

**OEHHA Domoic Acid Webinar: Evaluating Low-Level Exposure
and the Implications for Human Toxicity
30 July 2019**

The following transcript has been modified for clarity and readability. Each speaker is identified with their last name in brackets prior to the first word each time they speak. Speaker names and affiliations are in the agenda below.

AGENDA

Tracy Cook, GoToMeeting event coordinator

09:00 – 09:10 Opening Remarks: Webinar Goal *Lauren Zeise, Office of Environmental Health Hazard Assessment (OEHHA)*

Moderators: Susan Klasing and Shannon Murphy, OEHHA

09:10 – 09:25 Derivation of the Action Levels for Domoic Acid *Susan Klasing, OEHHA (Sacramento, CA)*

09:25 – 09:30 Brief Q & A

09:30 – 09:45 Recapping the State of the Science: Reflections on the 2017 Workshop *Shannon Murphy, OEHHA (Sacramento, CA)*

09:45 – 10:15 Maternal and Offspring Effects of Chronic, Low-level Domoic Acid Exposure in a Nonhuman Primate Model *Thomas Burbacher & Rebekah Petroff, University of Washington (Seattle, WA)*

10:15 – 10:20 Brief Q & A

10:20 – 10:50 Toxicokinetics and Physiologically-based Modeling of Domoic Acid Exposures *Nina Isoherranen, University of Washington (Seattle, WA)*

10:50 – 10:55 Brief Q & A

10:55 – 11:05 BREAK

11:05 – 11:35 Low-Level Domoic Acid Exposure and Everyday Memory *Lynn Grattan, University of Maryland (Baltimore, MD)*

11:35 – 11:40 Brief Q & A

Moderator: Pam Lein, University of California (Davis, CA)

11:40 – 12:40 Discussion Panel *Thomas Burbacher, Lynn Grattan, Nina Isoherranen, Rebekah Petroff; Audience Q&A*

12:40 – 12:45 Acknowledgements and Wrap-up *Lauren Zeise, OEHHA*

0:00 [Cook] Hello everyone and welcome to today's webinar, "Domoic Acid Research on the Effects of Repeat Low-level Exposures and its Implications for Human Toxicity". Before we get started, I'd like to go over a few items so you know how to participate in today's event. You may have joined the webinar using your computer's speaker system as the audio, which is the default. If you prefer to join over the telephone, just select telephone in the audio pane and the dial-in information will be displayed. For technical difficulties, please use the "questions" box of the control panel. You can submit questions to today's presenters by typing your questions into the "questions" box of the control panel. You may send in your questions at any time during the event. We will collect these and address them during the brief Q&A sessions following each presentation or during the panel discussion at the end of today's session. We have included handouts for today's session. You can download them from the handouts panel. Today's webinar is being recorded.

1:00 I would now like to introduce Lauren Zeise, Director of the Office of Environmental Health Hazard Assessment or "OEHHA"- Lauren. [Zeise] Thank you, Tracy. On behalf of OEHHA, I'd like to welcome everyone to today's webinar on domoic acid. OEHHA is one of six Departments of the California Environmental Protection Agency. Our mission is to protect human health and environmental health through the scientific evaluation of risks. We're the lead Agency for assessment of health risks posed by environmental contaminants, including domoic acid in seafood.

1:41 OEHHA reviews data on domoic acid levels in seafood and, when they're too high, in consultation with our Public Health state public health department, we recommend to the California Department of Fish and Wildlife closures of the affected fisheries. We also make recommendations to reopen fisheries when levels have cleared. Today's webinar builds on the 2017 Workshop we held with the University of California, Davis on the state of the science of the health effects of domoic acid.

2:16 Today, we are updating our understanding of domoic acid exposure by examining the very recent science on neurological and developmental effects associated with repeat exposure to low levels of domoic acid. As we listen to the presentations, we'll keep in mind a couple of points – first, how the recent findings from our non-human primate studies and human studies may shape our understanding of vulnerable populations, what their potential risk for domoic acid toxicity may be, the types of neurotoxicity we might be concerned about, as well as levels of concern. And, second, what the research might suggest about the vulnerability of high-frequency shellfish consumers who may be repeatedly exposed to low levels of domoic acid. So, with that, thank you everyone for joining us. I'd like to now introduce and turn the webinar over to our webinar organizer. Dr. Shannon Murphy. Shannon is a staff toxicologist in OEHHA's Fish, Ecotoxicology, and Water Section- Shannon. [Murphy] Thanks, Lauren. Let me add my welcome. Today's speaker session will begin with two short presentations by OEHHA. The first is a primer on how the current federal action levels for domoic acid were derived,

followed by a recap of the OEHHA-UC Davis State of the Science Domoic Acid Workshop of 2017.

3:40 Just a reminder, that the brief question-and-answer period following each presentation is intended for short clarifying questions. More in-depth questions may be addressed in the extended panel discussion session. Our first speaker is Dr. Susan Klasing, Chief of the Fish, Ecotoxicology, and Water Section at OEHHA. Welcome, Susan. [Klasing] Thank you and I'd like to welcome everyone again for joining us today. As noted, I'm going to be giving just a brief explanation of how the action levels for domoic acid were derived.

4:23 First, what are the action levels for domoic acid? Just to quote FDA, "action levels and tolerances represent limits at or above which FDA will take legal action to remove products from the market". My Office and the California Department of Public Health use FDA domoic acid action levels to make closure, delay of opening, and reopening recommendations to the California Department of Fish and Wildlife. Those action levels are 20 parts per million in all fish, which include finfish, crustaceans, and mollusks, except, the action level is greater than 30 parts per million in Dungeness Crab viscera. Our authority to make such recommendations comes under Fish and Game Code Section 5523.

5:07 Just a little background on how we first learned about domoic acid toxicity in humans. There was a poisoning incident that originated in Prince Edward Island, Canada, in 1987. Initially, three patients presented with rapid onset of disorientation, confusion, and memory loss over a few-day period. All of these individuals had eaten cultured muscles that originated from Prince Edward Island. At that time, domoic acid was not known to be a marine biotoxin, and so it wasn't on the radar when they were looking for a causative agent.

5:42 These exposures were acute, most likely a single meal in many cases. There were more than 250 reports of illness associated with consumption of mussels. 107 people met the case definition. 19 people were hospitalized. 16 treated in the ICU. Three died while hospitalized, and a fourth severely ill patient died of a heart attack a few months later. That person is often included in the mortality statistics for this event. Symptoms began within 15 minutes to 38 hours after consumption.

6:17 There were two types of symptoms. Milder symptoms were gastrointestinal in nature. They generally began within 24 hours: nausea, vomiting, cramps, and diarrhea. The more severe symptoms were neurological in nature: seizures, coma, and irreversible memory loss. They typically began within 48 hours. It's the irreversible memory loss that led to the moniker for this disorder of "amnesic shellfish poisoning" or ASP. They determined that the most sensitive populations during this outbreak were older, tended to be men, and those with pre-existing medical conditions, such as diabetes or kidney disease. Just a little background on risk assessment for those of you who don't do it for a living. One of the things that we're looking for is a dose-response curve, the dose plotted against whatever

adverse outcome that we are concerned about. A typical classic dose-response curve looks something like this.

7:16 And two key things that we're looking for are referred to as "points of departure". The first is a "NOAEL" or no observed adverse effect level, and that is the highest dose that is not significantly different from the control. The control would not have been exposed. Another is the "LOAEL" or lowest observed adverse effect level, and that is the highest, I'm sorry the lowest, dose that is significantly different from the control. So there may be other doses - higher, lower, intermediate - but those are two key points that we're looking for. It's important to know that these values are based on the experimental data that we have - whatever doses there were. The true values for the NOAEL and LOAEL for the population are likely unknown.

8:02 So the risk assessment that was done for domoic acid after the Prince Edward Island event. There was a dose-response estimation that was based on data that they had for 10 individuals for whom they had a sample of uneaten mussels. Those individuals ranged in age from sixty to eighty-four years. It was nine patients, plus one subject who did eat the mussels, but did not get sick. Three of the patients were hospitalized.

8:26 They collected uneaten mussels from these individuals and found them to contain between 310 and 1280 parts per million domoic acid. The estimated individual consumption rates range from 35 to 400 grams of mussels. When they combine these values, the individual estimated domoic acid consumed ranged from 20 milligrams in the person who did not get sick, to 290 milligrams in two individuals who were the sickest. So this table is adapted from Perl et al., who published a paper in the New England Journal of Medicine in 1990, and several other papers, as well. And you can see that this shows the estimated amount of domoic acid consumed in the second column, rated from the lowest to the highest. So 20 milligrams in the person who did not get sick and 60 milligrams was the lowest dose that showed any symptoms. In the four right columns, you'll see the four types of adverse effects that they were looking at: GI effects, memory loss, were they hospitalized, and were they in the ICU. And you can see a clear positive dose-response here. The higher the amount of DA that people were exposed to, the more likely they are to have one or more of these adverse effects.

9:48 So we plotted that data in a way similar to a dose-response curve with the dose along the x-axis and the type and number of effects, the "0, 1, 2, 3, or 4" on the y-axis, and you'll see that it plotted to be somewhat similar to the classic dose-response curve that I showed you a few slides ago, except there was one individual that had more severe effects than would be predicted based on a typical dose-response curve.

10:17 So, I'm going to show you now how the domoic acid action levels were derived. And if you look in the literature, you'll find that there are various ways of

deriving the number. Different agencies and different countries and they use different body weights, different consumption rates, different uncertainty factors, but they all came up to the same number. So I'm going to show you the way that FDA has explained this in some of their documents. First, 240 parts per million was determined to be the lowest effect concentration in mussel meat and that's the 60 milligram lowest dose that caused an effect, divided by an estimated 0.250 kg (kilos) serving size. This was divided by an uncertainty factor of ten, leading to 24. Just as an aside, uncertainty factors are always part of a risk assessment and are used to account for data deficiencies, such as inter-individual differences, small sample size, lack of chronic exposure data, and using a LOAEL versus a NOAEL in the calculation.

11:15 Uncertainty factors for human data typically range from 10 to 300, so that led to an action level of 20 parts per million (ppm) in seafood. Additionally, a tolerable intake was derived taking that same 60 milligram lowest effect dose divided by the same ten-fold uncertainty factor, leading to a tolerable intake of six milligrams of domoic acid.

11:43 So Health Canada originally derived the Action level and it was adopted by FDA, and then, subsequent to that, it was determined that there should be a separate Action level for Dungeness Crab viscera. Viscera tends to accumulate more domoic acid and there's less of it in a crab. So data were submitted to FDA, and FDA made several assumptions. One is that a person eats one crab, including the viscera in an eating occasion, that 300 grams is a reasonable estimate of crab meat yield from one crab, and that 6 ppm would be a potential concentration of DA in crab meat. So using that 6 ppm with the 0.3 kilo, they estimated that there would be approximately 1 milligram of DA contribution from the crab meat, and you'll see that there's rounding in all of these equations.

12:42 They further estimated that there was, would be a hundred and fifty grams as a reasonable estimate of crab viscera from one weight.

12:51 So, if one milligram would be an estimate of contribution from the crab meat and a tolerable intake is 6 milligrams, then that would allow five milligrams to be the contribution from crab viscera.

13:05 So that with that five milligrams being the tolerable amount of domoic acid in a hundred and fifty grams of crab viscera consumed in one eating occasion, that came out to 33, which is rounded to 30 parts per million as the tolerable amount of DA in crab viscera, and the Action level became greater than 30 parts per million in crab viscera.

13:26 So why are we all here today? We've had recent domoic acid events in California. In 2015-16, we had an unprecedented Pseudo-nitzschia bloom that affected the US Pacific Coast. Certain strains of Pseudo-nitzschia produce domoic acid under certain conditions. At that time, the California Dungeness Crab fishery

opening was delayed up to five months in parts of the state. It resulted in a fishery closure resource disaster and a commercial commercial fishery failure declaration for both the Dungeness and rock crab fisheries for the 2015-16 season. We've had closures and delay of openings occurring during subsequent crab and lobster seasons, and our recreational razor clam fishery has been closed in Humboldt and Del Norte counties since April of 2016.

14:15 Subsequently, there have been publications and workshops related to domoic acid. In 2016, the California Ocean Science Trust published two documents, an FAQ on harmful algal blooms in California Fisheries; another document framing the scientific opportunities on harmful algal blooms in California Fisheries. In 2017, it's already been noted that OEHHA and UC Davis co-sponsored a one-day workshop to evaluate the state of science on domoic acid and the implications for human toxicity, and, in 2018, the California Ocean Protection Council and California Ocean Science Trust co-hosted a HAB workshop on how the scientific understanding of HABs can inform domoic acid monitoring for seafood safety. And that's the end of my talk.

15:01 [Murphy] Thank you, Susan. We have a couple general questions from the audience. Some of you have asked if the PowerPoint presentations will be available for sharing. What I would recommend is you go ahead and email the organizer Shannon Murphy, myself, at Shannon.Murphy@oehha.ca.gov. And I will put you in touch with the authors of each presentation and you can contact them directly for to make that arrangement, if they so choose. Also, too, we are recording this webinar and we intend to post it at a future date and we're exploring the technical factors associated with that. So please stay tuned.

15:38 We do have another question. This is for you, Dr. Klasing. How was DA, or domoic acid, identified as the active agent in the first ASP event? [Klasing] There's a really excellent article on it, which I have not re-read recently, so I can't give you the details, but I can say that there was a very concerted effort that went actually very quickly on how they were able to rule out other potential causative agents and focusing on finding that it was domoic acid. [Murphy] We have a second question here. Can you explain the relationship between Pseudo-nitzschia and domoic acid? [Klasing] Well Pseudo-nitzschia is a marine diatom that under certain conditions and certain species will produce domoic acid.

16:26 [Murphy] Thank you very much. Not seeing any more questions, we'll go ahead and move forward. Just a reminder that attendees can submit questions at any time during the webinar through the "question" pane of the control panel. Also, another note is that we have handouts available. And, if you look at the control panel under "handouts", you'll be able to download those there.

16:56 In this next presentation, I will provide an overview summary of our 2017 domoic acid workshop. A key aim of today's presentations and discussion is to build upon the 2017 state of the science workshop. A copy of the 2017 workshop agenda,

including a list of all the featured presenters, is available on OEHHA's website. Before we dive into the new science, let us revisit some of the historical domoic acid highlights.

