Speaker's Notes

Slide 1 --- None

Slide 2 --- None

Slide 3 --- None

Slide 4

- Polychlorinated biphenyls (PCBs) were manufactured and used for a long time in the US and around the world . A PCB congener is any one of 209 chemicals (known as congeners) that contain:
    - biphenyl backbone
    - 1-10 chlorine atoms
- Domestic production banned in the late '70s . PCBs were produced as commercial mixtures which contained hundreds of congeners.
- Historically, Aroclors were used as dielectric fluid in transformers and in capacitors and as plasticizers in adhesives and caulks. But, There were many other uses for PCBs and products that contained them
- Also, it's been discovered in recent years that a number of PCBs are currently produced inadvertently during certain manufacturing processes, most notably pigment production.
- Humans are exposed to PCBs as diverse mixtures of congeners; these vary in structure, stability, toxicity and MOA depending on the number of chlorines attached to the biphenyl and the positions of those chlorines.

Slide 5

There are currently two noncancer reference values for PCBs in the integrated risk information system (IRIS). One is for the commercial mixture Aroclor 1016 – it's based on data from a study in rhesus monkeys that reported decreased birth weight; the other is for Aroclor 1254 – it's based on data from another study in rhesus monkeys that reported immunotoxicity as well as some dermal and ocular effects of exposure.

Importantly, there are currently no reference values for PCB mixtures as they exist in the environment today; environmental PCB mixtures are often dissimilar from Aroclor 1016 and 1254, making it challenging to decide which reference value is most appropriate to use at some contaminated sites.

Slide 6

PCB congener mixtures in the environment are highly variable. They are different from the original source mixtures, such as Aroclors due to processes like

- selective accumulation
- Selective degradation
- addition of congeners produced and released inadvertently.

In terms of general rules, higher chlorinated congeners tend to be resistant to metabolism and can bioaccumulate in the food chain, such as fish whereas lower-chlorinated congeners are more volatile and may undergo transportation in the air.

Slide 7

As we learned in the first session, and as stated in EPA Guidance, the IDEAL is to use health effect and dose-response data specific for the mixture that people are exposed to in their environment. However, it is increasingly rare to find contexts in which humans are exposed to unaltered Aroclor mixtures. A promising alternative is to assess health risk from exposure to an environmental mixture using health effect and dose-response data on a "sufficiently similar" surrogate mixture for which data are available, which in the case of PCBs, could be data on Aroclor mixtures.

U.S. EPA currently uses a simplified sufficient similarity approach in cancer risk assessment for PCBs; there are three cancer risk values divided into three categories: (1) "high risk and persistence", (2) "low risk and persistence", and (3) "lowest risk and persistence". The dose-response data used to develop these values derived from studies of liver cancer in rats exposed to Aroclors 1254, 1242, and 1016, respectively.

Slide 8

Before we begin discussing the mixtures modeling methods, we'd like to take a moment to quickly define a few key terms that will be important in the upcoming slides. We will be discussing more about BMDs and Eds and how they relate to mixtures similarity testing later in the presentation.
(read definitions)

Slide 9

To assess sufficient similarity of PCB mixtures, EPA has developed a Microsoft excel based tool (with contract support from ICF) to facilitate sufficient similarity analyses for mixtures. This is called the mixtures similarity tool (or MiST) and it implements a modified methodology from a peer reviewed publication by Marshall et al. The tool uses equivalence testing methodology to compare distance between benchmark dose estimates for mixtures. While the tool was developed specifically to support the goals of the PCBs assessment, the approaches can be applied more generally to mixtures broadly. Note that the tool will be the subject of the fourth talk in this session while the focus of the current talk is on the principles of the methodology implemented in the tool.

Slide 10

Before getting too deep into the modeling strategy, it'll be important to understand a few more key terms. We will be discussing these terms in greater detail in the subsequent slides

- **Reference mixture**: A mixture for which estimated effect levels (e.g., benchmark doses (BMDs)), along with variance information for these estimates, can be or have been derived. In Talk 1, this was referred to as the "tested mixture"
- **Candidate mixture**: A mixture selected for risk evaluation that will be compared with a reference mixture to determine sufficient similarity; a candidate mixture might lack adequate dose-response data for deriving estimated effect levels (e.g., many environmental mixtures). In talk 1, this was called the "mixture of concern".

