**EPA Tools and Resources webinar**

**Prioritizing Contaminants for Monitoring and Management transcript**

**Speaker: Lisa Matthews**

Ok, so I apologize for the technically difficulties today, so let's just jump right in. Our presenter today is Dr. Dan Villeneuve, who is a research toxicologist in EPA's National Health and Environmental Effects Research Laboratory. He is based out of Duluth, Minnesota. Before Dan starts his presentation, I'm going to turn it over to Dr. Tina Bahadori. Tina is the National Program Director for EPA's Chemical Safety for Sustainability Research Program, and she's going to provide some brief opening remarks and some context for today's topic. Thank you.

**Speaker: Tina Bahadori**

Thank you Lisa, good afternoon everyone. First, let me thank you for the opportunity to speak to you and for the opportunity to present one of our most innovative and collaborative projects to you, which, while Dan and his presentation is going to specifically focus on the nature of the project and the types of data and tools that will become available as a result of the research, I wanted to contextualize the work and explain how this type of work is very foundational to all of our Chemical Safety for Sustainability research. As background, I just wanted to tell you that this is one of the six National Research Programs in the Office of Research and Development, and it is the one program that is primarily focused on evaluating the safety of chemicals. That chemical space includes pesticides, chemicals, nanomaterials, existing chemicals, emerging materials, we're going to soon be looking at biotechnologies as well. So it is that entire universe of over 100,000 chemicals, not even counting the nanomaterials. For those of you who operate in that space, [you] know that maybe with the exception of pesticides and a handful of high-volume chemicals, there is actually very little information about the chemicals, whether you're looking at hazards properties, whether you're looking at exposures whether you're looking at prevalence, whether you're looking at manners to evaluate them. So in this program [that's] now very clearly propelled by the modernization of ToxCast under the New Ladenburg Act we've been given a lot of momentum to really advance these methods for measurement monitoring modeling, and this program is really focused on that. The research that Dan will describe to you today has a number of sort of global overarching priorities associated with it. One is that there really are too many chemicals, and they occur in the environment in numerous and sometimes people say in infinite combinations – although that isn't really true – so in this program we're trying to do a lot of work to get out of the pure laboratory perspective and in the filed begin to understand: what is the nature of the occurrence, what is the nature of the occurrences of individual chemicals, chemicals in the context of other chemicals related and nonrelated in different partitions in the environment; how do those interact to biological systems – whether they are ecological species or human species; and how do we begin to look at the effects of these exposures. So the methods that Dan is going to describe in this research – while developed in an ecosystem context and in collaboration with federal partners – is being directly translated and led by his research group into work that we're doing on the human health side. There's a lot of interaction in that domain. Also, in this research Dan and his team are beginning to demonstrate how some of the computational toxicology work – how some of the high thorough-put testing, how some of the toxic genomics work – can begin to be incorporated into the traditional monitoring tox-

testing and even chemical evaluation and maybe even risk assessment. So this project in the way Dan is going to present it to you is almost a perfect experimental environment for a number of priorities of EPA. So I'll stop with that and let Dan present, and I'll be around if you have any questions now or if you want to follow-up with me after the meeting is over – I'll be glad to answer any questions. Lisa, back to you.

**Speaker: Lisa Matthews**

Thank you Tina. So Dan, let's just jump right into the presentation. Thank you.

**Speaker: Dan Villeneuve**

Alright, thanks Tina and Lisa, and thanks to everyone joining the webinar today. So I mentioned I'm going to be talking about 21st century toxicity testing tools and how those can be brought to bear on this question of how we prioritize the limited resources we have available for monitoring and management relative to contaminants we have in the environment, and how we can use these new sources of data and new types of tools to help us do that.