17:25 2017 marked the 30th anniversary of the 1987 Prince Edward Island domoic acid event. That public health episode spurred teams of scientists, health agencies, and policymakers to identify, understand, and manage this mystery agent we now know as domoic acid. Health symposia and workshops directly followed, with the 20 ppm action level for domoic acid in seafood adopted by the FDA in 1992, and later by the European Union in 1997. During the next two decades the Pacific coast witnessed sporadic episodes of bird deaths and marine mammal illness and strandings attributed to domoic acid toxicity. These events were a prelude to the massive harmful algal bloom event of 2015-2016, where high levels of domoic acid impacted fisheries along the whole Pacific Coast, closing the entire California coast to Dungeness Crab fishing for most of the fishing season.

18:13 This season of "the Blob" launched a new series of workshops and public meetings, thrusting scientists, health officials, marine resource managers, and the fishing community into direct confrontation with domoic acid. A challenge that continues with our discussion today.

18:41 Among these events was the 2017 state of the science Workshop hosted by OEHHA in collaboration with the University of California-Davis, which featured a broad spectrum of presenters from experts in domoic acid toxicity. Here are the highlights of that conference.

18:57 Dr. Tasker of Prince Edward Island University, a researcher and "ground zero" witness to the seminal domoic acid event, opened the day with a first-hand recounting of November 1987. Applauding the valiant efforts of those scientists and health officials, Tasker outlined how these individuals worked in record time to identify the cause and minimize the impact of the crisis. The early years shed light on domoic acid toxicity with the naming of a syndrome, defining of the symptomatology, establishment of the Action level, and further description of domoic acid impact beyond the acute effects, setting the stage for a more complex profile of neurotoxicity.

19:40 Dr. Gulland of The Marine Mammal Center of Sausalito, described the effects of domoic acid toxicity on marine mammals, particularly sea lions. Called "Sentinels of the Sea", California sea lions exhibiting signs of DA intoxication can indicate the presence of a recent or active bloom, and the potential health implications for human exposure. Dr. Gulland described both the acute and chronic neurotoxic syndromes, highlighting that the fetal compartment, especially the amniotic fluid, acts as a "sink" or storage depot for domoic acid accumulation. These findings suggest that the fetus may represent a vulnerable population.

- 20:21 From the sea to the shore, Dr. Grattan of the University of Maryland School of Medicine, examined the impacts of low-level DA exposure in humans from consumption of razor clams through her efforts with the CoASTAL cohort study involving tribes of the Pacific Northwest. Neurobehavioral assessments and neurocognitive scoring suggest that these low-level exposures are associated with memory decline in high-frequency razor clam consumers. We will welcome Dr. Grattan later in the webinar for an update on this research.
- 20:56 Dr. Ramsdell of NOAA delivered a primer on the interesting pharmacokinetics of DA, noting that this glutamine receptor compound acts as a partial agonist with high affinity for kainate receptors. DA induces neuroexcitotoxic effects, in part, by failing to effectively desensitize the glutamate receptor. DA's kinetic profile of poor absorption and rapid elimination, make it a challenging compound to model. Age, the presence of kidney disease, and pregnancy complicate one's susceptibility to DA's toxic effects. Dr. Isoherranen's pharmacokinetic work in the context of pregnancy will further this discussion later in the webinar.
- 21:41 Dr. Faustman of the University of Washington expanded the discussion about low-level DA exposure during various developmental stages, critically examining them spectrum of neurotoxic effects associated with *in utero* exposures in mice.
- 21:56 Altered motor coordination and changes in startle response observed in the offspring of dams exposed during pregnancy, coupled with changes in the kinetic profiles of pregnant versus non-pregnant adults, and concentrated levels of domoic acid in the amniotic fluid, model effects similar to those observed in the offspring of DA-intoxicated marine mammals.
- 22:19 The work of Dr. Doucette of the University of Prince Edward Island further advanced the conversation about early life exposure and adverse neurodevelopmental effects, noting that rat pups exposed during early postnatal life demonstrate a varied spectrum of disrupted or delayed neurocognitive development.
- 22:39 Depending on the duration and the timing of exposure, aberrant behavior, indicative of changes in learning and memory, may remain latent for a time, manifesting later in life during another key window of development. We will revisit this concept of early DA exposure, and the implications for neurocognitive development, during Dr. Burbacher's presentation today.
- 23:05 Finally, Dr. Lahvis of the Oregon Health Sciences University fostered a stimulating discourse on the similarities between DA-induced neurotoxic effects observed in rodent models and marine mammals with elements of the neurobehavioral profile observed in children with autism spectrum disorders. Altered brain connectivity and aberrant social behaviors observed in exposed rodents, and disruptions in highly coordinated social behaviors and increased aggression noted in

intoxicated sea lions, mirror behaviors often noticed in children with these autism spectrum disorders.

23:42 As we prepare for today's discussion, we note that with this increased frequency and duration of these harmful algal bloom events, the intoxication syndromes observed in marine mammals, the neurodevelopmental implications from decades of work in laboratory models, and the emerging associations from social science studies, that ,collectively, the 2017 state of the science workshop highlighted the need for further examination of low-level domoic acid exposure, the nuanced spectrum of neurotoxic effects, and their nexus with identification of potentially vulnerable populations. Hence, the focus of today's webinar.

24:32 And with that, we'll have a look at any of your questions. Okay. It looks like we just have a quick question about the handouts and it looks like our organizer has addressed that. But, once again, we have a couple of handouts for people to look at, including the agenda and biosketches of the speakers.

24:57 All right. So moving ahead. I would like to now to introduce. Dr. Thomas Burbacher who is going to discuss Maternal and Offspring Effects of chronic low-level domoic acid exposure in non-human primates. Dr. Burbacher is a professor of Environmental and Occupational Health Sciences, Division Head of Reproductive and Developmental Science, and Director of the Infant Primate Research Laboratory at the University of Washington's National Primate Research Center in Seattle. His research investigates changes in brain development and function caused by perinatal exposure to neuroactive substances and the adverse human health implications, including developmental disabilities related to exposure to environmental contaminants. Dr. Burbacher is joined by Ph.D. student Rebekah Petroff, whose efforts contribute to research exploring the neurological effects of low-level chronic domoic acid exposure using behavioral observations and MRI studies in the non-human model. Welcome Ms. Petroff and Dr. Burbacher.

26:07 [Burbacher] Thank you. Thank you very much. Welcome everyone. I'm going to begin by giving you an outline of what we're going to talk about this morning- want to make sure that we talk about the study goals. Why did we, what were the main reasons that we initiated this study, talk a little bit about the non-human primate model, and then get into our original study design and procedures that we used and go over the results- the published results for those that we have so far, and then we're going to talk about a bit of additional research that we were able to do with a supplemental grant once we found out some other results from the initial study. And, then discuss what we're working on now in terms of publications, and then talk about potential future collaborations that we're looking for.

27:07 So let's let's start here. Okay, there we go. So the study goals we wanted to use a non-human primate model to provide data related to chronic exposure to low-level domoic acid. We focused a lot on the toxicokinetics thinking that you know, one of the major issues with exposure studies in humans is to actually get a really

good assessment of exposure. So with non-human primate model, with an animal model, you can actually get a lot better, a fuller, idea of what the dose response is for exposures. We also wanted to look at whether or not metabolism excretion of domoic acid changes- if it was different based on whether or not you're exposed for the first time, as opposed to chronic exposures.

28:07 And then we also wanted to see if there were differences in kinetics with pregnant versus non-pregnant females. And then, lastly, we wanted to look at what were the differences in kinetics of DA when you look at the maternal versus the newborn blood levels, but the major focus of the study was on looking at low-level toxic responses. We really focused a lot of our study on reproductive effects in the adult females. We did look at, sort of general overt toxicity signs in the mothers, but we assume that given our doses, which were one-tenth to even less than one-tenth of the toxic dose, that we wouldn't be seeing overt toxicity.

29:00 And then again the the real focus of the study was on prenatal exposure and infant development. Okay. So let's go forward here. So in terms of the non-human primate model, we have a primate Center here at the University of Washington and I've done non-human primate work for all of my career and we use a non-human primates on just selective types of problems that we feel are major problems for human health in terms of exposures to environmental contaminants. We do know that the toxic dose for DA is similar when you compare monkeys and humans, and there's a lot of characteristics of the primate in terms of their structure and function that mimics the human better than other animal models. So we chose *Macaca fascicularis*, which is monkey model that's been used previously in several studies looking at DA exposure and effects.

30:03 All right. So, in terms the original study design, we had 32 adult females that we separated into three different groups. We had 10 in a control group, 11 in our 0.075 milligrams per kilogram or lower dose, and then another 11 in our 0.15 milligrams per kilogram per day. So, those two doses sort of bracket the estimated acute tolerable daily intake for DA. As I mentioned, our exposures are oral. A lot of the previous investigations in animals have been using IV or IP, so we felt that oral was the best route to go. They were dosed every day. We had clinical assessments of the adult females. They were bred with non-treated males.

31:02 And we looked at various reproductive outcomes and then looked at the neural development of the exposed and control infants. So this gives you a little more detail about the study design and well, I don't want to go through this. It's a fairly busy slide, but I think what you can tell from this is that it's when you're doing non-human primate work, it takes a long time to get these studies done. We had a two to four month period where we just train the animals to do all the different assessments. We actually looked at all of our outcomes for two months prior to doing any dosing and then we did a kinetic study the first day that we had that we had dosing and then another eight weeks afterwards. We've bred the animals, and it took from one to seven months to get the animals pregnant, and then we dosed

them- exposed them- all through pregnancy and did an additional two kinetic studies during pregnancy. The additional studies that we did actually were along the postpartum period on the adult females where we did some MR scans and Rebekah will be talking about that. And then we have the outcomes from the infant testing. So Nina's going to talk about the different kinetic study results in her talk later on in the webinar.

32:28 Okay, so results so far that we've published- if you look at maternal plasma concentrations, and as I mentioned Nina will talk about this in more detail in terms of the kinetic modeling. If you look at this slide, if you look at the first column, we should have, it should be the first two on that column are of the pre-pregnancy period, and then the next one is during pregnancy, and then you'll see that again for the 24 (hour). It's pre-pregnancy and pregnancy. So you can see that you see a dose-response relationship in terms of maternal blood levels. You see a lot more scatter in the higher dose than you do in a lower dose and there weren't any really major changes across the time. So, basically, what you're looking at is the dots of each blood level from a female at that time of exposure.

33:26 So this is sort of a scatterplot of all the data. If you want to see the summary, it's in the next table where you can see that at the 0.075 animals were about one nanogram per mL in terms of the 5-hour sample, and about a little over three nanograms per mL for the 0.15. So a doubling of the dose more than doubles the blood level in the animals, and again, Nina will be talking more about the kinetic.

34:05 Alright next- maternal weight. This is at the the weights of the animals during all the different time periods- baseline, pre-pregnancy, and then during pregnancy, and we didn't see any differences across the three dose groups in maternal weight. So there wasn't any effect of the DA at these doses on maternal weight or maternal weight gain during pregnancy.

34:33 Okay, as I mentioned we did some observations of the adult females throughout the study looking for any kind of overt signs of toxicity, like seizures or vomiting. We did some work looking at visual following and reaching and pick up to look at motor coordination and there weren't any differences in terms of the pickup and the visual orientation. We really didn't see any shaking or seizures or vomiting at these levels. So we didn't see any very easy overt signs of toxicity. In other mention, we weren't really expecting to see that but what we did see when we were doing this reaching task, which requires the animals to reach out and extend their arm to pick up a small apple bit, we did see some some slight tremors in the adult females, and I think the next one will show you these tremors ***[Editor's note: video excluded from the recording at the speaker's request.]***

35:40 Okay. Okay. I have I hope for all back so that may have been difficult to see if you look carefully, carefully you could see that at the end of the extension, when they were just picking up the apple bit, you could see that there was a sort of a very high frequency, fast tremor in the animal's hand. So what we looked at is that

response over time and these graphs that you're seeing show you that as the dose gets higher, the one on the right is the highest dose, we saw a lot more of the animals showing these signs.

36:24 We had actually one control animal that was trembling pretty much throughout the study, and we actually found later on upon necropsy that the animal had a lesion, brain lesion. So if you look at the tremors we looked at to see what kind of differences there were and if you look at test comparing the three groups, there's a significant tremor, increase in tremors for the highest dose group, the 0.15 group. Although we do see it in some of the animals at 0.075. So this was a surprise and then we're going to have a little discussion about what we did in terms of some supplemental research looking at the basis for these tremors.

37:12 Okay, in terms of maternal reproduction, we looked at how many- the number of breedings to conception, the conception rate, C-section rate, and live birth delivery rate, and none of those were changed by DA exposure. Each of them had about two breedings to conception but the variability across the animals and all the groups is quite high. The conception rate was quite high for all the groups, 90-100%. The live birth delivery rate was high for all the groups, as well. We only had we had one animal that was born in a breech position that didn't survive out of out of all the animals in this study. We did have a higher C-section rate for the animals.

37:59 This was based on decisions that were made by our veterinarians at the at the Primate Center in terms of any kinds of signs that they feel that are indicating that there's some problem with delivery. If you look at the birth characteristics of the infants, we did see the normal differences in males and females, with the males being a little larger than the females, but we didn't see at these levels of exposure any effects on newborn size in terms of birth weight, crown-rump length, and head size.

38:40 We looked at their newborn behavior, looking at muscle tone, physical activities, skin color, using an Apgar test. One of the benefits of doing this research with monkeys is actually you can adapt different studies, different types of procedures that are done on human infants and you can do them with the infant monkeys. So this is a common test that's done on newborn infants and they're rated in terms of 1 to 2 on each of those things. So a perfect score would be 12. We did see some differences between the C-section and the vaginal delivered animals on this study, which is actually seen in in human infants, as well, due to the anesthesia that's provided during C-section deliveries, but that doesn't last very long. By the second test, everybody looks pretty much the same.

39:40 But, we didn't see any significant differences between the domoic acid groups and those controls, so with these basic reflexes and their status at birth, everyone looks pretty good.

39:54 During the neonatal period during the first three or four weeks, there's also a test that they do with human newborns called the Brazelton Neonatal Assessment procedure. And we've adapted that for doing that kind of work looking at reflexes and behavioral states, muscle tone, how they respond to different stimuli for our monkeys, and we did that every other day for 21 days and we didn't see any differences between the domoic acid-exposed animals and the controls in any one of these four activities that we look at. So they look pretty good during the neonatal period. So one of the first cognitive assessments that we look at, looking at visual recognition memory. And again, this is a procedure that's used with human infants and it's been used in quite a few studies looking at different types of exposures in human studies, and has been actually sensitive to various exposures that people have looked at in the past.

40:55 We've also used it in some of our previous studies and it's a fairly sensitive indicator of how the memory is functioning in these early, early months. So how this works is that you show the stimuli to the infant one on the left one on the right and they're the same, and then you can take that away and then show them the same picture again and then a novel picture; and if everything is working well, the newborns the infants will look at the novel stimuli.

