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Mixtures Modeling: Methods Considered for the Assessment of Polychlorinated Biphenyls (PCBs)

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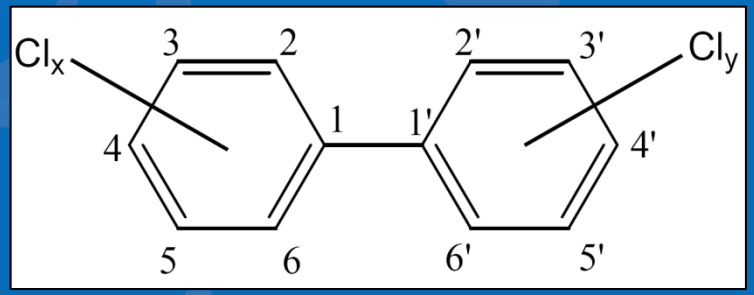
March 16, 2022



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Disclaimer

- The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.
- We have no conflicts of interest to disclose.

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Public Webinar on PCB Mixture Assessment Methods

- **Introduction to EPA's human health risk assessment practices for chemical mixtures**
  - *Glenn Rice, U.S. EPA*
- **Mixtures modeling: methods considered for the assessment of PCBs**
  - *Jeff Gift and Laura Carlson, U.S. EPA*
- **Methods for estimating relative potency values**
  - *Grace Patlewicz, U.S. EPA*
- **Overview of the Mixture Similarity Tool (MiST)**
  - *Graham Glen and Joanne Trgovcich, ICF*

Polychlorinated Biphenyls (PCBs)

U.S. Manufacture and Production:

- Manufactured as Aroclors from 1929 to 1977
- Total U.S. production >600 million kg

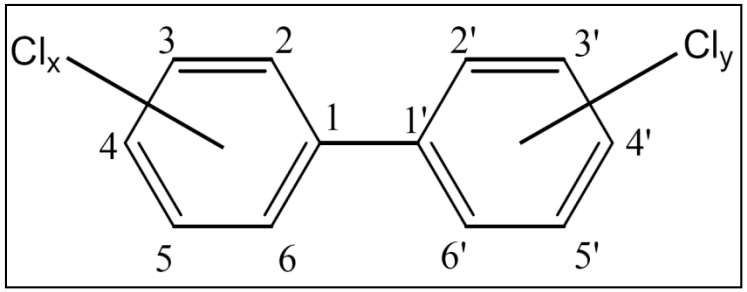
Legacy Uses:

- Dielectric fluid in transformers
- Electrical devices/appliances containing PCB capacitors
- Fluorescent light ballast capacitors
- Adhesives/caulks

Current Releases:

- Inadvertent congener formation in manufacturing processes (e.g., pigment production)
- PCBs 5, 8, 11, 12, 13, 15, 35, 36, 40, 52, 56, 77, 206, 207, 208, 209

Humans are exposed to PCBs as diverse mixtures of congeners.

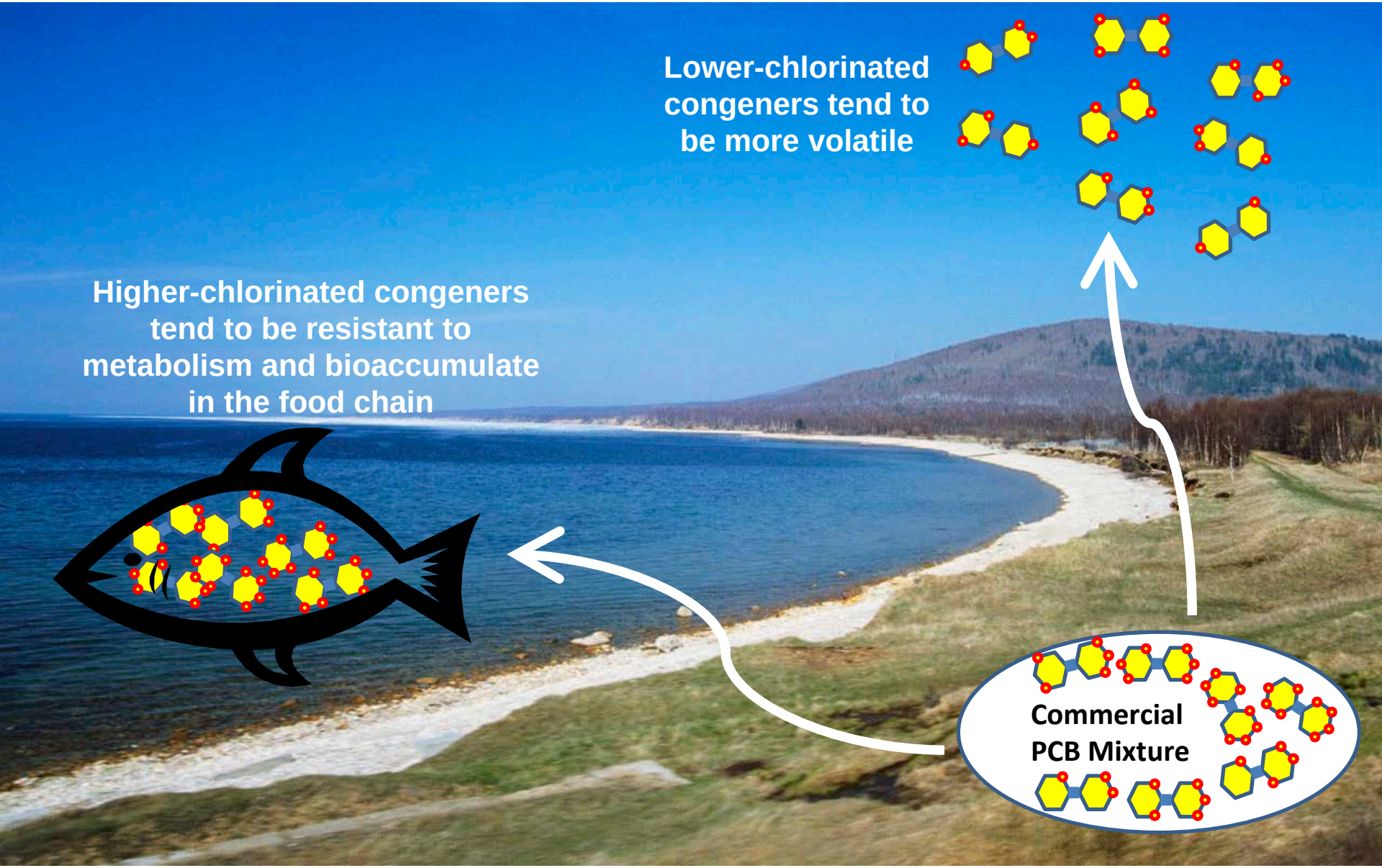


Congeners vary in structure, stability, toxicity and mode of action (MOA): these properties are determined by chlorine number and position

