before Dr. Petreas starts? We'll have time for discussion afterwards, but if there are clarifying questions from Panel members.

Dr. Wilson.

PANEL MEMBER WILSON: Thank you, Chair. And again, congratulations also. It's really help -- I'm glad to see the lab publishing your methods and so forth. So I had a clarifying question on the facts -- the FOX results for metals in urine. And maybe I didn't -- maybe I didn't understand your explanation, but that the -- you had findings of mercury in 100 percent of the participants. And that's against NHANES, where there are -- there was no mercury detected. And just a little more explanation would be great. And do you have a sense from that study -- you know, from this work what the source of that mercury would be in this population?

DR. SHE: That's an area I'm scared you're asking. For the analytical result part, I think as the party prepared more on this part and mercury result is this -- Alanna, you want to talk.

Sara is not here, but Alanna works with Sara, so
she may have some.

MS. VIEGAS: Hi. I'm Alanna Viegas, sample management for Biomonitoring.

We were unable to compare our FOX study to NHANES, because the detection frequency for that year was unavailable and so it wasn't appropriate to include the numbers. The fear was that the detection frequency was below 50 percent, and so the geometric mean would not make sense to calculate and put up there. Does that answer your question?

PANEL MEMBER WILSON: You mean the geometric mean for NHANES?

MS. VIEGAS: NHANES, yes.

PANEL MEMBER WILSON: Okay. Thank you.

DR. SHE: I'm not sure about the source. Has anyone in our team analyzed the source of mercury or reconsidered the sources?

So if no one knows for sure at this moment, I guess we take notes about this to see if we're able to finish this to analyze the questionnaire data and the survey results to find out where the possible source of the mercury comes from.

CHAIRPERSON LUDERER: Did you do blood mercury as well in the FOX, remind me?

DR. SHE: Yes, we did it, and then we tried to do
a correlation analysis. And then I ask Alanna, and we tried to do it, but we are told we cannot present that. I did -- I think that's publication issues that the PIs prepare. So we tried to look for correlation between the blood in mercury and the urine.

CHAIRPERSON LUDERER: Right, because comparing the blood and the urine can be helpful, because the blood is more indicative of organic mercury exposure, so -- which would be most likely to be from seafood. So that can be helpful in trying to sort out what the source of the mercury is.

DR. SHE: That's very good input. I think we'll work with PI. We need to finish this correlation analysis. So maybe give -- shed light what the source of mercury is.

CHAIRPERSON LUDERER: Thank you.

Dr. Fiehn, another clarifying question.

PANEL MEMBER FIEHN: Yeah. You know, whenever you change methods, and they become more sensitive and more robust and more automated which is a great thing to do, because it also will lower costs and increase throughput and all this, but it's very important to also validate and establish that method blanks are appropriately used. And I'm sure you've done it, but I would love to know, you know, what types of method blanks
have been used and what the results are.

DR. SHE: Yes, and that's very important for the lab, as you pointed out. Every time when we switch a method, we need to do equivalence test. We need to revalidate. For a minor change, we do the partial revalidation, but since this is a major change from off-line to the on-line, we did the complete validation again.

Regarding the method blank, we used the laboratory solvent process in the same way as we process the samples, and that's how we do the laboratory blank. Is there any -- we also did -- since this is on-line preparation, we specifically focused on the carryover, because this on-line -- off-line method we use disposable SP cartridge. So one sample one cartridge. We do not need to consider the SP carryover.

But since this one, we tried to reduce the cost, we use the cartridge multiple times, so our focus also on the how to prevent carryover from previous sample to the second sample. So beyond the method blank and the PT samples.

CHAIRPERSON LUDERER: Okay. Thank you, Dr. She. We'll move on to Dr. Petreas' presentation, and then we'll have time for more Panel questions and comments afterwards.
Dr. Petreas.

(Thereupon an overhead presentation was presented as follows.)

DR. PETREAS: Good morning. So I'll give you an update of our laboratory at the Department of Toxic Substances Control since last time.

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DR. PETREAS: So I'll go through and describe two new methods that we have now in our repertoire, which expand their capabilities and capacities for the analysis. I'll give you an update on where we stand with sample analysis, and bring up some activities that directly or indirectly benefit the Program.

So first, we have two new methods which really expand our capabilities. The first one we're measuring persistent organic pollutants with a new method now using a triple quadrupole MS/MS technique. And the benefit here is that we're using a single injection as opposed to our traditional method, which had two separate injections, one for the PBDEs and the other for pesticides and PCBs using the high resolution mass spec.

So this obviously improves the throughput, cuts down the time of data reduction and so forth. We're using this new method for samples from two studies, which are folded under the biomonitoring umbrella here. And I'll
talk more about them. They are the 3 Generation Study, or 3G Study, and the Childhood Leukemia Study we're doing with UC Berkeley, and I'll talk more about those.

The other method is again another breakthrough. We transferred our method of measuring hydroxy PBDEs to an LC-MS/MS method. Again, we have improved throughput, but more importantly, we eliminated the derivatization step that was used before when we used GC. And this avoids using the diazomethane which is explosive and carcinogenic. So it's toxic. Using this method, we're able to complete all the MIEEP samples, and we'll hear more about that later.

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DR. PETREAS: So a little more detail here for the new method with the triple quad GC-MS. We use it with the daughter's serum from the 3G Study. And I'll talk more again later. But the daughters are contemporary women. So it's what they have now, exposed to now. And we're also using this with the UC Berkeley Leukemia Study to look at the mother's serum. This is in response to an RFI we had issued in 2012, and that study was selected.

But also we are looking at children's whole blood. These are the cases with leukemia, and the blood was extremely precious, not much was left. But this method really used just 100 microliters of whole blood, so
it's really a breakthrough, not only for the leukemia study, which is all we could afford to get from them. But for future studies, there are specimens that have been archived and they have very small volume. So this is quite a breakthrough and we feel very happy for that.

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DR. PETREAS: And I want to acknowledge that this was presented at the dioxin meeting in Korea last September, August. And I want to acknowledge Dr. Crispo-Smith, Sabrina, who's here. Wave. She's one of our CDC funded staff who did this great work.

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DR. PETREAS: And I also want to acknowledge the other paper, again presented in Korea, about the hydroxy BDEs. And Dr. Petropoulou, who is DTSC funded staff, did this work. Actually, I think it's a good time, if I can take a break, and introduce many of our staff who came here. You never see them, because seldom do we all come together. And they didn't come for me, they came for Dr. Fiehn's presentation.

(Laughter.)

DR. PETREAS: Sabrina Smith, Sissy Petropoulou. In the back row, Miaomiao Wang, and Dr. Tan Guo, and of course Dr. June-Soo Park, who is a supervisor of all of them plus many more people and does a great job for the
lab.

So the importance of having this hydroxy BDE method being very sensitive is very important, because we know that levels of PBDEs and, of course, hydroxy BDEs are dropping.

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DR. PETREAS: And I want to remind you, if you have seen that or you haven't seen that, a couple of months ago, we had a paper - Ami Zota is the first author from UCSF - describing the temporal comparison of PBDEs, PCBs and the metabolites in serum from women from San Francisco General Hospital. It's the same catchment area, same cohort sampled about three years apart.

And what we saw was that really the PBDE levels are dropping, which in one hand shows the power of biomonitoring to gauge regulatory actions and how, you know, they reflect on body burdens, but also showed how levels are dropping.

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DR. PETREAS: And adapted from the paper, I put up here comparing, in the first top left, is the total PCBs in the serum with the women on the earlier and the later cohort. And the bottom quadrant is the comparison of PBDEs -- I'm sorry of hydroxy PCBs, again between the two groups. And the differences are not significant.
On the other hand, when we looked at the PBDEs and the metabolites, the differences were significant. Levels are dropping, which is great. And the levels of hydroxy BDEs are dropping, you know, more. So a six-fold, I think, reduction. And we think that has to do with the half-lives being shorter for the metabolites. So that's why we need to have better and more sensitive techniques to see dropping levels. So we're happy to have these methods on board.

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DR. PETREAS: Moving on with progress with our studies, a little reminder about the Teachers Study. This we're doing in collaboration with the Cancer Prevention Institute of California, UC Irvine, University of Southern California, and City of Hope.

And we're funded by the Breast Cancer Research Program to do a substudy measuring chemicals as risk factors for breast cancer. The recruitment is still going on. In fact, we're extending to 2014 to acquire enough cases, because the great news is that I guess incidence is dropping and it's hard to recruit people with breast cancer.

So the plan is to have about a thousand cases, and over -- almost 1,500 controls from the entire state. Were analyzing PCBs, PBDEs, perfluorinated chemicals, and
we're sending out to a clinical lab to get lipids and thyroid hormones. These are rather older women. I think the median age is about 65 and the oldest is 94.

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DR. PETREAS: And where we are with that as of the beginning of this month, we have a little over 2,400 samples in our lab, of which we have aliquoted, which is the first phase of dealing with the samples, dividing small volumes to different vials, about 1,792. And then each one goes in a different channel to be processed for the PFCs, for the PBDEs, and then the PCBs and pesticides. And you can see here that in comparison with the previous update, we have made progress with the PCBs, and of course we have extracted -- we have aliquoted many more samples which are ready to be processed. So we're marching along at a different pace with different techniques, but we're moving -- going at good progress and we're on schedule.

And because the Teachers Study now is under the biomonitoring umbrella, we are posting results on the website, so we're expanding the information we have on populations from California.

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DR. PETREAS: The other study, again funded by the Breast Cancer Research Program, which we are going to
put again under the umbrella of biomonitoring is the Three Generations Study. And this is a very special cohort of 15,000 pregnancies in the early sixties from Kaiser Oakland. And in addition to the women who were pregnant, now their offspring, and particularly the daughters, are recruited for a study. And these are the ones — the contemporary daughters are the ones that we will include in our biomonitoring.

The median age is 50. And if you look at the race breakdown, we have 50 percent black, and a little over 45 percent white with some Latinas, Asians.

So it's a very interesting cohort and we're happy to get this data. We're still working on analysis. So of the 300 daughters, we have completed all the PFCs and released this to the PI. And we're still working on the PBDEs, pesticides, OCPs and the hydroxys. So again, gradually we're making progress.

The plan is to — because the results of this study will be returned to the participant as part of the study, and assess the reaction to receiving the results, we won't be posting any results, even aggregate results, before that study is completed. So this we expect it to be in the spring of 2014. So once the study is completed, we will be posting the results onto our website.

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DR. PETREAS: And this is an example of working with other collaborators for having the synergy and sustain the program beyond our budgets.

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DR. PETREAS: The other collaboration we have with UC Berkeley now is the California Childhood Leukemia Study. And for that, I mean the purpose is to look at environmental and genetic risk factors for childhood leukemia. We have done a lot of work with dust from those homes of the children with leukemia and without leukemia. And now we're expanding to look at the blood of the cases. Only cases have blood not the controls.

So we have 195 children. These were sampled between '97 and 2008. As I said, we only have 100 microliters of whole blood, so we cannot measure lipids given the small volume. Nevertheless, in terms of sensitivity, we can detect, even with this very little volume, many of the major congeners of the different classes. And you can see the detection frequency goes from 100 percent for DDE - we all have DDE - down to 55 for one of the PCBs.

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DR. PETREAS: So the intent with this single injection method, we want to compare the blood levels and the patterns to the house dust levels and patterns. So
for that reason, not having lipids it's not so important. And also, we want to investigate the hypothesis that young children being closer to the ground, having more contact with dust, so this age hypothesis.

So also, we have serum from 50 of the mothers. This was a small study in response to the RFI we had issued in 2012. And again, we will compare the mother's serum to the patterns in the house dust and also to their children.

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DR. PETREAS: Progress with this. We are almost completed -- we have almost completed the mothers. And we're finishing the children. Well, things are in review before they get released to the PI and then get posted.

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DR. PETREAS: So other activities. We have presented and published method papers. But here is the one manuscript. The first one, which is describing the comparison of the blood drawing tubes for the analysis of POPs and lipids. And this has been submitted for publication. This is a very important paper, because this is what we use now for the BEST collection, and this is what we use for the Teachers, using these serum separator tubes, which facilitate collection and processing in the field, where we don't have the facilities of a clean
environment to work with.

We're also trying to have some of -- disseminate some information from the actual studies. And we are working on the serum persistent organic pollutants from the FOX study. So June-Soo Park is the lead on this. And we want -- we also tried to finish a study on measuring the same POPs and PAHs in dust comparing the levels in the firehouses versus some residential houses. And this is part of Beverly Shen, who is a master thesis student, that was part of her thesis and now she's published this.

We're also contributing on the publication on serum PFCs and blood metals that Sandy McNeel is the primary author.

So hopefully, some of the FOX data will be out soon.

In addition, I want to mention some new methods that we primarily want to have for DTSC needs. Our geologists are very interested, for example, about PFCs, perfluorinated chemicals, in groundwater plumes and so forth. Especially, differentiating between linear and branched isomers, because these can relate to sources and identify sources.

Also, PFC precursors of fluorotelomer alcohols. Again, identifying routes and pathways. So these are work that's not directly related to biomonitoring, but can be
easily adapted and used for biomonitoring.

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DR. PETREAS: So we're almost ready to make a recommendation of what chemicals -- what instruments we want to buy for identifying unknowns, because we understand that many non-targeted chemicals that can be identified by non-targeted screening may be very important candidates for biomonitoring to be nominated.

So at this phase, we have almost completed our exploratory work, checking the vendor specifications, visiting their spaces, sending them blindly samples, comparing prices. So we're going to discuss and finalize. This will be bought by the CDC last year's budget.

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DR. PETREAS: So we feel that we are still looking for the known chemicals under the lamp post, even though we have a brighter light bulb and we can see a wider radius of chemicals. And I'm talking about both labs, really expanding our capabilities. But we really need to go beyond that and look at the chemicals beyond the lamp post.

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DR. PETREAS: And that's why we're very interested in Dr. Fiehn's presentation and, you know, working together as a Program to give this capability to
the Program.

So for that, if there are any questions.

CHAIRPERSON LUDERER: Thank you very much, Dr. Petreas, and congratulations also to you on the presentations and publications.

And it was very exciting to hear about the development of the new quadrupole GC-MS/MS method for the various different organochlorine compounds and the PCBs, PBDEs and OCPs.

Do we have any clarifying questions right now from the Panel before we take public comments? And then we'll have time for more Panel discussion afterwards?

Dr. Wilson.

PANEL MEMBER WILSON: Thank you. Thank you, Dr. Petreas.

Do you know the age range for the 195 children that you had the PBDE findings for on the childhood leukemia study?

DR. PETREAS: No, but I can get back to you.

PANEL MEMBER WILSON: Okay. All right.

DR. PETREAS: Three to 14.

PANEL MEMBER WILSON: Age 3 to 14?

DR. PETREAS: Yeah.

PANEL MEMBER WILSON: Great. Thank you.

CHAIRPERSON LUDERER: All right. Do we have any
Thank you, Dr. Petreas.

No?

All right. Then we'll move onto the Panel discussion. Any questions or comments from Panel members?

Dr. Fiehn.

PANEL MEMBER FIEHN: Yeah. I wanted to come back to the arsenic analysis, so where you showed that the highest level of arsenic were found in -- as arsenochochine and arsenobetaine speciations. In recent publications it's been shown that regular choline is turned over by a gut microbiota bacteria into TMAO. And I wonder what is known about arsenochochine, is it also turned over to TMAO and what happens then to the arsenic species?

DR. SHE: I assume I need to refer this question to someone in the audience or the people who may have knowledge. I really do not know this, how to -- so you said there's some bacteria --

PANEL MEMBER FIEHN: Yeah, you know, usual nutritional choline is turned over by gut microbiota, specifically from people who are not vegans to TMAO pretty much efficiently. And TMAO is the most important health factor in cardiovascular risk. There are a couple of publications this year in Lancet and New England Journal of Medicine and so on, when you take out cholesterol. So
the next most important risk factor is TMAO, and it's all coming from choline.

So now I'm wondering if the same bacteria in the gut would also work on our arsenocholine, and what would happen then, what type of arsenic species would come after that kind of transformation? So that's -- because it's the most important, you know, part that you found in arsenic species.

DR. SHE: Yeah, I think possible we will follow up with you and to see this arsenocholine follow the same pathway. So we'll follow up with papers to -- yeah.

PANEL MEMBER FIEHN: Okay.

CHAIRPERSON LUDERER: Actually, I had a question about the arsenic as well, which relates not to the organic arsenic species that you found, but to the five individuals who had the elevated inorganic arsenic. I know you said that you're doing additional contacts with them to try to understand what the source of the inorganic arsenic is. I was wondering if there are any obvious similarities, like were they all from the same fire station or something like that that might indicate a common exposure?

DR. SHE: There's some suspicions. I'd like to Duyen or someone else to comment. You want to comment? So far, we think -- you ask the possible source?
CHAIRPERSON LUDERER: (Nods head.)

DR. SHE: And it's based on only five people. It's very small numbers. So as I mentioned, the data is still under review, but I can reveal a little bit with -- we thought, we suspect maybe rice eating, but this needs to be confirmed. And we'll check all of the data sets to see people who do not eat rice, even in low levels, so have negative controls. So I think the works need to be done more. And Duyen, you want to comment?

MS. KAUFFMAN: I'm Duyen Kauffman from the biomonitoring CDPH side. And just to clarify that there were five people identified, but we were only able to contact two so it's an even smaller number than that.

DR. SHE: So definitely need more work.

CHAIRPERSON LUDERER: Thank you.

Any other Panel comments, questions? Yes, go ahead. Did you hit your microphone?

Hit the button.

PANEL MEMBER QUINTANA: I had a question for Dr. Petreas. It had to do with the childhood leukemia study slide, which was slide 15, I think. And you mentioned that on the 100 microliters of whole blood, which is really a technological achievement, and I congratulate you, that you're not able to measure lipids. And I'm just wondering if you could just comment briefly on the amount
of variability that not being able to measure lipids might contribute to samples that you express, normally relative to lipid -- relative to the variability among the children.

DR. PETREAS: It is true. Usually, we always report persistent organic pollutants on a lipid basis, but given what we have and talking with the principal investigators and the other colleagues there, they decided it was worth doing the unadjusted for lipids just to compare with the profiles of the dust.