41:31 You want to, we actually show it again changing the orientation or changing the direction of where the novel stimuli are just to make sure there's no side bias. So you can do this with either objects, or you can do it, if you look on right side, you can do it with pictures of, for our tests, it's monkey faces; for humans, they use human faces. The human and monkey faces are much more difficult to scan and to remember than the objects. So it's a, it's a more difficult task. If we look at the results from this study for infant visual recognition memory, if you look on the left side, that's the visual recognition memory scores with using objects and they're right about where they should be. They are sixty percent or over, which means that they all prefer the novel object, which means they basically remembered the object from the first time they saw it and could distinguish the novel one from the familiar one.

42:45 If you look at the right side of the graph, or the table, this is using the more difficult stimuli, the social stimuli, and the highest exposed infants were the only group that couldn't, did not, show a novelty preference using the more difficult social stimuli, which indicated that they basically couldn't distinguish the novel one from the familiar one. There wasn't a novelty preference. This could, this could be due to a lot of different things. They may not have remembered it or they may not have actually processed all the details of the stimuli during the first period that they saw it to know whether it was novel or familiar. So we saw some changes in early memory processing in the highest exposed infants. I'm, we're getting some feedback that the first video didn't work that well, but we're going to go ahead and show a second one ***[Editor's note: video excluded from the recording at the speaker's request.]***

43:45 Okay, so we're I think we're back. We did get some feedback that the first video may not have showed the tremors very well, but we did see some tremors,

similar tremors that we saw in the adults, in a few of the infants that were from the highest exposure group, as well. If you remember that that video of the the infants at this time, were doing a reaching task and they are not they are uncoordinated. So you'll see a lot of uncoordinated activity, but those high frequency tremors that you see are not something you usually see. That's okay, I think we're running out of time. So, so, so we did see, so we so we did see some of those tremors and we only saw them for the first month or so.

44:36 So basically they kind of grew out of them as they got older. We didn't continue to see these tremors once they got over a couple of months of age. But, we did see them early on when looking at our, when doing our reaching tasks for the infants. Okay. So, let's see where we- are we back?

44:58 Okay, so I mentioned given that we saw these tremors in the adults, we talked with our our funding agency, which was the National Institute of Environmental Health Sciences, and we thank them for their support. We did talk with them about these tremors. We initially had not included any kind of post evaluation of those animals, but we did get some supplemental funds and Rebekah Petroff, my doctoral student, took the lead on writing the grant for that and we did get the money, the funds for it. So now she is working on this area of research for her dissertation. So she's going to take over and talk about her findings that she's had so far. [Petroff] Thank you. [Burbacher] Rebekah. [Petroff] So as Tom mentioned, we wrote a supplemental to mostly look at kind of the *in vivo*, and also post-mortem, effects of domoic acid in the adult.

45:58 So that's where all my research is focused on. So the first study that we have been able to publish is on magnetic resonance imaging- MR imaging- and we used a type of MR imaging called diffusion tensor imaging, or DTI, and just really briefly what DTI actually looks at is how water is moving in tissue. So it measures the directionality and the speed of how that water is able to kind of flow in tissue, and the concept behind this is that in the brain, we have bundles of axons in these really white matter major tracts and what happens is that we can pulse a magnet in different directions to look at how this water is moving, both along the gradient and against the gradient, and when we pulse it against the gradient we'll have a pretty slow diffusion rate of that water. Whereas, we pulse it along the gradient so the water will want to flow with the axon. So we can use this to then measure how the tissue is structured in the brain. So this is all just a structural measure of *in vivo* brain white matter.

47:17 So for this part of the study, we chose a subset of the adult female. We had a total of N, a total N of 12, with six control animals, two in our low-dose group, and four in our high-dose group, and all of the animals underwent a single, sedated, magnetic resonance scan with diffusion tensor imaging and a T2-weighted scan. And a T2-weighted scan just shows us a really brief image that we can look to see if there are any severe brain lesions. In the sea lion literature, we know that repeat

exposures, especially at those higher doses that sea lions get, can lead to really severe brain lesions.

48:05 So with that we looked at this DTI measure, which again measures the flow of water in the tissue, as well as if there were any brain lesions in our animals who were trembling. For the DTI, we use a cluster-based permutation and correlated this permutation with both dose and tremors. And one of the measures that we can use from this DTI is called fractional anisotropy, which measures the directionality of water, which was found to be significantly correlated with our tremor score. And just reiterating, that what we found was a decrease in this fractional anisotropy which indicates that there is less restriction of water, which suggests that the major white matter tracts are somehow decreased, and this could be for a number of biological reasons - some of which could mean an infiltration of brain immune cells, like astrocytes or other glial cells, but it can also mean that the white matter tracts and the axons of those neurons are damaged, too.

49:20 So on the bottom here, you can see the image from our animals as to where we found significant differences in the brain and I want to point out this major white matter tract kind of in the first two panels. All those little red and yellow dots are significantly different voxels, we call them, which are just individual little panels, each with their own unique numerical number that we can use to estimate this measure of fractional anisotropy.

49:50 So this first major white matter tract that we see affected is called the internal capsule, which is a white matter tract that connects both the thalamus and the pons, and it holds a lot of your fibers that are really important for motor coordination, which makes sense when we start to think about our tremors and how we saw those. And then one of the other major white matter tracts that you kind of see in the fifth and sixth image down towards the bottom of the brain is called the fornix, and that particular white matter tract stems from the hippocampus, which is the major area of the brain that's affected by domoic acid when you have those really high doses of domoic acid. We also saw some smaller effects. So you can see kind of in the last two panels in the corpus callosum and the pons, and those are really important structures for motor coordination, but also connecting the brain across the two hemispheres. So this is a lot of data to look at, so if we just kind of look at the really big structures that we saw differences in we can pull out those individual voxels and look at how the tremor scores actually relate to this DTI measure of fractional anisotropy.

51:14 So for each of these graphs, you can see the tremor scores on the bottom, and then the fractional anisotropy is going to be over on your left axis. And you can see how the tremor scores are related to fractional anisotropy, both in the internal capsule, which is shown on the top two panels, and the fornix which is shown on the bottom two panels.

- 51:42 So that's just a really brief overview of what I was able to do with the MR imaging so far, and I'm going to then just talk a little bit about our working papers and what we're continuing to look at right now.
- 52:02 So the first part which is what I'll be really spending my dissertation focusing on our continuing the research with the the moms who are trembling. So we have additional data that we're looking at with our MRI imaging where we can actually measure the tracts and look at other measures outside of that DTI imaging. We can look at this "tractography", it's called, where you actually look at how the tracts are connecting between different structures in the brain, and this is really important for understanding potential not just areas that are affected structurally, but also kind of how the fibers are in a functional way.
- 52:48 I'll also be looking at some EEG data. So we did *in vivo* EEGs on the moms, as well, or we can look at some measures to look at how not just the brain structure is changing in relation to domoic acid and the domoic acid tremors, but also the brain function. So, and then the final thing that we'll be looking at, and we have these data kind of churning right now, is differences in gene expression given the domoic acid status of these animals.
- 53:25 We're also working on some early infant manuscripts, including some data on early development up through about one and a half years. So this includes measures on growth as well as socio-emotional measures and more data on cognition. We have EEG use from neonatal ages, including as early as two days where we can actually go back and look at how the infants were sleeping and look at measures of REM and different quality markers there. And, then we have EEGs on one and two year olds where we can look similarly to the adults about functional power in the animals that were exposed and not exposed.
- 54:17 I'm going to pass it back to Tom to just talk about some of our continued plans here and we'll wrap it up quickly. [Burbacher] Thanks. Thanks, Rebekah. That was great. So we wanted to just end with letting folks out there know that we do have actually some tissue from these animals that we would be very interested in collaborating with other researchers. We unfortunately weren't successful in renewing this grant so we had to, we had proposed to do some additional functional assessments of the of the offspring now that they're getting a bit older, but that that was not funded. So we're going to actually have frozen and fixed tissue from both- we do have right now the frozen and fixed tissue from the mothers- and one of the things that we'd be interested in is looking at the DA distribution across the tissue from these animals that were chronically exposed.
- 55:14 Our other outcomes and measures of DA toxicity on the adults and then we will we will be beginning fairly soon necropsies of the offspring and we'll also have frozen and and fixed tissue for those to look at different parameters of toxicity in the offspring. So we just want to let folks know that we will be having a repository

of tissue that we will be sharing with people that are interested. And, I think that's it for us. Yep.

55:55 So acknowledgements - this took a long time, takes a lot of people, and a lot of effort. There's a lot of students that were involved in this over the several years that this grant has been going on, and I'd also, again, like to thank the funding agencies that we had, both at the NIEHS and some local funding that we got. So with that, we'll take any quick questions, if there are any. [Murphy] Thank you very much, Dr. Burbacher and Ms. Petroff. We do have a couple questions, one - the first question is, were the cage side observers blinded to the treatment groups? And then the second part of that question is, is there a way that the scorers were blind to previous scoring results, perhaps you had multiple scorers?

56:55 [Burbacher] Yes to both of those. The scorers were blind which which actually increases the difficulty of doing studies like this because you have to have separate staff doing the adult, working with the adults who, and the infants because all the infant testing was done blind, as well. So yes, we did have blind testers who were blind to the treatment. We did have multiple testers, and but I mean there really isn't any way unless, I just don't, I wouldn't know any way that you could actually separate multiple testing over time for all that we did have different testers. The results were the same no matter what the tester was but they all actually saw the animals over time. [Murphy] Great. Because we do have quite a variety of attendees today with varying scientific backgrounds and some from the lay community, can you just briefly explain what you mean by blind?

58:00 [Burbacher] Oh, sorry. That means they don't know what what exposure the animals had. [Murphy] Thank you. [Burbacher] The people who do the exposing are completely separate from the people who do the testing. [Murphy] Very good. A couple more questions for you. Were any of the fetal fluids tested for domoic acid concentration? [Burbacher] Yes, we did have some C-sections so we collected some amniotic fluid. I'll let Nina talk about that and we have some cord blood as well and we have some newborn blood. [Murphy] Okay. [Burbacher] I think that's going to be more in Nina's just discussion. [Murphy] Sure. Another question- did the neurological effects resolve when the DA challenge is discontinued?

58:51 [Burbacher] We did have a couple of adult females that we took off of the DA prior to necropsy and those animals continued to show the, the tremors.

59:06 [Murphy] So just to clarify for others in the audience, that means that the tremors persisted even when they were no longer being actively exposed to domoic acid. [Burbacher] That's correct. [Murphy] Okay. One last question for you - does captivity have an effect on brain development and if it does how do you control for this in a laboratory setting? [Burbacher] Well, the all the animals are nursery-reared. So we're not comparing nursery-reared animals to wild animals in the wild. There, there are some studies that would indicate that a nursery-reared animal is not the

same as an animal in the wild, but that's not the comparison. The comparison is for all across all the infants that are nursery-reared in this study.

1:00:02 [Murphy] Thank you. Very good. Thank you so much. We do have a couple general questions that I'll go ahead and address. One question came in from the audience asking about the 2017 Workshop. That was an in-person conference, all day conference at the University of California, Davis. And, unfortunately, that was not recorded, so there is not a file with those presentations to share. However, the agenda is on our OEHA website if you look at oehha.ca.gov/fish and then click on "Domoic Acid" page, there is an agenda there. So if you're interested in seeing who those presenters were, I encourage you to visit our website. Another question came through asking about whether or not domoic acid is destroyed by cooking. So for just a general announcement as we analyze the domoic acid levels in seafood, mainly done by the laboratories at the California Department Public Health, the domoic acid levels are measured in cooked crab.

1:01:02 So the crabs and and other species are prepared and cooked and then the animal, the levels are analyzed. So, those are the final levels in a cooked crab. It is also the recommendation of the California health agencies that when these seafood items, like crab and lobster, are cooked and water that the cooking water that was used to boil and cook the crabs is thrown out and not retained for making sauces or rues or stews because, as the seafood is cooked, domoic acid can leach out or come out of the critters and into the cooking fluid. So it's a way to possibly reduce the level of domoic acid exposure if you discard the water, and so that's the general recommendation there.

1:02:03 Oh, one note - the bivalve shellfish are not cooked prior to domoic acid testing, but the decapods, the lobster and and crabs, are. Okay. Very good. Well, as we move on, our next speaker is Dr. Nina Isoherranen, Professor and Associate Chair of Pharmaceutics and Research Affiliate at the Center for Human Development and Disability at the University of Washington in Seattle. Her research focuses on pharmacokinetics and dose-exposure relationships, including drug disposition, pharmacokinetic modeling, and drug-drug interactions. Dr. Isoherranen is presenting on the Toxicokinetics and Physiologically-based Modeling of Domoic Acid Exposures.

1:02:53 Welcome. [Isoherranen] Thank you. And thank you for inviting me to be part of the webinar. I want to start with just acknowledging the team, like Tom said earlier on, these things take a really big team, and especially with the various blinding strategies that we had on this study, we had a lot of people involved. So I want to acknowledge two, actually three graduate students, two graduate students and one research scientist from my laboratory at the Department of Pharmaceutics, Sarah Shum, Jing Jing, and Ariel Topletz, who really were instrumental for developing the LCMS assays, doing all the validation, figuring out their dosing, dosing solutions for this study that we basically QC'd every solution for every monkey throughout the entire study. And, then, really doing the modeling and the

analytical work and then the group that Tom already mentioned, who really were part of the non-human primate portion of this study at the behavioral assessments and then obviously NIH for the funding that has supported the work.

1:03:50 So what I'm going to talk about first is to cover really the fundamentals of the toxicokinetics of domoic acid and what we found out in this study using single dose administration, both intravenously and orally, and then go from there and how we use that data to develop a physiologically-based model for domoic acid, first in the non-human primate, and then in the extrapolated model to really predict what happens in humans and what the plasma concentrations, and even brain concentrations, might be a few moments after oral consumption of domoic acid. And then, finally as the third part of this talk I want to talk about what we found out in terms of the toxicokinetics during pregnancy and maternal-fetal transfer of domoic acid in the non-human primate model.

1:04:56 So just want to start with from a pharmaceutical scientist's or pharmacokineticist's perspective on how we look at domoic acid and its physicochemical properties. So it is a very polar compound and part of why it's very difficult to work with, as an analytical chemist, is because it has a lot of ionizable groups and really has very poor permeability. So when we look at it as a compound you would never expect it really to be absorbed from the gastrointestinal tract, as such, because it really isn't permeability enough to get across the intestinal cells. What we found out also in our studies is that it does not bind to any plasma proteins. So all of the domoic acid that we find, either in human or animal plasma, is free or unbound and it does not partition into the red blood cells.