U.S. EPA. 2019. Systematic Review Protocol for the Polychlorinated Biphenyls (PCBs) Noncancer IRIS Assessment (Preliminary Assessment Materials). EPA/635/R-19/201

Current IRIS Noncancer Reference Values for PCBs

- Reference Doses (RfDs) for PCB mixtures
  - Aroclor 1016 (**70 ng/kg-day)**
    - Reduced birth weight observed in rhesus monkeys exposed during gestation
  - Aroclor 1254 (**20 ng/kg-day)**
    - Immunotoxicity in adult rhesus monkeys exposed for 55 months
  - **NO reference values** for environmental PCB mixtures
- Risk assessors not always clear on which reference value to use


Lower-chlorinated congeners tend to be more volatile
Higher-chlorinated congeners tend to be resistant to metabolism and bioaccumulate in the food chain
Commercial PCB Mixture

Mixtures Risk Assessment Review

- Whole mixture approaches are preferred to component approaches
- When toxicological data are not available for mixtures as they occur in the environment, EPA mixtures risk assessment guidance recommends using toxicity data from a “sufficiently similar” mixture as **surrogate**
- For example: Current IRIS PCB cancer risk for PCBs uses 3 values based on sufficient similarity (congener grouping is qualitative)
  - High risk/persistence (2.0 per mg/kg-day)
    - food chain, soil, dust exposures, dioxin-like congeners; Aroclor 1254
  - Low risk/persistence (0.4 per mg/kg-day)
    - water soluble & volatile congeners; Aroclor 1242
  - Lowest risk/persistence (0.07 per mg/kg-day)
    - =<4 Cl; Aroclor 1016

U.S. EPA. 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. EPA/630/R-00/002
Cogliano. 1998. Assessing the Cancer Risk from Environmental PCBs. *Environ Health Perspect* 106:317-323

Definitions (part 1)

- **Benchmark dose (BMD):** the dose of a chemical associated with a specific level of effect. For example, the dose associated with a 10% extra risk of experiencing cancer or liver damage or some other effect.
  - If the benchmark doses for two chemical mixtures are close to each other, that indicates that the mixtures are similar in toxicity

- **Effective Dose (ED)**: the dose or concentration that represents a distance from the BMD that is deemed biologically or statistically significant; it could be the dose associated with an effect level above the response level used to derive the BMD. For example, if a BMD is set based on a 10% response, the ED might be based on a 20% response.
  - a mixture with a BMD within this bound could be considered similar to the tested mixture while another mixture with a BMD outside the bound would not be close enough to the tested mixture to be considered similar

Sufficient Similarity Testing

- EPA has developed a Microsoft Excel® based tool to facilitate sufficient similarity analyses for mixtures
  - Mixtures Similarity Tool (MiST)
  - Implements a modified methodology from *Marshall et al. 2013 "An empirical approach to sufficient similarity: combining exposure data and mixtures toxicology data" Risk Analysis 33:1582-96*.
- Uses equivalence testing methodology to compare distance between benchmark dose estimates for mixtures

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Definitions (part 2)

- ***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.
- ***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)
- ***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.

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Mixtures Similarity Tool

- EPA has developed a Microsoft Excel® based tool to facilitate sufficient similarity analyses for mixtures
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  - Implements a modified methodology from *Marshall et al. 2013 "An empirical approach to sufficient similarity: combining exposure data and mixtures toxicology data" Risk Analysis 33:1582-96*.
- Uses equivalence testing methodology to compare distance between benchmark dose estimates for mixtures
  - Is a given reference mixture "sufficiently similar" to the candidate mixture such that the reference mixture could be used as a toxicological surrogate?
  - If more than one reference mixture is "sufficiently similar" to the candidate mixture, which reference mixture is the most appropriate toxicological surrogate?

Defining a Similarity Bound (Critical Value Δ)

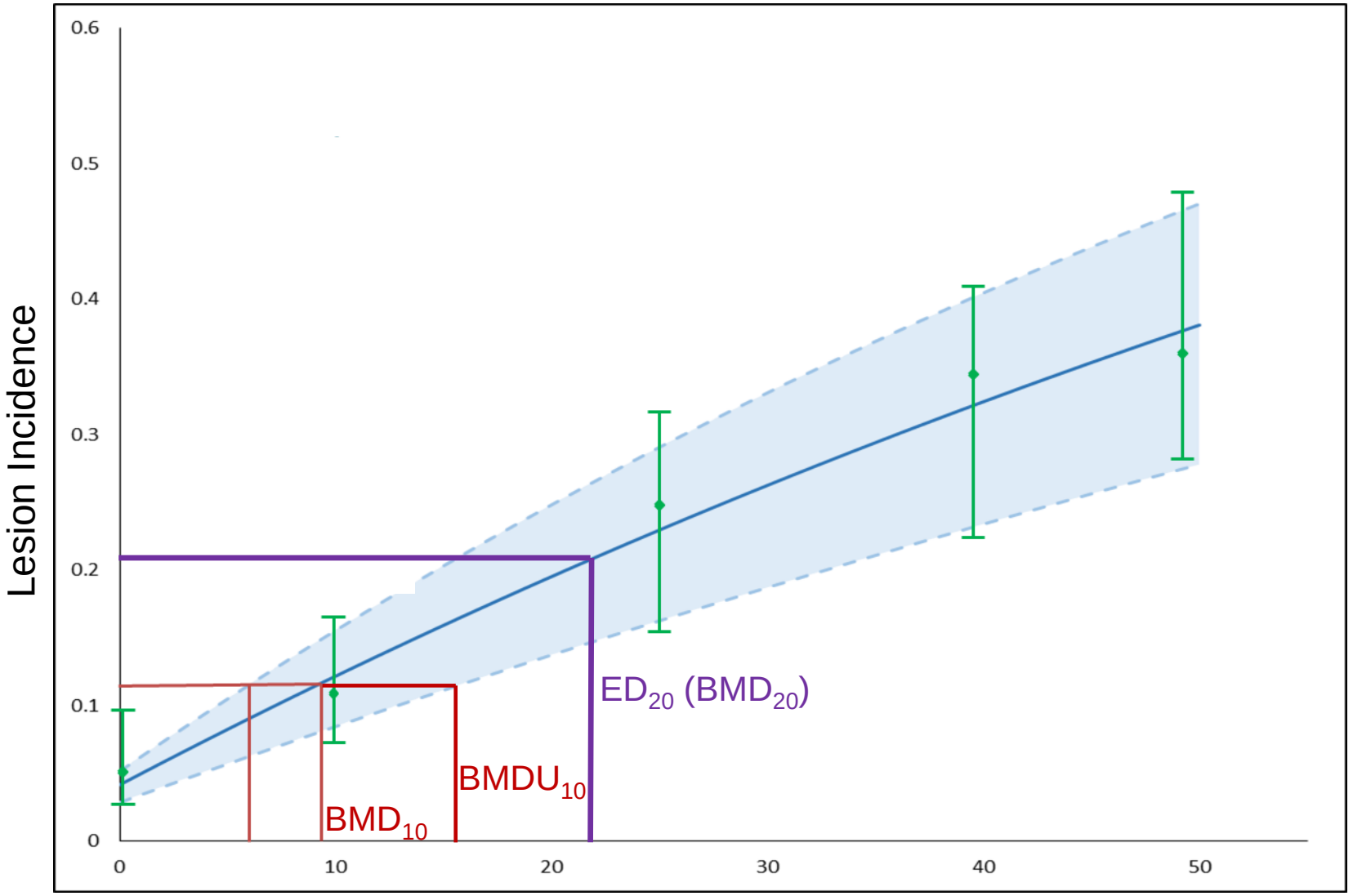
- **1) Data rich:**
  - BMDs are known for both reference and candidate mixtures.
  - Calculate Critical Value (CV or Δ) based on fitted dose-response functions for **both** reference and candidate mixture (PCB) using the benchmark dose (BMD) and effective dose (ED).