So this was like an add-on to our major dust study that we have. So we have characterized the homes with the dust and now have children. So trying to see which -- I don't -- the profile won't change whether it's adjusted or not. You won't be able to compare with other populations maybe, but again it's very rare to have young children's data anyway to compare. CHAMACOS have some work, but not too many. NHANES doesn't have younger -- young children.

So we thought it was worth doing that. But you're right, I mean, ideally we want to have a little more volume to do the lipids.

PANEL MEMBER QUINTANA: But it sounds like the variability among homes might be greater than the small error that's introduced by the lack of adjustment, that's
what you're saying, or are you just looking at the profile overall?

DR. PETREAS: I didn't hear. The variability between homes?

PANEL MEMBER QUINTANA: I'm just saying is the percent of variability that's introduced by not being able to characterize lipids, it might add another 30 percent to variability or something.

DR. PETREAS: I don't think it would be that much.

PANEL MEMBER QUINTANA: Or I wasn't sure about the magnitude, if you had an estimate.

DR. PETREAS: Yeah, but the decision was to ignore that and just look at the profile for this particular purpose.

CHAIRPERSON LUDERER: Thank you, Dr. Quintana. Any other -- yes, Dr. Kavanaugh-Lynch.

PANEL MEMBER KAVANAUGH-LYNCH: I am glad to see that you're exploring the instrumentation and methods for non-targeted screening. We will in the next couple of months be releasing an RFP for a study on non-targeted screening of drinking water throughout the State. So you may have a customer for that methodology soon, or maybe you'll be the customer.

(Laughter.)
CHAIRPERSON LUDERER: Any other questions from Panel members, discussion?

Okay. Thank you very much, Dr. Petreas and Dr. She.

So then we will move on to our presentation by Dr. DiBartolomeis, who I see has arrived. And so Dr. Michael DiBartolomeis is going to provide us an update on the Biomonitoring California activities.

(Thereupon an overhead presentation was presented as follows.)

CHAIRPERSON LUDERER: Welcome, Dr. DiBartolomeis.

Glad you were able to make it.

DR. DiBARTOLOMEIS: Well, thank you and good morning. And let me apologize for the entire Bay Area. Actually, this was one of those mornings that you know doesn't happen very often, but when it does happen you're not surprised.

My problem -- the problem with me is I couldn't get on the freeway. Once I got on, I was fine, but it was so backed up, I couldn't get out of my driveway, and people were just being really rude and it was incredible.

So I'm happy to be here, and I'm just going to go ahead and dive right in.

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DR. DiBARTOLOMEIS: This is actually a fairly
simplified Program update. I'm just going to update on
the three projects, with the caveat that for when we get
to the third bullet, which is the biomonitoring exposure
study, I'm going to have -- spend a little bit of time
because we're going to talk not only about what we've been
updating you about over several meetings, but we're going
to talk about the expanded BEST so sort of BEST part 2.

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DR. DiBARTOLOMEIS: So just to refresh people's
memory. Back on the last meeting in August -- excuse me,
let me just get my little cheat sheet here.

We had just returned the second set of results to
participants, and we were still in the process of
analyzing the final panel of chemicals, which are the
hydroxy BDEs or the diphenyl ethers -- brominated diphenyl
ethers. And where we are now is that that analysis is
complete, and -- excuse me. And I do want to say this
marks -- I guess this particular project, and this
happened -- started long before I came here, is the first
project for the Biomonitoring Program. And really by
completing this analysis, this really completes the
project, in a sense. I mean, we still have some analyses
to do and some additional products to deliver, but this is
a real milestone.

And I do want to extend my appreciation to the
staff, the complete staff, past and present, of the Biomonitoring Program that worked on this. And also I wanted to extend our appreciation to our two collaborators, Dr. Rachel Morello-Frosch from UC Berkeley and Dr. -- I always get this mixed up. And then Dr. Tracey Woodruff who is with UCSF.

So we do have some analysis that's ongoing. We will be mailing the -- we anticipate sometime in the next couple months to get the results returned. So this should be very near completion.

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DR. DiBARTOLOMEIS: And I also want to say that I'm very happy to announce that -- take a good look at this slide, because this is the last time you're going to need to see this slide. We have hit the completion all the way down, so I know you'll miss it, but say goodbye to it.

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DR. DiBARTOLOMEIS: So let's move on into the Firefighters Exposure Project. And we've already heard quite a bit actually from Dr. Petreas about where we are with this, but I wanted to kind of remind people where we were. We had -- we were in the process of analyzing -- we had two things going on. One was a survey of participant understanding, and the other is that we are continuing
evaluating data. We had just submitted the second set of
analytes to the participants.

And I also should just mention that this is
collaboration again, just to remind people, with Dr.
Leslie Israel at UC Irvine. And again, many people who
have not -- who are not even part of the biomonitoring
project, like Dr. Rupa Das, are key in having gotten this
up and running.

So the -- I just want to mention again that the
participant understanding piece is a survey of the
firefighters, and it is -- we used a SurveyMonkey.

So if you look at the next slide --

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DR. DiBARTOLOMEIS: -- we actually completed that
analysis -- not only the survey, but the analysis. And I
just want to just spent a couple seconds on this.

The number of firefighters that we had -- that we
had email addresses for, because this was sent -- this is
a SurveyMonkey sent by email, were 92, so 92 out of 101.
And we received back a little less than 10 percent of the
surveys or nine. So -- yeah, nine out of 92.

So one might say, well, that's kind of low. And
it is kind of low. No matter how you look at it, it's
low. But when you're using a SurveyMonkey, you do expect
a lower rate of return. Plus, we also understood before
we started this that the population might tend to not be responsive to these sort of things anyway.

Because the response rate was so low, even though we've analyzed the data and we've sent it off to Dr. Israel, we're not going to make it public, because the numbers are just too small for us to draw any conclusions.

I do want to say, however, that the results we did get were very positive. So the package of information, those people who did respond thought it was really good and they got, you know, solid information out of it. So for what it's worth qualitatively, what we did get back was positive. And Dr. Petreas already mentioned that there are some publications in the works, three of them.

I can just tell you that the Dr. McNeel paper I've reviewed already, and it's in our CDPH chain of review, so it should be submitted fairly shortly.

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DR. DiBARTOLOMEIS: Let's go ahead now and move onto where I really want to spend a little bit of time. I want to -- I'm going to actually step back a little bit, and this is for the purposes of anybody who hasn't been on the Committee for a while or hasn't -- who are in the room who haven't seen these slides over and over again. So Pilot BEST was what it sounds like, it was a pilot aimed
at looking at a representative population of -- you know, a representative California population.

And it's in collaboration with Kaiser Permanente. And I want to just -- Northern California division. And I want to again shout out to the collaborators, Dr. Stephen Van Den Eeden, who is the co-principal investigator Amethyst Leimpeter -- I hope I'm saying her name correctly -- who is the project manager for Kaiser and Denise Hodges, who is the recruitment coordinator.

So Pilot BEST involves six counties in central California, Central Valley. We are -- we attempted to achieve an equal recruitment across race and ethnicity, gender, and age. And participants were recruited by mailing a letter. And the samples were collected by a phlebotomist who went door to door or went to -- not door to door, but did home visits. And we'll talk a little bit about the pros and cons of doing that. Using this approach, we enrolled 112 participants.

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DR. DiBARTOLOMEIS: So on the next slide, I just want to summarize what the target goals were, and then where we -- and then what we achieved.

So out of 112 participants, you can see that we were, again on the target side nice equal numbers trying to get the four races that we were -- and ethnicities we
were aiming at were Hispanic, Asian and Pacific Islanders, White and Black. And we wanted to get about an equal number. And if you look at the number of enrolled participants, we did fairly good job of achieving those numbers.

For the male, female gender split, we again were pretty close to 50 percent with slightly more males. But again, generally we were on target with our enrollment.

One thing you will notice is that on the age profile, we tended to be a little bit on the older side. Although, I have a hard time believing that 55 is really that old. I'm sure everybody in this room probably -- a lot of people feel that same way. And we attest -- we -- the reason -- probably the primary reason for this, and there is somewhat of a selection bias here, when you have a phlebotomist going to somebody's house during the daytime between 8:00 and 5:00 p.m., you're going to get certain people at home. Whereas, if you visited them at their workplace, you might have had a different spectrum of age breakdown.

So it's something that is an artifact probably of the fact that we were going to them, rather than they going some place else, and not being available on the weekends.

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DR. DiBARTOLOMEIS: On the next slide, I just -- now going back to our familiar looking slides for -- in terms of the updates. Just to remind you where we were in August, we were ongoingly analyzing the second set of chemicals. We were -- we had not yet returned the results of course, and there was some data analysis ongoing.

The current slide looks pretty much the same, but I've got to tell you that there's a lot more behind those green boxes as you'll see on the next slide.

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DR. DiBARTOLOMEIS: We've -- actually, the labs, I give a lot of credit for, they've really stepped it up here and have, as you can see, many of the panels now are complete. And, in fact, the once, like perchlorate, that not complete, they're in review. So a lot of progress was made over the past couple of months. So I just wanted to, again, shout out to the labs who really produced quite a bit during the last couple of months on this. And so I won't dwell on this.

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DR. DiBARTOLOMEIS: Now, I want to move into the Expanded BEST, which is really -- I don't know how to say this, if it's just sort of BEST part 2, or if it's really a separate project. You know, we can kind of -- a question of semantics. So I'm just going to say that this
is a continuation of BEST, but with methodologies that are quite different, such that probably you won't be able to compare the results, per se. Although it's really not clear.

One thing that we did is we added one county in the Central Valley. But in this particular case, we wanted to oversample for Hispanics and Asian and Pacific Islanders, because that is more representative of the California population, and I'll get into some of the other pros about doing that.

We purposely targeted to include more monolingual Spanish speakers. We had -- in the Pilot BEST, we did have Spanish speakers, but they were also English speakers, and so it wasn't really getting at true -- a population truly that was only Spanish speaking. And we were trying to again have an equal distribution across age and gender.

The other main difference is that instead of using a phlebotomist, we wanted to take advantage of the Kaiser Permanente clinical system, which allows -- I think there's 50 different locations or labs where people could go and donate. So this opens it up to hours that are not just 8:00 to 5:00 and weekends. So we thought that we would get a different spectrum and increase our diversity that way as well. It does raise some problems, too, which
I'll get into later, but for the most part these were --
this is where -- how we set up Expanded BEST.

We did -- within the race and ethnicity groups,
we did stratify random samples on gender and age.

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DR. DiBARTOLOMEIS: So our target was to hit 420
enrolled participants with, as you can see, oversampled
Hispanic and Asia Pacific Islanders, and of course try to
achieve the 50/50 split for gender and age. If you look
at the enrolled participants, and as of October 23rd --
and by the way, enrolled participants mean that they've
signed up, completed the consent form, and have donated
samples at the lab. So I just want to point that out.

303. We still have an opportunity to increase
that number. And the other thing that we did, by the way,
with Expanded BEST is the questionnaire was available
on-line, so that also facilitated folks signing up and
enrolling into the program.

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DR. DiBARTOLOMEIS: And just to continue on,
recruitment activity. So we stopped the recruitment
activities, but we haven't stopped the enrollment process,
because people can still have the opportunity until about
the end of the month to actually get the consent forms in
and do their samples. So we're hoping that that 300
number will go up.
And I do want to say that in terms of the sample
collection and the challenges, because Kaiser is primarily
clinical and we're primarily investigatory, or some people
might say research or public health application oriented.
Sometimes you don't exactly have things matching. For
example, a urine that's collected for clinical use may not
have to have the volume that is needed for biomonitoring
use, and we did run into a little bit of that problem.

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DR. DiBARTOLOMEIS: So this is the first time
you've seen this slide. And this looks like some of the
other slides that we've had, but this is now something
that will be embedded in our update presentations. So
this is just basically where we are right now in the
Expanded BEST process. We are still collecting blood and
urine. We are involved in extracting information from
medical records, and the analyses have not yet started,
which is what you would expect.

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DR. DiBARTOLOMEIS: I want to close this
conversation -- or this discussion here about Pilot BEST
and Expanded BEST with a comparison table, just to kind of
put it all into one piece of paper. And for sure, we'll
be able to regurgitate this in the future if we wanted to
spend a little bit more time on any of these particular
differences.

Location, same. Study period, obviously Expanded
BEST is coming after the Pilot BEST. We are slightly --
we have more participants in Expanded BEST and we are
hoping to even get closer to our target of 400 or 420.

Languages. Again, probably with BEST is
primarily English. Although, we did get Spanish speakers.
But in this case, for Expanded BEST, we are having a
targeted monolingual Spanish enrollment.

Recruitment oversampling. Whereas Pilot BEST was
to achieve some equal distribution. In Expanded BEST, we
wanted to oversample for Asian and Pacific Islanders. And
let me just again say that the advantage of this is
California is a diverse population. It doesn't look like
any other state in the country, and federal biomonitoring
efforts really are not representative too well of
California.

So this is really exciting, because BEST does
represent the closest we have to anything that would be
more or less a random sort of representative sampling,
even though it is Central Valley, which is a very specific
region in California. So getting these results into the
future are going to give us an idea -- a better idea at
least of what we might expect as sort of representation of
what the California population would look like.

I do know that CDC is very excited about seeing this, because they can't do something like this. So this is the -- one of the benefits of having a Biomonitoring Program in a State like California.

The recruitment method we used in Pilot BEST was a letter in the mail with a follow-up phone call, which exactly probably tends to have a more personalized approach, and you might get a higher percentage rate of enrollment versus a letter in the mail, which is kind of like a mass mailing, you just hope people are responding. But we sent out a lot of letters for Expanded BEST.

And in terms of the consent form I've already mentioned, the -- well Pilot BEST was an in-person, again personalized, survey or interview. And whereas with the Expanded BEST they would go on-line and do it on-line.

And then finally, one of the bigger differences is sample collection phlebotomists at home visits for a Pilot BEST. They go to the lab or the clinic in Expanded BEST.

Make sure I covered everything. Otherwise, my staff will go "Michael, you forgot something". Although, I think I did pretty well there. I'm sure you might have some questions.

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DR. DiBARTOLOMEIS: But before we do that, I'm going to close by saying I know that you've mentioned this morning, and I missed it -- I know that Dr. Alexeeff paid tribute to Dr. Lipsett in his retirement, but I want to say some personal things on behalf of the entire Program, staff who are here presently in Biomonitoring Program, and those who are possibly listening, but who have since moved on to someplace else.

You know, Michael is -- and I know -- he might be listening actually. But if not, he can read the transcript.

You know, Michael's contribution, besides the fact that he's just, you know, all around great guy to have be -- to work with, is that he -- you know, he had a major role, if not one of the major roles in starting up this program.

And I don't know how much Dr. Alexeeff mentioned this, but he wrote the proposal, the first -- and with a couple of staff, and with the labs' input on getting the CDC funding. And I don't think we would have been where we are now if we hadn't been able to achieve that. So that was huge.

He's the primary author on other materials, and he is the principal investigator, and he's -- or outgoing principal investigator. And served as the CLIA
coordinator for the entire department, I guess, or at least for the labs. And I do know personally he -- running a Branch, he probably put 50 to 60 hours on the average per week, which meant -- which means that he probably could have retired about five years ago if you can count all those extra hours for longevity.

So he did recruit me to take over for the lead of the Biomonitoring Program about a year ago. I am thankful that I had this year to work with him. I was expecting to have more than this, but we'll have to move on without him.

I want to add that he has not only been a colleague of mine, but he has been a really good friend of mine for over 25 years. So if there's one positive note about Michael leaving it's that I guess hereafter, maybe starting in January, I no longer will have to be Michael D. and he Michael L. It will just be Michael.

(Laughter.)

DR. DiBARTOLOMEIS: Until such time we hire. So anyway, Michael, hopefully you're listening, thank you for everything. We're so proud of your accomplishments, and we wish you the best in your retirement.

Thank you.

CHAIRPERSON LUDERER: Thank you very much, Dr. DiBartolomeis for the update and the -- I think all the
Panel members also agree that we wish Dr. Lipsett all the best. And we are very thankful for all of his contributions to biomonitoring in California as well as his many other contributions.

And so we can take some questions from Panel members.

Dr. Quint.

PANEL MEMBER QUINT: Thank you, Michael, and thank you Michael L. Just a great person to have in any department and program. So very much appreciate all your work.

And great results. So lots of accomplishments. I just wanted to ask whether or not it would be possible, at some point, to get the project leads for some of these great studies to come and present back to the Panel? I mean, it's nice to see all the completes for the different analytes and, you know, to hear about the results. But like for the MIEEP program, I know that there was a really -- a good questionnaire that accompanied, that was a part of that study. So I was curious as to whether or not there was any pattern between, you know, what was collected on the questionnaire and the results.

It also -- I think, CDPH had a little piece of that, wanting to pilot a questionnaire that could be used as a part of a medical record. So I don't know if that --
you know, they continued with that -- you know, to test that hypothesis. But it would be just nice to have the whole project presented to the Panel, if that's possible with these great, you know, pilot projects.

DR. DiBARTOLOMEIS: So let me just ask you. By project leads, did you mean having Tracey and Rachel come?

PANEL MEMBER QUINT: Yes, exactly.

DR. DiBARTOLOMEIS: We'll definitely look into that.

PANEL MEMBER QUINT: I mean, I know there's two pieces. There's test -- you know, trying to understand participant's understanding of the results, which is a separate, you know, wonderful thing to do. But then there's just, you know, how many people worked versus -- I mean, was there a pattern between what they found, an occupation or not or you know that sort of thing. I think just the questionnaire versus the results. I mean, even hearing about that would be very interesting. So it would be good closure, I think, for the Panel to get that.

And I had a second question about the FOX study. The SurveyMonkey didn't produce great -- you know, you didn't get a great return. Are you thinking about other ways to tap in to get participant feedback, other than -- I know the -- it's very labor intensive to, you know, do the kind of study that Rachel and others are doing. But
is there something in between?

Because it would be really great to compare the understanding of the FOX participants versus the -- you know, the people who were enrolled in MIEEP, because they're two very different cohorts.