So I just want to start out by reiterating the basic problem that many of us face, and that is the increasing range of chemical contaminants that are being detected in the environment. This ranges from pharmaceuticals to personal care products, current generation pesticides, perfluorinated compounds, flame retardants and many others that Tina mentioned in her introduction. We face a lot of challenges related to these. Just to give an example, we frequently hear something like – we've just detected a particular chemical in 30% of the surveyed surface waters in your state, and this gets picked up by local newspaper or television station [then] pretty soon you get citizen action committees and several of your state legislators knocking on your door wanting to know if this is of concern, wanting you to provide guidance on what the potential effects are on human health or impacts on wildlife and the environment. Unfortunately, all too often you go to look for guidance and what you find is that there are no existing water criteria or standards for that compound. In many cases there's little or no toxicity data available in peer review literature or through other sources. In general, there's been very little legal authority to collect those data outside of specialized cases like pesticides. One of the reasons for this, is that most of the toxicity data we've generally relied upon has come from whole organism toxicity testing. Whole organism toxicity testing while can be effective, is certainly costly and time consuming, particularly if we move beyond just standard acute and chronic legality [and] move into trying to assess impacts on reproduction, development [and] cancer formation in humans. All these things as we try to cover more and more of these possible toxicity outcomes – it's an incredible resource load in terms of the amount of testing, the amount of animals and so on that would need to be used to provide that that kind of characterization. Likewise, in many cases if we have the chemical structure, we can try to use quantitative structure activity relationships to infer something about the toxicity of that chemical, but it's well known that in order to effectively and appropriately apply quantitative structure activity relationships, it's important to know something about the mechanism through which that chemical is acting to cause toxicity.

So this general problem of the lack of chemical safety and chemical hazard information available for the majority of chemical in commerce was recognized recently in President Obama's remarks when he signed the 2016 TSCA reform legislation. If you read the drivers for the REACH legislation in Europe, many of the same themes resound throughout that in terms of the lack of information that we have

about most chemicals. There's been a recognized need to try to do address this, and do it in a manner that is efficient in terms of resources and doesn't create a demand for a tremendous amount of additional animal testing.

So that was basically a charge put to the US National Resource Council back in about 2005. They were charged with this problem and trying to come up with a vision and strategy on how we might do regulatory toxicity testing differently in the 21st century. What that group basically advocated in their 2007 report is that we transform toxicity testing from a system based primarily on whole animal testing to one founded much more heavily on in vitro methods that use suites of predictive, high-throughput assays that assess critical mechanistic endpoints that are involved in the induction of overt toxic effects rather than measuring the effects themselves.

In response to this vision, a number of federal agencies, including EPA and also in partnership with FDA, NIEHS, NCGC and other organizations world-wide, have generated some of the first programs in what can be referred to as high throughput toxicology. One of those prominent programs that is based in EPA's Office of Research and Development is the ToxCAST program. To date, ToxCAST has tested well over 2,000 chemicals, probably approaching 3,000 chemicals now, in well over 6,000 different pathway-based assays. Screening these chemicals for activities like their ability to inhibit particular enzyme activities, to bind to specific receptors, to activate transcription factors, to cause overt cytotoxicity, [and] even in some cases looking at some in-vivo responses to things like zebra fish embryos, sea eloigns and some other small in-vivo models that can be run in a 96 well or a 384 well plate format dosed with robotics and so on. These data are generated at a cost of about $20,000 per chemical, which to put that into context is less than the cost of a single fish early life-stage test. Likewise, the Tox21 program is very similar, using high throughput techniques, robotics, to test chemicals in dose-response format. It uses a more limited number of assays, but Tox21 program has already tested well over 10,000 chemicals in a large number of pathway based assays. Both of these programs have demonstrated the ability to very rapidly and cost-effectively screen chemicals for the kinds of biological pathways they can perturb, and identify the relative concentrations at which they perturb them.