$$\Delta = absolute\ value\ of\ max\{(BMD_r - ED_r), (BMD_i - ED_i)\}$$

- **2) Data poor:**
  - Candidate mixture BMD is unknown.
  - Calculate Critical Value (CV or Δ) based on fitted dose-response functions for **reference mixture** (PCB) using the benchmark dose (BMD) and effective dose (ED).

$$\Delta = absolute\ value\ of\ BMD_r - ED_r$$

Setting the ED: The Impact of Study Quality



How MiST Works: Three Basic Steps

- **Step 1:** MiST calculates the Euclidean distance between the *user-specified* Reference Mixture BMD and the *user-specified OR assumed* Candidate Mixture BMD ($\mathbf{D_w}$).
- **Step 2:** MiST estimates upper one-sided 95% confidence limit on the distance between Candidate and Reference mixture BMDs ($\mathbf{D_wU_{95}}$)
- **Step 3:** MiST compares the $\mathbf{D_wU_{95}}$ to the similarity boundary defined by the **critical value** (i.e., Δ); For the two BMDs to be considered sufficiently similar

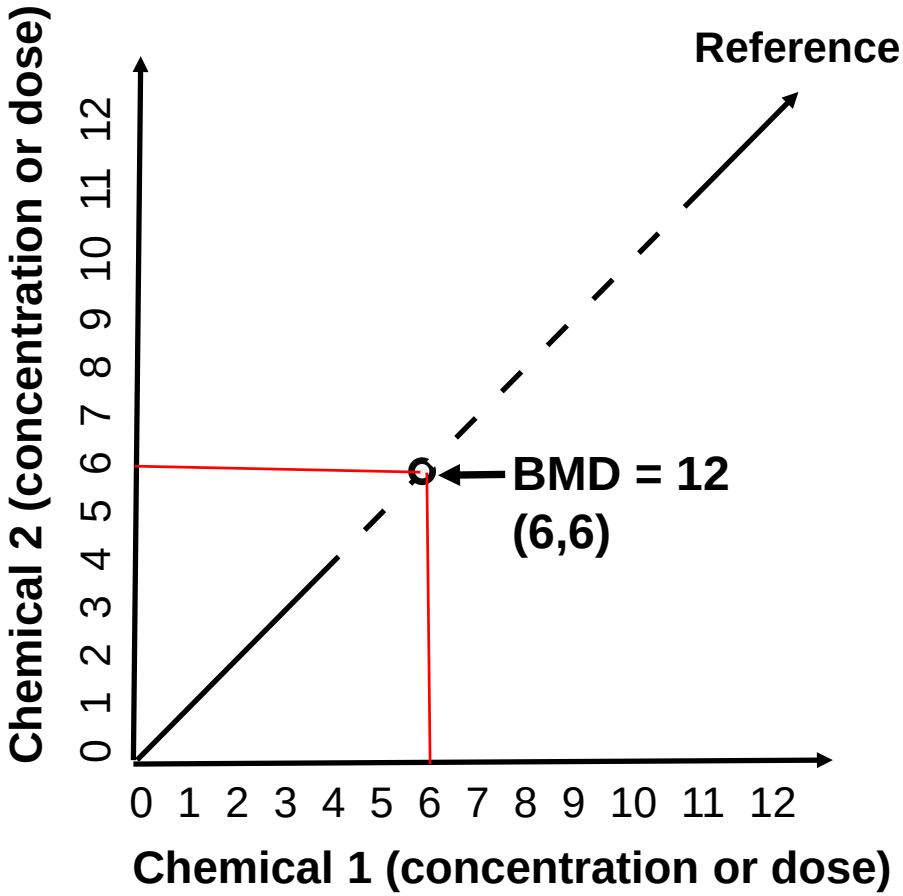
$$\mathbf{D_wU_{95}\ must\ be} \leq \Delta.$$

How MiST Works: Defining the BMD

1. A mixture's BMD is defined by its chemical composition

2. The chemical Composition is represented by a plot line (vector) in C dimensions, where C is the number of mixture components