DR. DiBARTOLOMEIS: Thank you, Dr. Quint. That's a really good question. I don't know what the official response is of the Program at this point. Personally, I do think -- I am advocate of evaluating survey -- you know, approaches, getting feedback from participants, as well as stakeholders. I mean, I think that that's really important to do. I don't know exactly what is the in between. Having done evaluations in the past for other -- in other -- for other things that I've done, it's sort of just a case by case. You almost don't know exactly what the best process is going to be.

The firefighters were a special population. They -- you know, probably unless we went there and showed up on their doorstep, they probably weren't going to be too interested in spending more time in responding to something on paper or whatever, but you never know.

So I'm just going to -- I don't see anybody sort of jumping to answer that question any differently. So why don't we just get back to you, because I think it is a good question, and it's worth us considering as a Program.
CHAIRPERSON LUDERER: Thanks.

Dr. Bradman.

PANEL MEMBER BRADMAN: Yeah. I also want to just extend my thanks to Michael Lipsett. And I've also known him for many years. And I really can't overstate his contributions to this Program and public health in general in California.

To go specifically to your presentation and the information with respect to Expanded BEST. And I think that's really a great contribution and extension of both the existing program and also, as you said, in achieving the goals of the Biomonitoring Program.

What are the prospects for expanding this beyond say the Central Valley and perhaps working with Kaiser or maybe other, you know, health maintenance organizations or other providers as a mechanism to, you know, expand the geographic representation in California?

I know we've talked in the past this might be the best way to align with some of the specific goals in the legislation. And maybe that's a direction that can also develop further. And I assume that, you know, you've all been thinking about that, and maybe there's some discussion warranted.

DR. DiBARTOLOMEIS: Well, yes, Dr. Bradman. That definitely -- it's not, we finish and then we move on. I
mean, we definitely are giving some thought to this. There are a lot of different considerations. One is even if Kaiser is wanting to participate as a collaborator into the future. I mean, we have a very good relationship now. And, in fact, we have a second where we're providing service for -- I don't know if, Dr. She, did you mention the bisphenol A work with Kaiser?

DR. SHE: No, I don't.

DR. DiBARTOLOMEIS: Okay. We'll probably hear about this in the future, but we're doing a smaller work, not with the exact same collaborators within Kaiser to look at some bisphenol A levels and metabolites and analogs.

So there seems to be some really positive relationships with Kaiser. I don't know about other HMOs. I don't know to what extent this program has even looked into that feasibility in the past.

Resources is, of course, a concern. And we're not -- we didn't spend -- I did not spend any time at this meeting to tell you about sort of updates. But, you know, just keep in mind the CDC funding is over on August 31st, 2014. So that's a big chunk of the Biomonitoring Program's funding.

So we're still in the process of discussing and look at options to -- you know, what might be available to
replace that funding. So all those considerations. But technically, the labs certainly have the capacity and capability, whether they can do expanding, you know, into other projects without shifting priorities or adding staff or whatever, I mean, because at some point you reach your capacity for throughput, and you have to, you know, consider all that. So there are those types of things in the works.

I also might want to just mention and remind you that we've also extended a -- or we're looking into the feasibility of using samples from the Genetic Disease Screening Program in CDPH. And we've made actually some -- I didn't mention this as part of the update, but we've made contact with them and it looks like that starting some time early next year samples will be available. And we are working out the details for how the Program can obtain some of those.

And again, that's not quite a representation as the BEST study, but it is another way of getting at representative samples. So we're -- and we do know that, you know, Dr. Petreas last -- at the last meeting mentioned that at least for the serum samples, it looks pretty good that we can look for specific analytes.

PANEL MEMBER BRADMAN: Right. Yeah, no, I think that's very important. A person to contact at Kaiser, I
don't know, is Kathy Gerwig. I don't know if you've been in touch with her. She's their environmental safety manager, and she's been instrumental in changing medical materials to reduce exposures to toxic substances. So she might be somebody worth talking to, perhaps could facilitate some of the relationships with them.

CHAIRPERSON LUDERER: Dr. Wilson.

PANEL MEMBER WILSON: Thank you. And thank you for the presentation, Michael. And I had a question and then a comment. The first question was on the MIEEP slide. You know, the analyses are all completed here. And I'm wondering if you have a sense of the numbers of different congeners, you know, of these different substances that have been identified? And if so, what the total number is?

DR. DiBARTOLOMEIS: Okay. So I'm going to have to turn to staff here.

EHL does between 50 and 60 different analytes for -- and then -- so I guess that's sort of the congener analytes.

Did you want -- Myrto, did you want to say something to that?

DR. PETREAS: Yeah. For the PFCs, there are 12, right? We're doing 12. PBDEs or the POPs. 30 PCBs. With the hydroxy, 36 come together. And the pesticides we
have six major pesticides and 17 PCBs.

PANEL MEMBER WILSON: So what is that, a couple of hundred?

DR. PETREAS: So that's the one that we are targeting, but some of them may not be detected in everyone.

PANEL MEMBER WILSON: So it's -- just -- I didn't quite catch the numbers as you were going by. It sounds like about 200 --

DR. DiBARTOLOMEIS: About 150.

PANEL MEMBER WILSON: -- of congeners all together.

DR. DiBARTOLOMEIS: Well, I mean, it's hard to say what the definition of congener is or whatever. But I think overall it's about 150 different analytes. Is that right?

Not quite 200. So somewhere a little less than -- probably around 150.

PANEL MEMBER WILSON: Okay. Great. And do you have a sense from Rachel or for Tracey Woodruff when the -- when those results will be available?

DR. DiBARTOLOMEIS: Boy, these are tough questions.

PANEL MEMBER WILSON: I'm sorry, Michael. From one Michael to another.
DR. DiBARTOLOMEIS: Well, the simple answer is no, I don't, but I do know that they've resumed activity. There was a little bit of a lull period, but we've made contact, and they are actively working on it. So I can't give you a specific date or even a target, and I don't even think it's fair to put that out there on a transcript.

PANEL MEMBER WILSON: Okay. Sure. Okay. Thank you. And I guess the comment on the survey results. You know, the nine percent it doesn't surprise me. But just one suggestion -- and maybe you did this. I'm not sure, but, you know, they -- that population will tend to be responsive to the union leadership. And if the -- you know, if in communicating with the union leadership that you make it clear to them that this is a high priority, and you want their membership to respond to this survey, they will get that word out to their members, and that will bring your response rate up, I think.

If it's -- if you're -- you know, if this is something you want to revisit, if -- you know, it sounds like very interesting information that could be useful going forward, just as a suggestion. I don't know if you did that or not.

DR. DiBARTOLOMEIS: I actually don't know if we did that, but definitely is duly noted. And if we --
the future when we work with another population. I don't know if we're going to go back for this particular -- in this particular instance. But when we're working in occupational settings in the future, I mean that's a really good idea to not only bring the labor in at the end, but you know, start at the beginning. Have them involved from the very beginning. Explain why this is really important.

I've learned that from my training. So I don't know exactly to what extent that was something that was part of the protocol way back when FOX was started, but in the future that is a very good suggestion.

PANEL MEMBER WILSON: Yeah, okay. And just I want to extend my congratulations to Dr. Lipsett as well. He was someone who I looked up to from -- in the mid-nineties in my graduate studies, so for many years.

CHAIRPERSON LUDERER: Okay. We have Dr. McKone.

DR. DiBARTOLOMEIS: You need to turn your microphone on.

PANEL MEMBER McKONE: There. So I want to begin with a compliment rather than at the end to the Michaels. But I think it's really great that, you know, the Program is in your capable hands, but we will miss Michael L. a lot. I think he was -- I mean, for me, it's the same thing, he's just been around in so many meetings that I've
been to and had so many interactions. It's kind of hard to imagine not coming up here and seeing him wandering around somewhere in the audience.

But my question concerns I guess on some of the future uses of the information, particularly for something like BEST, are there plans for putting that in context, such as looking at how it compares with NHANES? Is there any timeline or plan for, you know, again not only just making the data available, but some studies to put in context health issues or in -- you know, for me, I think it would be very interesting to see a comparison to NHANES in some way how that relates to what's observed nationally now that we have some State monitoring.

DR. DiBARTOLOMEIS: I'm going to turn the question over to Dr. Fenster who -- and I think I might even have the answer, but I want to hear her answer and see if we're the same.

DR. FENSTER: I'm really excited about the BEST population, so I thought I'd get up and say some hip hip hoorays, because I think it's one of the first times where we've been able to use their lab infrastructure to really reach a diverse population, and as Michael said, allow people to come to collect their samples on non-traditional working hours. So I'm very excited about that.

We're just starting to analyze the lab data that
you saw, in terms of Pilot BEST. We've just got an avalanche of data that the epi biostat staff are working on. And we have plans to -- we're already starting to compare to the appropriate NHANES population, we're going to be looking at the questionnaire, so we're very -- we have plans to pursue that as well as when we've completed enrollment for Expanded BEST. And the numbers have already gone up in Expanded BEST. Probably they're about 333 approximately. Oh, 337. Yea. So as we speak, people are donating more samples.

CHAIRPERSON LUDERER: All right. Thank you. I actually have a few more clarifying questions about BEST. I'm very excited about it as well.

And one of the things, my recollection was that -- because you mentioned, Dr. DiBartolomeis that there was an equal distribution across ages, but it's adult only, isn't that correct? So it's over 18.

DR. DiBARTOLOMEIS: (Nods head.)

CHAIRPERSON LUDERER: Right, okay. I just wanted to...

DR. FENSTER: I want just to also add onto the possibility to look at health endpoints within Kaiser. I think that could potentially be a way to apply for grants to look at some of the -- particularly for the chemicals with -- that are associated with different endpoints in
the Kaiser population, whether it's diabetes or thyroid
disease. That's a possibility. And we would need
resources, but it's also, I think, very attractive to
funding agencies, given it's Kaiser and there is -- there
are the medical health records.

CHAIRPERSON LUDERER: And then I just wanted to
follow up on the comment about the comparison of the Pilot
BEST and the Expanded BEST. Is the questionnaire that's
being used identical, other than one was an interview and
the other one is on-line questionnaire?

DR. FENSTER: Right. And I did want to mention
that the Expanded BEST, if people didn't feel comfortable
answering a computer-based survey, they also had the
option for a hard copy questionnaire.

We tried to keep the questionnaires comparable,
so that we could potentially, with different caveats,
given the method, the design differences, we could
potentially combine the populations for different -- you
know, when we were examining potential routes of exposure.
So they're very similar.

CHAIRPERSON LUDERER: And so then the analytes
are also going to be the same analytes for the two or are
there differences?

DR. FENSTER: They're only going to get better.

(Laughter.)
DR. FENSTER: Because, as you saw, the labs are expanding their analytes. And so, again, they're similar, but there will be some potential differences.

CHAIRPERSON LUDERER: Great. Thank you.

I know we have at least one public comment. Have we gotten any other public comments?

Just one. All right. Well, we'll take time now for the public comment, and then we have some time after that for some more Panel discussion.

So our comment is from Davis Baltz of Commonweal.

MR. BALTZ: Davis Baltz, Commonweal.

Dr. Luderer, Dr. Alexeeff, Panel members, nice to see you again. And let me also add my congratulations to Michael Lipsett on his retirement. Unlike some other people who've spoken, I didn't have the honor of being hired by him --

(Laughter.)

MR. BALTZ: -- but I have worked with him since the inception of this Program, and he has been accessible and resourceful, obviously hard working, and has done a lot of the heavy lifting that's gotten the Program to where it is today. So wish you all the best on your retirement, Michael. If you're listening, with any luck, maybe we can figure out a way for him to come back and contribute in new ways to the Program. He's obviously
already a respected graybeard.

(Laughter.)

MR. BALTZ: I was very pleased to hear the updates from the labs and the Program. Lots of progress since the last meeting. And so I think there's a lot of exciting developments happening. The collaborations with the California Breast Cancer Research Program, I was encouraged to hear those. The Three Generations Study that the Environmental Chemistry Lab is undergoing with the Breast Cancer Research Program, I think that's important. And as have been mentioned, the UC Berkeley Childhood Leukemia Study and the work with Kaiser and the BEST study. I mean, that's a very wonderful way to capture some efficiencies to piggyback on their sampling lab infrastructure, so that it saves the Program resources in that regard.

So the new results that have been posted have been circulated among my networks. And the new methods development, of course, will lead to more results being published in the future. So I just, you know, give my thanks to the staff once again for all of the work hard that has gone on under not always easy circumstances with budgetary constraints and so forth.

But as I've said to the Panel several times over the years and the Program's been in existence, getting
results out where people can see them I think is critical, so that advocates and others can talk about the successes of the Program and how it's contributing to public health and environmental health in the state, and that the legislature, among others, also becomes aware, to the degree that they maybe haven't up until now, the value of this Program. And this is especially important as the next year approaches and the funding prospects for the Program are once again somewhat up in the air.

So again, thanks for the continued work. And to echo what Dr. Wilson had said, looking at workplace exposures I think is an important activity that should be explored further, as it can mobilize and activate another sector of important populations in this state who can then support the Program.

And as a final note, Dr. Bradman mentioned this, Kathy Gerwig at Kaiser Permanente who's their vice president for workplace safety as well as their environmental stewardship officer, we worked with her. She was involved in the beginning from Health Care Without Harm. She's very creative and deeply knowledgeable about environmental health and encourage the Program to contact her and see how she might have some ideas on how BEST could be expanded and improved.

So thanks again.
CHAIRPERSON LUDERER: Thank you very much for those comments.

Do we have any other discussion or comments, questions from Panel members?

Dr. Quintana.

PANEL MEMBER QUINTANA: Hi. I wanted to congratulate you on the BEST study. I think it's very exciting. I did have a clarification question or maybe a suggestion for future BEST activities. When I was looking at the results and the recruitment strategy, it looked a little bit old fashioned, in the sense that California is different than the rest of the nation. And one way it's different is the number of people identified as mixed race. But there's no category in your recruitment for people identified as mixed race.

And it seems to me that what you're trying to achieve in BEST is to make sure that our population is represented. And some populations that are sometimes overlooked are not overlooked in this case, but it may be good to include additional categories, perhaps based on income, based on being born outside the United States or other issues that would also make sure we capture the populations that you're trying to capture.

And I also understand that -- I think I'm correct that the BEST study is only Kaiser patients. So, of
course, we're missing perhaps the currently uninsured or people outside the system. And I was just curious if they, in the future, might be gracious enough to even offer their labs for other populations that you might sample, which is probably a pipe dream, but just a thought, because I just feel like it's the idea of inclusiveness that you want to achieve, correct?

   DR. FENSTER: I'll just mention, I wish I had picked up the hearing aids that I'm supposed to get yesterday. I think I canceled that.

   I couldn't quite hear all of your questions, but I did hear that you're encouraging the Program to look at getting the tails of the distribution of a population that may not be, first of all, Kaiser members, and then secondly, the issue of trying to, in terms of the race ethnicity categories that served as recruitment.

   My guess is that there are mixed race within those. I'll have to do, you know, more exploring. We did add on some acculturation questions that Dr. Van Den Eeden has examined previously which are very interesting in terms of relationship to health outpoints, and I imagine in terms of exposure assessment as well. So was there another question you had that I didn't cover?

   PANEL MEMBER QUINTANA: That basically captures it. Sorry, I guess I need to lean forward a bit more.
CHAIRPERSON LUDERER: Actually, I had another related question to that, which is whether you were able to, for the Pilot BEST and/or will be able to for the Expanded BEST look at the -- be able to compare those who were actually recruited to the people who were contacted via letters, you know, just sort of demographic characteristics to see if there are any major differences between those who were recruited and those who were not.

DR. FENSTER: We are -- we're looking at -- we will be able to look at who responded and -- for example, in Expanded BEST, who used the on-line survey versus who requested a hard copy. We'll be able to look at recruitment in both of those. Although, I have to say part of the benefit of working with Kaiser is that it has so many members, and it has such a diverse membership, both race, ethnicity, and income, male, female. So we tended to kind of bombard that population within our stratification design, rather than say in a typical epi study where you really pursue and be considered about your participation rate, per se.

That's a limitation, but also the strength of Kaiser and our resources, in terms of the first study having a phone call. I want to say Expanded BEST, the recruitment now that we are getting into closing the study, one of the Kaiser staff has been calling people
that have consented and filled out a questionnaire --
completed a questionnaire, but haven't yet donated their
samples, alerting them that the study will be closing.
You know, in many cases, they've lost their lab slip or
they've been too busy. But we are actually contacting as
many of those partial participants as we can, as the study
moves towards closure.

CHAIRPERSON LUDERER: Thank you. Any other
comments, questions from Panel members?

All right. Well, thank you very much for those
very interesting presentations this morning.

So we're -- looks like we're finishing a little
bit early. So we have -- I think we will allocate an hour
and 25 minutes for lunch, as we had planned. So that
would mean we would come back at 1:35. And -- no, sorry.
I'm looking at the clock wrong. 1:20. 1:20.

And I just also wanted to say that prior to
breaking for lunch, Fran Kammerer, who is the staff
counsel for OEHHA, is going to give us a reminder about
Bagley-Keene upon returning from lunch and call the
meeting back to order. Upon returning to lunch, we'll
call the meeting back to order and then I'll introduce the
next agenda item.

So, Fran.

STAFF COUNSEL KAMMERER: Good morning. I'd just
like to remind the Panel members that this Committee is 
subject to the Bagley-Keene Open Meeting Act. So I'd like 
you to refrain from discussing Panel subjects, if 
possible, that have been discussed or will be discussed 
today, and try to wait and discuss them here, so the 
public can participate in that.

    Thank you.

CHAIRPERSON LUDERER: All right. Thank you.
Then, everyone, enjoy your lunches and we will 
see you back at 1:20.

    Thank you.

(Off record: 11:47 AM)
(Thereupon a lunch break was taken.)
AFTERNOON SESSION

(On record: 1:28 PM)

CHAIRPERSON LUDERER: All right. Now, that we're all back from lunch, I'd like to call the meeting back to order. Welcome you all back, and introduce the next agenda item.