- **Toxicological surrogate**: A chemical or mixture with toxicological data sufficient for use to support risk assessment of a related chemical or mixture for which data are limited or unavailable.
- ***Critical Value (CV or Δ):*** Maximum difference allowed between Reference and Candidate mixture BMDs for the mixtures to be considered toxicologically similar. Note that Selection of a critical value balances statistical and biological significance and requires expert judgement. Different selections for a critical value can alter the results of an analysis. We'll be discussing this more in the following slides.

I'm now going to hand over the discussion to Jeff Gift, to discuss the MiST methodology.

Slide 11

Now that we're familiar with the terminology, we can talk a bit more about the equivalence testing methodology and the questions it's intended to answer.

I'm going to talk about how EPA implemented the approach described by Marshall et al. in an Excel based Mixture Similarity Tool we call MiST.
Some example questions might include:

- Is a given reference mixture "sufficiently similar" to the candidate mixture such that the reference mixture could be used as a toxicological surrogate for the candidate mixture?
- If more than one reference mixture is "sufficiently similar" to the candidate mixture, which reference mixture is the most appropriate toxicological surrogate?

Slide 12

One of the most critical aspects of making a similarity judgement is defining your similarity bound, which is the critical value that we discussed earlier. The critical value is defined slightly differently for the two scenarios that MiST can address, Data Rich and Data Poor scenarios. In a data rich scenario, the BMDs are known for BOTH the reference and candidate mixtures, and the critical value can be calculated by using the benchmark dose and the effective dose (absolute value of the maximum of BMD-effective dose) for both candidate and reference mixtures. In the data poor scenario, we only have a BMD for the reference mixture, and the candidate mixture has an unknown BMD. In that case, the critical value is based on the BMD and effective dose for the reference mixture only.

Slide 13

As we've mentioned, there are both biological and statistical considerations in establishing what effective dose (ED) to use in setting the critical value. Time does not permit a discussion of biological considerations. However, we can briefly describe an example of an important statistical consideration having to do with study quality. When the BMD is derived from a high-quality study (e.g., in a study that uses a large number of animals per dose group), confidence in the BMD will be high and the BMDU will be relatively low (1st animation). If we set the ED to the BMD20 estimate (2nd animation) the upper end of the "similarity range" would be well above the upper confidence limit estimated for the BMD. WITH THIS ED, we would accept candidate mixtures with BMD estimates as high as around 22 mg/kg-day as being similar to the reference mixture, even though we have high confidence that the actual BMD for the reference mixture is much lower, less than 16 mg/kg-day. It might be more appropriate to establish an Effective Dose that is closer to the BMDU in this situation.

Slide 14

Now let's talk about how MiST uses the critical value or Delta. MiST performs three basic steps. First, MiST calculates the Euclidean distance between the Reference Mixture BMD, which must be specified by the user, and the Candidate Mixture BMD, which can be either specified by the user (we call that a Data Rich scenario) or estimated by MiST (we call that a Data Poor scenario**)**. We will refer to this as **Dw**. For those wondering how MiST estimates the BMD for the Candidate Mixture in the Data Poor scenario, hold on, we will get to that shortly. In Step 2, MiST estimates the upper one-sided 95% confidence limit on the distance between Candidate and Reference mixture BMDs. We will refer to this as **D_wU_{95}.** For those wondering how the **D_wU_{95}** is estimated, hold on, we will get to that shortly also. Finally, in Step 3, MiST compares the **D_wU_{95}** estimate to the similarity boundary defined by the **critical value or Delta**; For the two BMDs to be considered sufficiently similar the **D_wU_{95} must be less than the critical value or Delta.**