3. BMD is a point on the line; dashes reflect uncertainty (BMDL – BMDU range)

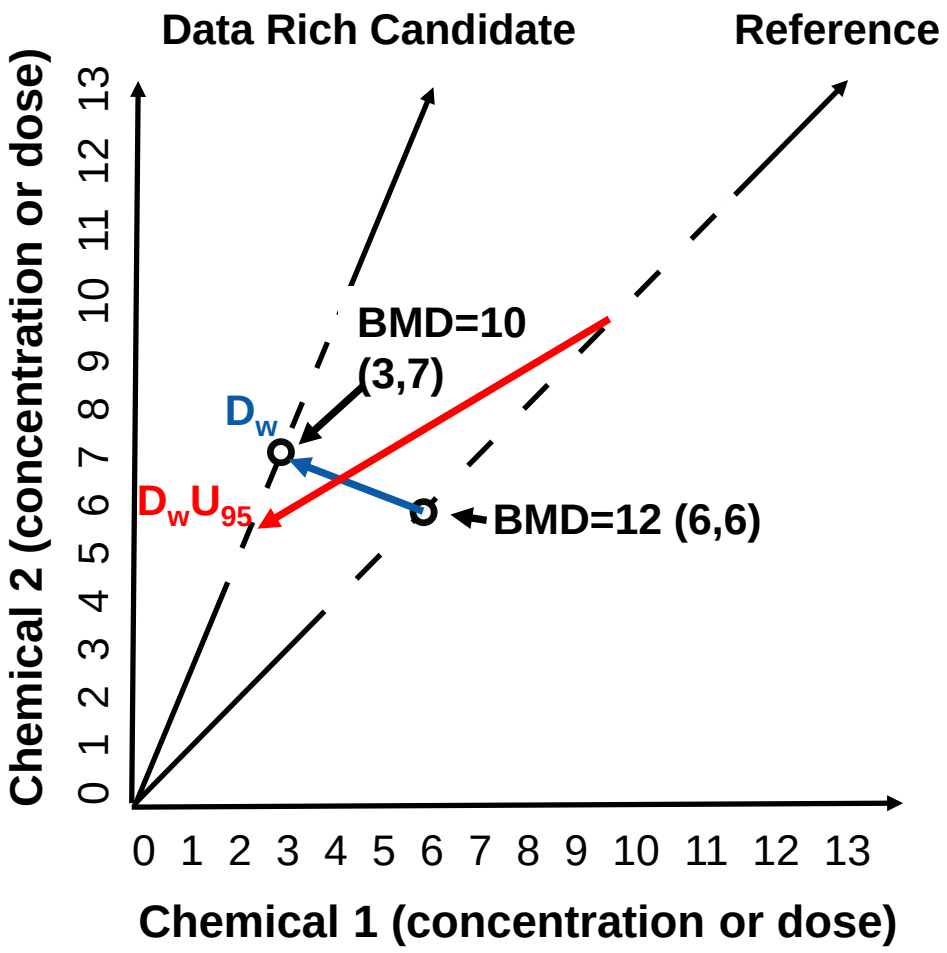


How MiST Works: Estimating D_w and D_wU_{95}: Data Rich

1. Data rich = BMD available for all compared mixtures

2. MiST estimates the Euclidean distance between the median BMDs (D_w)

3. MiST also estimates 95% upper bound on D_w (D_wU_{95}) using the two confidence intervals and Monte Carlo (MC) sampling method