So the next agenda item is going to be consideration of selected aroma chemicals as potential designated chemicals. And I'd like to induce Dr. Gail Krowech, who's a staff toxicologist with OEHHA.

Dr. Krowech.

(Therupon an overhead presentation was presented as follows.)

DR. KROWECH: Good afternoon. Is it on now?

Okay. So I'm going to present work that Laurel Plummer, Sara Hoover, and I've done on these potential designated classes of chemicals.

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DR. KROWECH: First though, just to go over what designated chemicals are. And they're chemicals that can be considered for biomonitoring by the Program. They consist of chemicals that are part of CDC's National Reports on Human Exposure to Environmental Chemical Program, and chemicals that the Scientific Guidance Panel has recommended be added to the list of designated
chemicals.

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DR. KROWECH: As background for today's discussion, at the November 2012 Scientific Guidance Panel meeting, the Program presented four -- a screening of four classes of synthetic musks and a structurally related aroma chemical, Iso E Super. And that meeting, the Panel requested documents to support consideration of these aroma chemicals as potential designated chemicals.

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DR. KROWECH: For today, we are going to consider two classes of chemicals. They're structurally related and would have a common analytical method. The synthetic polycyclic musks and the class tetramethyl acetyloctahydronaphthalenes, which is the class of chemicals to which Iso E Super belongs.

In terms of the other classes not under consideration today, we did some research on nitro musks, and think that there will be very low -- there is very low or no current use. There's probably low use of musk ketone. Musk xylene was identified as very persistent and very bioaccumulative under REACH and banned by the International Fragrance Association and then banned by the European Union.

There are methods in the literature that look at
polycyclic musks and nitro musks using the same method. And so if in volunteer specimens the laboratory finds nitro musks, then we can come back and consider them -- bring them forward for potential designation at that time. Macrocyclic musks and alicyclic musks will be looked at at a future meeting.

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DR. KROWECH: So I'm just going to show example structures of these two classes now. These are examples of polycyclic musks: HHCB, and AHTN. The names at the bottom of this slide are names that correspond to the abbreviations. The full chemical names are in the documents.

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DR. KROWECH: This is a tetramethyl acetylactahydronaphthalene, which is structurally similar to some polycyclic musks. And here comparing it to AHTN.

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DR. KROWECH: Before going further, I just want to list the criteria for the Panel to recommend designated chemicals. They are: exposure or potential exposure to the public or specific subgroups; known or suspected health effects, based on peer-reviewed scientific studies; the need to assess the efficacy of public health actions; the availability of biomonitoring analytical methods, the
availability of adequate biospecimen samples; the
incremental analytical costs. And we always add that
these criteria are not joined by "ands".

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DR. KROWECH: Now, I'll start going through
polycyclic musks, and then follow that with the other
class.

Polycyclic musks are widely used in personal care
products and in some cleaning products. They were
introduced as replacements for nitro musks. And their use
increased in the nineties as nitro musks started to
decrease. We highlighted in the document two polycyclic
musks, HHCB and AHTN, which have been the two most
commercially important musks.

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DR. KROWECH: This slide shows other polycyclic
musks that have been in use. And again, the full chemical
names are in the documents -- in the document. I just
want to point out that the musk on the bottom right AETT
was prohibited by IFRA, the International Fragrance
Association, in 1977, and was prohibited because of
toxicity.

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DR. KROWECH: This slide looks at
import/production volume. The levels are from -- are what
was reported to U.S. EPA. And you can see with HHCB these levels look fairly consistent, with HHCB being a high production volume chemical since 1994. And the specific number in 20 -- reported in 2012 has to do with the new reporting rules.

In terms of AHTN, it's a little harder to get the story there. It was reported as CBI to U.S. EPA in 2012, confidential business information. We were able to get the volume of use for North America from IFRA, the International Fragrance Association. And I've also included DPMI, another of the polycyclic musks here.

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DR. KROWECH: These are examples of the use of polycyclic musks in personal care products, just to give a flavor of where they're used.

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DR. KROWECH: And here's more use in household products. Also, I just want to point out, that not only are they used to provide a fragrance, but they can also be used to mask odors. And if something says unscented, it doesn't necessarily mean that these chemicals aren't in there.

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DR. KROWECH: This is from a paper that looked at specific levels of HHCB and AHTN in personal care and
household products from the U.S., from Albany, New York. And you can see the levels are quite high. One of the things that was found in this study was that a number of products had both HHCB and AHTN in it.

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DR. KROWECH: And this is from another study that looked at many of the similar personal care products and household cleaning products. And so I'm just giving more of a flavor of what was found in this study as well and the ranges. And also, this study looked for DPMI and found it in a couple of products.

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DR. KROWECH: This study shows house dust that was part of the Canadian house dust study with samples collected between 2007 and 2010. And as you can see, both HHCB and AHTN were detected in 100 percent of the households. And also, I should mention that both HHCB and AHTN have been found in indoor air.

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DR. KROWECH: The main environmental source is effluent from wastewater treatment plants. And HHCB and AHTN have been detected in fish caught in effluent waters in sewage sludge, in some drinking water, and -- oops, how do I go backwards?

There we go. And in one study they were both
found in runoff from agricultural fields irrigated with treated wastewater.

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DR. KROWECH: Polycyclic musks have been detected in biota, most notably in bivalves from the San Francisco Bay. Not only HHCB and AHTN, but ADBI and AETT were detected. They've been detected in fish, and the levels have been dependent on location, and on metabolism and lipid content of the fish.

They've been found in low levels in marine mammals. One study was interesting in finless porpoises in Japan. This was published in 2005, and they looked at eight porpoises. And for three of those porpoises both the porpoise and its fetus -- and in one -- two, they were not able to measure the fetus because they were so immature. But in the third, the level in the fetus was comparable to the level in its mother.

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DR. KROWECH: And this slide I'm just going to point out some of the indications of biological activity. There have been indications of endocrine activity, weak estrogenicity in reporter gene assays, inhibition of estrogen, androgen, and progesterone receptor activity.

Also in reporter gene assays.

In vivo, there's -- using the same type of
reporter gene assay, they showed anti-estrogenicity in transgenic zebrafish. In a recent study, looking at steroidogenesis - it's a 2013 study - found that there was decreased progesterone and cortisol synthesis based on down-regulation of the enzymes responsible for their synthesis.

In terms of other biological activity, another recent 2013 paper found that -- reported that AHTN caused changes in the activation of certain signaling pathways in mouse embryonic stem cells. One paper looked at several polycyclic musks in efflux transporters in mussel gill tissue. And found that the musks inhibited the transporters. These are regard -- transporters are regarded as a first line of defense limiting absorption of foreign chemicals.

One interesting note about this paper was they showed that low levels of several musks could inhibit together -- combined, could inhibit the efflux transporters to the same degree as a higher level of a single musk.

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DR. KROWECH: Polycyclic musks all look fairly lipophilic. And you can see the list of them right here, or all the ones that we could find, in terms of the Log Kow. And so you would think that they would...
bioaccumulate, and they do have potential to bioaccumulate in some species. The bioaccumulation factors and bioconcentration factors have a wide range however, and it -- you know, from one study, it really looks like whether or not these chemicals bioaccumulate is dependent on both the lipid content and whether -- and the degree of metabolism in that particular species. So low metabolism, high lipid content, you would see bioaccumulation.

There's some indications of persistence.

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DR. KROWECH: There are a number of biomonitoring studies that are detailed in the document. And most of them are from Europe and Asia. Very few studies are from the U.S. Of the studies that looked at use of personal care products, most of them reported that increased levels of personal care products was associated with increased -- with higher levels of polycyclic musks.

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DR. KROWECH: And this is a summary from -- or the details from one -- I think the one study in breast milk from the U.S. And you can see that HHCB was detected in 97 percent of the individuals. This study -- the samples were collected for the study in 2004, so it's almost 10 years old.

One of the things that the study found was
that -- they found an association between use of products and higher levels. They did not find an association between age and higher levels of musks, which again makes it -- sort of reinforces the point of the study that I mentioned that looked at bioaccumulation and metabolism.

There was a relationship between -- although it was not significant between number of children breast-fed and levels, so that levels were lower in women who had previously breast-fed a child.

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DR. KROWECH: Now, I'm going to switch gears and talk about tetramethyl acetyloctahydronaphthalenes. This chemical structure is for OTNE. OTNE is used to refer to a mixture of isomers, and sometimes just to one isomer, the beta isomer of this class. It has a woody, floral and amber fragrance. And it's widely used in personal care products and in some cleaning products.

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DR. KROWECH: This is what has been reported for the four isomers of -- four isomers that we have identified so far, in terms of the production/import volume that was reported to U.S. EPA.

So the beta isomer is definitely high throughout, has increased -- has shown an increase, and we can't really tell for the other isomers based on the CBI in

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DR. KROWECH: Again, these are examples of use in personal care products and cleaning products. And like the polycyclic musks, the tetramethyl acetyloctahydronaphthalenes also can be used to mask odors.

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DR. KROWECH: This is from the same Canadian house dust study. And it shows the house -- the levels of OTNE in vacuum cleaner dust with a detection of 82 percent. And so I've put the levels of the polycyclic musks, so that they can be compared to OTNE. And OTNE was the third most important fragrance that -- in terms of levels in dust in the study.

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DR. KROWECH: In terms of environmental occurrence, the main environmental source is effluent from wastewater treatment plants. OTNE has been detected in influent and effluent wastewater, in sewage and sewage sludge with levels comparable to the polycyclic musks, HHCB and AHTN.

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DR. KROWECH: In terms of bioaccumulation and persistence, the chemicals seem fairly lipophilic.
Bioconcentration factors do not suggest bioaccumulation and generally they are below 1,000. There are few published studies on persistence. And based on the data that we've seen so far, there doesn't seem to be evidence of persistence.

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DR. KROWECH: There are also few toxicological data for this class of chemicals that are publicly available. And structural -- it's structurally similar to AHTN, which has shown some potential for endocrine and other biological activity.

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DR. KROWECH: So the next two slides are summary slides for the polycyclic musks. They are in high levels in personal care and household cleaning products. They have a potential to bioaccumulate in some species. There's a potential for endocrine and other biological activity. They've been detected in various environmental samples, including house dust. And they've been detected in human blood, breast milk, adipose tissue samples.

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DR. KROWECH: In terms of the tetramethyl acetyloctahydronaphthalenes, OTNE is a high production volume chemical. There has been an increase over time in the use of OTNE. It's been detected in dust, wastewater...
treatment plant influent and effluent, and in biosolids, and is structurally similar to AHTN.

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DR. KROWECH: In terms of laboratory analysis, the methods for analysis of some of these chemicals are available in the literature. The laboratory, and that would be the Environmental Chemistry Laboratory, would develop methods to measure polycyclic musks and tetramethyl acetyloctahydronaphthalenes in serum samples. Analysis of these two classes could likely be bundled.

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DR. KROWECH: In terms of the need to assess the efficacy of public health actions, there's widespread use of these aroma chemicals in California and in the U.S. Biomonitoring would determine whether these chemicals are found in California residents and at what levels, and would also allow us to track these levels over time.

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DR. KROWECH: In terms of options for the Panel. The Panel can designate synthetic polycyclic musks as a class; can designate -- the Panel can designate tetramethyl acetyloctahydronaphthalenes as a class; the Panel can postpone a decision; or, the Panel can decide against designating.

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DR. KROWECH: And I'm happy to take any questions.

CHAIRPERSON LUDERER: Thank you very much for that interesting presentation, and also for the very thorough documents that we received for review. Why don't we start out with any clarifying questions that Panel members might have? Dr. Quint.

PANEL MEMBER QUINT: Yes. Thank you, Gail, for a great report and presentation. You mentioned that xylene musk was banned for toxicity reasons. What was the toxicity?

DR. KROWECH: Actually, it was banned because it was very persistent and very bioaccumulative.

PANEL MEMBER QUINT: Oh, okay. Great. Thanks.

DR. KROWECH: Can I continue my response?

PANEL MEMBER QUINT: Sure.

DR. KROWECH: You might have been thinking of the polycyclic musks, is that what you were --

PANEL MEMBER QUINT: Oh, right. Something was banned.

DR. KROWECH: Right, was prohibited from use.

PANEL MEMBER QUINT: Prohibited from use.

DR. KROWECH: Right. And that's one of the musks. Let me see if can I get to it.
PANEL MEMBER QUINT: The polycyclic musks were not used as much in Europe, right? Is that declining?

DR. KROWECH: There is definitely declining use there, but it does not appear to be declining here.

PANEL MEMBER QUINT: Right.

DR. KROWECH: I'm coming to it.

There we go. The one at the bottom right, this was. From what I understand in routine tests, dermal tests in rats, in the seventies they notice -- late seventies, they noticed a blue color on the rats on the skin. Then did further investigation, and internal organs were also blue. And what they basically -- the pathological basis for this was basically there was a demyelinization of nerves that was occurring.

PANEL MEMBER QUINT: That's interesting. Thanks.

CHAIRPERSON LUDERER: I just have a clarifying question about the tetramethyl acetyloctahydronaphthalenes, which is a mouthful. So the four OTNE, those isomers that you mentioned, in addition to those, are there a lot of other chemicals in this class that would, if it were designated as a class, would also be included or is it just those four?

DR. KROWECH: Those are the four that we have identified so far. And so I can't really answer that. I'm not sure. Those are the ones that we were able to
look up production -- import/production volume for. We
don't know. There might be other -- there clearly are
other isomers, but whether they're in commercial use, we
don't know. And it seems like they are a mixture. And so
they have -- they're in different portions for different
products. And I think these are the main ones. The U.S.
EPA workplan listed those four isomers, so we consider
them to be the main ones.

CHAIRPERSON LUDERER: And so those four isomers
do kind of have different uses, because you were just
mentioning that they used different proportions of the
isomers, depending on the product?

DR. KROWECH: I think that there is some --
usually the beta isomer is the one in the greatest
proportion. But there is some commercial use to -- you
know, something is enriched in a certain isomer. It's
hard to get all the information. So that's as much as we
have, I think.

CHAIRPERSON LUDERER: Dr. Quint, you had a
question.

PANEL MEMBER QUINT: I just had a quick follow-up
to that. So I was intrigued by the fact that it
was -- they claimed CBI for three of the isomers and not
the -- so are they made by different manufacturers or
something? I mean, are they all beta isomers made -- I'm
trying to figure out why you would report the beta isomer, how much is made -- manufactured, and then claim CBI for the others? It doesn't -- I mean, unless -- you have no clue --

DR. KROWECH: I don't have the answer to that.
PANEL MEMBER QUINT: Yeah, so it's one -- it's just one manufacturer --

DR. KROWECH: No.
PANEL MEMBER QUINT: -- involved here?

DR. KROWECH: No, there's not.
PANEL MEMBER QUINT: Okay. So that --

DR. KROWECH: Oh, yeah. Okay.
PANEL MEMBER QUINT: It's confusing.

DR. KROWECH: I'm trying to remember. I think there's several for different -- for the different isomers.
PANEL MEMBER QUINT: Isomers. Okay. We're not talking about the same. Got it.

DR. KROWECH: Right.
PANEL MEMBER QUINT: And what is the basis for including these in the EPA workplan? What's the basis? This is just because -- because I know they are interested in fragrances.

DR. KROWECH: Right.
PANEL MEMBER QUINT: So -- but it's the state of
toxicity --

DR. KROWECH: Well, I know high use was part of that.

PANEL MEMBER QUINT: What?

DR. KROWECH: I know high use was part of that.

And it might have been concerns about persistence as well.

PANEL MEMBER QUINT: Okay.

DR. KROWECH: I'm pretty sure that's what it was.

PANEL MEMBER QUINT: Yeah, because the EPA Design for the Environment has worked with a group and come up with criteria for fragrances. And I'm not sure where this all fits in. I didn't get a chance to really look at their criteria. But are you familiar with what -- because they're trying to come up with safer fragrances.

DR. KROWECH: Yeah, right.

PANEL MEMBER QUINT: And they have some criteria for what a fragrance shouldn't, you know, be in order to be considered safe. And I was just wondering how all of this fit in with that?

DR. KROWECH: I'm not familiar with that.

PANEL MEMBER QUINT: Yeah, I'll figure it out.

CHAIRPERSON LUDERER: Dr. Fiehn.

PANEL MEMBER FIEHN: Thanks. I'm interested in understanding better how these compound classes compare to other classified or designated chemicals, in terms of
their bioactivities. So I see here that there's very little evidence for biological activity. Few studies seem to have been carried out, although they are widespread used.

And I'm wondering, you know, if we look at these classes in comparison to say BPAs or phthalates and so on, where there is an abundant literature on biological activities. So do we have here enough evidence, or how are the reports relating to the designated chemical classes?

DR. KROWECH: Well, I think it's true. There have not been, you know, very many studies on these chemicals. And for some designated chemicals we definitely have a lot of information. There are others that we have designated where we have had some information on biological activity and a lot of information on exposure, and have designated based on that, because we don't really know, and there is evidence -- there's so much of it, and we've designated on the basis of the indications of biological activity and the exposure.

And I can think of triclosan being one of -- not triclosan. I'm sorry, triclocarban, and some of the class of brominated and chlorinated flame retardants where we had obviously a lot of information on some, but not on other members of the class. And I guess similar to that,
we designated the class of non-halogenated aromatic
phosphates, where again there wasn't a whole lot of
information.

CHAIRPERSON LUDERER: Dr. Wilson, then Dr.
Bradman, then Dr. McKone.

Dr. Wilson.

PANEL MEMBER WILSON: Okay. Thank you, Dr.
Krowech. It's just a clarifying question, and that -- and
maybe it's what Dr. Quint was asking, but that -- there
was -- the toxicological data for the OTNE, the OTNE one,
is a little bit more limited. But the International
Fragrance Association has placed limitations or
restrictions on its use. And I'm just -- I'm curious if
that's -- what was the basis for that action by the
Association?

DR. KROWECH: I'm not sure.

PANEL MEMBER WILSON: Was it a toxicology
question or sort of it's -- yeah, we don't. Okay.

DR. KROWECH: Oh. Okay. Laurel just told me
it's dermal sensitization.

PANEL MEMBER WILSON: Okay. Thank you.

