Empirical Whole Mixture Approach for Risk Evaluation: Combining Exposure and Mixtures Toxicity Data

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Overview

- Motivation
- Component-based approach vs. whole mixture approach
- Testing FOR sufficient similarity:
  - Data rich case
  - Data poor case
  - Illustration: mixtures of pesticides in child care centers
- … a word on selecting which mixture(s) to evaluate with a case study
- Discussion

Setting the stage…

- Humans are exposed to a diverse and complex array of chemical mixtures (83K in production in U.S.)
- Regulatory agencies and legislative acts are generally focused on specific categories of chemicals or products
  - e.g., EPA office of pesticides, FDA, CPSC; FQPA, Safe Drinking Water Act
- First step in cumulative risk assessment is identifying group of chemicals for combined evaluation
  - Co-occurrence of chemicals
  - Common adverse outcome

Component-based approach

- Default assumption of additivity –i.e., the effect of a mixture can be predicted from the individual dose-response curves
- Dose addition
  - Common mechanism/mode of action
  - Common adverse outcome
- Independent action assumes stochastic independence models
- Hybrid - Integrated addition

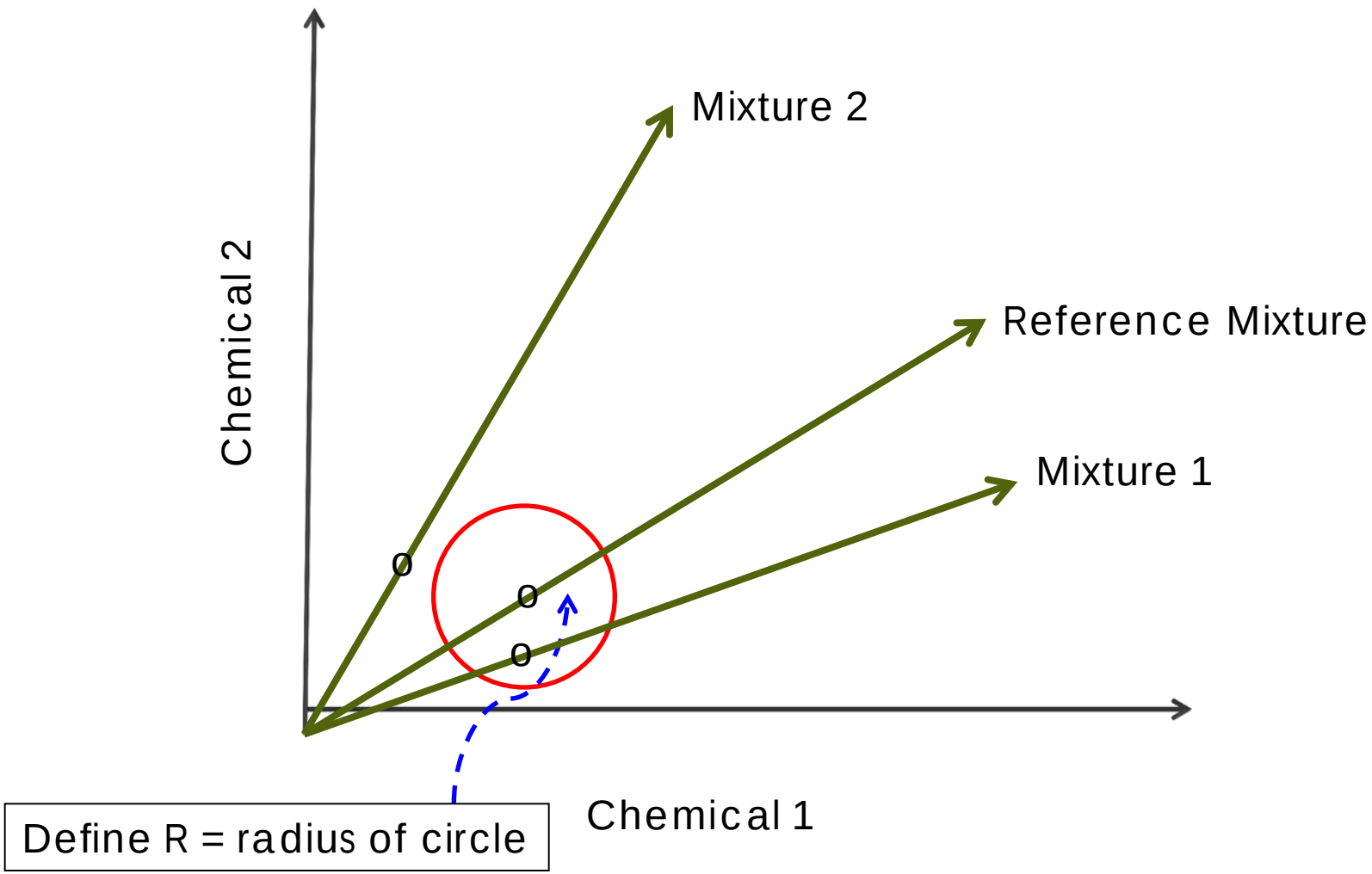
Whole mixture approach

- At first glance an impossible problem, BUT…
- Mixtures may be intentionally formulated/generated by processes derived by humans – reduces the number of relevant exposures (Tornero-Velez et al, 2011)
- Guidance documents allow for 'sufficiently similar' mixture(s) to act as surrogate(s) for other mixtures
- Perhaps a manageable number of mixtures may be thoughtfully selected and evaluated which are sufficiently similar to a wide variety of human exposures

Whole mixture approach

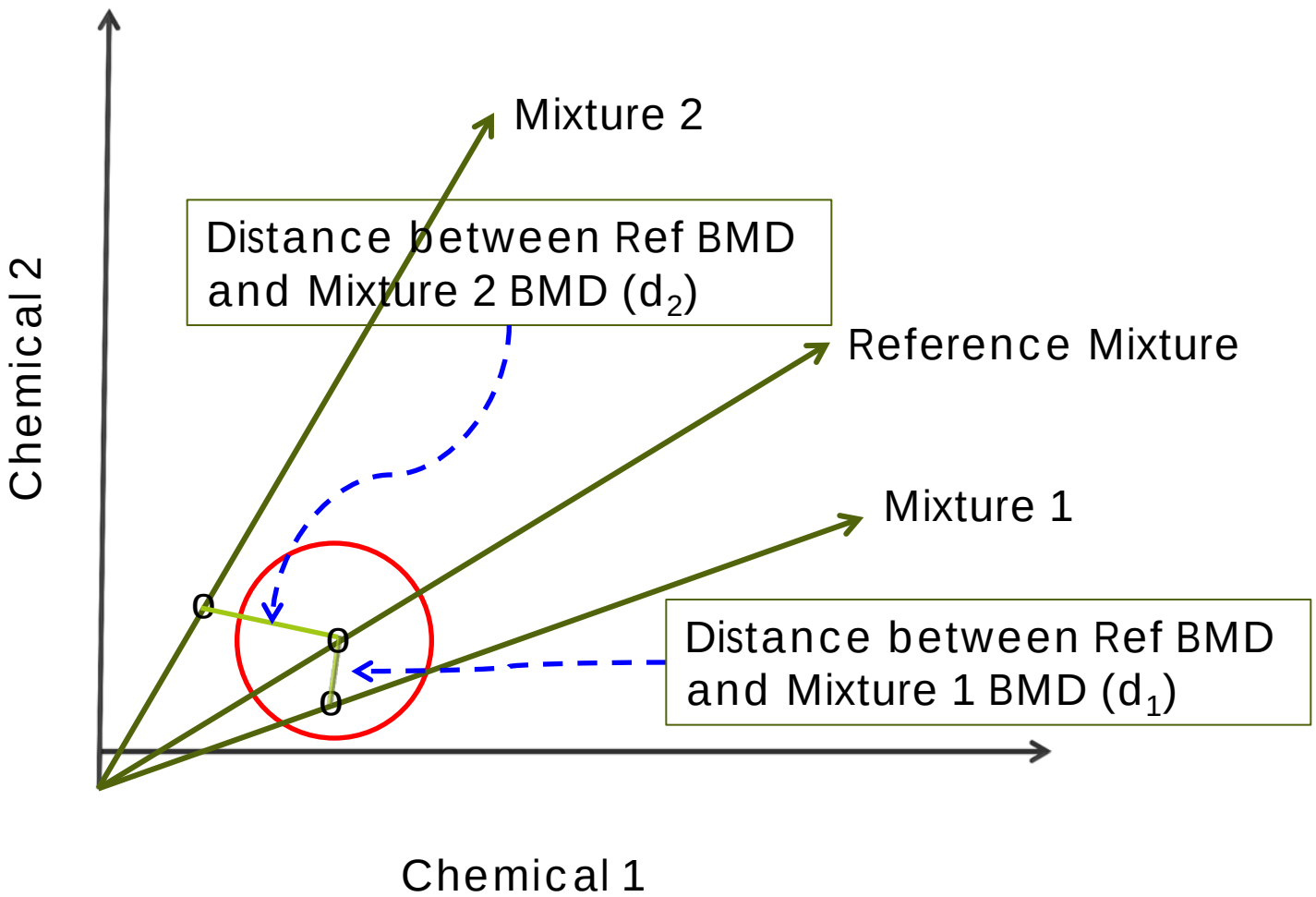
- Whole mixture approaches do not require default assumptions of additivity.
- We define sufficient similarity, without assuming additivity, using equivalence testing methodology comparing the distance between benchmark dose estimates for mixtures in both data rich and data poor cases.
- 2-dimensional schematics are readily generalizable to higher dimensions