This gives us an incredible new resource of data that we can access to provide us information about potential mechanisms of action through which chemicals can cause toxicity. Those data are all publically accessible, you can access them today if you go to the URL that's shown here on the screen. This is the iCSS dashboard [it] has all the ToxCAST and Tox21 data for the chemicals that have been run to date. If we take the example of the hypothetical chemical in our story problem, what we would pull up in searching for that chemical is a bio-activity profile. You can see that that chemical elicits a variety of responses in these pathway based assays. Many of those responses occur at or around concentrations close to those that cause overt cytotoxicity. This can be thought of as fairly generalized, sort of systemic toxicity, not acting true to a very specific mode of action, but due to bulk interaction with membranes, reactivity with proteins and so on, then this has been termed the cytotoxic burst and this is a common phenomenon in this type of these high throughput screening. What you also see for this compound is a variety of activities that occur at concentrations well below that cytotoxic burst that suggest more specific interactions of this chemical with target biomolecules and potential to perturb pathways in very specific ways. For this particular chemical, we see multiple lines of evidence for the activity of this chemical as an aromatase inhibitor, aromatase being an enzyme activity, and we also see additional evidence that this is an inhibitor of hepatic cytochrome P450s that can be involved in phase I metabolism. So again, this is a new resources of data that's available for a large number of compounds

that we can read across and start to extend that to even more chemical structures that may exhibit similar types of bioactivity. This is a resource again that we can start to use immediately. Unfortunately, it's also a little bit hard to interpret. We don't regulate enzyme activity, citizens don't care about receptor binding, so it's important that we're able to translate this type of mechanistic data and information about pathway preservations into something that's meaningful in terms of impacts on human health or on ecosystem functions and services that we value from a management perspective, from a societal standpoint.

To address that challenge, we've been working with the international community to develop and formalize what's called an Adverse Outcome Pathway Framework (AOP). An AOP is a conceptual framework that portrays existing knowledge concerning the linkage between some direct molecular initiating event – so where a chemical interacts with [or] reacts with some molecule in the body of an organism that causes a perturbation to its normal biology. If that perturbation is severe it can cascade across levels of biological organization, and it can culminate in an adverse outcome, at a level of biological organization that we consider relevant to risk assessment. So an AOP simply helps us to organize what we know about biology and toxicology, and make more effective use of these types of pathway-based or mechanistic data in risk-based decision making.

So an example of an AOP that relates to this aromatase inhibition that we saw for this particular chemical is shown here (on slide). This is the kind of information you would find in an AOP description, basically laying out the fundamental biology that aromatase plays a role in. So in this case, we know the enzyme aromatase is rate-limiting for the production of 17 beta-estradiol, which is an important reproductive hormone in female vertebrates. That hormone in oviparous vertebrates stimulates the production of egg yolk precursor proteins that are produced in the liver. Those proteins circulate to the developing oocyte and are accumulated in that oocyte to provide for proper development and growth for the oocyte as well as provide nutrition for the developing offspring once spawning and reproduction occurs. So based on this normal function that aromatase plays in reproductive physiology, we feel it's plausible that if we inhibit aromatase activity, we could potential impair reproduction in oviparous vertebrates. In addition to establishing the plausibility of that based on our fundamental biological understanding, the AOP also captures evidence from studies that have actually looked at aromatase inhibitors and examines this expected profile of responses, and [it] showed that testing multiple aromatase inhibitors we see that predictable patterns of response - reductions in circulation estradiol, reductions in circulating E2 concentrations, reduced accumulation of those yolk proteins and failure to ovulate and spawn leading to reduced cumulative sucumdudy, which ultimately would translate to a declining population trajectory. So the AOP captures those studies that were done, presents that evidence in a very transparent manner [that] makes it accessible to somebody who wants to make that connections, and in addition to providing that plausibility and evidence, technical experts review the support for that association and developers as well as the reviewers go through and evaluate the level of confidence that we have in these relationships depictured and identify relevant uncertainty's and potential gaps that need to be filled in. That weight of evidence evaluation then informs how we might use those relationships; how much confidence we have in the types of regulatory decisions we think we could support based on those relationships.