CHAIRPERSON LUDERER: Dr. Bradman.

PANEL MEMBER BRADMAN: I just had a quick
question was it the -- I sent the DfE, Design for the
Environment, criteria on fragrances the link to you and to
you and to the Biomonitoring Program. So my question is
other than EPA, has any of the CDC, NHANES, any of the
other federal agencies expressed an interest in this? And
is there any method development work or biomonitoring
being considered at the CDC?

DR. KROKECH: CDC is not doing the biomonitoring.
I know that the New York Biomonitoring Program has done
some work on this. I don't think they're doing anything
now, but they did -- the people who did the work in the
mid-2000s were from that group. And I think they've done
some work in dust.

But I don't know about CDC. They actually did
some methods work about 10 years ago and didn't pursue it.

CHAIRPERSON LUDERER: Dr. McKone.

PANEL MEMBER McKONE: Yeah, I want to sort of
reiterate your point, first, about how we designate in
terms of toxicity, and because we're not -- you know, we
don't designate based on risk, or really even hazard,
although, if something is of interest for potential.

And I think it goes back even as far as the
cyclic -- maybe the cyclic siloxanes(sic) were the first
case where, you know, this issue came up about well,
there's not clear evidence of toxicity, but the reason we
thought they were important for designation was large
production. There was clearly widespread use, and I think
a belief that we were likely to see something in terms of
exposure that would be important, whether or not it was
toxic -- as long as -- I mean, if something is no evidence
of toxicity at all, you probably wouldn't be interested in
it, but I think it's probably sufficient.

So I guess the question -- sort of a comment, but
really a question is it looks like these are chemicals
that certainly meet our criteria, or implicit criteria,
that they're likely to be -- we're likely to learn
something important by designating them and looking at
them.

I mean, anything that is, you know, produced in
large volume, used in -- intimate to the consumer, right,
or they're used in the home, they're used in care
products, so there's a high likelihood. And there are a
lot of questions about patterns and trends that we
probably would be interested in. So, I mean, again, I'm
sort of thinking, is that what seems to come up with
the -- I think the full set of compounds kind of meets
this criteria that something that would be high priority
for wanting to track what's going on because of their use,
likely persistence, and they're -- you know, high
production and use in a case where they're very close,
very proximate to the exposed individual.

DR. KROWECH: Yes. Thank you. Glad that you
remembered to bring up the cyclosiloxanes, because it slipped my mind.

PANEL MEMBER McKONE: Yeah. As I recall, that might have been the first time we really had to deal with something that was not on the CDC list, and it was not so much because of historical concerns about exposure, but because it was something that was a rising trend and we wanted to make sure the Biomonitoring Program could not only look backwards into what we already knew was of concern, but also look forward to things that are entering the marketplace and have characteristics that we thought would be important for attracting them.

CHAIRPERSON LUDERER: Dr. Quint.

PANEL MEMBER QUINT: Yes. I was going to make that same point about the siloxanes, because it was the chemical. And it's basically the same type of thing, indoor air. It's -- you know, these chemicals are used -- I mean, they're inside the home, personal care products, and cleaning agents.

I was also struck by the fact that in some of the studies it was the younger age people -- you know, people of younger age seemed to have, you know, the most exposure, so people who use personal care products a lot. And it followed that trend. So I think it pretty -- it is, you know, very similar. You know, and the data that
we do have, I think is even of more concern than the
siloxanes, you know, because of the estrogenic activity.
So I think both sets of chemicals certainly fit our
criteria for designation.

And like Dr. McKone, I think we can learn a lot
about, you know -- and the other thing that strikes me is
unlike a lot of chemicals that -- and I say this -- I
mean, it seems that we don't have to have these fragrances
in everything. That we do -- that we could have products
without fragrances. It's not -- so functionally, I don't
think they are high priority chemicals. You know, I'm
sure to the people who make them they are, but in terms of
having them in everything, you don't even -- if they're in
unscented things that we don't even know they're there,
then I think it's a real problem.

CHAIRPERSON LUDERER: I actually wanted to second
what Dr. Quint and Dr. McKone said. And I think also to
add that the other thing that was striking was for the
polycyclic musks that there have been quite a bit of
biomonitoring studies that you presented, and the
prevalence, you know, was 100 percent in many of them, you
know, whether they were looking at blood or breast milk or
adipose tissue. So I found that quite striking. It was a
little bit less so for the OTNE. But even there, there
was a fairly high prevalence in some of the few studies
that there were.

And another thing that I found striking was in
addition to the estrogenic activity, it was interesting
that they had looked at anti-estrogenic, anti-androgenic,
anti-progestogenic activity. And I was really struck by
the nanomolar concentrations for the effective
concentration 50 for -- I can't remember if it was one of
the polycyclic -- yeah, so I think I'm definitely -- I'm
in agreement with Dr. Quint and Dr. McKone.

Before we move on to talking about making a
motion, do we have any public comments on -- I know we
actually have one public comment from before lunch, which
I will read, and it looks like we have a public comment
here as well.

DR. DiBARTOLOMEIS: Michael DiBartolomeis. This
is actually a question -- a clarifying question, more or
less, but you started bringing this up, Julia. I thought
I heard you say just because something says fragrance free
doesn't mean that these chemicals aren't still in those
products. Did you say something like that?

Unscented. Okay, unscented fragrance free. So
is there another function for these chemicals besides
fragrances? That's my question. So if a chemical -- if a
product has an odor, they can be put in there as sort of
anti-odor, interesting.
So I hadn't -- because that can I've just made me wonder why you would put these in there, if you don't want to have a fragrance. So there is another functionality apparently, which -- so it could be such an onerous odor, that people wouldn't buy the product if you didn't have something in there to counter -- anyway, I just throw that out there for why possibly these are being used, even though they're not, you know, meant to be, you know, a fragrance.

PANEL MEMBER McKONE: Could I make a follow up to that point? I mean, I think it's interesting that we're getting into questions of the utility, which I think is interesting. But in terms of designation, I don't -- even if it were an essential product, right, we're not trying to decide whether it belongs in -- or whether any chemical belongs in a product, but if it's of interest for our criteria that it has some health concerns and it's persistent. It's in large volumes, I think we would -- I we're kind of neutral as to whether it's essential to commerce or essential to something else, but we're just mainly driven by the curiosity, the need to really -- not the curiosity, but the need to track these for health studies for trends, for exposure assessment in general.

I mean, although I think it might -- you know, again beyond our Panel's goals, somebody would want to
bring in some if something were essential to nutrition or make a product, you know, more acceptable in some way. That might come into somebody else's decision, but I don't think it really enters into our decision about designation.

CHAIRPERSON LUDERER: Dr. Quint.

PANEL MEMBER QUINT: I agree with that, that it doesn't enter into our deliberations here, as to whether or not we designate. I think I was wearing my green chemistry safer alternatives hat, because in that process when we were trying to, you know, think about which chemicals need to have a safer alternative, one of the questions we ask in that arena is whether or not, you know, it's essential to function.

And so I was wearing that hat, but I would agree with that. But in some cases, masking -- if this is a cleaning product, and you're masking an odor, I think it can be harmful to workers, because, you know, it -- one of -- you know, the warning properties of chemicals are a big deterrent. I mean, a protective measure in a lot of workplaces.

So if you have a chemical that has an odor, even though odor is not toxicity, it is -- it does provide a warning that you're having some exposure. So in cases like that, if it's used to mask, you know, something, then
that could be harmful, not if you're putting it on your body, I mean I guess, if you don't want it to smell bad. But for occupational purposes, masking odors could be not a good thing.

CHAIRPERSON LUDERER: Dr. Wilson.

PANEL MEMBER WILSON: I'm just sort of picking up on that point from both Dr. Quint and Dr. McKone that specifically with regard to the Panel's criteria for recommending designated chemicals, you know, one is exposure and potential exposure. And I think Dr. Krowech's document and her presentation provide, you know, really striking evidence, in that we have a high production volume set of substances here, not withstanding the CBI claims. They're the most important used commercially. They're used in multiple products, and I would add, would include worker exposures, and primarily domestic workers.

And also on the also exposure side, the evidence of bioaccumulation and persistence -- environmental persistence I think is reasonable, and in some cases that you cited is pretty compelling. And the -- and what I think is striking evidence of exposure potential in the dust samples that were reported, and that the exposures are likely occurring during fetal development. And that they -- the substances, I would think, would -- you know,
appear to be transmitted to an infant in breast milk. There was some limited evidence to that effect.

So I think, you know, you've put together a very, I think, strong case for -- on the exposure side, and there's also the evidence of the -- you know, the endocrine activity, the hormone signaling effects, and so forth.

So I think you've done a great job here, and I commend you for that work. And so I think we have a set of substances here that very clearly meets the criteria for designation.

CHAIRPERSON LUDERER: Thank you. Do we have any additional Panel comments before we take the public comments?

Okay. All right. So we have two public comments, one of which is actually from before lunch. It came in late via the internet and it's actually from Sandy McNeel regarding the FOX study. So I'm just going to read that now. It relates to one of the questions that was brought up during the discussion.

She said -- Titled, "Union Involvement within FOX Study". A combined firefighter union/Orange County Fire Authority management committee was involved and updated throughout the FOX study. I don't believe that we asked their direct input to encourage participation during the
results communication email survey, so that suggestion is
a good point for the future. Thanks to Dr. Wilson for
bringing this up".

All right. Kind of backtracking a little bit,
but that clears up one of the questions that we had.

All right. And then we have a public comment for
this session. And the commenter is Nicole Quinonez -- I'm
not sure if I'm -- Quinonez -- from the International
Fragrance Association of North America.

MS. QUINONEZ: Good afternoon. It's Nicole
Quinonez, so you were very close. So I'm here
representing the International Fragrance Association of
North America. Unfortunately, I'm not a technical expert.
They're are all located over in Washington D.C. area. And
they apologize they couldn't be here today, but definitely
wanted to extend the offer to come out at a later meeting
and present. It sounds like you guys have some really
good technical questions I know that they would like to
talk to you about.

So I just wanted to use today as an opportunity
to kind of talk about the Association, as well as the
Research Institute for Fragrance Materials, and highlight
how we've been working with the Biomonitoring Program
since last November when you guys first started looking at
the synthetic musks.
So IFRA North America represents the fragrance materials industry here in the United States and in Canada, but they are a part of IFRA Global, which their membership supplies 90 percent of the global market for fragrance compounds. And the primary concern or goal of the Association is to ensure the safety of fragrance ingredients in the industry's products. Our member companies are strongly committed to the IFRA Code of Practice, which is the highest safety and environmental standards for fragrance manufacturing and fragrance ingredients.

These standards amount to 174 substances which have been either banned or restricted in their use in fragrance products. This is a self-regulating program. All members of IFRA are required, as a condition of membership, to observe the IFRA Code of Practice.

IFRA North America and the Research Institute for Fragrance Materials or RIFM, are both internationally recognized as experts on fragrance materials, partly because of the Code of Practice and their research that they do.

So since the late 1960s RIFM has been at the core of the safety and evaluation program used by the fragrance industry. They gather and analyze scientific data. They also produce their own testing when they recognize that
there's a data gap. They have an independent panel of academic experts that reviews their data, and then encourages uniform safety standards related to the fragrance -- use of fragrance ingredients.

The fragrance industry has put much time and effort into the evaluation of musks and Iso E Super. And there is significant information and data available on exposure, use, and hazard of these materials, some of which were mentioned in Dr. Krowech's presentation. We've also provided unpublished studies, which we understand is not used in your consideration for designating.

So we do have a history of working with federal and State agencies to provide the relevant information on fragrance materials for use in their assessments. And shortly after the biomonitoring meeting last November, the fragrance industry began proactively sharing that information and research with OEHHA staff.

Members of IFRA and RIFM have made themselves available as a resource to the Biomonitoring Program. They've proactively submitted dossiers and general uses information on representative musks and Iso E Super.

Recently, published and unpublished information on HHCB and Iso E Super was provided to the U.S. EPA under Toxic Substances Control Act workplan. We provided that same information to OEHHA staff along with additional musk
data, including the volume of use information that Dr. Krowech reported.

By submitting information to the Biomonitoring Program, including these volume of use data, IFRA was making confidential business information public. So just to reiterate, you know, we've -- to staff, we've expressed our intention to serve as a resource as they move forward in this process. We also want to do that -- you know, extend that invitation to the Panelists. And we definitely appreciated staff's willingness. They've taken a lot of our calls and answered a lot of our questions, particularly Sara Hoover, who is not here today and Dr. Krowech.

So with that, I just want to thank you. And I can attempt to answer any questions, but as I offered before, you know, maybe it's a better place to have a follow-up expert come out.

Thank you.

CHAIRPERSON LUDERER: Thank you very much.

Dr. Wilson, do you have a question?

PANEL MEMBER WILSON: I really appreciate your comments and appreciate you coming today. And we certainly think -- I'm speaking -- if I could speak on behalf of the Panel, appreciate, you know, a proactive industry association stepping up and sort of working with
these issues.

And my question is on the safety side that the Association has taken some kind of action on about 174 ingredients, if those -- if your experience has been that those ingredients and the actions that have been taken on those have been primarily for acute effects, sort of the dermal irritation or eye irritation, and maybe, you know, dermal sensitivity, where someone would get a rash or something from an ingredient? That's the first part of the question.

And then the second part is, is the Association equipped or sort of -- should I say -- what's the word? It's sort of willing and able to look at these more subtle chronic effects that we're discussing here on the Panel around hormone signaling and endocrine disruptive kinds of -- or you know, endocrine activity that's less obvious in terms of a health effect, but is nevertheless of public health concern?

MS. QUINONEZ: Sure. Well, just to take your second question first, because I don't think I have a good answer for that, but I would definitely like to take that back to the research -- RIFM, the research arm. I know that they are constantly reviewing their ingredients, but I don't know to what level that they're specifically looking at.
As far as kind of the use restrictions, I think maybe what you're asking is they can vary from being very prescriptive to, as you mentioned, if it's a dermal exposure problem, restricting the use of those fragrance material in, say, a body lotion, because we know that's going to be rubbed on the skin or on a lipstick, because it might be, you know, ingested.

So they do -- can range from an all out ban to a very specific use restriction that also includes in, you know, what levels, what amounts in a formulation.

PANEL MEMBER WILSON: May I follow up?

But is the Association looking at these sort of more subtle longer term health concerns, as far as you know?

MS. QUINONEZ: As far as I know, I do not, but I will definitely get back to you on that.

DR. WILSON: Okay. Thank you very much again.

CHAIRPERSON LUDERER: Dr. Bradman has a question as well.

PANEL MEMBER BRADMAN: I have a question and a couple comments. Again, I want to underscore what Dr. Wilson said of just the appreciation of your taking the time to come here and the proactive kind of involvement of the industry.

Some of my concerns about these compounds would
be similar to other things used in personal care products, like phthalates and BPA, where there's a very clear pathway between use of these materials and exposures in people, including, you know, adults, teenagers, and very young children.

Has the work done by your group also looked at, you know, environmental fate? We heard a lot about contamination related to these materials in -- potentially in effluent, in other environments. We've also heard that they're in house dust. We know, for example, from lead that when there's something in house dust, it's guaranteed to get into very young children. So I'm just curious about what other pathways have been looked at and considered by your organization.

MS. QUINONEZ: Sure. I know specifically that wastewater effluent has definitely been studied extensively. I do not know about dust or other sort of environmental factors, but I'd be happy to look into that as well, and just get a better sense of kind of across the board what they're looking at, but wastewater absolutely.

PANEL MEMBER BRADMAN: Okay. Thank you. And also underscore that this group and this Program is not a Risk Assessment Program. You know, it's a Biomonitoring Program. And the risk side of it kind of is another arena. And really, I think the interest here is trying to
understand what exposures are and what the trends are.

MS. QUINONEZ: Yes.

CHAIRPERSON LUDERER: Dr. Quintana.

PANEL MEMBER QUINTANA: Hi. Thank you for your willingness to come here and presenting your information. I had a question. Do you happen to know about the manufacturing facilities? It sounds like your manufacturing is very proactive as well. And I'm wondering if you know if they have any internal biological monitoring data on workers or internal standards on the workers that they use internally in those factories.

MS. QUINONEZ: I do not know, but I can find out. I mean, I know the Association does represent the manufacturers, and they do also look at worker exposure. That is part of their assessment, and restrictions can deal with in the manufacturing process, as well as the end, like consumer product use.

PANEL MEMBER QUINTANA: Thank you.

CHAIRPERSON LUDERER: Dr. Quint.

PANEL MEMBER QUINT: Yes. This is Julia Quint. I want to add my thanks for your coming and spending time with us to clarify some issues. Very much appreciate it.

The State -- the California State Water Resources Board is starting to -- is deciding to look at contamination of water by these chemicals. Do you know if
you are working with them at all? Is there any activity?

MS. QUINONEZ: No, not specifically on fragrance issues, but --

PANEL MEMBER QUINT: Because I guess some people are able to get it out of the effluent and others are not. So I was just wondering if you were working on that issue as a separate issue from just --

MS. QUINONEZ: No, I'm not, but thank you for letting me know. I'll definitely contact them.

PANEL MEMBER QUINT: Thanks.

CHAIRPERSON LUDERER: Okay. Yeah, thank you again very much. We really appreciate your coming to share information with us.

MS. QUINONEZ: My pleasure. Thank you.

CHAIRPERSON LUDERER: All right. Do we -- Dr. Wilson, you had earlier mentioned that -- now, let me just clarify, too, from Dr. Krowech that these are two separate classes that we're talking about designating. So the Panel could designate neither, both, or one or the other.

DR. KROWECH: Exactly.

CHAIRPERSON LUDERER: And so the two classes are the polycyclic musks. That's one class, and the other class is the tetramethyl acetyloctahydronaphthalenes.

So, Dr. Wilson.

PANEL MEMBER WILSON: I would like to recommend
and move that the Panel list synthetic polycyclic musks as designated chemicals of the California Biomonitoring Program.

CHAIRPERSON LUDERER: All right. So Dr. Wilson has proposed a motion that the Panel recommend that synthetic polycyclic musks be added to the designated chemicals list for the California Environmental Contaminant Biomonitoring Program. Do we have any seconds?