Slide 15

Now let's dig into some of the details of MiST's inner operations. **1st Animation**: First you must understand that MiST defines every mixture's BMD by its chemical composition. **2nd Animation**: A mixture's Composition is represented by a plot line or vector in C dimensions, where C is the number of mixture components. In this simple example we assume that our mixtures only have two chemical components, or **congeners**, as they are referred to in the PCB world; For PCBs, there can be as high as 209 congeners in a mixture. We'll also make one other simplifying assumption. **In MiST** each component (congener) can be assigned separate toxicological potencies/weights, and while that is ideal, it is a more complex analysis and not always possible. We'll talk a little more about congener-specific toxicological potencies later in this talk and a lot more in the final two talks of this seminar, but here we'll make the simplifying assumption that our mixture components (congeners) have equal toxicological potencies (i.e., the toxicological contribution of each component is totally dependent on the fraction of that component in the mixture and not toxicodynamics). **3rd Animation:** So, with that said, for this simple two-dimensional plot of a 2 chemical Reference mixture with a 1/1 mixing ratio, the mixture is represented as a vector line with a slope of 1. **4th Animation:** And the user-determined BMD of 12 is a point on the line where the sum of the chemical individual concentrations or doses is 12, in this case 6 units from chemical 1 and 6 units from chemical 2. And the confidence interval about the BMD is represented by the dashed portions of the vector line.

Slide 16

Now let's talk about how the D_w and the D_wU_{95} are estimated in a Data Rich scenario, where BMDs are available for the compared mixtures. This is our Reference Mixrture, **1st Animation:** and let's assume we have a Data Rich Candidate mixture with a mixing ratio for its two chemical components (congeners) of 3:7 and a BMD of 10 (so the BMD is represented as point 3,7 on the reference mixture vector line). **2nd Animation:** MiST estimates the Euclidean distance between the median BMDs **(blue line),** which we are referring to as Dw (we will show how Euclidean distances are calculated later). **3rd Animation:** next MiST estimates the 95% upper bound on the D_w **(red line),** which we are referring to as the D_wU_{95} using a Monte Carlo sampling method. We use a different approach to deriving this D_wU_{95} confidence bound then what is described in the Marshall et al. paper so that we can take advantage of the way the EPA BMDS reports BMD confidence intervals (i.e., as a CDF). You'll hear more on this MC sampling approach in the last talk of the seminar, but it basically involves using the confidence intervals reported by BMDS or other software (represented here as the dashed portions of the plotted lines), to estimate 10,000 possible Euclidean distances and then find the distance higher than 95% of the estimates.

Slide 17

Now let's talk about how MiST determines whether these two mixtures are "sufficiently similar." **1st Animation:** Let's assume that, from our BMD analyses for this Reference mixture, we have estimated an ED20 of 22, which we will use to determine a critical value or delta of 10 (22-12), shown on the plot with a green text and arrow (as described earlier, MiSt would also consider the critical value for the Data Rich Candidate mixture and use the higher of the two; but for simplicities sake, we'll assume the Reference mixture's critical value is highest here). **2nd Animation:** As you can see, the DW falls well within this critical value distance (blue circle), but that's not what the similarity determination is based on. **3rd Animation:** The similarity determination is based on the D_wU_{95} which, in this case, also is less than the critical value distance because it falls within the green circle representing the critical distance from the Reference BMD starting point. Thus, these two mixtures would be considered "sufficiently similar."

Slide 18

Now let's examine how the approach is applied in the Data Poor scenario, where a BMD is not known for a candidate mixture that has a mixing ratio for chemicals 1 and 2 of 1:3. Again, we're assuming that the toxicological potencies of the components are equal, so their toxicological contributions to the BMD are totally dependent on their mixing fractions. Based on the assumption that mixtures with similar mixing proportions will have similar BMDs, the total dose associated with the candidate mixture's BMD (TDc) is assumed to be the same as the total dose associated with the Reference mixture's BMD (TDr), which is 12 mg/kg-day, and the confidence intervals (the dashed lines) are assumed to be the same. **1st Animation:** Thus, for a Data Poor Candidate Mixture with a mixing ration of 1:3, the TDc is represented as the point 3,9 point (because 3+9=12) on the mixture's vector line. **2nd Animation:** D_w (blue line) is the Euclidean distance from the Reference TD to Candidate TD, **3rd Animation:** and the 95% upper bound on D_w ($D_wU_{95;}$ red line) is calculated as before using the Monte Carlo approach. The determination of sufficient similarity is made as described for the data rich scenario, by determining whether D_wU_{95} is less than the Reference mixture's critical value.