This information on AOPs is being deposited into an internationally harmonized AOP knowledgebase. The URL for that is shown here on the slide, and again you could go to that URL today and look at the existing AOP descriptions. As part of our EPA research effort, we are developing links between the iCSS

Dashboard and the descriptions of the assays that are included in that testing battery and linking that to the AOPs that are relevant. So for an assay that's relevant for measuring aromatase activity, if you go to the assay information [and] look at the AOP Wiki tab there's a link to the AOP descriptions in the AOP knowledgebase that are relevant to that particular biological activity. So you can link directly to that information that I just showed. This is a steadily growing resource of AOP descriptions, and again made available through an internationally harmonized platform.

So this is all great, these are all things we can do to characterize individual chemicals using these new high throughput toxicity data and AOP descriptions, but we also face additional problems. We're not always just dealing with the chemical of the week, but often are faced with a whole laundry list of chemicals that we're detecting in surface waters and soils and other matrices and media in our states and regions. We've got limited resources available for monitoring and assessment, and it's important that we be able to identify from these long list of chemicals what the highest priorities might be in terms of specific chemicals we might need to be focus on, sites at which those chemicals may be occurring at concentrations that may be adverse, and identifying the types of effects or hazards that those chemicals may cause.

One of the ways that we are approaching this for these long lists of chemicals is using a risk-based screening and prioritization tool called the Exposure Activity Ratio (EAR). EAR is very similar to a hazard quotient that's been traditionally used in risk assessment, where essentially what you're doing is comparing exposure concentrations out in the environment to the concentrations that are known to produce effects in organisms, and looking to see essentially if those concentrations overlap. The only difference is that here, instead of our effects being an apical effect measured in an in vivo toxicity test, instead we're using the effect concentrations measured in these pathway based bio-assays, so this gives us a sense of whether the concentrations in the environment are approaching those that may elicit these pathway-based bio-activities. Importantly, this does not necessarily mean that we would see effects in vivo at these concentrations, there are other considerations that would need to come into play in terms of the absorption, distribution, metabolism and elimination of the chemical, in vitro to in vivo extrapolation or reverse dose-symmetry that allows us to translate the effect concentrations we see in a 96 well or a 96 well plate in vitro verses what you might expect in the tissue of an organism to elicit that type of response, but what this does at this level is [it] allows us to account for two critical factors – the relative concentration of that chemical in the environment and its relative potency to act on these specific targets. Again, this is a very simple concept and a simply calculation, you're just dividing the concentration detected in the environment by the effect concentration. [It's] easy, you can do it on the back of a napkin; however, it's not quite as simple when you have a matrix of 300 chemicals and 650 different assay endpoints and have 195,000 calculations that you need to do.

So in order to facilitate this kind of analysis, we've been developing software tools that have a nice, friendly user interface. [It] allows you to input the chemical monitoring dataset, compares that directly against the ToxCast assays and data that are available for the compounds - all the compounds that are listed or have been run from that dataset, and then [it] allows us to visualize and interpret this EAR data for these large data matrices. We can take something the maximum or mean EAR values, plot that on a map of different sites, and based on that start to identify sites that may be of particular concern or have got multiple chemicals at concentrations that may exceed those that are causing biological activities.

Similarly, we can look across the variety of sites and start to identify those chemicals that tend to be present at or near bioactive concentrations at a large number of those sites. This can help us focus in on particular chemicals, and we can use this approach to start to think about the integrated effects of multiple chemicals on these various assays and targets. As we see biological responses in these various assays, we have multiple chemicals that are impacting these, we can actually sum them or sum their EAR to get an assessment of what the integrated impact may be on that particular target. So even where there's no single chemical at a given site that may have a high EAR that might elicit concern, when we sum multiple chemicals together we may find that we're approaching a level that again would be expected to elicit some bioactivity.