1:05:53 So when we look at blood or plasma concentrations essentially, all of the domoic acid is in the plasma water and, based on that, we really it is sort of surprising that after eating or consuming domoic acid orally it gets absorbed or we can find it present in the plasma. So we started by looking at some of the past pharmacokinetic studies that had been done in non-human primates and that the data that existed from the 1990s was following an intravenous dose of domoic acid and what this figure shows is the serum pharmacokinetic or toxicokinetics of domoic acid after a 0.05 mg per kg dose of domoic acid intravenously.

1:06:49 And what was found in this study was the domoic acid had a very short half-life of one to three hours, approximately, and in the non-human primate, unlike what had been seeing previously in rodents, about 60 or up to 90% of majority of these animals about 60% of the domoic acid was excreted into urine. What I'm noting here is that in the rodents you pretty much find hundred percent of the dose, which is intravenously administered, in the urine, but in the non-human primate, it actually seems that there are other elimination pathways already based on this data. So one of our questions, initial questions, was whether this toxicokinetics that was established in the 1990s, with the fairly high dose of domoic acid that caused acute toxicity would translate to the kind of doses that we looked at in the non-

human primate model. So what we did is we repeated the intravenous study in three, sort of what we call “pilot” animals.

1:07:55 What you see on the left hand side of the slide is the intravenous dose in pharmacokinetics. You may note that this is a 10 times lower dose of domoic acid than what was done in the previous study. So in these animals, we did not observe any acute toxicity. This is actually an intravenous dosing study of that was done in two separate occasions in the same animals.

1:08:21 So this is essentially a mean of three animals done twice in each animal, and what you see is that the half-life after the intravenous dosing is very similar to what was observed previously, 1.2 hours. What we saw when we looked at urinary excretion of domoic acid in these animals was very similar to the previous study, about 40% of the dose was excreted into urine, so that's the A_e in the table, amount excreted in the urine, which is 42%. What was very surprising when the intravenous data really reproduced the work done earlier with much higher doses- what is really surprising to us was what we then found out in the oral dosing study. And, what you see on the right hand side is the oral dosing kinetics after the two different dosage levels that were ultimately used on the larger study that Tom just presented, 0.15 mg per kg and 0.075 mg per kg, orally, given in a sugar water, essentially, to the animals. And what you see here is that when we give domoic acid orally, the half-life that we observe, what we call an “apparent” half-life, is actually much, much longer than what we observed after the intravenous administration. So you see that we see approximately 10-hour half-life in these animals and we call, call this “flip-flop” kinetics.

1:09:56 And partially why I emphasized the low permeability of domoic acid earlier as the this is not completely unexpected for a compound that is as polar and as ionized as domoic acid. What happens is that the absorption from the GI tract is actually so slow that it's slower than the elimination would be and, hence, this half-life becomes rate-limited by the absorption rate. And that is kind of surprising because it really changes our expectation of how the actual plasma concentration time profile is going to look like after oral exposure, in comparison to what we would have predicted from the intravenous data alone.

1:10:41 Another piece of information that we saw from here is that, of the oral dose, 4% ended up as excreted unchanged in the urine and, from the data, because these are the same animals dosed both intravenously and orally, we could calculate the oral bioavailability of domoic acid, as well. And in these animals, it was 6-7%, which is shown there in the table. So from these pilot animals, we actually gained a fair bit of information of the dose concentration relationship of domoic acid, meaning that we have a good handle on the bioavailability of domoic acid. We gain the information about the half-life and we see that there is a dose-exposure relationship, meaning that when we give a higher dose, we'll also see higher plasma concentrations of domoic acid.

1:11:36 So just to summarize the key findings from the single dose PK studies, one of the things that we did observe is that after the oral doses the toxicokinetics are very variable, the plasma concentrations both within an individual animal and between animals had a very high variability, likely due to the very highly variable oral bioavailability. The other very important finding was the flip-flop kinetics, meaning that, after oral absorption, the elimination is absorption rate-limited and what that means is that we cannot predict the time to peak concentrations or the time course of domoic acid concentrations from the intravenous data. And the presence of domoic acid in blood is actually much more prolonged than what we would expect from IV data. But also, that the peak concentrations of the oral doses are going to be lower than what we would expect, based on the intravenous administration.

1:12:37 One of the interesting aspects that we found out mechanistically from this study was that the renal clearance or the renal elimination of domoic acid was only 30% of what we would expect from creatinine clearance. So this is in terms of the renal clearance being we can compare it to the glomerular filtration rate of the unbound domoic acid, and we see that only 30% of what is filtered ultimately ends up in the urine. And what that tells us is that there is likely active transport that happens in the kidney that is responsible for reabsorption of domoic acid. And if that translates to human it could actually have a major impact on the overall elimination of domoic acid in humans.

1:13:26 We also found out that the only about 30 to 50 percent of the dose was ultimately excreted in urine after the intravenous administration, and that really suggests that in the non-human primate, there are other elimination pathways for domoic acid. We did look at actually feces in these monkeys and we did find domoic acid in the feces after intravenous administration, suggesting that there is biliary secretion in the liver of domoic acid. We don't currently know if this if the monkey here is the best model for human domoic acid elimination, but it's something that probably should be looked at if biliary secretion is relevant in the human situation because that would also implicate that various conditions in the liver would effect plasma concentrations and elimination of domoic acid in humans.

1:14:23 So with that I want to move on to the next part which is really the physiologically-based modeling of domoic acid disposition. Would you see here in the grey boxes is the standard physiologically-based pharmacokinetic model. The arrows in the model really refer to blood flows going from arterial blood to venous blood and we can essentially adapt this to either the non-human primate or to the human, based on the physiological sizes and blood flows of these organs. It can also be applied to various different physiological conditions by changing, for example, the placental-fetal unit that you see in the bottom, changing the expression level of enzymes in the liver, changing the kidney function formula filtration, and so forth. So it's a very powerful method to predict changes in toxicokinetics in various physiological conditions.

1:15:22 In terms of the domoic acid model what you see on the right hand side of the slide is the sort of overall list of things that we used for the nonhuman primate model. So we populate the model with the basic physical chemical parameters, which help us simulate the absorption from the gut we can actually put in the permeability values and predict how the absorption is going to happen. We include the blood to plasma ratio and then the partition ratios, which is referred to as KP values, into the individual tissues based on the properties of the drug to essentially predict how well domoic acid gets into the brain or all the other various organs. In terms of the liver and the kidney, in the liver we used in the model the biliary secretion that we characterized in after the intravenous dosing and that's really we populate that with the biochemical parameters in the liver.

1:16:22 And then similarly for the kidney, we used the renal clearance values that we have gotten from the intravenous studies to simulate in this model the kidney clearance. And then based on that we can also scale this using standard interspecies scaling from once we know what's happening in the monkey to human and predict what the disposition going to be look like in the human. So just to show you how the model turned out, for this modeling we used the commercial software called Simcyp that has a ready-built non-human primate model already there, so the physiology was set and we really built a drug model in that platform but one could adapt this model to any other PBPK platform that one would want to. And what's shown in these figures here in the bottom is the individual data points are on the left from our intravenous data, where we dosed domoic acid intravenously in each one of the animals, and on the right-hand panel is the study that was done by Truelove and Iverson at 1994.

1:17:22 So we did two independent intravenous domoic acid dosing studies with tenfold different doses and then simulated those so the continuous line is our simulated mean and the dotted lines are the 90% confidence interval for the plasma concentrations of domoic acid. And what you can observe from this graphs is that the physiologically-based model really captured the intravenous dosing of domoic acid quite well, it is possible that, in the 1994 study, the half-life of domoic acid is somewhat longer, but it's very difficult to compare this because one has to remember that our study was done with LCMS system and a different sample preparation than what was done in the original study.

1:18:22 So some of this could just be assay variability from study to study, as well. But what you can say is that overall we are capturing the systemic toxicokinetics of domoic acid fairly well with this model. We then proceeded to test the model with our oral data and this one shows you the two studies that we did after oral dosing of domoic acid. So again, each one of the individual symbols and the bottom panels shows individual data points of the three animals that we did the two different dose levels. So left-hand side is the 0.15 mg **[Editor's note: corrected to milligram (from microgram) based on follow up email with the presenter]** per kg oral dosing and the right-hand side is the-sorry- the left-hand side is the 0.075 and the right-hand side is the 0.15 dose level of domoic acid.

1:19:05 And what you see here is that surprisingly also the mechanistic physiologically-based model predicts the flip-flop kinetics and the longer half-life after oral dosing, just the same as what we see in the observed data. So as we look at the same 95% confidence interval and the mean simulated data, we can conclude that the model fairly well captures the oral absorption and the toxicokinetics of domoic acid and what we typically would call from here is that our physiologically-based model is now verified based on this. In addition, we took one of the data points that was one of the few data sets in the literature that have shown domoic acid concentration following multiple doses orally to animals. So what you see here, this is again from Truelove from 1997. We captured the plasma concentrations from the published data.

1:20:13 The individual circles on this show the observations of domoic acid concentrations in plasma, and then the continuous line that you see going through up to 16 days shows the simulated multiple dose kinetics of domoic acid in the monkeys that were studied in the 1997. And what you really see here is that, regardless of whether we look at 20 years ago, what today's data the physiologically-based model fairly well captures the oral the plasma concentration time course of domoic acid after the oral dosing regardless of single dose or multiple dose, so we were fairly happy with the model at this point. The next question was done because this is a physiologically-based model. Can we predict the brain concentrations or the acute toxicity using this model?

1:21:13 And what we did is we looked at essentially the reported onset of symptoms after intravenous and oral doses of domoic acid in monkeys and then try to look at that duration of effect and the onset of symptoms whether that matched what the physiologically-based model would predict in the brain. So what you see here is that, after intravenous dosing, the toxicity symptoms start fairly rapidly within couple of minutes from the dose. The duration of the symptoms is longer, the longer the intravenous dose, but still even with the 0.5 mg per kg dose, the duration is only about two hours after intravenous dosing that the animals will vomit and have the various CNS toxicities. In comparison, when you look at monkeys after oral dose of domoic acid, the onset of the symptoms is much later, as we would predict from the slow absorption.

1:22:21 So from 60 minutes to 720 minutes, and then the duration can actually be longer, a fair bit longer, up to 400 minutes following the oral dosing which would also be consistent with what we would expect from the flip-flop kinetics and the much longer half-life after oral exposure to domoic acid.

1:22:37 So what we went and did is we simulated the brain concentrations using the physiologically-based model. And what you see in this graph, the red line on the graph is the simulated brain concentrations in the following intravenous dosing that was used in the previous studies. The gold and purple lines that you see at the

bottom are the predicted brain concentrations in our pharmacokinetic study after oral dosing, meaning that these were subacute doses.

1:23:14 We predicted that the animals should not have any acute toxicities and indeed when we look at the predicted brain concentrations, we would not expect to see any toxicities. And then the black line that is simulated is essentially the simulated brain concentrations of domoic acid following the oral dose that was observed to cause toxicities. And what you see, the two dashed lines on the figure really are what we are looking at threshold of acute toxicity concentrations. What would be the brain concentration that would need to be reached to observe acute toxicity and then how long would the concentrations that we're simulating in the brain stay above that line. And indeed, what do you see when you look at the time about the dotted lines for the oral dosing we would truly expect a much longer duration above the toxic threshold in the brain than after the intravenous dosing. So this was quite encouraging and then based on this data, we then went on and actually simulated what should happen in humans. Now, I do want to say that we do not have any data to really verify that any of this modeling truly applies to humans.

1:24:38 We do not really know for sure, or certain that humans demonstrate the same flip-flop kinetics as we see after in the monkeys, but if we assume that the monkey predicts what happens in the humans, this is what we would simulate based on the physiological principles for human plasma concentration time course, after the 0.075 mg per kg dose. So the top panels are the plasma domoic acid concentration and the bottom panel is the predicted brain concentration in the humans. And in the left-hand side panels, A and C show the 0.075 mg per kg dose, and then on the right-hand side what you see is actually doses that were observed in the first domoic acid intoxication case that was discussed in the very beginning of the, of the day.

1:25:31 So these are what we are predicting should be the plasma and brain domoic acid concentrations in the individual that suffered from the domoic acid intoxication in the Prince Edward Island case that happened. I'm really showing this just to illustrate the feasibility of using physiologically-based modeling and to give an idea of what we expect the concentrations in the brain or plasma would be when you take a acute toxicity doses of domoic acid versus what is currently considered relatively safe for domoic acid. So we can basically predict or what we would expect to happen in humans that really remains to be shown by some experimental data that this applies. So now to come back to then the real pregnancy study at the maternal-fetal transfer of domoic acid, and I hope I will answer some of the questions that were asked earlier on about domoic acid in pregnancy and maternal-fetal transfer.

1:26:36 So I just want to show this one figure from the literature that shows essentially when domoic acid is given to pregnant rats intravenously what is observed in the rat fetuses, and what do you really see here. This is just to show that in the rat, domoic acid gets across the placenta, it gets into the fetus, and it gets into

the fetal brain. And we also see a fairly long-term exposure in the fetal or long-term concentrations in the fetal brain after the IV dose. Now, the question is can these findings be extrapolated to lower doses, or lower doses all along maternal exposure?

1:27:23 So this is just to summarize again the overall, overall study design for our pregnancy study that Tom already showed, really the overall study design is running there on the golden line. The red ones are the ones that I'd like you to focus on for the part of my talk, which means that we basically did a first day in before these animals got pregnant a toxicokinetic sampling, which was a full 24-hour pharmacokinetic study after oral dosing, and collected urine as well on that day. Then 56 days later, we did another full toxicokinetic study for 24 hours, which is our pre-pregnancy baseline steady-state domoic acid toxicokinetics. The animals were bred after that and then as they got pregnant, we did the second trimester, third trimester, and postpartum, six weeks postpartum, another full 24-hour toxicokinetic study.

1:28:30 And, in addition at delivery thanks to Tom's wonderful team, we got maternal and infant sampling at delivery. So I'm going to show you some of those concentrations and then we also analyzed amniotic fluid and so forth that was mentioned already before. So here is the overall toxicokinetics observed in the pregnant and pre-pregnancy animals. What you see here clearly is that the plasma concentrations are higher with the 0.15 mg per kg dose than the 0.075. You see that as we go along from the pre-pregnancy to second trimester and third trimester, there is some level of decrease in the AUC of domoic acid, and what you see when these animals are chronically exposed, that essentially we see exactly what we would have expected from the flip-flop kinetics that there is almost no fluctuation on the concentrations of domoic acid over the 24-hour dosing interval.