Slide 19

Now let's talk about how the Euclidean distance is calculated, including how we take into account relative potency weights for the mixture chemical components (congeners) if they exist. Basically, for the mixtures being compared, we sum the squared difference between the congener contributions to each mixture's estimated BMD weighted by a congener-specific toxicological relative potency weighting factor; we then take the square root of that value to get the Euclidean Dw. In this first equation, the Wj is the toxicological relative potency weight given to the jth congener and the thetas are the individual dose contributions of each congener to the total dose BMD for the reference (r subscript) and candidate (i subscript) mixtures. If your like me, you like to see examples, so at the bottom of the slide, we've included the Euclidean distance calculations for the simplified Data Rich and Data Poor examples we've just gone over. For these examples, the relative potency weights are set to "1" for each congener (i.e., there is no toxicodynamic difference between the congeners; equal doses are assumed to contribute equally to the BMD). Also, this is just calculating one distance, the Dw, which is the distance between the mean BMD values. That distance is shorter for the Data Rich scenario we covered, but that may not be the case when we calculate the DwU_{95} for each scenario, because the MC estimation of the DwU95 accounts for uncertainty (dashed portion of the lines) which as you may have observed were different for the candidate mixtures of our two scenarios.

I'll now pass it back to Laura Carlson who will showcase a few case study analyses.

Slide 20 --- None

Slide 21

Now that we've discussed the methodology of the tool, we will present a couple of case examples so that hopefully you all can get a better understanding of how the tool may be used to support the IRIS PCB assessment. The first case example will be a "data rich" analysis, meaning we have dose response data for both reference and candidate mixtures. We will evaluate neurotoxicity data from 4 Aroclor mixtures, and will also utilize congener relative potency estimates that were based on in vitro neurotoxicity data and derived for untested congeners using quantitative structure activity relationships. Note that the third talk in this session will focus on methods for relative potency factor estimation, including quantitative structure activity relationships.

The second case example will evaluate similarity of an environmental mixture, in this case a simulated fish mixture, relative to Aroclor 1254 or Aroclor 1016. It will not utilize relative potency data, and we do not have dose response data for the candidate mixture, making it a data poor analysis.

Slide 22

The case example data are taken from a 2000 publication from Freeman et al where adult rats were dosed with 2-3 concentrations in diet of various Aroclors for a year, and evaluated for a variety of neurotoxicology endpoints. The animals were evaluated for the functional observational battery which included a variety of domains.

Slide 23

For this particular pilot example, we performed BMD modeling on one endpoint, landing foot splay, and calculated the BMD and CDF using the reported data and EPA's BMDS Software. Landing Foot Splay is a measure of motor function and can be an indicator of neuropathy and gait abnormalities
We did perform a weighted data rich analysis by using congener toxicological potency values for neurotoxicity which were developed in collaboration with scientists in ORD's center for computational toxicology and exposure. I've included the citation information, if you wish to learn more about the neurotoxicity potency values here from a 2019 publication. We then assessed the similarity between a candidate mixture, which in this case was defined as Aroclor 1254 and three reference mixtures (Aroclors 1016, 1242, and 1260).

Slide 24

Lets review the needed mixtures modeling inputs, including required and optional modeling inputs from earlier in the talk, including mass fraction information for the reference and candidate mixtures, benchmark dose estimates for the reference mixtures, and an effective dose level for the reference mixture.
For this analysis, we had all the required information available as well as dose-response information on the candidate mixtures and relative potency values for a relevant health effect, so we had the optional information available as well.

Slide 25

These bar charts are a comparison of the percent abundance of PCB congeners in the various Aroclor mixtures. The congener number is on the x-axis, and the percent abundance is on the y-axis. As you can

see, the congener profiles differ across mixtures, with Aroclors 1254 and 1260 containing more heavily chlorinated congeners, and Aroclor 1016 having more of the lighter chlorinated congeners. I realize that the small bar size is hard to resolve, but the intent here is to show the great diversity of congeners represented in the different mixtures.

Slide 26

This slide shows the BMD calculations from the Freeman paper for the different Aroclor mixtures- you can see AR1016 was least potent, with the highest BMD value. The BMDs are entered into MiST as a CDF distribution in this example.