PANEL MEMBER FIEHN: I second that.

CHAIRPERSON LUDERER: All right. And then for the designation, we don't need to take a formal vote as I recall, or do we?

We do. Okay.

PANEL MEMBER WILSON: Chair, if I could amend that for one second. My apologies. I would -- I'd like to state it as synthetic polycyclic musks as a class, just to be clear. So if I could restate the motion.

CHAIRPERSON LUDERER: I mean, I think that that's -- it's clear.

PANEL MEMBER WILSON: Okay.

CHAIRPERSON LUDERER: Thank you. All right. We'll start with Dr. Quint.

PANEL MEMBER QUINT: Julia Quint, aye.

PANEL MEMBER WILSON: Mike Wilson, aye.
PANEL MEMBER BRADMAN: Asa Bradman, yes.
CHAIRPERSON LUDERER: Ulrike Luderer, aye.
PANEL MEMBER FIEHN: Oliver Fiehn, yes.
PANEL MEMBER KAVANAUGH-LYNCH: Mel Kavanaugh-Lynch, yes.
PANEL MEMBER QUINTANA: Jenny Quintana, aye.
PANEL MEMBER McKONE: Tom McKone, yes.
CHAIRPERSON LUDERER: All right. Unanimous opinion on the Panel for designation of synthetic polycyclic musks.
Do we have any Panel members that want to express opinions about designating the other class, the tetramethyl acetyloctahydronaphthalenes?
Dr. McKone.
PANEL MEMBER McKONE: My only comment was I would make a motion, but I don't know if I could pronounce it.
(Laughter.)
PANEL MEMBER WILSON: Give it a shot, Tom.
(Laughter.)
PANEL MEMBER McKONE: All right. So I would move that the tetramethyl acetyloctahydronaphthalenes be designated as a class. Sorry.
(Laughter.)
CHAIRPERSON LUDERER: All right. Dr. McKone has made a motion that the Panel recommends -- now I have to
say it again -- that the tetramethyl acetyloctahydronaphthalenes be added to the designated chemicals list for the California Environmental Contaminant Biomonitoring Program.

Do we have a second?

PANEL MEMBER QUINT: I second.

CHAIRPERSON LUDERER: Shall we start on this end this time?

PANEL MEMBER McKONE: Thomas McKone, aye.

PANEL MEMBER QUINTANA: Jenny Quintana, aye.

PANEL MEMBER KAVANAUGH-LYNCH: Mel Kavanaugh-Lynch, aye.

PANEL MEMBER FIEHN: Oliver Fiehn, yes.

CHAIRPERSON LUDERER: Ulrike Luderer, aye.

PANEL MEMBER BRADMAN: Asa Bradman, yes.

PANEL MEMBER WILSON: Mike Wilson, aye.

PANEL MEMBER QUINT: Julia Quint, yes.

CHAIRPERSON LUDERER: All right. Another unanimous recommendations from the Panel. So we have next on the schedule is a break, which was 15 minutes. I think we'll keep it to 15 minutes, so we'll come back at a quarter of. And please remember, to the Panel members, that these microphones may still be on after -- during the break.

DR. KROWECH: And the video.
All right. If Panel members can make their way back, I'd like to call the meeting back to order.

All right. I'd like to welcome everyone back from the break, and call the meeting back to order. And the next agenda item is going to be a presentation by one of our newest Panel members, Dr. Oliver Fiehn, who is a professor and director of the National Institutes of Health West Coast Metabolomics Center at UC Davis. And he's going to present, "Identifying Novel Compounds in Untargeted Metabolomic Screens".

Dr. Fiehn.

(Thereupon an overhead presentation was presented as follows.)

PANEL MEMBER FIEHN: Thank you for asking me to present some concepts of metabolomics. How it's approached, what pitfalls there are, and how we can then identify interesting compounds that popped up as being important in one or the other ways statistically significant, or indicating some health effects, so that is the goal here in the next 30 minutes today.

So UC Davis has been designated as one of the six...
NIH funded metabolomic centers in the United States, and the only one west of the Mississippi. So that gives us a lot of responsibility and work.

There is also a national data repository for metabolomics data, not only for the six Metabolomics NIH Centers, but also for all other investigators who are NIH funded that is located in UC San Diego.

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PANEL MEMBER FIEHN: So I'd like to start off by just giving a little idea about what metabolomics is actually meaning, apart from being a novel fancy word that people like to pop it into their grant proposals. So the idea was that we have 200 years of chemical analysis. This was historically always targeted analysis. People would designate a chemical that might be interesting, and then usually -- or one or a few compounds, and then these would be screened for, in different samples, different matrices with the idea of very high accuracy. And we have today seen several splendid examples how this is done.

The problem with that approach is, of course, is you only find what you seek for. You don't get a bigger scope of other chemicals that might be around. So with the advancements of computers in the 1990s, novel mass spectrometers came onto the market, novel software solutions came onto the market that could better be used
for screening all the small peaks that usually people would discard, and say they are not important in analytical chemistry profiles.

Based on the platform people would use, this is then called metabolite profiling or chemical profiling based on, you know, the properties of the method that was applied or can be applied.

And by logical extension, metabolomics is then the idea to go with very high scope, ideally all small molecules that are present in a certain sample. Metabolites could be endogenous metabolites, the compounds that are done by enzymatic conversions in a cell, but also, of course, exogenous metabolites from drugs to chemicals -- exposed chemicals, including foods. So metabolomics is, in a way, a larger chemical approach with a giant scope.

Now, the problem that is whatever method an analytical chemist chooses, you will introduce a bias. So for some compound classes, it will be better suited than for others. And all the universe of small molecules have many, many different physical chemical properties, some of them we have discussed like lipophilicity and hydrophilicity, and volatility. We had it with the musks odors just a half hour ago. Size. Of course, some can be very, very large, others are very, very small.
In addition, if you want to do this, you not only need more than one platform, you also need chromatography, if you really want to distinguish isomers. If you now have 20,000 samples, and you have different platforms of 200,000 samples, say, how do you do this?

And some people said then you need a very quick screening tool to classify the most drastically different samples using direct infusion mass spectrometry, or using NMR's spectroscopy, or infrared spectroscopy, then use classification tools multivariate statistics to say these samples are all similar or they're grouped. Let's look at these outliers, so that you basically first screen 200,000 then you go in more detail and say 2,000 samples. So that is called metabolomic fingerprinting or metabolomic.

Okay. So that's the idea.

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PANEL MEMBER FIEHN: In Davis now, we have the NIH Metabolomics Center, where we, in one lab, in our lab in the Genome Center, we have 15 mass spectrometers. In six other labs, there's an additional 20 mass spectrometers, and five NMRs, including, for example, the NIEHS Superfund laboratory is headed by Bruce Hammock. So there is also a long history in Davis on small molecule analysis.

What you can see here is how we break up the
different parts of endogenous metabolites into, you know, brackets. And the numbers indicate the numbers of compounds that we typically identify in a given sample, for example, in blood plasma or in liver. Of course, depending, of course, on the actual numbers on what type of sample you look at.

So, for example, for primary small metabolites, we can detect up to a 500 small molecules, out of which we identify 200 in polar and neutral lipids. In blood plasma, for example, we easily detect something like 800 to 900 different features, of which we can identify 350 unique lipids or complex lipids and so on. So you can also look at volatile with SPME fibers or we use here twister absorption bars, where you can again like go to 150 identified compounds.

So we break it up basically based on the physical properties of those compounds, and then look for the platform that can best address a large survey of a certain chemical class.

So take-home message from this is you cannot have one type of method, one type of platform, and hope to get the metabolome, rather it's a combination of methods.

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PANEL MEMBER FIEHN: Now, pitfalls. Many people try -- and this is a public data set that I downloaded
from the data center in San Diego. So this is something
that people would have, so you have 800, or whatever,
features. And then you have these sample sets where there
is lots of missing values. As you can see here on the
right-hand side -- and I cannot see this here. I don't
have a pointer. So you go to the right-hand side of that
image, and you see some numbers. And these are the
intensities of chemicals, but you see often that there's
missing values or almost missing rows.

And now for statistics. That places a huge
problem because your power analysis goes down. You don't
know why that chemical wasn't detected. Was it not
detected because it wasn't there, or was it not detected
because some parameter settings were wrong?

So this is the problem of using software and
using the adequate software, and also adequately using the
correct software. So that is not easy to do, even if you
have nice instrumentation.

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PANEL MEMBER FIEHN: So there's another one. If
you do very large studies, like we do at UC Davis, you
come up with thousands and thousands and thousands of
samples. So these are actual data from our lab, and
usually I, of course, don't show this, but just to see --
get, you know, an idea of pitfalls you run into. This is
a study also funded by the NIH from the TEDDY consortium, The Environmental Determinants of Type 1 Diabetes in the kids, TEDDY, where we look over 12,000 samples. It's a multi-national consortium, Finland, Germany, UK and the U.S.

And you see there is, of course, drifts on the left-hand side. You see there's always some drifts and scatters of the total intensities of all the identified compounds. These are lipids here. And you then define, you know, upper and lower intervention limits. And you define the derivations, and you keep it in a certain order. And you say well that's the order. That's the magnitude of raw data intensities that we allow. But on the middle panel, you see that there was a 10-fold drift between one type of a batch to the others. And if you only rely on ratios or on internal markers and you don't look at the absolute intensities, you would not see that.

So that means if you want to do this in an untargeted manner, you have to control also your absolute machine sensitivity. And, in this case, we ran those failed batch again.

And you also see then some kind of temporal drift on the right-hand side for another platform, where you see some kind of, you know, upwards or downwards trends. These can be corrected as long as they are not outliers.
like the three samples that you see there. So it's important to have these kinds of quality control measures when you do metabolomics.

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PANEL MEMBER FIEHN: Then there -- an LC-MS, and people are very fond of using LC-MS, but the point is one compound will always come up with different ions at the same time. And this is an example for the exact same lipid that we now screened over many, many different runs. And use on the left-hand side, you see different adduct ions for these lipids. One is an ammonium adduct and the other one is a sodium adduct. On the left panel, you see it's almost one to one. And on the right-hand panel, you see that the ratio of sodiated to ammoniated species is more like one to three.

So you cannot just rely on one adduct, but you have to combine those to get a clear representation of the total abundance of a specific lipid. You can't just -- you know, and the same is true for other chemicals as well. You cannot just rely on the RT and MZ values, or features as they are called, in these untargeted metabolomics.

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PANEL MEMBER FIEHN: So pitfall 2 is, of course, data processing. So you need to, you know, define what a
true peak is. And these are three samples here from women's breast tissues. The green and the blue were women who had breast cancer tumors, and the red one was non-malignant tissue.

And you see now that there are certain peaks. Two of them were identified, two of them are unidentified. They get the number. And they, of course, then, after statistics, good targets for compound identification. But there are more peaks, if you -- you know, this is just one extracted ion chromatogram.

You see there are more peaks and you have to define what is your threshold at which you define a peak to be integrated into your analysis strategy, into your statistics. And the point is, you know, specifically for exposure, they may not be all the time there. We have just had exposure of 100 percent. Well, is 50 percent exposure not relevant? Is it only relevant if exposure is in high abundant peak or is it, you know, maybe a very potent and very dangerous compound at low abundance? So these are all decisions you have to make, and that you have to use software appropriately.

Now, on the right-hand side, we now see the reality. There is many -- there are many, many, many, many peaks found and many of them are overlapping, so this is unavoidable, because we can't just purify a compound...
like we do in targeted analysis. And then you have to find those peaks and they have to be unique, and they have to be able -- we have to be able to selectively screen for them also in the next 1,000 samples we run, and we have to have criteria which define which peaks to carry forward, and which peaks to exclude, because they're too low, abundance too noisy, or not selective enough to give us adequate intensity issues.

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PANEL MEMBER FIEHN: Good. So next pitfall. Mass spectrometrists are very fond of their mass spectrometers. On the left-sand side, you see a perfectly nice peak eluting after an HPLC with an accurate mass. And we then, on the right panel, you see a so-called tandem mass spectrometry that can be used for identification.

But if you look more closely into the tandem MS, you'll see these are actually two different triglyceride lipids would have the exact same elemental formula, but different position of the bonds. So they appeared together at the HPLC. They had the exact same mass. And if you have the exact same mass, no mass spectrometer can separate them, but you, in principle can separate them on the MS/MS level, but you have to be looking very closely.

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PANEL MEMBER FIEHN: Another example. Here is another blood plasma lipid that appears as a signal peak using a specific instrumentation here, is the Agilent 6530 at 10,000 resolving power. If we use now higher resolving power mass spectrometers, we see that this is not one peak, but in fact, two different lipids that have different elemental compositions, but slight -- but only slightly different masses. The mass difference here was only 40 millidaltons.

And you really need this high resolving power mass spectrometers to actually discern those. And again, you need then good software to define that there are actually two peaks. So resolution, and the type of instrument, really matters.

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PANEL MEMBER FIEHN: The third pitfall is that people who don't do it very often don't know how to do the statistics right. And again, many metabolomicists who are starting are very fond of the use of multivariate statistics. On the left-hand side, you see so-called unsupervised principal component analysis. You see four groups, a green, a yellow, blue, and red group.

And there's quite a significant, you know, overlap of the yellow group with all the other three groups. So with PLS-DA, which is a supervised statistics
method, you can easily now say, well, I can separate the yellow group from all the other three groups. It's all getting much clearer, and then you can define certain markers that are different in the yellow group to the blue, red, and green group.

And this has been published many times. The problem is it's overfitting. If you have many, many variables, there will be some variables that appear to be different in the yellow group to the -- compared to the others, but they are not true. It's really the wrong method if you don't use independent validation control samples. So this is called data overfitting. And especially if your study is underpowered, you run into this problem in multivariate statistics.

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PANEL MEMBER FIEHN: So now let's come to the next 14 minutes on -- let's assume we have done everything right. We have perfect data sets. All the data processing and all the statistics is good. Now, we know which peaks we want to look at. Now, we want to identify them.

The best way to do it is if you use structure de-replication it's called. So basically use mass spectral databases. There are a couple of public databases around, like the NIST 12 library has been
largely expanded, the Wiley Registry. These are for --
mostly for GC-MS data. The NIST now also has about 12,000
LC-MS/MS spectra in it. But overall, these are, for
example, not having retention time information.

So without retention time or retention index
information, you can't do a lot. For that reason, we have
developed quite a number of libraries where we also
standardized the recording of the mass spectra together
with the recording of the relative retention time.

In our databases, we have over 150 million
experimental mass spectra. We have done it on volatiles,
on primary metabolites, and most recently we published
papers on complex lipids, where we have over 200,000 MS/MS
spectra for lipids. And the idea there was -- and we now
go to the different kinds of compound classes including
acylcarnitines, acyl-CoAs, flavonoids, and so on.

The idea there is you cannot possibly buy all
compounds. You have to predict how mass spectra will look
like, otherwise we will never look at the overall
universe.

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PANEL MEMBER FIEHN: So the overall universe at
PubChem, which is the Congress-sponsored public repository
of all chemicals that are known to human kind accounts for
about 40 million compounds.
Now, if you look at the compound spectral libraries that are available in the public for LC-MS/MS spectra, there are less than 40,000. So for every compound that is known, in terms of chemical structure, you know, for every 1,000 compounds that are known, there's only one spectrum. That's bad. So that's the reason why, if you buy a mass spectrometer, most compounds you will see will be unknowns.

In GC-MS, it's a little better. People have done 60 years ago standardization efforts, so accumulated now over 250,000, maybe 300,000, electron impact ionization spectra, which gives much better confidence in terms of identification. That's why GC-MS is much easier for starters.

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PANEL MEMBER FIEHN: Okay. So, now let's assume we get -- the important compounds you have identified are not known. And we have published here three papers, and a couple more actually, that we give there just as a literature reference if you want to know more about it. Then you have to follow a certain workflow, assuming that this is a compound that may be known to human kind. It may be part of the 40 million compounds. You just have to find the best candidate fitting it.

Okay. So the first thing you to have do is you
have to determine elemental formula. And you can only do this if you have an accurate mass spectrometer that I showed before. Then with that accurate mass formula, you have to go into large databases like PubChem, and retrieve all compound structures. Usually, per formula you get five net different structures on average.

Then you have to look at those structures and say what is the most likely structure? You have to have filter -- different types of filter, for example, on substructure constraints, but also on prediction where it would elute in the chromatography to say these are -- from the 500 possible candidates, these are the 10 most likely ones, and then you can look at the MS/MS similarity.

So this was all not easy. So what you see now on the right-hand side is the computation generation of all chemically possible structures from up to 300 daltons. And you see that, you know, there are many, many, many, many structures and elemental formula possible. So not -- even determining the one and only correct elemental formula is not easy in an untargeted way.

So not only that, but also people exaggerate the accuracy of current mass spectrometers. So the vendors would say that our -- their mass spectrometer would have an accuracy of 1 ppm. This is, on average, true, but they forget the deviation. And since it's an unknown, you
don't know the true value. So you have to look for the error.

And, you know, classically if you want to have 95 percent correct ones, you have to use something like a two sigma window, which is, on most instruments, something like 3 ppm. If you then look at all the compounds in PubChem, all the elemental formula in PubChem, you see that even at 200 daltons, you will already have two possible elemental formula. And at 900 daltons, you would have 1,000 different formula that all would fit your experimental data. So, for that reason, mass accuracy alone is not enough to give you a unique elemental formula.

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PANEL MEMBER FIEHN: So instead, you have to use isotope abundance. So all the different elements have different isotope abundances as we know. So, for example, for carbon, there's a 13 carbon isotope that has roughly 1.1 percent abundance. And you can accumulate this information and then use it to constrain your elemental prediction. And if you that, you see on the right-hand side, that the same table that we have seen now -- before at 3 ppm mass accuracy is now much better to the level of, let's say, 600 dalton, where you have then only four different formula.
Now, what we then did -- oh, this is just an example. So this is an example where we just looked at the structure that is given there, and looked at for all other elemental -- other structures in PubChem that would have the same accurate mass. And now this little red window would define those that have the same isotope ratio given on the left-hand side again with some kind of instrument error assuming.