1:29:30 What it says that essentially the concentrations increase slowly after the dosing and then stay high across the dosing interval. And this is a very busy table, but essentially summarizes all of this pharmacokinetic data that we collected. It shows you that by the third trimester, the AUC actually significantly decreased after the 0.075 mg per kg per day dose, dosing group, in comparison to the pre-pregnancy, the top part of this table is the low dose and then the bottom part of the table is the higher dose of domoic acid. So we see that the oral clearance is increased at the third trimester compared to postpartum, and this is most likely due to the increase in the renal clearance of domoic acid.

1:30:30 We would expect that, based on what we know, both in humans and monkeys, that the kidney functioning filtration goes up significantly during pregnancy, which we see in the creatinine clearance values in this table. So we see at least a doubling of the creatinine clearance and that proportionately translates to increase in the renal clearance of domoic acid. We also see that the fraction of domoic acid excreted unchanged in the urine is very comparable between the two with the pilot data that we did. One of the things that I do want to note is that there is

some differences between the lower and higher dose in this table. So if you look at the very right most column, which is the fraction experiment change in terms of the percentage, you see that it looks like it's about two-fold higher, the fraction of domoic acid excreted unchanged in the urine in the high dose group compared to the low dose group.

1:31:30 Similarly, when we look at the oral clearance of the dose-normalized AUC, we see that that is actually significantly decreased in the high dose group compared to the low dose group. So that is the third column from the left, that is the clearance over F. If you look at the third trimester number for the low dose group, it's 420 and in the high dose group it's 190. So we see about 50 percent decrease in the oral clearance going to higher dose. It seems that that is probably our hypothesis is that that is probably because of some impact on the absorption with the higher doses, but we do not have enough mechanistic data to explain what really is the driving force for both.

1:32:27 How about, by and large, the variability of the domoic acid kinetics within and between individuals is larger than what the observed pregnancy effect in the changes in the kinetics is, suggesting that really non-pregnant individuals can be used to predict what happens in terms of pregnancy to the toxicokinetics of domoic acid. Now, last part then is what happens to the maternal-fetal transfer of domoic acid at the fetal concentrations. And what you see here on the left-hand side, is the concentrations in the infant plasma at delivery. And on the right-hand side is the maternal plasma concentration at delivery. The numbers given to each one of the dots is an individual animal in both of the groups, the low dose and the high dose group. And the numbers above give you the average concentration in the group.

1:33:26 So if you compare the 0.075 dosing group on the left panel to the right panel, what you see that the infant plasma concentrations are a fair bit lower than the maternal concentrations, and the same applies to the higher dose group. In addition, what you see as that the higher dose group also the infants had higher plasma concentrations at delivery. Now, what is somewhat surprising is that after the higher dose, the 0.15 mg per kg per day dosing, we saw a significantly higher maternal-to-fetal DA concentration ratio than what we saw after the lower dose.

1:34:14 So the maternal-to-fetal DA concentration ratio is 0.6 after the after the high dose and 0.3 after the low dose. And it is possible that there are some transporter function in the placenta that contributes to that ratio, but this study was not designed to test dose-dependent differences maternal-to-fetal DA concentration ratio. So I would not read too much into that, and based on these these data, we can also predict putting together a physiologically-based modeling of this data that the fetal brain concentration should actually be 40 to 70 percent lower than what we observe into maternal brain, because the placenta does actually limit the exposure or the passage of domoic acid into the fetus. So consistently, the fetal or infant plasma concentrations are lower than what the maternal plasma concentrations are.

1:35:16 So with that I'll conclude so we're just to summarize all what I've said. So number one, I think one of the big important findings that we had was the flip-flop kinetics after oral dosing, that really does affect the shape of the plasma concentration-time curve after oral exposure. Another important finding was that really what was found about the domoic acid elimination pathways in the rodents didn't entirely apply to the monkeys. What we found is that only part of domoic acid is cleared by the renal route, and we really expect that biliary secretion is an additional clearance mechanism. We tried some metabolic studies as well, but could not find any metabolism of domoic acid, either conjugation or oxidative metabolism.

1:36:05 One of the really key findings that I hope I've alluded to is that all our data from the study, whether it's the intravenous dosing, oral dosing, the pregnancy of the maternal-fetal, maternal-infant ratios suggest that active transport really plays a very important role in domoic acid disposition. We have some hypotheses to work the transporters at play, maybe, but I think we really need to invest some time and effort into identifying the critical transporters for domoic acid, so that we can understand which species really will translate to humans, and what the relevant elimination pathways are in humans. One of the things I hope I explained as PBPK modeling can really be used to predict the domoic acid concentrations in humans following different exposure scenarios- both low-dose, high-dose, single-dose, multiple doses, and so forth.

1:37:04 But I think we do need some data from human plasma concentrations after known exposures to verify the model. In terms of the DA absorption, it's very variable and I think that will really contribute to variability in individual responses to domoic acid, and the pregnancy effect is less than that variability between individuals. And then the saturation of the a transport is a possibility that it actually plays a significant role in dose-dependent kinetics of domoic acid, but we don't have enough mechanistic data to, to elaborate on that. And with that, I will conclude and I'll be happy to take any questions.

1:37:51 [Murphy] Thank you, Dr. Isoherranen. We have a couple questions for you. So you've talked quite a bit about the variability that you've observed. Can you elaborate just a little bit more on that in this relationship to the absorption, and kidney clearance and reabsorption, and then what might that imply for sensitive humans? [Isoherranen] So in terms of the kidney clearance, what's quite surprising is when we do the intravenous dosing there's very little variability from animal to animal, and if you look at even the data from 1997 or 1994 of what we did now, the intravenous kinetics is incredibly reproducible. In comparison, the oral absorption is really, really variable. So I think majority of the variability we observe comes from the oral absorption. We don't know if that has to do with ionization status whether that has to do with you know, we actually did some pilot studies with fed versus fasted, for example.

1:38:53 We never really observed a food effect, for example, in the monkeys. Doesn't mean that that wouldn't happen in humans. So I think that that part of

variability is going to be enormous. How the domoic acid, both the rate and the extent of absorption from the GI tract, that doesn't mean that if an individual has renal impairment or kidney disease, or in my opinion, we should probably even think about liver disease based on the findings of the biliary secretion, that's going to have a big impact on inter-individual variability and the clearance. We did not have that in this study because we did not have monkeys that would have had an organ impairment *per se*.

1:39:43 So I think in a human population- that's going to contribute significantly to inter-individual variability. So if somebody has a decline in kidney function, that is going to contribute to much greater plasma concentrations per dose than in a healthy 25 year-old individual. [Murphy] Thank you. [Isoherranen] Does that answer the question?

1:40:02 [Murphy] Yes, so in tandem with that, we noted that the IV dose levels were significantly lower than those used in for the oral dosing. The question is how do you reconcile the two?

1:40:19 [Isoherranen] So we basically did not want to have any acute toxicity in the animals. And if you imagine that in the oral dose only three to five percent actually ends up being absorbed. We basically wanted to have roughly the same plasma concentrations after the oral dose as after the intravenous dose. Because if there is any kind of saturation ongoing for example, you want to have the same concentrations to compare one to the other, so we had to *a priori* adjust for that expected 95% of the oral dose that does not get absorbed. And that's why we used a much higher dose orally than intravenously to essentially achieve the same kind of plasma concentrations.

1:41:09 [Murphy] Thank you. Next question. Although domoic acid has multiple charges at physiological pH, is it passing through the blood-brain barrier? Does that mean that amino acid transport is used to cross the blood-brain barrier?
[Isoherranen] That's a really good question. You know when you look at the molecule you really wouldn't expect it to get to the brain. It was surprising to us.

1:41:35 But when we do the physiologically-based modeling, we actually do not include any transport processes in that. So when we simulated the brain concentrations in our modeling, we did not include any active transporters, so some of it can actually get across, regardless. I don't think that it's impossible that there is an amino acid transporter. I think in particular, probably expect that to happen in the intestine. So I think there is very, there is a very real possibility that there is, in addition to active transport, and we would really need to do some brain concentration studies to get a good handle on what the true brain concentrations are, and I could even envision that that's not different parts of the brain. There's going to be different concentrations of domoic acid due to different expression of transporters and so forth. So our data cannot refute or verify that there is a transporter.

1:42:52 [Murphy] Okay, very good. Another question is, Are the offspring from the pregnancy study available for MRI, and will they be studied? Or are these monkeys shared with perhaps the Burbacher work? [Isoherranen] These are the same monkeys that were done in the Burbacher work. [Murphy] Very good. Okay. Another question. Can you speak to the number of subjects, both maternal and fetal, that were lost to follow-up and how you accounted for that? [Isoherranen] What do you mean that were lost to follow-up?

1:43:25 [Murphy] Not sure, perhaps that person can send in a clarifying question. [Isoherranen] These are the same monkeys that were followed then in all the studies that Tom just presented. So I don't think we lost any monkeys to follow-up. We did fetal, like the infant's, blood draws were done later on. So we have actually, you know, followed their domoic acid concentrations up to I believe a week after birth. We can detect domoic acid in some animals, but we did not lose anybody to follow-up. [Murphy] Okay. She clarified and she said that, for example, in the event of an unplanned death or miscarry.

1:44:10 Did that occur? [Burbacher] We did have, we did have one, we did have one female deliver a breech baby, which died during the delivery. Okay. So that's the only one that we lost. [Murphy] Thank you. Looks like final question here. Is it possible that DA accumulates at sites within the body, so that not all of the dose is eliminated?

1:44:41 [Isoherranen] Theoretically, it is possible that it accumulates somewhere and that's why we recover only 40 to 50%. I think there is a long-term elimination phase for the DA. We actually did a study that I didn't present, where we followed urine, we collected urine for, or I should say, Tom's group collected urine for weeks after the last dose in an animal, and we could see the domoic acid for a really, really long time and the urine samples. It's incredibly low levels. So I don't think it will ever account for the hundred percent of the dose, but I think there is a portion of the domoic acid that sticks around for a very long time.

1:45:34 [Murphy] Thank you very much. Not seeing any further questions, we are going to take a short break. We're running a little bit ahead of schedule.

1:45:43 All right, everyone. Welcome back. Our final speaker was also a featured presenter and panelist at our previous Workshop. Dr. Lynn Grattan is a Professor of Neurology, Epidemiology, and Public Health. She's a Director of the Neuropsychological Diagnostic and Research Laboratory, and Director of Clinical Neuropsychology at the University of Maryland School of Medicine in Baltimore. Her research examines, the neuropsychological impacts of exposure to marine harmful algal bloom-based toxins, including a key partnership with Northwest-based Native American tribes for the epidemiological study known as the CoASTAL cohort, which she will now describe. Welcome, Dr. Grattan.

- 1:46:35 [Cook] Lynn, I believe you're self-muted. Lynn, you may need to go to your control panel. And unmute yourself. [Grattan] Oh, sorry. [Cook] Thank you. Okay.
- 1:47:04 [Grattan] This is Lynn Grattan, and it's an honor and a pleasure to have the opportunity to share our work with you today. And when I say "our", I truly mean "our". There are number of collaborators involved in this study. They're listed on this slide.
- 1:47:31 The main findings of the study that I'll be discussing at the end have been, have been published, in *Toxins*. And I'd also like to acknowledge the two NIH-funded projects that helped us bring these studies to conclusion. Okay. First of all, I'd like to just give you a brief overview of the background of the study, and what I'll be presenting today.
- 1:48:14 First, I'll be discussing the general background. Secondly, I'll briefly summarize the overall cohort studies through which this initial study is taking place. And, finally, I will get to the meat of the talk, which is the specific impacts of exposure on everyday memory.
- 1:48:37 Okay, we all know from our earliest presentation today that high levels of domoic acid is neurotoxic. It's neurotoxic to humans, to wildlife, sea lions, and, in Dr. Burbacher's Laboratory, it's neurotoxic to monkeys. The greatest exposure risk to humans in the Pacific Northwest is through razor clam consumption.
- 1:49:06 And this is largely because, in the razor clam, the toxin domoic acid accumulates through feeding and then is extremely slow to deplete, deplete. A crab may deplete the toxin out of its viscera within a couple of days. A razor clam can hold toxin in its meat for up to a year, regardless of whether or not it's been frozen, canned, or otherwise preserved, and it might even hold it longer than a year. Except, Dr. Jack Wekell, who did these studies, concluded it at the end of one year. So that makes the razor clam perhaps the riskiest potential source of domoic acid exposure in the Pacific Northwest.
- 1:49:56 Currently, as previously discussed and described, the regulatory levels for harvesting shellfish is 20 parts per million, and in the 12 years during which I've been, over which I've been conducting all of these studies, there have been no reported cases of acute poisoning in the cohort I've been studying.
- 1:50:20 Now, while we know there's been no acute impacts of exposure of a single acute exposure or severe illness, impacts of repetitive or chronic low-level exposures remain unknown.
- 1:51:05 Therefore, the overall overarching research question for all of our studies is whether or not is repetitive or chronic exposure to low-level domoic acid by a razor clam consumption associated with memory decline.

1:51:37 I think I may need someone to take over the slides. [Cook] Certainly, Lynn. [Grattan] Thank you next slide, please. So right now I'm going to jump into the summary of our cohort studies. Next slide, please.

1:51:55 Basically, razor clams are a very valuable natural resource to the Pacific Northwest Tribes. It's an important part of Native American history and culture. It's a source of protein in their diets. It's important to the local economy, as they distribute these clams, and they can them and harvest them, and they sell them all over the world. It's also important to tourism- would be between 250 and 400 thousand harvesters a year come to the Washington coast to try their hand at digging razor clams. And, once again, even the non-native American industry is in many ways dependent on their ability to sell the razor clams. Next slide, please.

1:52:49 Okay. Razor clams in the Pacific Northwest are eaten quite regularly, and when you're there, you can have them for breakfast, as you can see on the far left; lunch- you can have a cup of soup for lunch and dinner. Now these slides are taken from meals at a fine dining restaurant associated with one of the tribe casinos. Not everybody has their clams prepared exactly this way, but these are certainly, they can be quite appetizing and delicious. Next slide, please.

1:53:25 Okay. Now let's talk about amnesic shellfish poisoning, which was the syndrome named and associated with the major outbreak in Montreal, back in the late 80s. Now Memory falls on a continuum in humans. It can become totally amnesic, which many of their patients were, which means pretty much everything goes in one ear and out the other- now sort of like a replay of the movie called, "Groundhog Day", you they learn something new and 20 minutes later, they completely forget it. That was the amnesic shellfish poisoning. Now memory, you know people just don't go from no memory to full amnesia. They can have mild impairment, moderate impairment, etc., as we can see often times with people with dementing illnesses.