Slide 27

This slide shows the results from the mixtures similarity testing. We have shown the example output from the tool, which includes the information supplied and calculated by MiST (the BMD, Delta, Dw, and upper 95th Dw, along with the tool's conclusion and suggested rank). You can see that for this particular analysis, the tool concluded that Aroclor 1242 was sufficiently similar to Aroclor 1254, but not to Aroclor 1260 or 1016. This is based off an analysis that estimated a BMD10 and the ED30.
I also want to point out that in Talk 4, we will see a more detailed demonstration of how to input data and run analyses using the draft excel tool.

Mixture distance (dw)
Delta: this example uses the candidate/reference mixtures BMD and ED are based on fitted dose response function (absolute value of the difference of BMD & ED to derive the delta/critical value)
SE: standard error of estimate dw- from BMD distribution
95th: compare upper on sided 95% confidence limit against similarity boundary (delta), and the two mixtures are similar if 95th dw < delta
Conclusion: provides the similarity testing results based on the parameters supplied in the analysis.

Slide 28

We will now move into the 2nd case example, where we explore a comparison of an environmental mixture to Aroclor mixtures.

Slide 29

We will now compare a representative environmental mixture of Fish Tissue (Wisconsin). The Examples is based on a Fox River Fish Mixture with the congener profile information from Kostyniak et al. 2005. We will then test for similarity to Aroclor 1254 or Aroclor 1016.

(OPTIONAL)
The fox river fish mixture is actually a mixture of several Aroclor mixtures, and was characterized using the congener profile data in the ATSDR tox profile on PCBs. Agency for Toxic Substances and Disease Registry (ATSDR). 2000. Toxicological profile for Polychlorinated Biphenyls (PCBs). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Slide 30

As a reminder, In order to perform a mixtures modeling analysis, certain key information is needed.

For this particular analysis, we lack the benchmark dose information for our candidate mixtures (fish mixture) and we lack relevant congener potency information. As environmental mixtures don't often have dose response information, this is a data poor example.

Slide 31

This slide again shows the results from the mixtures similarity testing for the fish mixture. You can see that for this analysis, the tool concluded that Aroclor 1254 was sufficiently similar to Fox River Fish mixture. This indicates that AR 1254 could be considered an acceptable surrogate for the fox river fish mixture

Slide 32

We will once again explore some congener profiles of the tested mixtures. This slide again shows the congener profiles for AR1016 and 1254 on the left hand side and fox river fish profile on the bottom right in red. As you can see, the fish congener profile overlaps more with the congener profile of AR 1254, which intuitively supports this analysis' conclusions.

Slide 33

While we have presented several example analyses in this presentation, its important to discuss some remaining challenges with environmental mixture analyses.

It is relatively rare for 209 congener analyses to be performed, as they are expensive and it can be difficult to analytically resolve all congeners. Similarly, congeners that are below the method detection limit may impact results. For this particular analysis, we utilized 209 congener analyses when possible and when relevant, coeluting congeners were estimated by dividing the value by 2. Values below the limit of quantitation are treated as zeros. Any assumptions or modifications made to your analysis would need to be disclosed.

Finally, environmental samples are heterogeneous- samples will be location dependent and not necessarily generalizable to other locations or other matrices. For example, fox river fish samples are not generalizable to fish samples from other locations.

Slide 34

So, how does this method complement the IRIS PCB assessment? These mixtures modeling methods support evaluation of similarity across PCB mixtures. They can be used to group datasets for similar mixtures to develop reference values. The methods can also be used with the posted assessment to apply reference values to similar mixtures in the environment. And, importantly, The methods will be described in the final assessment but also published in the peer reviewed literature prior to assessment release.

Slide 35

I know we have covered a lot of content today, but I'll try to briefly summarize what we've discussed. EPA has extended the mixtures modeling methods that were developed by Marshall et al. 2013 to facilitate sufficient similarity analyses for comparing PCB mixtures. These sufficiently similarity approaches can be used to identify suitable dose response data to apply in risk assessments of environmental or untested PCB mixtures. The subsequent presentations in this session will discuss

potency estimation approaches and provide more details on how analyses are conducted using the MiST Tool.

Slide 36 --- None