We can utilize this EAR approach to deal with a large number of chemicals that we can identify to analytical chemistry, measure the concentrations, and [that] have been assessed in ToxCast. Of course we know that that's just the tip of the iceberg of chemicals that are actually out there in the environment. The fraction that we can detect and quantify to chemical monitoring, the fraction that have actually been run in the ToxCast platform are still just a small fraction of the totally number of chemicals that are out in the environment and that appear in these samples. How do we address these unknowns, these the other parts of the number of chemicals that are out there? One of ways that we can do this, and a way that's been traditionally used is for example in the whole effluent monitoring program, where you've got a very complex effluent composition [that] isn't completely characterized, so the way we've traditionally dealt with that from a toxicity testing standpoint is to directly test the effluent in a bio-assay and look to see whether it kills fish or kills daphnia to determine its toxicity. We can do something very analogous to that but using these high throughput screening tools. For example, we can take an ambient water sample, extract that sample and get a mixture of chemicals that includes both some of those things we could quantify but also many of those unknowns. We could test it using those same types of platforms that are used for ToxCast allowing us to evaluate the activity of this mixture in a wide variety of biological assays. Based on that bio-activity that's observed [then] link that to the relevant adverse outcome pathways, allowing us to predict what kinds of hazards may be associated with that mixture, identify what types of taxa may be impacted by those pathway perturbations, and identify appropriate endpoints for targeted monitoring and follow-up.

This is an example of some results from some pilot work we did in the St. Louis River using this type of direct analysis of water sample extracts, using one of the subsets of ToxCast assays. Here we see a number of sites upstream at a waste water treatment plant site near the discharge and then downstream. What we see again [is] increasing bio-activities as we get closer to the wastewater treatment plant, we can identify what those specific bio-activities are [that are] associated with that site specific mixture. In this case we see that much of the waste water near the treatment plant shows things like aero-hydro carbon receptor activation, estrogen receptor activation [and] a number of other activities here. Again, we have adverse outcome pathways for these AOPs linking AhR activation to potential developmental effects, AOPs linking estrogenic activity to reproductive and developmental toxicity. This gives us an idea of the potential hazards we might want to look for in resident species at that site, gives us ideas of what endpoints we might want to use. If we were to go out and say use caged fish studies, put caged fish out in these environments [then] what types of endpoints would we look at to get a handle of the spatial and temporal distribution of these bio-active contaminants. [It] gives us insights into other types of targeted bio-assays we could employ to get a more extensive bio-effects based monitoring, and identify potential sources. We've been applying these types of tools to a whole

variety of case studies around the country. We've done a lot of work along these lines in Great Lakes tributaries and near-shore areas. In collaboration with USGS in a nation-wide mixture study they conducted, we've evaluated water from 38 streams across the US, including all 10 EPA Regions. We've done a number of studies out in Region 8 in effluent dominated and in very low flow sort of western streams, and in a variety of other systems across the country. We continue to partner with USGS as part of their stream quality monitoring efforts and other large scale sampling efforts to bring these types of biological activity measurements to bear and integrate that with the chemical monitoring that they are able to accomplish.

Overall, as far as take home messages, pathway-based bio-effects data are being generated at a very rapid pace, and we have the legislative drivers in place for that to continue. AOPs offer a formal framework for linking these pathway-based bio-effects to hazards of concern for both ecological and human health risk assessments. Combination of pathway-based data and AOPs can provide data regarding the hazards associated with chemicals, to which traditional toxicity data are lacking.

Using modern computational tools, simple concepts like EARs can be applied to large data matrices. EARs can be used to help prioritize sites at which management actions might be needed, hazards/effects that we might want to monitor for resident populations [and] chemicals for which standards/criteria should be developed. EARs can be summed to consider the integrated impacts of site-specific mixtures, and high throughput screening can be applied to environmental samples for early warning of potential effects, even for chemicals that are not measured.

So overall, when faced with the challenge of detecting chemicals of unknown toxicity or trying to assess impacts of mixtures, states can use these tools and approaches to make effectives use of new pathway-based data streams for decision making, identify relevant hazards associated with individual chemicals or mixtures, and rank and prioritize chemicals, sites and hazards to help optimize resource investments.

So with that, I'd like to thank you again for the opportunity to present, and I would be happy to take any questions you have today. My contact information is here if we don't get a chance to get to your question today, I would welcome you to follow up.

Thank you!