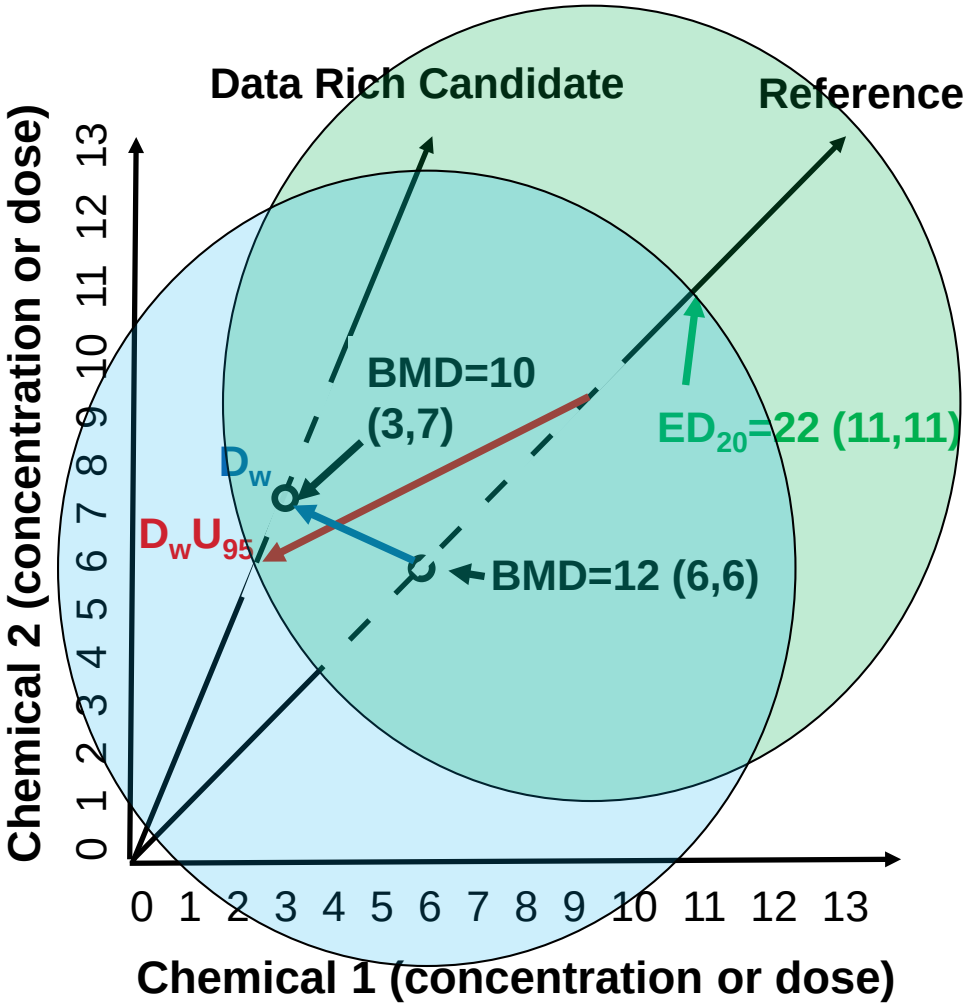


How MiST Works: Data Rich Comparison

1. Using ED_{20}=22, Δ =10 (maximum absolute value of the BMD–ED values)

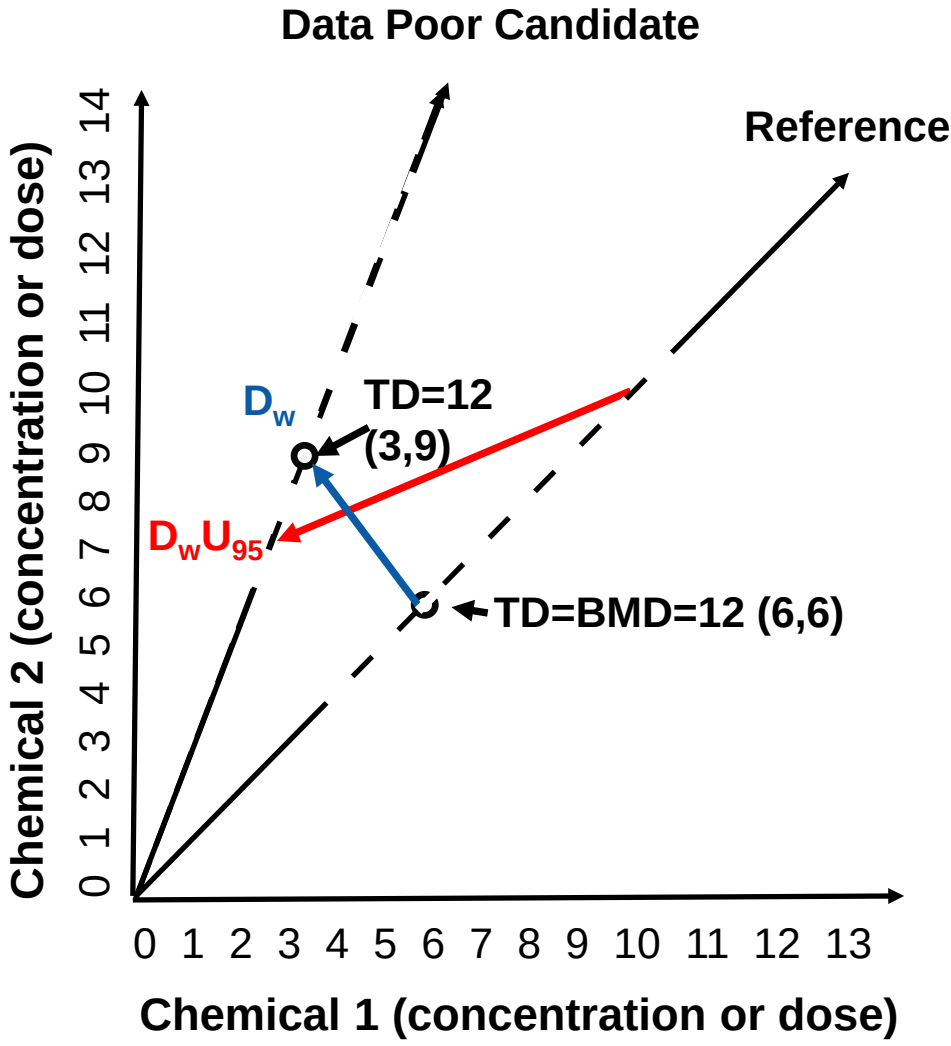
2. In this data rich comparison of Candidate and Reference 2 mixtures, D_w < Δ (blue circle)

3. <u>Also</u>, D_wU_{95} < Δ (green circle). Therefore, MiST determines that Candidate and Reference <u>are</u> similar



How MiST Works: Estimating D_w and D_wU_{95}: Data Poor

1. Data poor scenario = BMD not available for Candidate with mixing ratio 1:3
2. Assume Candidate and Reference Mixture Total Dose (TD) & distributions are the same (TD_c=TD_r)
3. D_w is Euclidean distance from Reference BMD (TD_r) to Candidate TD (TD_c), where TD_r=TD_c
4. MiST estimates 95% upper bound on Dw (D_wU_{95}) by Monte Carlo (MC) method



Weighted Estimation of D_w

Estimate *Euclidean distance* using weighted mixing ratios.

$$D_w = \sqrt{\sum_{j=1}^{c} W_j \left(\theta_{jr} - \theta_{ji}\right)^2}$$

D_w -- the weighted distance estimated from available dose-response data

θ – Contribution of each mixture component to the total dose BMD

W – weighted relative potency of each chemical component (congener)

Subscripts r, i represent reference and candidate mixtures

Subscript j represents the jth of C mixture chemical components (congeners)

D_w estimates for our simplified examples, assuming relative potency weights of 1:

Data Rich $\sqrt{1 * (6-3)^2 + 1 * (6-7)^2} = \sqrt{10} = 3.2$

Data Poor $\sqrt{1 * (6-3)^2 + 1 * (6-9)^2} = = \sqrt{18} = 4.2$

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Case Study Example: Sufficient Similarity Evaluation of 4 Aroclor Mixtures with Neurotoxicity Data

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Case Study Examples

Two Case Examples of PCB Mixture Similarity Testing

1. Assess sufficient similarity of 4 Aroclor Mixtures (*Data rich; uses congener relative potencies*)
   - Rodent assay assessing neurotoxicity after chronic exposure to 4 Aroclor (AR) mixtures in adult animals
   - Congener relative potencies based on *in vitro* neurotoxicity data and derived for untested congeners using Quantitative Structure Activity Relationships (QSAR)

2. Assess sufficient similarity of an environmental mixture compared to Aroclors (*Data poor; no relative potency data*)
   - Simulated fish mixture compared to Aroclor 1254 or Aroclor 1016

Aroclor Comparison Analysis

An Assessment of Neurotoxicity of Aroclors 1016, 1242, 1254, and 1260 Administered in Diet to Sprague-Dawley Rats for One Year

G. B. Freeman,*,[1] R. A. Lordo,* A. W. Singer,* A. C. Peters,* B. H. Neal,† E. E. McConnell,‡ and B. A. Mayes§

Battelle, Columbus, Ohio, 43201; †JSC, Inc., Arlington, Virginia; ‡ToxPath, Inc., Raleigh, North Carolina; and §General Electric Company, Environmental Research Center, Schenectady, New York 12301

Freeman et al. 2000 Tox Sci 53:2:77-391

7-8 wks age
N=10/sex/dose group

DOSING (52 weeks)

Aroclor 1016 (50, 100, 200 ppm)
Aroclor 1242 (50, 100 ppm)
Aroclor 1254 (25, 50, 100 ppm)
Aroclor 1260 (25, 50, 100 ppm)

EVALUATION
(Functional Observation Battery)

Autonomic
Muscle tone/equilibrium
Sensorimotor response
Central nervous system
Physiological

Case Example Analysis

- Performed BMD modeling on one endpoint (landing foot splay) at 26 weeks of exposure
  - Calculated BMD and CDF using EPA's BMDS Software
  - Used congener toxicological potency values for neurotoxicity from Pradeep et al. (2019) Integrating Data Gap Filling Techniques: A Case Study Predicting TEQs for Neurotoxicity TEQs to Facilitate the Hazard Assessment of Polychlorinated Biphenyls. *Regul Toxicol Pharmacol*. 101:12-23

- Assessed similarity between a candidate mixture (Aroclor 1254) and three reference mixtures (Aroclors 1016, 1242, and 1260)

Mixtures Modeling Inputs

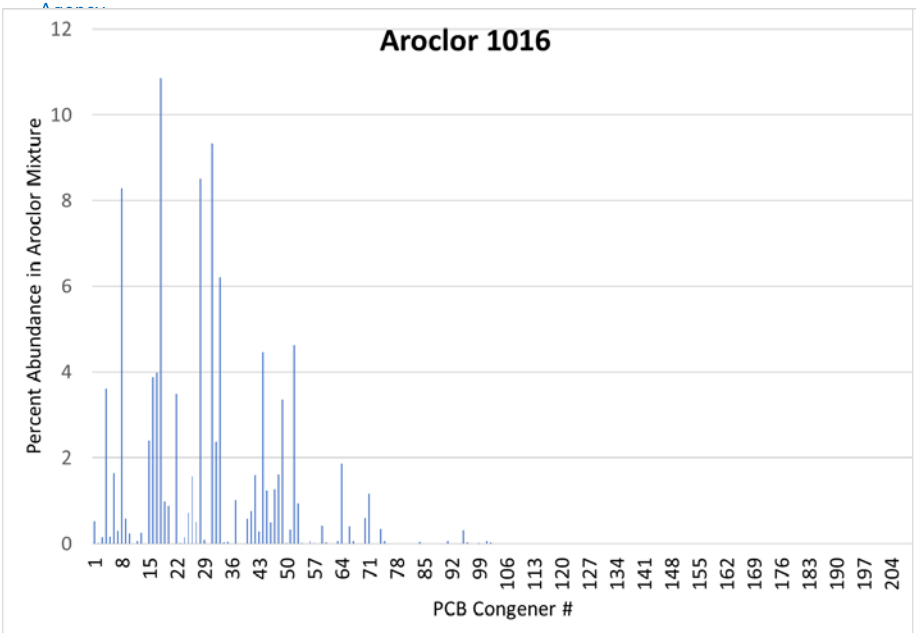
Required information:

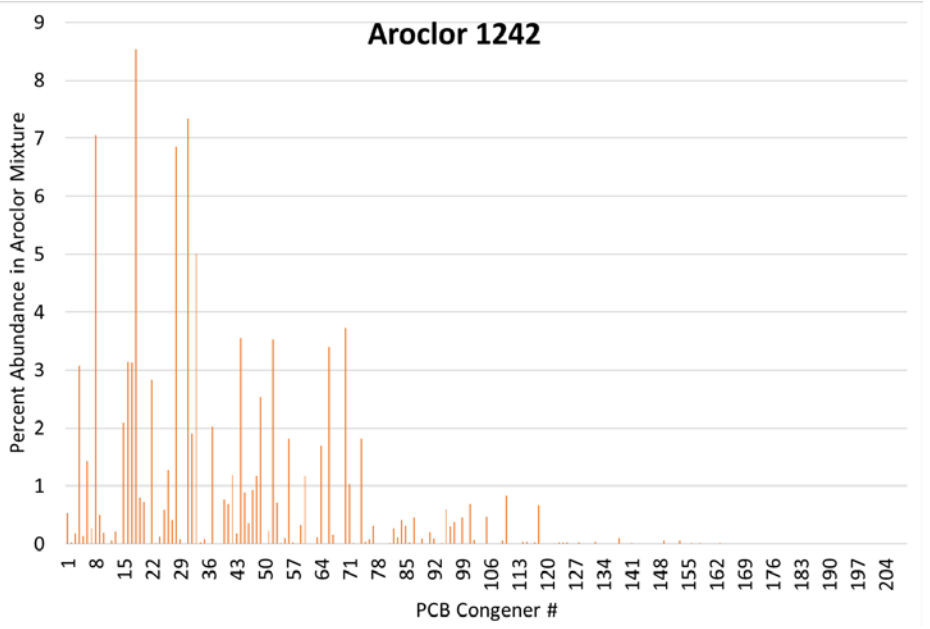
- ✓ Mass fraction of each congener (reference mixture)
- ✓ Mass fraction of each congener (candidate mixture)
- ✓ BMD ± SD or BMD CDF (reference mixture)
- ✓ ED (reference mixture)

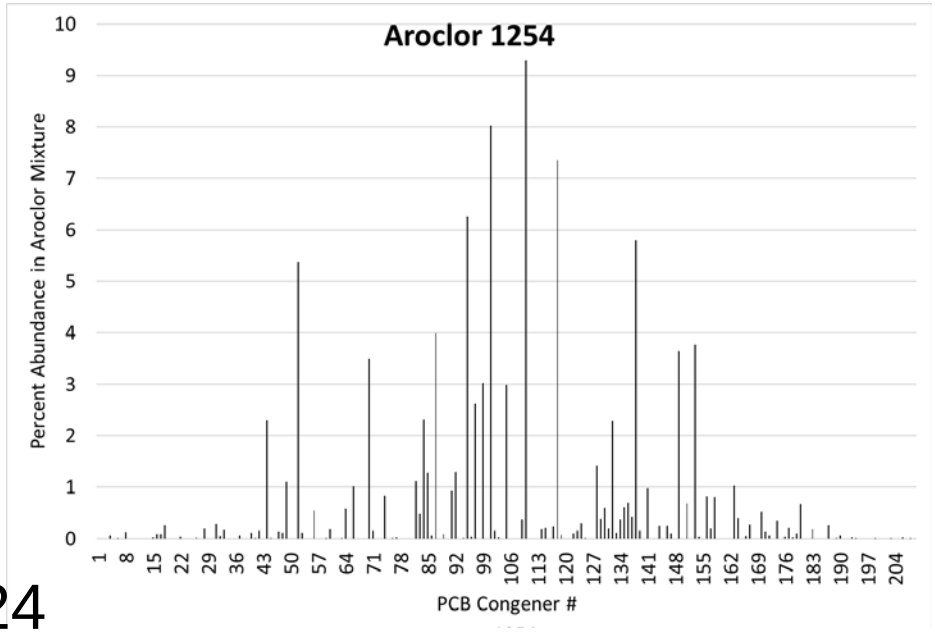
Optional information:

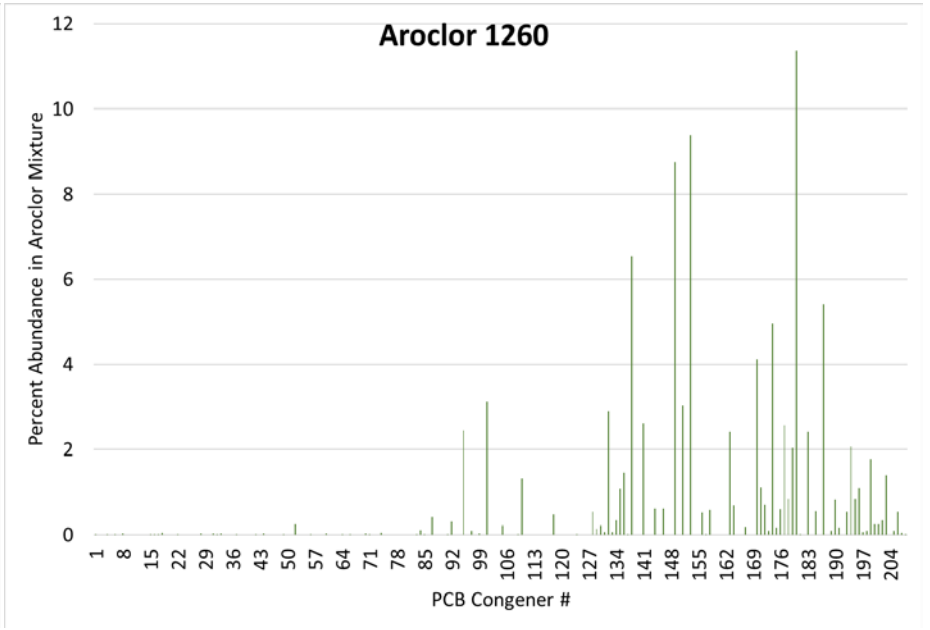
- ✓ BMD ± SD or BMD CDF (candidate mixture)
- ✓ Relative toxicological potencies of congeners
  - For this case study, neurotoxicity equivalency factor values (NEFs)