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PANEL MEMBER FIEHN: So you see just having the isotope error included is a major constraint value. You see this also on the structure below, that is about 700 dalton, and again the same idea. All the dots given there would have all the same accurate mass, given the 3 ppm mass error, and the little red window would have now those that have this isotope pattern that includes sulfur compounds.

So if you are able to detect those isotope patterns, you already know that your compound must have a sulfur atom in it. And that massively restricts your search for the correct elemental formula.

If you do this on a broad scale, and these are 100,000 -- actually, sorry, 50,000 different compounds, including peptides, but also drugs, and pesticides downloaded from PubChem. If you then just plug now the
isotope ratio for the first isotope on the X axis and the second isotope on the Y axis, what you see there is in an untargeted screen, it's very easy to see if your compound of interest has bromine or chlorine elements in it.

This is very -- has very, very clear isotope patterns, and therefore, the ideal candidates for untargeted chemical screens in environmental exposure studies. Whereas, if you go down to the other elements, it's much harder to even detect the correct compounds.

Good.

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PANEL MEMBER FIEHN: So let's now say we have derived the correct elemental formula. We go to the next level. We retrieve the structures from PubChem.

Oh, first of all, we actually have more rules I forgot to say. This graph here gives, in red, if you don't apply rules and how many different elemental formula -- this is on the Y axis, how many different formulas you would get if you don't apply any rules, and in green, if you apply the so-called seven golden rules that we published. The software is freely available from our website. Some mass spectrometry vendors have included that into their software.

If you now retrieve the structures from PubChem, the question is how often do we get the correct hit as a
top hit, because that's what you want to have. You know, even if you have four elemental formulas, you want the correct one to be in the top it. And if we don't apply any database, just ask for anything that is chemically possible, that's a red line, and depending on the mass that you look at on the X axis, it can still be something like 80 to 90 percent correct as a top hit, but 10 percent, you know, being one of the lower hits.

If you restrict now your compounds to elemental formulas to only those that actually are known to humankind in PubChem, you get at least 90 percent accuracy. And if you do have a target library, I am only interested in pesticides, or I'm only interested in drugs -- these are very small target libraries -- then you get a much better hit rate, in terms of that your unknown elemental composition is actually present there, but that, of course, you have a targeted question.

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DR. FIEHN: So the next thing is let's assume now that your unknown -- you have the elemental composition, and now you have several structures downloaded that could be possible. You need to know about substructure constraints.

If you use GC-MS, you do derivatizations to increase volatility, and you can also do derivatizations
on different type structures like carbonyl atoms. That's what you see here. So with derivatization, you can determine the number of acidic protons your unknown has and you can determine the number of carbonyl groups you have.

And, in principle, if you use other types of chemistry, you can determine other types substructures as well. In MS/MS or LC-MS/MS, you could do similar strategies, for example, knowing about neutrolosis, that a certain neutral loss or a certain fragment clearly defines a certain head group, for example, choline and lipids -- and complex lipids.

So these are different types of substructure constraints. The more the better to say of the 500 structures I can sort out 400 that don't have the correct number of substructures like acidic protons and others.

You then need to -- maybe you have still 100 compounds left over or structures left overall, all the same elemental formula, all the correct number of substructures, and types of substructures. But some of them may elute much earlier and some of them may elute much later. So you can do retention time prediction software.

For GC-MS, it's done by NIST. We have improved that for derivatized compounds that you can see there and
it's all published. But, of course, there's a deviation. You also see that. So that's something where more research needs to go in.

And for LC-MS, it very much depends on the chromatography you use, on the solvents you use. Much harder to do an LC-MS, these predictions. But, of course, there are some papers already out.

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PANEL MEMBER FIEHN: Lastly, then you can do the mass spectrometry prediction. You see there, for example, an experimental spectrum with accurate masses. And then you see the prediction of software. This is Mass Frontier here. But there are other solutions, of course, where in different structures, isomers, what you put in. And what you see there in green is you would have the same nominal masses so 116, 132, and 141, but all of them would have been different elemental compositions, different accurate masses, so they would not fit the experimental data. Only the right structure would fit all the experimental data. So you can use mass spectrometry prediction tools these days to further filter or rank your most likely isomer. So it's not impossible.

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PANEL MEMBER FIEHN: Giving you one idea how it works in practice, here is a compound that we found in a
combination of four studies that were all involved in type 2 diabetes and muscle mitochondria oxidation. This unknown would have accurate masses. We put it in the GC/Q-TOF first in electron ionization to see if we find the same compound that we had found in the screening that was done by low resolution. And then we do two different types soft ionizations. We determine the elemental composition, and then we go the workflow that I outlined to you.

And then we come up eventually with this mass spectrometry prediction and annotation of all the different ions, and do they fit the fragmentation pathways that are predicted by the software. And then you come up with a possible candidate. In this case, it was 2-keto-3-deoxygluconate.

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PANEL MEMBER FIEHN: Then you have to synthesize this or you have to leave it as an annotated compound and go from there.

So there is -- this is a painful and very manual project. You cannot do it for thousands of compounds, at least not today. In 10 years, you may have better software, but this is where pretty much the state of the art is.

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PANEL MEMBER FIEHN: So conclusions. Well, we have larger Metabolomics Center here in California that is reaching out to investigators. By the way, we have pilot projects, so anyone who is eligible to submit an NIH grant can also submit grants to us. This year we have funded 12 pilot projects up to $50,000 of worth in services. So this is definitely -- we have also one that looks at, for example, DDT exposure and the expected -- so there are environmental studies already done or carried on in our center, so we can give grants out in a way.

We have -- I've shown to you that there's metabolomic data. It's not easy to get high quality data, but it's possible. Different pitfalls have to be avoided, but they can be avoided. And by the way, we do course too for training. And then compound identification has spent quite a bit, so the first and best approach is -- you know, it is part of libraries, of existing libraries. And then you'll -- you know, you can fit your unknown mass spectra to the mass spectra you found.

And the much harder way is to identify your unknowns is by this workflow, going from elemental compositions to substructures and retentions and then to the mass spec interpretation.

--o0o--

PANEL MEMBER FIEHN: So I'd like to thank my lab,
of course, Gert Wohlgemuth and Tobias Kind are the ones who did cheminformatics. Mass spectrometry, several other people. And I'd like to thank all the funding agencies, and especially the NIH. Thank you for your attention.

(Appause.)

PANEL MEMBER FIEHN: Thirty-two minutes.

CHAIRPERSON LUDERER: Thank you very much for that very interesting presentation and overview of a complex topic.

Do we have any questions?

Dr. Quintana.

PANEL MEMBER QUINTANA: I had a question that's not about what you do with the sample once you get it, but it has to do with the samples themselves. And I've heard -- and I don't do metabolomics, but I've heard that it can be quite sensitive to sample storage and preparation, and so thinking about potentially using it for biological monitoring. In some case, we've heard about analysis of previously archived samples. Do you have any comments on how they have to be collected or treated the same or even frozen and thawed the same number of times and that kind of thing?

PANEL MEMBER FIEHN: Yeah. This comes back to the topic that I've showed as a second slide. For some compounds, any -- whatever method you use, there will be a
bias. And for some compounds, it will be okay and for
other ones it will not be okay. So you will never have
one single method that will be okay for everything. So
you can only be as gentle as possible, and as
comprehensive as possible. And then you look really at
the -- you had to compare different methods.

And that's, for example, what we've done on blood
plasma. We looked at different blood withdrawal
strategies. And there's, of course, lots of papers also
out asking the question which sample gives us the most
reproducible most robust and most comprehensive view on
metabolome. And surprisingly, this was serum, if the
serum is done in always the same manner. So always the
same type -- time of coagulation, and then the same time
of freezing after you let the blood clotting occur.

So most clinics, however, don't trust the serum.
They'd rather go for plasma and do the centrifugation.
And again, you shouldn't have the centrifugation speed too
high, but then there's different anti-coagulant that all
would have a different effect on the metabolome.

So we tested heparin plasma to EDTA plasma to
citrate plasma. And this is actually heparin plasma seems
to be the best in terms of using it for metabolomics
studies. Now, some studies and repositories have decided
on their protocol 20 years ago and biobanked them since
then. And you can still use those. It just means that
life gets harder.

For example, for the TEDDY study, we used citrate
plasma. Obviously, there's a ton of citrate, and you
cannot determine citrate then anymore, because no -- it's
part of the metabolome, right, citric acid, TCA cycle. So
that is, of course, something you cannot change often.
But if you go for prospective studies, of course, then you
should think about it.

And then again, for some compounds, if you
already have a hypothesis in mind like oxidized lipids or
so, they may need to be treated in a different way and we
have specialists, you know, advising on those different
types of chemical classes.

CHAIRPERSON LUDERER: Dr. Bradman.

PANEL MEMBER BRADMAN: That was a very
interesting conversation -- presentation, I should say.
I'm sure some of it went over my head, and suspect that --
PANEL MEMBER FIEHN: Yeah, it was very mass
heavy.
PANEL MEMBER BRADMAN: -- others are in the same
boat.

But that was very interesting, and I just have a
few questions. Three questions.

One -- and I'm going to ask them all, and then
you can decide which or how to answer.

One, it seemed like a lot of the metabolomic analyses are focused on examining biological processes. And how would we differentiate essentially a discovery process between looking at biological processes versus looking at exposure?

The next question -- and my note to myself is biomarkers of what? And given their emphasis here is on exposure, you know, how can we target that?

You also talked about using spectral libraries. It sounds like both like the NIST and other libraries, and then also it sounds like in your lab you've developed a whole set of, and others have, that are potentially usable.

How -- and then in particular that one slide, it seemed that if you target your metabolomic analyses on specific classes or subgroups, that you greatly improve accuracy. So does that suggest we should perhaps, as a first step, or maybe one first step might be to, for example, maybe use archived materials in some of the studies that have been done for the Biomonitoring Program and do targeted, you know, library analyses to see if we can identify peaks that warrant further analyses?

The other question, this is kind of for clarification. You talked about, you know, one part per
million as a possible threshold. And I wasn't clear there if we were talking about the concentration in a sample. And if it's not, should we perhaps identify a threshold that could then be a cut point, where we would look at peaks with an area under the curve that would exceed say one nanogram or one picogram. It seems like when we look at the lowest concentration of things, we generally study environmental studies. Right now, it's at the picogram per gram level.

And maybe we might start as having a natural cutoff and looking at things higher than that, and then see if we can identify environmental chemicals with exposures, you know, at that range or above.

PANEL MEMBER FIEHN: Yes. So I'll start with the last question. I'm sorry, I confused many people. Of course, the value of ppm in mass spectrometry is a mass accuracy. Parts per million is the mass accuracy, not the intensity or concentration. You know, so, yeah, these terms are used in different contexts in different manner.

The mass specs themselves have about -- these type of TOFs or so, they have about four orders of dynamic range. So if you don't want to oversaturate your mass spec, if you think that your very abundant compounds might also be interesting, that limits you to a 10,000-fold range.
However, if you say, well, I'm only looking at exposure, and that this will be the low abundant, you can voluntarily say I don't care for the big peaks. I only care for the small ones. So that is all about your -- you know, how much do you inject and do you allow your detector to be sometimes saturated for certain ions. And we say I don't care for these complex lipids, because they don't -- they are not exposure. They are endogenous metabolites.

So that was your -- I guess, your second question.

PANEL MEMBER BRADMAN: I think the question is kind of like where do we start? Where do we start?

PANEL MEMBER FIEHN: So the second question was that where do we start?

And I tried to make clear that I think for compounds that have chlorine or bromine atoms in it, these are the -- should be the easiest to find if there is exposure of non-classic chlorine and bromine metabolites, adducts, and so on, based -- if you have a mass spectrometer that actually can nicely discern isotopes and get good isotope accuracy. Not all mass specs do that, by the way.

So the next one I said sulfur compounds. Sulfurs are the next best classes of compounds that can be
discriminated by isotopes. Now, if you think about musks that we just discussed, that was no sulfur, no chlorine, no bromine. So you wouldn't detect those as exposure compounds in this manner.

So you -- somehow, once we have not designated chemicals, you know -- and, of course, the priority pollutants always were those with chlorine and bromine, but they're kind of fading out, yeah? So the novel compounds are all phosphorus, nitrogen. They look like endogenous metabolites that don't have any very specific markers anymore. So it gets harder to find those.

So what you can do, of course, is also still use then classic environmental toxicology approaches by saying well, we are interested. We designate chemicals, based on for example, bioaccumulation properties. So let's look at the lipid fraction, and maybe then discard the triglycerides to clean up the matrix a little bit from the very, very, very, very non-polar ones, yeah?

So you can do a little bit of sample clean-up, instead of saying I care for everything. So there are these classic strategies that can be used. Wherever, of course, you use them, some bias. We say like very lipophilic, we don't care, and hydrophilic we also don't care. You know, so that is, of course, always an intentional strategy you would then follow after
discussion or deliberation, because you can't do everything, I guess.

The first question was, help me again?

PANEL MEMBER BRADMAN: The first question was about spectral libraries and using targeted libraries like pesticides targets and what are we measuring?

PANEL MEMBER FIEHN: Yeah. Okay. So endogenous -- yeah, you said -- no, the first question was biological metabolomics seems to be biologically oriented and what about exogenous drugs?

PANEL MEMBER BRADMAN: And how do we differentiate?

PANEL MEMBER FIEHN: How do we differentiate?

So first of all, of course, metabolomics was done in the realm of genomics. So it was all driven by biology and health and biochemical modules and pathways and so on. So that's where the history comes from.

But, of course, we -- for example, my lab also look at drugs and drug exposure and drug efficacy and response of individuals to treatments. So, for example, in human lung tissue, we have found 37 different exogenous non-human compounds. So you can't see them by those libraries.

Usually -- well, it depends on the question. Usually, we ignore those, because they are not relevant to
understand biochemistry, unless you -- that -- unless it's exactly your question. What type of compounds do we find in which tissue or in which biological specimen?

So I was actually surprised to find so many drugs in the lung. Some of them were supposed to be there, cough medicine, for example, yea -- yeah -- which gives you the proof of confidence that, yes, I mean, the suspects that should be there are there.

These are done by libraries. These are the classic drugs and over-the-counter drugs, and the mass spectra are available. And the same for, of course, the pesticides, you know. The question is do we have all the spectra for all the household products, the musks or whatever? Do we have it all for the other designated chemicals?

Maybe that's something you want to find out.

So you know, can -- you know, do we have this list and do we then have hypothetical compounds -- and that's actually what my lab is doing as a research project. So assuming now that these are exposed, and we now metabolize those. We glucuronidate them. We hydroxylate them. We methylate them.

This can be done all computationally. So you can say let's do a hydroxyl on it. Which is the most susceptible area where you would have an epoxide hydroxyl,
or two of them, and how would then the mass spectrum look like? So you could have then these -- we call them virtual mass spectral libraries. But again, this is research. That's not done yet, or there's papers out, yeah, but it's not like as easy. And then you have to validate. But that's the idea of how to expand the -- from a certain group, let's say, of 20 designated chemicals -- well, let's have the human enzymes work on them or chemical exposure oxidation work on it, so that we get these hypothetical structures, and then these hypothetical mass spectra.

So that is the next wave, so to say, of research that's actually carried out right now. There was a nice paper last week, not from us. So that's, I guess, the little bit of an answer to that how we go from known compounds to all chemicals.

PANEL MEMBER BRADMAN: I do have a follow-up, but I don't want to dominate the questions.

CHAIRPERSON LUDERER: Questions from other Panel members?

Ask your follow-up -- Dr. Alexeeff.

DIRECTOR ALEXEEFF: Yes. Thank you so much for the presentation. Mine is not going to be as -- I think Asa made as complicated a question as your complicated presentation.
(Laughter.)

DIRECTOR ALEXEEFF: And I don't know if my question is going to basically defeat the purpose of a time of flight spectrometer, but -- so your presentation was talking about sort of to me and the way I was interpreting it, although we're looking for unknowns, the more you can kind of decide what parameters of that unknown you're looking for, the better you'll have luck in terms of actually identifying compounds?

And since we have designated and priority lists of chemicals, does it make sense to kind of come up with a library of spectra for all those compounds that we've designated or that we've prioritized and to look for those? Is that -- does that make sense to do or is that defeating the purpose of this type of analysis?

PANEL MEMBER FIEHN: No, that's fine. And that's what I also tried to say in the second slide where I said the overview of the idea. The idea was that you might have targeted questions. Is the so-in-so compound in this tissue? And it's a targeted question and it can be nicely done in a targeted analysis manner.

And then you can -- and I've seen it today pool some compounds, like we discussed in the mass center. Well, we have these methods and these methods, but they could be combined. They look like we can combine ODHT, if
I remember correctly, and the musks together, you know, in a single protocol. And that's, what we call, metabolite profiling, you know, where you say assume a dedicated or improved method for extracting and clean-up and one method for targeting those compounds could do more than what was published before. So we call this chemical profiling, if you like.

And that would be totally useful for this Program. And actually this is what was -- what was discussed an hour ago for these designated chemicals, you know. So the question now is can we actually put more into it, right?

So when we look at all the designated chemicals, how many of those would be with that much of error, whatever that is, right --

(Laughter.)

PANEL MEMBER FIEHN: -- still be found in a single shot, yeah? And it can be done in, A, liver, in B, blood plasma, in C, in saliva, and then 3 in urine, and then in house dust and in -- yeah?

So you would go then through the matrices, but it's certainly a very valued approach because it's more cost effective, because then you see, well, of the -- I have no idea how many we have -- 200 -- many of the thousand -- I just say thousand. You know, in this method
we would see 200 of those, and in that method we would see
another 150 of those. You know, if you just use these two
methods, we have already a third of our lists looked at.
And hey, you know, we only pay once. So it might be cost
effective.

And that is one of the ideas to combine
analytical strategies. That is essentially also behind
metabolomics, because biochemical analysis was always done
in the last 60 years. I mean, there's my grandfather's
and your grandfather. They've done this in HPLC-UV. And
so it's not news. The only news is that we have now
better machines and better protocols that can actually
find those peaks, discern those compounds and put them all
in different -- in a matrix for statistics.