1:54:20 Next slide please. There are a number of factors that potentially impact memory and, and let's just keep clicking ahead on slides, please. Age can affect memory, education can affect memory, occupation. Someone's work occupational status can affect memory. There's their sex, gender can affect memory.

1:54:51 The marital status, believe it or not, also affects memory. Their medical history, other medical factors can affect memory. Fatigue, being tired can affect memory. Drug use can affect memory. Depression can affect memory. Anxiety can affect memory. Generalized cognitive status can affect memory, and alcohol abuse can affect memory. Continue, please. And drug use, of course. Next slide, please. Oh, and effort, definitely effort.

1:55:28 When we're doing our exposure assessment- and please go back one (slide).

1:55:36 When we do our exposure assessments, basically, we do a dietary assessment. We look at what they've been eating and we developed and validated a shellfish assessment survey. Basically, this is a measure of how much shellfish, how many razor clams people are eating on a daily, weekly, monthly, or annual basis. We validated this measure against a couple of standard measures- the block dietary questionnaire, as well as 24-hour food diaries, to make sure that we were capturing the information we wanted to capture. We also looked at number of clams consumed by average monthly DA levels at particular source beaches. This latter method was a little bit too vague and general for us so we did not use it in our findings. Next slide, please.

1:56:26 All right, if you see the black oval on this graph, and these are, this is basically some data on the Washington State maximums for domoic acid from the years 1991 to 2015, provided by Dr. Vera Trainer from Northwest Fisheries Science Center and NOAA in Washington. And in the black oval area, of these were the DA levels at the time of our study. Interesting what sometimes happens when you have a study in the field- the toxin does not appear. But of course, we were asked to come in and look at this in the tribe after the higher levels as you can see, the elevated levels on the right (on the left). Now, if you look at the right, this is when we begin to have what was called the "Big Blob" that was, that wasn't talked about earlier. After the blob, the far right line and dot, the level started to come back down.

1:57:26 I just want you to keep in mind that as these levels were falling just below 20 parts per million, was when we conducted our everyday memory surveys. Okay, next time, next slide, please.

1:57:37 All right. I'm just going to summarize the findings of the epidemiological studies we did with the tribes. Overall, it was a 12-year study with data analyzed at four and eight years. We saw over a thousand participants or did a thousand exams during this time, was about 500 to 600 actually sticking with us throughout the entire study. So it's their data that we've, that we've analyzed and reported. Now, these individuals were randomly drawn from tribe Registries. They were cleared for a general intellectual status. And also, we made sure that they could see the stimuli properly and they could comprehend it properly. We found and we saw them once a year for the time of the study.

1:58:29 We found that if people ate 15 or more razor clams per month, over 12 consecutive months, after four years, we noted a mild memory decline. After eight years, we continued to have the exact same finding so we kind of replicated the study ourselves. Within that context, I'd just like you to know that 22% of the Native American cohort would be considered high consumers by this standard. So, you know just over 20% would be considered, let's say at greater risk, of having mild memory decline after multiple years. Next slide, please.

1:59:20 Now this is the big question- what is mild memory decline? Well, it's slight, we can measure it, but let's keep in mind that in a neuro-psychology laboratory, you

can measure just about anything, just depending on the sensitivity and specificity of your measures to what you're looking at. So, yes, we measured it and we found that it was a slight decline. However, whether or not it actually impacts daily life- was noticeable to the person, to the individual- where we get to determine like, is it meaningful? Next slide, please.

2:00:06 So right now I'm going to talk a little bit about the impacts of the domoic acid exposure on everyday memory. Next slide, please.

2:00:20 The overall research question for this particular study is, does, do the mild memory changes found in the domoic acid cohort over time actually make a difference in the daily lives of the coastal dwelling Native American people in the Pacific Northwest? Next slide, please.

2:00:42 So now, what is everyday memory? It's another construct that we actually have to begin to measure. Everyday memory is essentially functional memory or the frequency of memory failures in everyday life. For example, how often do you have to check something that you should have done?

2:01:03 How often do you forget to do things that you should have done? How often do you forget something that you were just told yesterday? How often do you forget to tell someone something that is important? You know, we all have some memory failures on a day-to-day basis. Most of the time it's due to inattention or, or stress or lack of sleep. But, in this study,- next, next slide, please- we took a look at this using a standardized everyday memory questionnaire.

2:01:36 Now in this particular study, it's a sub-study of the cohort. It was six, we looked at sixty adult men and women ages 17- ages 18- to 79 after a community harvest. This was the first community harvest after the opening of the beaches of the Blob, which means after, after they've been closed for months due to elevated DA levels. DA levels around that time, were not three to five parts per million. They were more like 8-14 parts per million. Okay, we administered the everyday memory questionnaire. I gave you a few examples of that questionnaire in the previous slide and the reference time-period was since your last razor clam meal. So, basically, they were asked to think back to their last razor clam meal and respond to the memory questions, accordingly.

2:02:36 For exposure, we simultaneously collected data on using the shellfish assessment survey. We looked at exposure in two ways. We looked at exposure for the prior week because it was up to seven days that they could have had their last meal right after the harvest, or the past year. What was their exposure over the past year, thinking perhaps we may find a difference between Target week and past year memory problems. All participants were reimbursed \$25 for coming in for our assessments. Next slide, please.

2:03:17 In addition to having two time periods, Target week and prior year of exposure, also was divided the group into exposure levels prior to our data analysis.

2:03:31 Low exposure were considered consider those folks who consume between none or 14 razor clams for the past 12 consecutive months. The high exposure groups were participants who conceived 15 or more razor clams over the past 12 consecutive months, the 15 cut-off was based upon the data from the prior cohort study that indicated that people who ate 15 or more clams may, have a higher, may have a memory problem a memory problem, we found with mild next slide, please.

2:04:11 Okay, and we had the exposure time-frames as I noted before but I'll review again. Once again, with a number of clams consumed within the week or seven days prior to the assessment, and the past year number clams consumed over the prior year. And here you see a picture of clam fritters. We are- preparation methods were taken into consideration with the amount of clams consumed, adjusted by our diet, by our dietitian on the project after a lot of cooking and making fritters and making chowder in terms of how much clam would actually be in each, actually be in each portion. Next slide, please.

2:05:01 Okay, and this is our first data slide and it's quite busy. I'll have a summary follow-up slide immediately following. In any event, what you can see is we've got an average age of 42, slightly more women than men, and when you come right down to it the high DA exposure group had more memory problems than the low exposure group, whether it was the Target week or the prior year. And let's go on to the next slide where we can just look at this in summarized form. Okay. So we've got the average age of women in the sample was 42 years, or the average age of the sample was 42 years, pardon me. 56% women, 51% reported at least one or more everyday memory problem. Let's keep in mind a higher score on the everyday memory scale means more of a problem.

2:06:00 There was no significant difference in age, sex, or education between the DA groups or between exposure groups at either time epoch, whether it's the Target week the week prior, or the past year. So whether it was a high DA exposure group or the low DA exposure group, they were not in any way different on these basic demographic variables. We did find, I mean just calculating descriptive t-tests, that the high DA exposure group had higher everyday memory problems and were more likely to report any memory problems than the low exposure group for both the Target week in the past year. Of course with that statistic, we did not take into account all of the possible intervening variables. Next slide, please.

2:06:54 And this is where we begin to take into a consideration age, gender, and education on, on these, in this data. And as you can see on this slide, we're beginning to see a, beginning to see a trend in education, but it comes in the inverse direction that you would think. People with higher education have more memory, reported more memory problems, but we're going to be discussing that closer to the

end of this presentation. Next slide, please so we can summarize all of this. Okay. DA exposure, from the prior slide, indicated that during the Target week, it was not associated with everyday memory score.

2:07:43 That means how many clients that ate the prior week, after controlling for age, gender, and education, had nothing to do with the everyday memory score if you do a linear regression and use your outcome and use everyday memory as a continuous variable for the outcome. Everyday memory score was associated with the participants' DA, domoic acid, exposure or razor clam exposure during the prior year. So the week before did not appear to affect their everyday memory school, but the amount they ate over the prior year affected this score.

2:08:16 We also found that higher exposure during the past year had everyday memory scores that were higher than the lower exposure participants. Next slide, please. And here we're going to see another busy slide. Basically, as we can see there is significance at both levels for Target week and past year in, in our models, and this is a logistic regression model, where the outcome is high everyday memory or low everyday memory, based upon scores. Next slide, please.

2:08:56 Okay. Now, in this logistic regression, we looked at the association between both domoic acid and the odds of having any high, having a high score, and we found that high consumers of razor clams, at more than 15 a month, were five times more likely to have more memory problems than the low consumers, whether you were talking about the prior week or the prior year. Okay. So high consumers are five times more likely to report memory problems or be in the high memory problem group than the low consumers. Next slide.

2:09:38 And this is the last logistic regression table. Again, we're finding significance at Target week, and have the impact of education rolling in there again, and this is where we'll get a chance to talk about that. Next slide, please.

2:09:55 Okay, so in summary of the previous slide, looking at the presence or absence of any memory complaint, we found that high consumers over the Target week were almost four times more likely to report any problem at all, than the low consumers during the week prior. There was a trend for people with higher educational levels to be three times more likely to report at least one memory problem than high consumers.

2:10:29 And this finding sounds, maybe counterintuitive or paradoxical, but the explanation for that is that, that reporting a number of memory problems that a person has on a daily basis requires an element of what we call "metamemory". That means being able to extricate yourself from where you're sitting right now, cognitively, and reflect back on what's been going on with your memory for the last week. And available data has consistently suggested that awareness or insight into the memory problems that you've had over the last week is associated with education. So then, it would not be surprising to find that people with higher

education have the tendency to report more memory problems. So it never reached significance when controlled for in the progression procedures, but it was, it certainly is worth noting. Next slide, please.

2:11:46 Okay, so, in general, I'm just going to summarize all of the everyday findings at this point with this slide. And, that is that high consumers, both the Target week and the past year, were five to six times more likely to have an elevated everyday memory score. High Target week consumers, that means high consumers right after the dig and before we tested them, are more likely to have at least one memory complaint compared to high razor clam consumers over the past year,

2:12:17 Is one memory complaint meaningful? Well, statistically it was but they did have one more complaint than the overall year, and then there was the trend for people with higher educational levels to be three times more likely to report at least one memory problem than those with less education. Next slide, please.

2:12:53 As a result of this study, of the findings for this study was shared with the State Health Department and the tribes prior to publication. The Washington Department of Health, put out a Statewide razor clam advisory, basically advising people, in general, to not be eating more than 15 clams a month, and specifically targeting vulnerable groups: pregnant women, children, then elderly, largely because we suspected there may be some special problems with this group during *in utero* exposures and possibly with older people and renal clearance. Though I'm going, I'm going to defer it to Nina to discuss those possibilities, but these groups we did choose to take into consideration and not. Next slide, please.

2:13:52 I'd like to review some of the limitations of this study because, in human studies and human epidemiological studies, there's far less control over exposure and measurement than in the lovely laboratory studies presented before me. Basically, we don't know their historical exposures.

2:14:17 We can ask how many years they've been consuming razor clams, but those memories will not be as reliable as, let's say the prior week, the prior month, or the prior year. So, they may have had a combination of high and low level exposures throughout their lifetime. Secondly, these findings may not generalize to infants, toddlers, or geriatric populations.

2:14:42 Thirdly, the findings may not even generalize to non-native, to non-native populations. And finally, there's always an issue of recovery. Do they eat a lot of clams and then they get back, they get back to baseline? And then they eat a lot more clams, then back to baseline? And once again, some of these questions are best answered in the laboratory settings. Studies, like Kathy Lefebvre suggest, that there is some recovery. However, we need to follow up, as Dr. Isoherranen had, find out what her studies will, are contributing, and will continue to contribute to this knowledge. Next slide, please.

2:15:31 Okay, future directions or places that we'd love to go. We'd like to do extended studies of vulnerable groups studying infants, children, the elderly, elders, and seniors as separate and individual cohorts, and be able to study them robustly. We believe we need to take into account some of the cultural aspects of exposure. So studies of non-native Americans would be a great idea and just to add we just wrapped up a study of non-natives in terms of a generalized risk assessment just to see how many clams they were eating. Do we really need to worry about the general population of Washington State razor clam harvesters being affected by eating a lot of clams. I mean how many actually eat 15 or more month?

2:16:23 We know 22% of the Native Americans do, but do the non-native recreational harvesters eat that many? And, our findings, which we just ran calculations on within the last two weeks with that, about 16% of licensed razor clam harvesters eat clams at we consider a high level, 15 or greater a month. Now we reached the most heavy consumers in the state because they're the actual razor clam harvesters and we got them right off the beach, if they were coming off the beach. We did a random sample of them. So they would be expected to be the highest harvesters in the state. In comparison, Dr. Ferris *et al.*, in *Toxins* found that about three percent were high consumers who were exposed to clams on the Washington beaches. The difference in the studies is that in, in the study we conducted, we focused on the highest consumers, which is the harvesters and took the right off the beach. In the other study, data was collected from people recruited off the beach to mail in surveys, reflecting up to six family members.

2:17:49 And so when you're adding in all the family members, in addition to the harvester, you may end up with fewer people eating a high number of clams, or possibly more, just vacationing harvesters. Okay. We also need to take a look at the reversibility. And I'm really not sure this can be done in human studies, At least not ethically. I think we need to look to our to our animal modellers, such as Dr. Burbacher and Dr. Kathy Lefebvre, to answer questions.

2:18:27 Because I think it would nearly be impossible to have a person eat clams and check their memory, eat clams , and we check them ever again. Also, we don't want to be encouraging people to be eating, over time, a clam that may be toxic or that we know is toxic to some people. But we're also working on the best way to promote effective health messages, prevention and management, if these are indeed a slight memory problem, there's certainly would contribute to a different message than if we were worried people were going, all going around and be amnesic if they ate too many clams. Finally, to be able to better assess exposure, the only way, in the best way, we could assess exposure to domoic acid was through the number of clams consumed in your diet, and at the self-report of that. Now we have no reason to believe that this group, we ran analyses for outliers, was doing any exaggerating or minimizing, but we certainly don't know exactly how much they were exposed to.

2:19:26 So identify a biological marker that could be applied in humans would be the best, the ideal way to move forward with looking at whether or not there's a direct relationship between the memory problems and exposure. Now, the first step of this was completed by Dr. Lefebvre *et al.*, including Nina Isoherranen, who had spoke earlier, spoke right before me, and it was a large group of chemists and biologists involved because, while I was collecting the everyday memory data from folks, we were also collecting blood and urine samples. Those samples went back with. Dr. Lefebvre who blinded colleagues and sent them and published the paper, basically, saying that they found a potential blood serum antibody associated with the DA exposure.