Aroclor Profile Comparison









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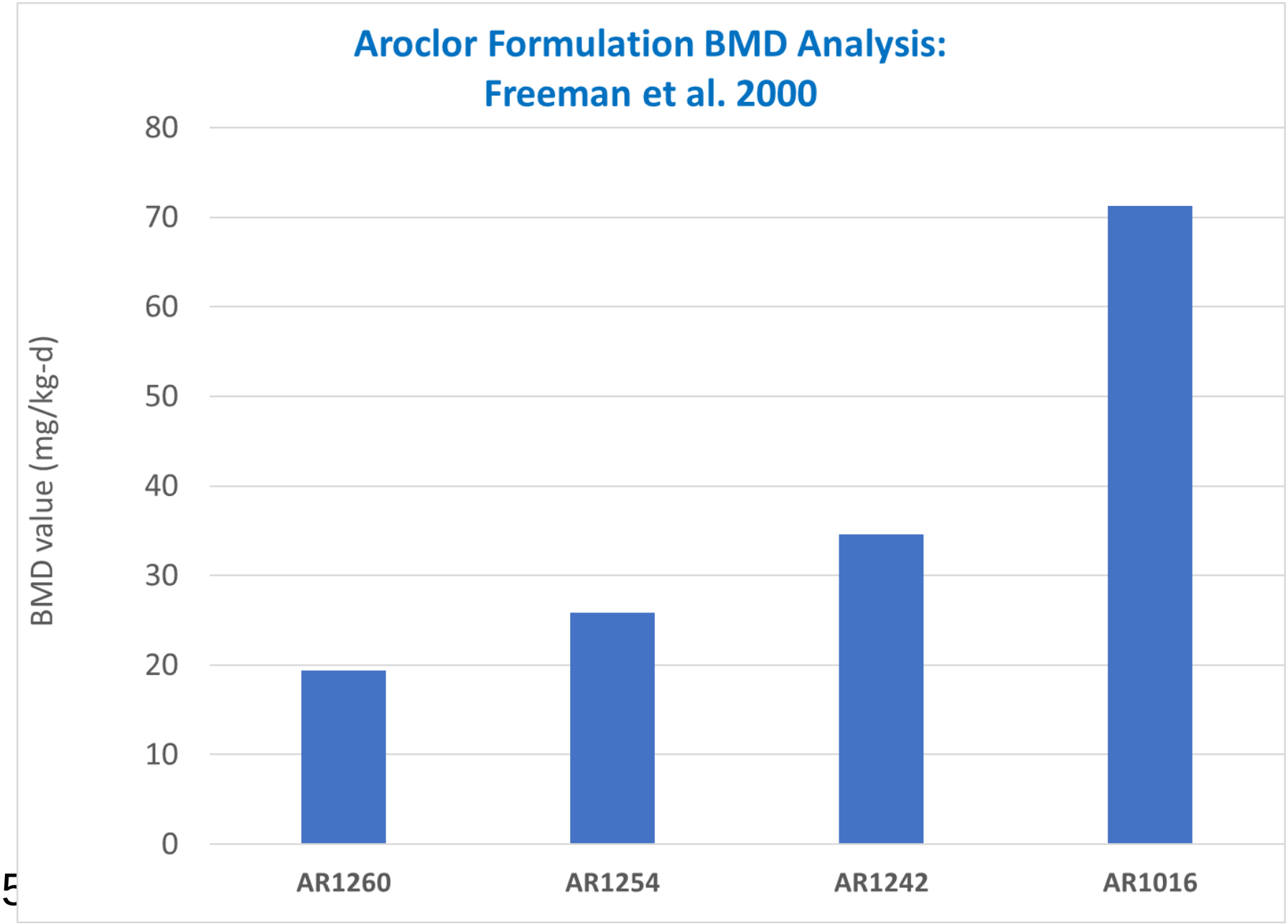

Mixtures Similarity Testing Results

Weighted Analysis of 4 Aroclor Mixtures				
	AR 1254 (candidate)	AR1242 (reference)	AR1260 (reference)	AR1016 (reference)
BMD	25.86	34.56	19.36	110.38
ED	77.56	103.69	58.09	331.14
Delta |BMD-ED|	51.71	69.13	51.71	220.76
Dw		15.07	39.04	3515.1
Upper 95th		58.67	309.61	34458.18
conclusion		acceptable	not acceptable	not acceptable
rank		1	2	3

- Thus, AR 1254 could be considered sufficiently similar to AR1242 (Dw upper 95th < Delta) but **not** to AR 1260 or AR 1016 (Dw upper 95th > Delta).
  - The BMD was estimated for 10% response level (BMD_{10}) and the ED was estimated for a 30% response (ED_{30})

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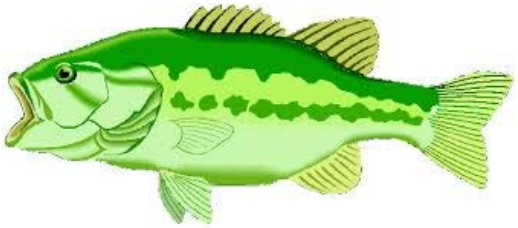
Case Study Example: Environmental Mixture Comparison

Comparing Aroclors with Environmental PCB Mixture

- Representative mixtures
  - Fox River fish mixture *(Kostyniak et al. 2005 Tox Sci 88:2:400-411)*
- Tested for similarity to AR1254 or AR1016 (based on congener profiles from *ATSDR 2000 Toxicological Profile for PCBs*)



Mixtures Modeling Inputs

Required information:

- ✓ Mass fraction of each congener (reference mixture)
- ✓ Mass fraction of each congener (candidate mixture)
- ✓ BMD ± SD or BMD CDF (reference mixture)
- ✓ ED (reference mixture)

Optional information:

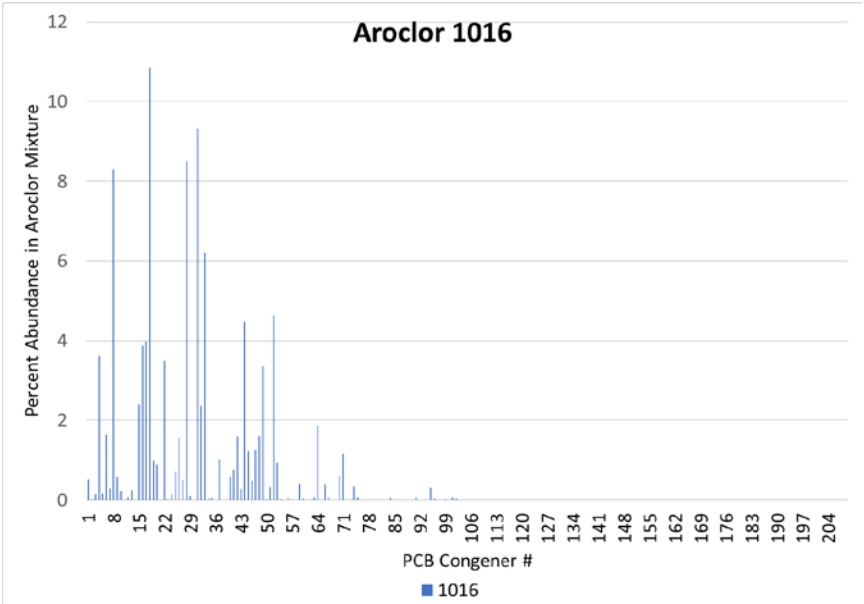
- ❑ BMD ± SD or BMD CDF (candidate mixture)
- ❑ Relative toxicological potencies of congeners

Environmental Mixture Testing
Fish

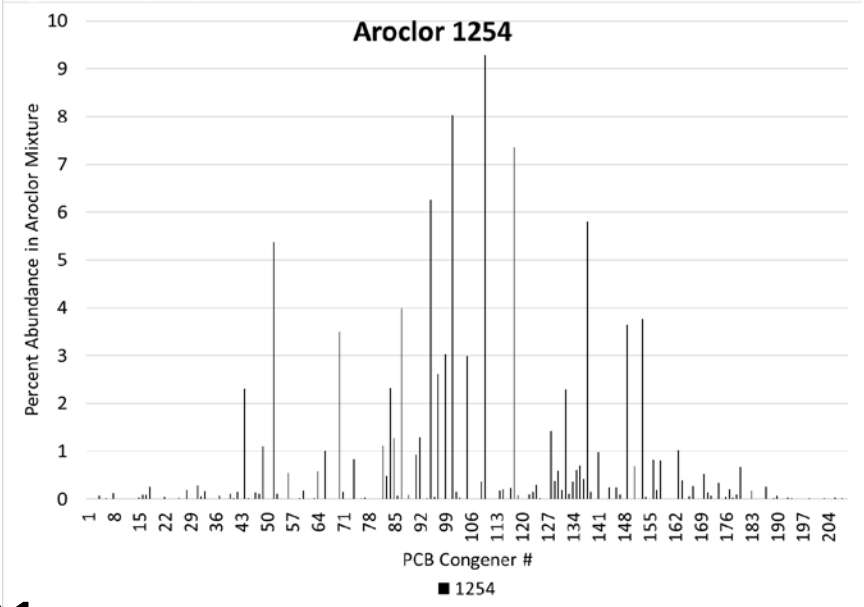
Environmental Mixtures: Unweighted Similarity Testing		
	Fox River Fish-Candidate	
	AR 1016-Reference	AR1254-Reference
BMD	110.38	25.86
Delta |BMD-ED|	220.76	51.71
Dw	5643.1	6.7
Upper 95th	38880.7	11.0
conclusion	not acceptable	acceptable
rank	2	1

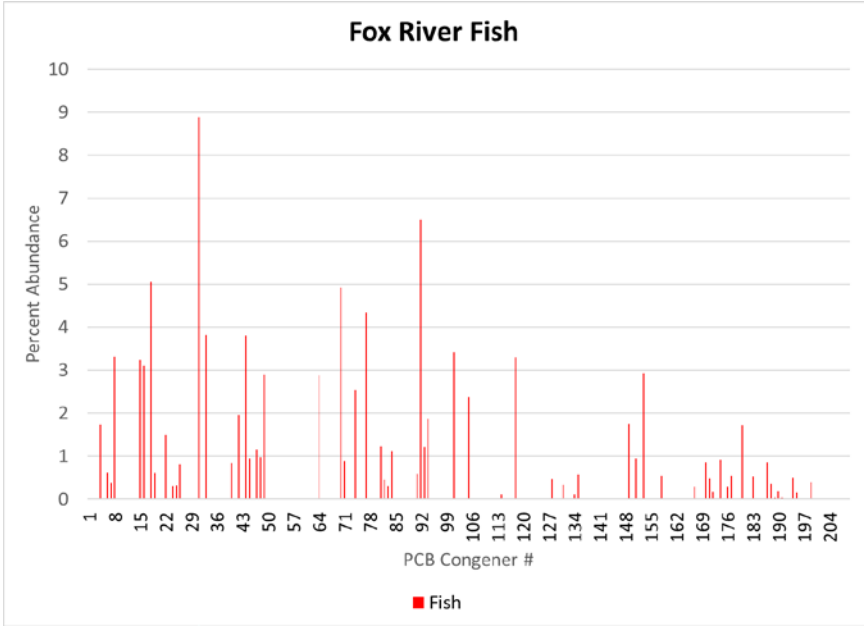
- Based on this example analysis AR 1254, but not AR1016, could be considered an acceptable surrogate for the Fox River Fish mixture

Congener Profile Comparisons



-fish profile overlaps more with congener profile of AR 1254 than AR 1016





Environmental Mixture Analysis: *Caveats and Challenges*

- 209-congener analyses are expensive and relatively rarely conducted
- Methods used to address congener co-elutions and values below the method quantitation/detection limit
  - For these case examples, co-elutions were treated as containing an even split of the congeners in the co-elution, and values below the limit of quantitation were treated as zero
- Environmental samples are inherently heterogeneous, samples will be location dependent and not always generalizable across matrices
  - Ex: fish samples from the Fox River are not generalizable to fish samples from other locations
  - Ex: soil samples are not generalizable to dust, water, or air samples

How Could This Method Complement the IRIS PCB Assessment?

- Modeling to support evaluations of sufficient similarity across PCB mixtures
  - Group datasets for sufficiently similar PCB mixtures to develop reference values
  - Use with the final assessment to apply reference values to sufficiently similar PCB mixtures in the environment
  - Methods will be described in the assessment but also published in the peer-reviewed literature prior to assessment release

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Summary

- EPA has extended the mixtures modeling methods developed by Marshall et al. 2013 to facilitate sufficient similarity analyses for comparing PCB mixtures
- Sufficient similarity approaches can be used to identify suitable dose-response data to apply in risk assessments of environmental or untested PCB mixtures
- Subsequent presentations will discuss potency estimation approaches and provide more details on how analyses are conducted using MiST

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  - Chris Gennings, Mount Sinai

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