Yeah, so that's, I think -- and the other one
would be, of course -- you know as I said, it could be a
totally untargeted screening where you then focus on
certain characteristics, the lipophilicity, or the
elements that are included, you know -- but that would be,
you know, saying well maybe apart from the designated
chemicals, maybe there's exposure that we should know
about, and that we haven't done in our screen.

CHAIRPERSON LUDERER: I actually have a question
kind of wondering whether we could use metabolomics in
the -- kind of somewhat in the opposite direction. So one
of our designated chemicals was diesel, but we didn't --
don't have a specific biomarker, you know, that we know is
specific for diesel, and would it be possible to use
metabolomics say, you know, comparing a population of
exposed and unexposed and trying to sort out whether
there's some good biomarkers to differentiate?

PANEL MEMBER FIEHN: Yeah, that would -- that's
fairly easy to do. It's fairly easy. And I would almost
say fairy routinely, you will see papers out there,
biomarkers of exposure. In nutrition -- I mean, I work
mostly with nutritionists and they say biomarkers of fish
eating versus meat eating. That's much harder, you know,
but biodiesel and so on is much easier.

And also some of that can be nicely done in the
laboratory. You could have animals exposed here, animals
exposed there. So in Davis, for example, we have
cigarette smoke exposed animals, many of them. I have
even breast milk from mice exposed and unexposed, yeah.
So, you know, these things can be done much easier than
say nutritional exposure.

CHAIRPERSON LUDERER: Dr. Bradman.

PANEL MEMBER BRADMAN: Just following up a little
bit on your comment with respect to diesel. And I should
say the question you asked was my next question.

(Laughter.)
PANEL MEMBER BRADMAN: So that solved it. But there's actually a group at the University of Washington that is developing or examining a biomarker for diesel. And I actually forwarded that to the Program. I think they're looking at 1-nitropyrene. And we actually have a situation in the Bay Area, San Francisco Bay Area, where we have Interstate 580 and Interstate 880. And Interstate 580 big trucks are banned, and Interstate 880 is full of big trucks.

And I wonder if there could be an opportunity there to actually try to look at differences possibly related to diesel. A little speculative right now, but this could be an interesting research project.

PANEL MEMBER FIEHN: Yeah. I comment on this 1-nitropyrene. In most studies we've -- actually, in all studies we've done, it always turned out better to look at the panel of compounds, not just this one. So, you know, glucose levels doesn't only tell about diabetes. And I'm fairly sure there will be other sources of nitropyrene than diesel.

So once you have a panel of say 20 compounds, you're much more on the safe side to say this is so much of say diesel exposure than if you only study one compound. Just as a general comment these panels are statistically usually more sound.
CHAIRPERSON LUDERER: Dr. Quintana.

PANEL MEMBER QUINTANA: My question is about sensitivity again.

   Sorry, my chair keeps tipping over.

Getting back to 1-nitropyrene. I'm working with the University of Washington at the U.S.-Mexico border, and we're finding that in femtomole concentrations in urine. And I'm thinking it wouldn't maybe pop up as your first screen some of these compounds. And so you're saying I think you need to look more widely at metabolomics, partly because of a sensitivity issue or just in general.

PANEL MEMBER FIEHN: No. What I tried to say is it all depends on your sample prep. If you take a litter and, you know, of -- you know, the sensitivity of the mass spectrometers are very extraordinary. So Thermo Fisher has come out, I think four years ago, with a study where they used their own triple quadrupole -- LC/MS triple quadrupole compared to their own accurate mass mass spectrometer and found that the sensitivity and selectivity of the accurate mass mass spectrometer was even better than triple quadrupole, because their selectivity is by the mass resolution.

   So the question is how likely is it that noise, at that time -- at that retention time, will have the
exact same accurate mass? And there, you go -- that's the idea of selectivity using a high resolving power. Whereas, the triple quadrupole historically has done -- has relied on the MS/MS transition. And MS/MS transition actually is also not so unique, as people think.

So, you know, otherwise the sensitivity issues is most related to sample preparation. The mass specs are exactly the same. And you can -- there's even people using triple quadrupoles for profiling, by the way.

CHAIRPERSON LUDERER: Do we have questions -- additional questions or comments from Panel members?

Let's see if we have any public comments?

None. All right.

Comment from a former Panel member.

CAL/EA EPA DEPUTY DIRECTOR SOLOMON: Gina Solomon, Cal/EPA.

Among the issues that you raised about potential pitfalls, one of the ones that worried me the most, I guess -- maybe I don't know if I was right to be worried the most by this one, but was that difficulty differentiating peaks that are extremely close together. And you showed one where actually the peak was mis-identified because there were two that were sort of merged. And so actually the number was wrong in the initial identification of that peak. And so one could
actually then proceed merrily along to identify a compound that wasn't even there at all.

And the only solution that you presented there was to use way more powerful instruments than I think we will have access to. So I'd just sort of like to get a little bit more of a sense from you about how likely that will be if we do what it seems like this Panel is thinking about doing, which is constraining the universe a little bit to try to maybe have a slightly higher confidence that we're identifying, what we think we're identifying. Are we still going to risk identifying completely the wrong things with any kind of frequency?

PANEL MEMBER FIEHN: Yeah. Metabolomics is the art of not doing sample clean-up.

(Laughter.)

PANEL MEMBER FIEHN: So therefore, you have very complex chromatograms. But it depends on your — how can I say — concentration scheme that you can also have very, very complex chromatograms under usual solid phase extraction clean-up procedures, if you not just look for your target compounds.

The idea of omics, in general, is hypothesis generation. So what I, not really, alluded to maybe in a half sentence is, whatever you find in your first pass screen has to be validated in a second pass with a
targeted method. So let's assume you would have some novel chlorine compounds. Three more that were seemingly there, and you then make big claims. Don't. Go for a second trial.

(Laughter.)

PANEL MEMBER FIEHN: And this is the same, by the way, in human clinical trials. We always go for two-thirds to one-third between discovery and validation sets. This is very important also I said when I talked about the PLS, partial least square, Where I said, well, it could be data overfitting. And similarly, it could be chromatography complexity driven, right.

So the idea is you have to have independent cohorts and maybe an independent method. Maybe you use another type of HPLC, which then will all of a sudden, you know, separate your compounds, right, of interest, or you alter the methods.

Of course, that is the only valid strategy to say, well, okay, the first thing is discovery, hypothesis generation. And then you validate your hypothesis in a second cohort or a second method or both. It doesn't --

CAL/EPA DEPUTY DIRECTOR SOLOMON: One other. If I may. A follow-up question is so what I think I heard you just say there is that you -- that the second phase is not -- does not require getting a purified sample of the
chemical --

PANEL MEMBER FIEHN: No.

CAL/EPA DEPUTY DIRECTOR SOLOMON: -- which is
often difficult -- would be difficult for us to do. And I
heard other investigators kind of say, well, if you find
it, then you have to actually, you know, obtain an
analytical standard and that would be tough.

But it sounds like you're talking about a
different type of validation, which is much more feasible,
so that's great.

And then the other question is fluorine,
fluorinated compounds, would those be as easy as
brominated and chlorinated, because there's a whole class
of perfluorinated compounds that are of interest to us as
well?

PANEL MEMBER FIEHN: Of course, these people who
have told you that it's great to have the analytical
reference compound are correct. I mean, this is the
mainstay of chemistry, have the compound and you can do
your recoveries, your sensitivity analysis, your precision
and accuracy tests, spiking into a matrix, getting it out
over the matrix. We love to do that.

However, as you say, it's not always possible,
and especially not if you're look at 1,000 compounds. So,
you know, here it was -- I meant to say that if you have
the concern that there might be some compounds involved or that your power of your cohort was not high enough, you then can validate other statistics, if you find it again. This is where the study was fail was -- genomics step analysis.

Or you can also do a different type of analytical method and find the compound again, because it will have the same accurate mass and same MS/MS. So even if you don't know, it should then again show up in a different method.

Now, the NIH has also funded two centers for chemical synthesis, specifically for that purpose. One is in Stanford, one is at North Carolina, because the NIH found that also kind of difficult to say we have all these new compounds popping up and how do we validate those and verify. So we have two designated NIH chemical synthesis centers that could help.

When we put in the -- actually, anyone can put in proposals and then they get -- there's a committee looking at those and so on. But we -- you have to argue about health effects or why is it important. You can't just say I found those, give me those compounds. You have to say something like in 100 percent of the mothers where the children get sick, you know, or something like that, you know, it's been found. So there has to be a good
argument.

So basically after the discovery phase, but yeah, I mean -- and the NIH will not go away. They are actually good guys. They think forward in these strategies. And this is -- by the way, I didn't say, these are the so-called NIH Common Fund, for those of you who are policymakers. And the NIH Common Fund funds about 100 different research areas, including metabolomics, which wouldn't fit in the typical column of a certain NIH institute, say NCI or NIEHS or so, but rather it's a common fund of all the different NIH institutes.

CHAIRPERSON LUDERER: All right. Thank you again very much for that wonderful presentation and very interesting discussion.

(Applause.)

CHAIRPERSON LUDERER: All right. The last -- second to last item on our agenda is the open public comment period. So this is the opportunity for members of the public to comment on anything related to the Program, not necessarily today's presentations.

Dr. She.

DR. SHE: I want to comment on Dr. Fiehn's presentation. This is really timely presentation, and based on your experience. I say it's timely because the Program is about to start unknown identification for
Biomonitoring Program, so your experience and especially
your lab's resource experience could be very helpful to
us.

So my comment is I listened to what you talk and
also the other comment, sounds like the Biomonitoring
Program should start with some kind of -- instead start
with unknown unknown. It's kind of more like targeted
unknown, which is a class of chemicals. For example, you
mentioned -- let's say, we look for the phenol BPA
analogs. They possible also go through the same Phase 1,
Phase 2 reactions. One of the Phase 2 reactions is they
combine with the same group of the -- due to -- so then we
can use mass spectra feature filters. Just look at, let's
say, neutrolosis or ions to combine with accurate mass,
isotope profiling.

We can be more easily successful. Also, use --
like Gina's questions, like fluorine compound. You
mentioned like bromine, chlorine. They're easy to
identify by the M plus 2 isotopes. Fluorine have very
unique features, a single element, and also matches the
deficiencies. So using the matched deficiencies it may be
able to help this.

So I'd like to get your comment if the Program --
we have the machine set to -- already set up. We thought
about it to start something smaller, kind of demonstrate
success, and then allow the question of how we expand it. So we thought maybe start with BPA analogs. And at the same time -- like the last time at the SGP, I talk about Derek Mueller or someone recommended 600 chemicals and published it in the EST. We look for all of this group of chemicals. And they make very significant for this kind of program.

So second thing is, as you know, whatever we put by a TOF or a trap we already have, we never reach your levels of equipment. So the collaboration is very important, not only between us, between the expert like you -- for example, like other question Gina mentioned, for example, we found two mass is so close we cannot tell them what they are. That's for the others.

Finding unknown is like playing puzzles. You put all of the substructures together to find the original structure. Mass spectrometer, just like a process -- you have a vase -- that adds to the people's -- you have vase. You smash them on the floor. Now, you pick up all the pieces. You have to put them together to say, okay, what's my original vase?

So many guess process, so we should require the cross validation. So with your laboratory's experience, we start kind of like infancy. What do you think is possible? For example, not routine, like at least, one,
we have a very hard question how we should put this piece
together, and then can come to you to ask, you know, some
help.

PANEL MEMBER FIEHN: Yeah. If I might respond to
that, please?

CHAIRPERSON LUDERER: Dr. Fiehn.

PANEL MEMBER FIEHN: So I think you are -- thank
you for your clarification on the fluorine elements. I
had forgotten to answer that question by Gina Solomon.

So, yes, I think what we have done here is we
have outlined different strategies that can be followed
from exposure questions. So, you know, that would be a
metabolomic strategy for diesel exposure or other types of
exposures, to asking questions, from starting from the
designated lists of chemicals or certain parts of
designated chemicals, and expanding those to non-targeted
screens on certain elements that might -- that are known
to be xenobiotics, and you know, historically being
important. So there are different strategies that can be
followed. You know, that is definitely, you know,
something I think we can take from our discussion today.

DR. SHE: Thank you.

CHAIRPERSON LUDERER: Okay. Thank you. So we
received one email comment. Were there other speakers as
well that are -- or is this the only comment?
Okay. All right. So this is from David Nuber, and relates very much to what you were just saying, I think. "I am still not clear about what the purpose of metabolomics will serve within the Biomonitoring Program. Can the SAG please elaborate?" I think perhaps SGP is what he meant.

So he's asking about what the purpose of metabolomics is within the Biomonitoring Program. You actually just were addressing.

PANEL MEMBER FIEHN: Yeah. I mean, just to replace metabolomics to chemical profiling, and then, you know, make it clearer. And if you then specify the chemical profiling to certain areas of interest, for example, designated chemicals or exposure programs, knowing what, you know, compounds -- new compounds that might be found in this manner by accurate masses. It could also be correlated, for example. So you'd say correlation of these compounds that correlates to this type of environment, you -- the interstates were mentioned before.

I think this is where untargeted or class-based targeted strategies would nicely fit into the target-only strategies that were historically followed. So like an extension rather than replacement, of course. So that's a good way to think about chemical screening or chemical
profiling.

And, of course, I mean -- well, we still have the biological component. And if you know that certain populations are more exposed, say a lot more different exposed, so these are the -- a lot diesel exposed, and now we have a match control who are not, you can actually try to use our type of metabolomics, which is biological driven and see if there are differences in the biochemical regulation of these cohorts.

So as I said, we do this for drugs, but replace drugs with other xenobiotics and you have the same idea, right? So that is, of course, something that would be yet another health-related component, rather saying we need for -- to wait for a health endpoint, we want to know if certain populations, where we know they're exposed to these types of chemicals, are there significant differences in biochemical regulation?

CHAIRPERSON LUDERER: Thank you very much. Do we have any -- Dr. Bradman, do you have a --

PANEL MEMBER BRADMAN: Just one -- ask that question too. I think the most simple terms that can really be useful for biomarker development, both in terms of effects and exposure. And to put it in maybe familiar terms from a decade or so ago, we kind of have no knowns, and unknown knowns, and unknown unknowns. And I think the
goal here is to bring the unknown knowns into the known
knowns, and move the unknown unknowns into something that
we can also know at some level. And I really think that's
kind of the -- summarizes where we can take this. And
that ultimately, it can be useful for the Biomonitoring
Program, which is primarily focused on targeted chemicals.

CHAIRPERSON LUDERER: We had -- since we have no
additional public comments, and we thought this would be a
good time, Dr. Quintana had something that she wanted to
bring up as a potential priority chemical to be considered
in the future.

PANEL MEMBER QUINTANA: This comment is for the
Panel members, at least those that remain. And it has to
do with should we perhaps give an emphasis on measuring
biomarker of tobacco exposure, secondhand smoke, and
active smoking routinely in our samples, which is not
currently being done, is my belief. I'm thinking
specifically of cotinine. Although, there are others.
And cotinine is a metabolite of nicotine, which has a
half-life of less than a day, which is fairly short, but
it tends to be quite stable in populations, because the
behaviors are quite stable of exposure to secondhand
smoke.

And so I had -- don't want to open that up for a
discussion today - it was not my intent - but to ask the
Panel if they would consider discussing this at a future meeting?

CHAIRPERSON LUDERER: So I might add that cotinine is already on the designated chemicals list, because it's one of the chemicals that the CDC NHANES program monitors.

Any thoughts or comments from Panel members regarding that?

Dr. Bradman.

PANEL MEMBER BRADMAN: Just, I once brought that issue up, and there was some concern about the laboratory commitments that would be necessary to do that. I don't know what the situation is now, but I think actually Dr. Lipsett responded about that. That was at the very beginning of the Program. But certainly, I mean, we know tobacco smoke is a very important public health issue.

PANEL MEMBER QUINTANA: I should add that there have been studies using the NHANES data on the chemicals that we are measuring, such as PAHs and metals that have found associations with secondhand smoke, so it may help interpret some of the variability in our data. But again, I don't want to open it up to discussion at this very late hour, but more to see if the Panel is open to discussing it in the future.

CHAIRPERSON LUDERER: Did somebody -- what Dr.
Bradman brought up, does someone from one of the labs recall that discussion and maybe want to comment on that?

DR. SHE: We like -- Panel noticed and CDC already monitor it, but I think they monitor it in blood serum, not in urine, right?

PANEL MEMBER QUINTANA: Serum mostly. Sometimes in urine.

DR. SHE: Serum, yes. And then CDC have a method. So for us to follow up, we need to -- I didn't check the method carefully, so we possibly need to look at the method. And also like you mentioned, this -- actually, we also feel important. Like, we recently look at some PAH datas. Definitely, that's -- you feel like you miss something to give an interpretation of what you found.

So laboratory, I think, either ECL or us, we will look at it and then come back for laboratory part in next meeting.

CHAIRPERSON LUDERER: Okay. Thank you very much. So it sounds like, you know, there would be some interest in discussing that further at a subsequent meeting.

Another public comment. Okay. Great. Thank you.

All right. We did get -- this is response from Dave Nuber to the response to his prior question. He
says, "Therefore we are looking more at the exposome than the metabolome".

PANEL MEMBER FIEHN: Yes, of course.

CHAIRPERSON LUDERER: Yes. That's the goal of the Program.

(Laughter.)

CHAIRPERSON LUDERER: All right. So then if we don't have any additional public comments or Panel comments, I'd like to go ahead and wrap up.

I want to announce that as always a transcript of this meeting will be posted on the Biomonitoring California website when it's available, and remind everyone that the next Scientific Guidance Panel meeting is scheduled on March 27th, 2014 in Oakland.

So thank you all for coming and the meeting is adjourned.

(Thereupon the California Environmental Contaminant Biomonitoring Program, Scientific Guidance Panel meeting adjourned at 4:03 p.m.)
CERTIFICATE OF REPORTER

I, JAMES F. PETERS, a Certified Shorthand Reporter of the State of California, and Registered Professional Reporter, 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 29th day of November, 2013.

JAMES F. PETERS, CSR, RPR
Certified Shorthand Reporter
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