2:20:32 This is in no way yet ready to become a marker, but I need to thank Kathy Lefebvre and her colleagues for at least starting that work so we can look forward to that in the possible future. Now, I am finished at this point with my formal talk. I'm done a little early. I'd be happy to answer more questions or turn it over to the whole group for questions regarding this entire webinar. Thank you very much.

2:21:09 [Murphy] Thank you. Dr. Grattan. We do have a few questions from the audience, you, several of them had to do with reversibility and looking at recovery, and some mentioned the zebrafish and mouse work from Dr. Lefebvre's lab. I think you've covered that. Another question comes here. Do these memory loss issues- are they permanent or temporary in the population that you've observed so far?

2:21:37 [Grattan] That I can't answer, that has to do with recovery. Like we found over four years and eight years, we saw mild memory problems occurring over time. They never reverse back to not having a memory problem, but I can't, I cannot speak to that recovery. Because, as I said, it would be too hard to measure in great, in any detail with humans. Thank you.

2:22:10 [Murphy] Thank you. Another question comes from, as with looking at any correlation with DA exposure, oral DA exposure, and increased incidence of other dementia-like symptoms and syndromes, such as Alzheimer's. [Grattan] Not based upon our cohort. We would like to look further into that and just study a cohort of Elders. However, based on our cohort, there was no evidence of general cognitive decline, and we used comparison measures of constructional praxis, language, and attention and concentration in our analyses, and none of them showed any decline. They were all stable over the time of the study. But that's an excellent question, and that's a direction we need to go in at some point in the future. [Murphy] Sure. A question comes from- what toxins were the clams tested for and were any of these participants tested for the toxins, as well? You'd mentioned that there were some biological samples taken. Do you know what the DA levels were in these clams that were harvested or can you speak to that, perhaps, what was known about the concentrations of the clams that were consumed?

2:23:27 [Grattan] Our best available knowledge, are the reported concentrations that are established by the Washington Department of Health, and then reported on

the website from the Department of Fish and Wildlife, and we stay in close touch with everyone after the studies are over. We're blinded during the studies, that meaning we're unaware of the DA levels during the studies, except the last round of study we did, when you knew the levels have to be below 20 for us to go out and test participants because they were eating. So basically, we believe the levels were somewhere between eight and maybe 14 parts per million.

2:24:08 [Murphy] Okay. Do you know if the razor clams were tested for any other contaminants, including mercury? [Grattan] I do not. However, what I can tell you is that we took 20% of our large cohort sample and got hair mercury levels established at aiming and sent them in to be tested for mercury. And it was considered, they were found to be having not a significant exposure to mercury. And that was through measuring the hair samples for the participants.

2:24:43 [Murphy] Okay, thank you. Another question comes from the audience- are razor clams the only significant source of domoic acid for human populations? Are there other shellfish that may not hold onto domoic acid as long as razor clams, but are eaten more readily and could pose a risk for exposure?

2:25:02 [Grattan] I think at this point the greatest risk for exposure in shellfish other than a razor clam, is the Dungeness Crab. And as it was so well presented earlier in this webinar, the viscera, crab viscera, can hold a lot of DA, a lot of domoic acid, as well as the crab meat, but the viscera holds the most. And, the domoic acid- the current DA levels for being allowed to harvest crabs and crab viscera is a viscera level of 30 parts per million, established by the FDA. It's a very old number, so at 30 parts per million in the viscera, you can buy Dungeness Crabs. Most people remove the viscera prior to eating. But, if they're boiling the crab in with the viscera, or if they're in certain cultures that eat the crab viscera or crab butter, they're at risk of higher levels of DA.

2:26:02 I have to say, the monitoring is very close, is exceptionally close in Washington, Oregon, and Northern California, but I can just say that the only other risk would be to the crab even though they don't hold it for a year. If you have an extended bloom of Pseudo-nitzschia which, which, which some of it produces domoic acid, they're going to be keep getting repeatedly exposed until that does, will present a problem.

2:26:37 [Murphy] Thank you. You mentioned at the beginning of your talk that different species have different depuration rates or rates at which they can get rid of the domoic acid, with crabs being on the shorter end of the scale and razor clams being on the long end of the scale. Do you have a sense about other bivalves, including mussels?

2:27:01 [Grattan] No, I think a shellfish biologist would be best to answer that. That's a great question. [Murphy] Thank you - going through our list here as more audience questions are coming through. It looks like most of these have been

answered. Thank you so much, Dr. Grattan. I think we'll be moving on to our panel discussion.

2:27:30 Next we'll have a discussion panel led by Dr. Pam Lein, Professor and Chair in the Department of Molecular Biosciences in the UC Davis School of Veterinary Medicine, a faculty member of the MIND Institute, and Director of the Counteract Center for Excellence. The Lein Laboratory studies cellular and molecular mechanisms by which environmental chemicals interfere with neurodevelopment and neural function, to contribute to environmentally-induced diseases. Our panel will feature our four key speakers: Dr. Tom Burbacher, Ms. Rebekah Petroff, Dr. Nina Isoherranen, and Dr. Lynn Grattan. Welcome, Dr. Lein. [Lein] Thank you, Shannon. It's been a really exciting webinar. I'm really glad I was able to join in from the beginning and hear all the presentations. So thanks to all the presenters.

2:28:22 And before we get into the actual panel discussion, I'd like to bring all the audience back around to the mission of this webinar that was introduced to us at the very beginning, which is really to focus on the science that's come forth over the last few years, really looking at neurological and developmental effects of chronic low-level effects or low-level exposures, I should say, to domoic acid. So with that, I'd like to lead off with the first question which is to go round robin through all our four speakers and get your personal perspectives based on the science you've both done and that you're familiar with in this area, and give us your sense of what you think is really the most sensitive adverse effect that occurs at the lower doses of human exposure.

2:29:04 So let's start with Tom. [Burbacher] Okay, in terms of what we know right now, you know, if I if I were if I were at EPA and trying to do risk assessment at this point in time, and sort of knowing what their risk assessment procedure includes, they typically would take the human data as the primary source, or what they usually call it, a critical study, and then use the animal studies to support that in terms of weight of evidence. I think that if you if you did do that, you would use the everyday memory outcomes as your most sensitive indicator at this point. [Lein] And do you think that the animal data supports that as one of the more sensitive adverse effects of domoic acid?

2:30:16 [Burbacher] Well, we didn't do we didn't do any memory assessments on our adults. We know we did do this observations for overt toxicity and found not overt toxicity, but more subtle effect on motor. We think motor, in terms of the tremors. The early memory scores for the infants would indicate that, so far, that's the most sensitive indicator in terms of infancy effects due to prenatal exposure. It's a different type of memory than I think what Lynn's talking about in terms of everyday memory, but you know, it probably is I would assume that it's targeting the same parts of the brain.

2:31:08 [Lein] Thank you. Rebekah, your thoughts?

2:31:12 [Petroff] I think Tom did a very good job of explaining kind of what we would think of in the human.

2:31:19 But I also want to reiterate that some of the studies that we did have never been conducted in humans. So it would be important to try to follow up what we did in our nonhuman primate model to understand what's translatable and what's not, and then look at those results to really understand what's the most sensitive marker of toxicity in humans. [Lein] Thank you. Nina, your thoughts?

2:31:51 [Isoherranen] Well, I don't know that that's necessarily my area of expertise, but I do find it kind of interesting how clear-cut the tremor data was that Tom presented. I don't know if we have actually tried to assess that in humans, but I think it would be interesting to see if that happens. You know, I agree with what both Tom and Rebekah said until now so. [Lein] Great, thank you. Lynn, and what do you think?

2:32:27 [Grattan] Well, obviously I thought memory would be a good in the first marker because that's where we started and that was the most profound effect with the acute cases in Montreal and even at post-mortem. They had atrophy neuronal, neuronal problems in CA1 area of the hippocampus, which has been associated with memory, and those same findings were found in sea lions and another species, but I cannot and I would not venture to say that that's the only problem. That's just what I looked at in humans and ruled out other cognitive problems, but I can't specifically address infants, small children, or maybe special problems associated with the elderly.

2:33:20 [Lein] Okay, thank you. So I'm going to ask a question of each of you in turn that's directly relevant to the presentation you gave, and but I would encourage any of the other presenters who are part of this panel to respond and I'll give everyone a chance to do that after the initial person I address the question to has their chance to answer. So Tom, I am going to start, start with you and I think the altered visual recognition in the nonhuman primate infants was particularly striking. I wondered if you could comment on whether you expect similar sorts of outcomes to manifest in human infants. And is there any evidence to suggest either in the nonhuman primate or in the human that these very early symptoms or effects in the infant will really translate into memory deficits later in development?

2:34:13 [Burbacher] Good question. So the, the early visual recognition memory effects that we've seen, or I think I mentioned, are very similar to ones that we've seen for other toxicants we've done studies with our animals with methylmercury exposure *in utero*. Methylmercury exposure and you know, it's a widely accepted developmental neurotoxicant. We saw similar results in those studies in developing this test when we adapted it from humans. We also found that other high-risk groups like premature infants, low birth weight infants, sometimes, also showed deficits in recognition memory.

2:35:02 So in our model, as well as there's actually quite a body of literature in human, human studies, indicating that it's a fairly sensitive indicator of early memory processing and there have been several human studies as well with other contaminants that show that there are effects on infant facial recognition memory. There's also another body of literature since this test is sort of been in active use, that shows that it's a fairly good predictor of IQ later on in life, unlike, unlike a lot of other tests that you know are done on human infants this young. So it's one of the hypothesis that it's picking up very early abilities to process information effectively, which actually is, would be expected to correlate better with later IQ than some of the others that we have.

2:36:10 We haven't, we haven't looked and, and again in our laboratory is probably been the most sensitive indicator of than the other assessments that we do a little bit later in life. They tend to be not as sensitive as this one. So I think there's, there's evidence that this this is a good test in terms of picking up differences related to exposures and it also has been shown to be a fairly good test in terms of predicting later cognitive functioning.

2:36:52 [Lein] That's pretty interesting. Do any of the other presenters have any follow-up to Tom's response?

2:37:00 Okay, thank you, Tom. [Grattan] This is Lynn. Okay. I just wanted to address a couple of things, and this is Lynn Grattan, and that is a number of the animal models, specifically mice and rats, showed some protracted problems emerging that and initially after birth, and in the juvenile animal, there wasn't a memory problem found, but then they found it later as they got older. And secondly, more related to my study, I presented some data at the American Public Health Association meeting last year on a series of infants that our team saw in collaboration with Dr. Kim Grant from Dr. Burbacher's lab and the data that the information that I found, now this is just a small number of cases, it was just thirteen like pilot cases, show that the kids, the babies that were exposed *in utero* to domoic acid, or, we looked at nursing also, but it looked like it was primarily *in utero*, higher level of *in utero* exposure, had delayed motor development, but they caught up by the time they were 3-5 years old.

2:37:56 And there was no evidence of delays by the time they were 7-10 years old. So I don't know if that delay in motor development in that very handful of infants we saw, would in any way be related to what Tom is seeing, but I would just like you to know that we're looking at it. We'd be looking at it further except my NIH funding was not continued. I'm disappointed, but still determined, so I will not give up answering that question.

2:38:53 [Lein] Great. Thank you. Any comments from Rebekah or Nina on the discussion, this far?

2:39:01 Okay, so moving on then. Rebekah, I was actually really fascinated by your MRI work - really cool stuff. And so one of the things I noted is that if you think at least the normal person who thinks about domoic acid, the non-expert in the field, you know, the hippocampus is always the brain region that you think of being most impacted, and of course, that's a lot of the data from the acute domoic acid neurotoxic studies, but you are showing that there is a number of other brain regions that appear to be impacted. So these, these white matter tracts in the brain and other brain regions that aren't typically thought of in the context of domoic acid neurotoxicity. So I wondered if you could sort of comment on whether this provides insight into mechanisms beyond neurotoxic excitotoxicity mediated by a kainate receptor stimulation, or if it basically may give us a way to identify additional vulnerable populations?

2:40:01 [Petroff] Yeah, so there's a lot in there. [Lein] Yes. I'm sorry. [Petroff] I think the first thing is kind of thinking about what I was actually able to look at with this study. So DTI only looks at white matter. The hippocampus is typically the major structure in the brain that is associated with domoic acid exposures, but it is mostly gray matter. So this particular method wouldn't actually be able to look at the hippocampus itself. That being said, the major white matter tracts that does come from the hippocampus, the fornix, was impacted in our study.

2:40:43 So it's parallel but not totally unexpected. The unexpected part was the internal capsule, which had never been looked at before, and I think this is really interesting and shows kind of what can be limiting in science sometimes because we kind of went with a whole brain approach rather than looking at pinpointed areas, which allowed us to have this new discovery. That's not to say it hasn't been changed in other cases throughout this whole episode. I also want to note that the white (matter) in monkeys is very different than the white matter tracts in humans. There are a lot of parallels in the structures, but that could certainly affect how these results translate to humans.

2:41:39 I think the second part of your question was thinking about new vulnerable populations, correct? [Lein] Yeah or how do your data inform what we know about susceptible populations or even mechanisms of action? [Petroff] Right, so the decrease that we saw is associated with kind of a couple different mechanisms of action, one of which could be activation of the immune cells of the brain and infiltration into the areas that we saw to be impacted. Another could be the axons of the neurons actually dying, or another one could be, and this is seen in some slice studies, so when you're actually looking at parts of the brain outside of the animal-full animal model - could be fiber sprouting.

2:42:36 So if axons start to generate additional fibers, that could change the measure that we saw changed in our MRI. All of these give us an idea of new areas to look at but don't necessarily pinpoint a single mechanism. In terms of vulnerable populations, I think what's really interesting with this finding is that we can start to think about how things like co-exposures, which was kind of mentioned earlier with

mercury, but we can think about other exposures in our environment, as well as other neurodegenerative diseases, can impact one's ability to withstand domoic acid toxicity.

2:43:12 But that's about all we can do with this particular data is think about those potential hypotheses and look to new research to really test those. [Lein] Great, thank you. Any additional follow-up from any of the other members of the panel to Rebekah's comments?

2:43:48 Okay, thank you. So moving on. Nina, we're in an area where I don't have a lot of expertise, which is toxicokinetics. I really enjoyed your presentation, but one of the questions that I did have for you is, you seem to make the point that in the acute toxicity exposure paradigms that really was the peak concentration of domoic acid, which is most of concern with respect to the acute toxic outcomes, but when we're thinking about the low dose exposures, is it your feeling that it's the peak concentration that's reached or the persistence of the exposure, that's going to be more important in determining the toxic outcome? [Isoherranen] I think that's a really, really good question.

2:44:43 And I think one would actually have to specifically design a study - you could probably do this in rodents, to see whether the peak or peak concentrations or the duration of the concentrations drives the toxicity because the shape of the curve is so different between the IV and oral. I completely agree with what you said that in the acute toxicity, it is the peak concentrations that is driving that. But I think when we talk about the effects that you know on memory or you know, the tremors or whatever not, I would tend to believe that it's actually persistence of the concentrations. But one would really have to get the same AUC, essentially, after an IV continuous exposure versus oral continuous dosing to truly answer that question. I could design the study. I do not have the data.

2:45:43 [Lein] Fair enough. Yeah, I think my gut feeling would also be it's the persistence rather than the peak, but you're right until we have the data we just don't know. Another question I did have, and then I'll open up to the rest of the panel, was this idea that there might be a transporter for domoic acid is fascinating, and I think also points to another area of susceptibility because we know there's well-known, well-described polymorphisms in the genes that encode transporters amongst humans, which could, of course, then impact disposition of domoic acid. But in terms of just the delivery from the maternal unit to the fetal unit, how is this possibility of an existence of a transporter going to impact that? [Isoherranen] Yeah. So I think the placenta what, you know part of our general research program that I'm part of at the School of Pharmacy is actually maternal-fetal transfer of various drugs and things like that.

2:46:43 So, we'll look quite extensively in terms of metabolism versus transport into placenta or the fetal liver. The conclusion collected from all the drug related work that we have done is that the predominant protective mechanism for the fetus

is for placental transporters. But the placenta really has very high expression of both uptake and influx transporters actually, so I think you know, I don't think the monkey placenta has been as well-studied, but what we know from the human placenta is that the transporter expression actually does change as function of gestational age, as well. And then, the different transporters are localized to the different parts of the placenta, as well. So, it is likely that there is a transporter that essentially pumps domoic acid from the placental cells back to the maternal unit.

2:47:43 And that's what's limiting the exposure because otherwise when we look at this in babies, they had been there certainly in what we call a steady state, the exposure had gone on throughout the pregnancy. So that lower fetal concentration of domoic acid compared to the mom cannot be explained by not insufficient time for the domoic acid to get to the fetus - hope that makes sense. [Lein] Interesting. Yeah, that's really interesting. So do you suspect or do you know, are there transporters in the fetal blood-brain-barrier that could also be influencing uptake into the fetal brain? [Isoherranen] My understanding is that the fetal blood-brain-barrier is not nearly as tight as the, you know, adult blood-brain-barrier. It is not my area of expertise though - the fetal blood-brain-barrier.

2:48:43 But, what I understand, it really develops sort of across pregnancy and it's not as tight. So one could imagine that probably in the fetus more of the domoic acid gets into the brain than in the adults.

2:49:03 [Lein] Great. Thanks. Any comments, responses from the rest of the panelists?

2:49:13 Okay, and then my last direct question and we'll go back to Shannon and see if there's any questions that have come in from the audience. But, Lynn, really fascinating presentation that you gave and so one of the questions would be that, if you think about the CoASTAL cohort data that you've collected in the context of the existing animal study data, what does it really tell us about dose-response in the low-dose range? Can you make any comments on that?

2:49:45 [Grattan] Well, my general perspective is that if you eat a whole lot, is that 20 parts per million may not be protective, or less than 20 parts per million, may not be protective if people have repeated high-level exposures [Lein] And by high level exposures you mean within the low dose range? [Grattan] Eating a lot of clams, and I say that after also looking at the animal literature. [Lein] Okay, great. Any comments from the other panelists?

2:50:31 I'm sorry, Lynn, did I cut you off? Did you have something else to add? [Grattan] No, thank you for asking. [Lein] Sure. So I did have one last question on my own personal curiosity and then I will turn it over to Shannon to listen and what the audience is asking but that is you know, the tremors in both the adult female monkeys, as well as in the infant monkeys, is pretty interesting. And, I wonder if either Tom or Rebekah would like to comment on your thoughts as to whether this is

really a CNS effect or if it's really an effect at the level of the skeletal muscle system?

2:51:20 [Burbacher] Well, both Rebekah and I are in agreement that it's a CNS effect. But, if you want to [Lein] okay [Petroff] I think that one piece of convincing information, which I'm hoping gets published soon, is from one of our colleagues out of Wood's Hole, Dr. Paleo, just finished her dissertation on, on domoic acid in zebrafish and they found a lot of effects with the myelin, in particular, which could be definitely associated with the tremors that we're seeing.

2:51:56 That being said, we're certainly interested in looking at a lot of different areas that the tremors could be stemming from. [Lein] Okay, cool. All right, Shannon. Do you have any questions from the audience for the panel?

2:52:14 [Murphy] Yes, and that tremor question was a great lead-in as we have one from the audience that I would like to put this to Dr. Grattan. Would it be feasible to go back and test for the tremors that were observed in the primate model in the cohort? Would be difficult to do that? Do you still maintain contact with people that have been participating in the CoASTAL cohort in the earlier years?

2:52:40 [Grattan] We still maintain communication with them, and I don't see any reason why we couldn't go back and look at it. I'd actually be even more interested in looking at it in aging folks in the cohort, but I think it's worth looking at in all age groups.

2:53:03 [Murphy] Thank you and Pam, this one is for the (pause) [Burbacher] Before we move on, so, trying to think how to say this, in assessing tremors, there's a high degree of background noise with this outcome, even in our monkeys. You probably could see that on the on the graphs that we have. So people tremor for a lot of different reasons, and, everybody kind of tremors more as you get old. I know that for sure, so it is, it is a difficult test to you know to use given the variability that you're going to get in probably your control group. Sort of depending on who you're looking at.

2:53:56 So I think you know, I think we've got to be careful. I mean it there's also, there's also several ways to assess tremors. What we did was, as I mentioned, part of a fairly quick and easy approach just to look at what their arms look like when they're reaching for something. If you really wanted to do it in a real organized, standardized way, you can actually use accelerometers to actually quantify people's tremors, which would be I think a much more sensitive indicator than what we did. So, so yeah, so you can do it. It'll be noisy. So you'll probably need a fairly large N to be able to show differences, depending on your exposures. [Petroff] Yeah. I also want to point out that we're really limited with our model.

2:54:52 It's amazing that we were able to pick up this really subtle tremor in the first place, just because we can't, unlike humans, we can't ask the monkeys to draw a

spiral or touch your finger to your nose. So it's really powerful that we did see this. But again, it's going to be quite different in humans just because you know essential tremor impacts so many different people, and comes from so many different areas of the brain, and can be caused by disease, other toxins, or toxicants, a whole host of unknown ontologies from this particular disease, it's really fascinating disease - trembling in adults - but really unclear right now in the literature.

2:55:46 [Burbacher] So we're not we're not indicating that you should - we should think about this, so we don't be too negative. But I mean there are there are issues. There are issues with it that need to be considered.

2:55:58 [Lein] So, this is Pam again. I mean, I think a lot of people, and I know tremors are not the same as seizures, but a lot of people do kind of associate chemical-induced tremors that may be chemical-induced seizures. Is there any indication that the individual animals that do exhibit tremor are more susceptible to the seizure-genic effects of domoic acid?

2:56:23 [Burbacher] I wish we knew, we don't know that. As I mentioned, a few of the infants, not all the infants were, you know, had tremors. We had probably three or four that had a transient tremor outlook. As I mentioned, by about two months for those are pretty much gone. Part of our proposals to study them as they got older was to see whether or not those early tremors were related to any kind of, you know, motor kinds of deficits later on in life. So hoping, we're hoping to look at that and possibly even do some seizure susceptibility types of tests, but we didn't do that. [Lein] Got it, would be a cool study, though. [Burbacher] Yep.

2:57:17 [Lein] Shannon, I turn it back to you. [Murphy] So, in continuation of this conversation with the human data versus the animal data, it's important to keep in mind that the messaging to the public is critical and we must be careful with that. For example, what is the transferability of the animal model data to humans? And, the questioner brings out the point that many folks who rely on shellfish and other seafood for subsistence dietary needs may be very impacted.

2:57:56 [Lein] Anybody want to take a stab at that? [Burbacher] Well, I think you know, I think that gets to the messaging in terms of how you want to interpret this data in terms of limiting any kind of seafood intake. I know that in talking with the folks from the Department of Health and, and in Washington and and then Lynn brought this up as well, that they have put out a provisional, what would you call it, warning? To limit, you know your consumption.

2:58:44 So I think, I think that, that the process, my understanding of the process that they used to do that, looked at what, what is the current sort of acute tolerable daily intake and put some order of magnitude on that process to bring it down to, you know, to where it was, I think the 12 to 15, you know that you could that you could eat. I think that it's probably when I probably I think it would be a good a, good idea to make sure that we you know also include the message that you know, seafood is

good for you and because you're limiting one type of seafood doesn't mean you have to limit other types and make sure that you know, we're not changing people's diets overall.

2:59:30 [Petroff] Yeah, and we acknowledge that the razor clam, in particular, is a very cultural, valuable food to a lot of populations here in the Pacific Northwest, including the Native American tribes on the coast, and I think we want to be really cognizant of that going forward and making sure that we're not really coming at this from a top-down perspective, but working with the communities to make sure that we are getting the message right and making sure everyone is protected, but also protected culturally, as well.

3:00:06 [Lein] Thank you. Anyone else on the panel have a comment? Okay, Shannon, any other questions from the audience? [Murphy] Yeah, so we have a question that just came in. Are there any known immunological or endocrinological effects associated with DA exposure?

3:00:29 [Lein] Anyone on the panel want to answer that? [Petroff] There are a couple papers. They're few and far between. We did look at some hormones and some peripheral blood markers of immune function and we did not see any differences in our nonhuman primate model.

3:00:50 [Lein] So, this is Pam. I know there is also literature in the sea lions indicating that at least acute domoic acid neurotoxicity can cause pretty pronounced and persistent neuroinflammation in the brain.

3:01:08 [Lein] Thank you, Lynn? Okay, then nothing from you. Okay, go ahead. Thanks Shannon, next question.

3:01:16 [Murphy] Another question comes through, similar to one that we talked about earlier in the webinar, are there ways to prepare razor clams that do degrade the domoic acid toxin? And we talked about, particularly from the standpoint of the California health agencies, that we recommend either boiling and/or steaming shellfish, so along with other types of seafood, to allow the juices to drain away so that you can discard the fluids in which you're cooking the razor clams. The DA is very heat stable and that cooking does not degrade the toxin, and however, there can be a leaching effect, where DA is coming out of the critter into the fluids. So, we recommend that the fluid is always discarded after preparation and not used for other dishes.

3:02:18 Okay, that looks like we're up to date at the moment. Did anyone else have any? Okay. [Lein] So I guess we'll just go around the panel one more time and ask if they have any closing thoughts or statements. So we'll go in reverse order this time, so, Lynn, would you like to start? [Grattan] Hey, well, first of all, thank you for allowing me to participate in this important webinar, and I look forward to continuing

to do research in this area and will keep you all posted on human health impacts as they arise. Thank you.

3:02:59 [Lein] Thank you, Lynn, Nina? [Isoherranen] Thank you on my behalf, as well, for including me on the panel. It's been very interesting and, I guess, stay tuned for the next steps. [Lein] Great. Thank you, Rebekah?

3:03:18 [Petroff] Yeah, first of all, thank you to all the organizers and lovely people who put that time in to organize this and dissemination of research, and I guess the thing that we really want to stress from our group over here is that, given all the research and given the way that the climate is trending, we need to consider domoic acid as a public health problem that impacts not just one isolated group of people, but can impact a lot of different groups. So I want to stress that and hope that there's continued research in this arena going forward. Thank you. [Lein] Thank you and good luck to you on your PhD research. It's a fascinating question. And Tom? [Petroff] Thanks, I am hoping to finish soon.

3:04:18 [Burbacher] And she will be looking for a post-doc if anyone's out there. So this is Tom. Yeah, I want to thank the organizers. This is done quite, quite well, very nicely and thanks everyone for all the attendees for listening. I guess once, the only thing and it's kind of self-serving, I just wanted to remind folks if there's any, you know, research labs out there that are interested in the domoic acid issues that, you know, would like to take a look at our tissue repository and do some collaborative work. Just send me an email. I hope my email somewhere on all this information that's going out. So if that's the case then go ahead send me an email. So thanks a lot. [Lein] Great and I think, Shannon, that we're finished with our panel discussion.

3:05:07 [Murphy] Thank you, Dr. Lein and panelists. A few other reminders - housekeeping items. If you are interested in seeing one of the PowerPoint presentations that were presented today, please go ahead and email me, Shannon Murphy. My address is on the screen here, and I will put you directly in touch with the authors and you can make arrangements therein. Also too, in the control panel under "handouts", we have a couple of handouts available, including today's agenda, complete with the titles of the speakers' presentations, a short bio sketch summary of all of the speakers and organizers, as well as a flyer of today's event. I will remind you as well that for those of you who are interested in the speakers and presentations that were discussed at the 2017 OEHHA-UC Davis workshop, we do have a copy of that agenda on our website that might be of interest, particularly some of you who expressed some interest in furthering discussion about marine mammals, harmful algal blooms, in general.

3:06:06 That would be a good place to start and certainly feel free to email me if you want to be put in touch with a particular presenter from that event. I encourage everyone to visit our website at OEHHA, especially our domoic acid page. We do intend to post this webinar at a future date, and are exploring the technical options to do so. With that, I'd like to take the opportunity to express our great appreciation to

all of our panelists: Dr. Burbacher, Ms. Petroff, Dr. Isoherranen, Dr. Grattan, and Dr. Lein, and to all the attendees for your contributions to today's webinar. We would also like to acknowledge our producer Tracy Cook, OEHHA scientists Beckye Stanton, Wes Smith, and Tran Pham, and the OEHHA IT Department, especially Diane Curtis and Alina Ketkhenesa for their critical contributions to the organizing of today's event. Tracy, our producer, do you have any further announcements before we make our closing remarks? [Cook] I don't, but thank you. I thought the session today was great.

3:07:13 [Murphy] Thank you so much for all of your work behind the scenes. Thank you, Tracy. Now here is our OEHHA Director, Lauren Zeise, with some final thoughts. [Zeise] Well my final thoughts - just a big "thank you all" for participating on the webinar, sending in your questions, and thank you to the panelists and the speakers and Pam Lein for such a masterful job in the last session, and I just want to close the meeting and thank our OEHHA staff very much for a job very well done. So, thank you all.

3:07:56 [Murphy] Thank you everyone, goodbye. [Multiple voices] Thank you. Bye. Bye.