DATA Rich Scenario Schematic



o depicts BMD
Red circle depicts the Similarity Region

DATA Rich Scenario Schematic



o depicts BMD
Red circle depicts the Similarity Region

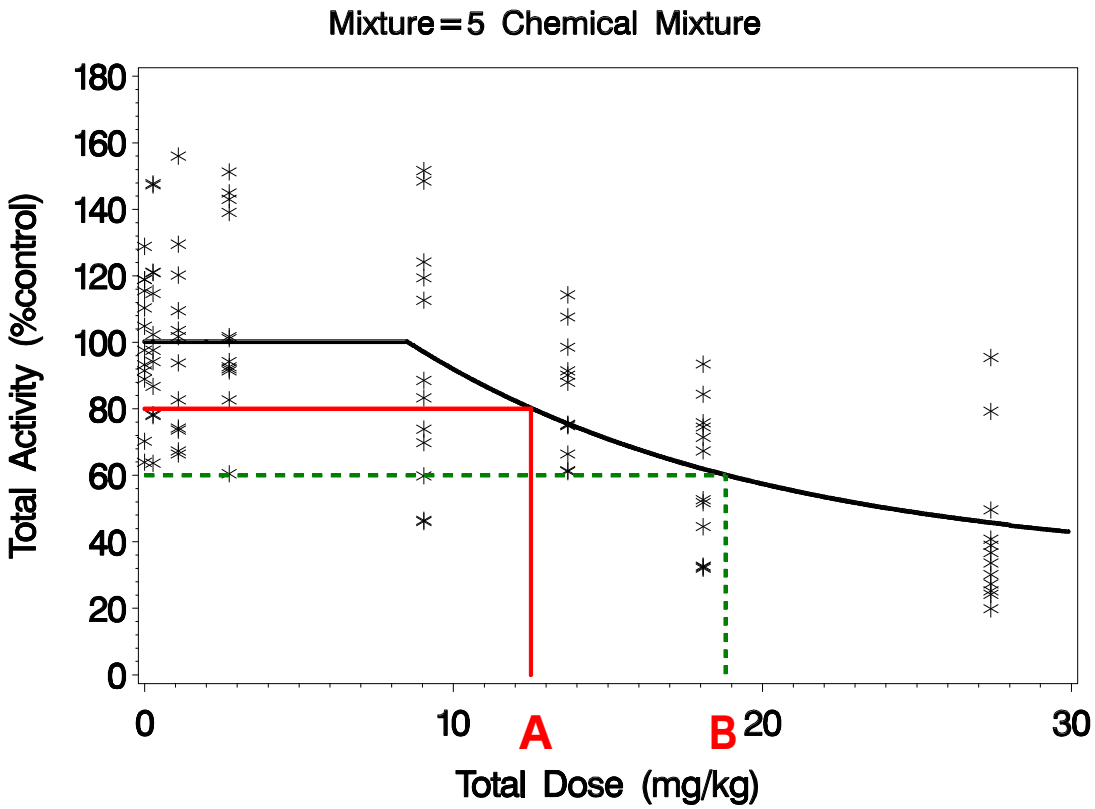
Test FOR Sufficient Similarity

- Define the radius of the similarity region to be R
- Define d_i as the distance between the BMD for the reference mixture and the ith candidate mixture (as specified from exposure data).
- The estimate for d is determined from the estimated BMDs.
- TEST FOR Sufficient Similarity:

$$H_0: \ d > R \text{ vs } H_1: d < R$$

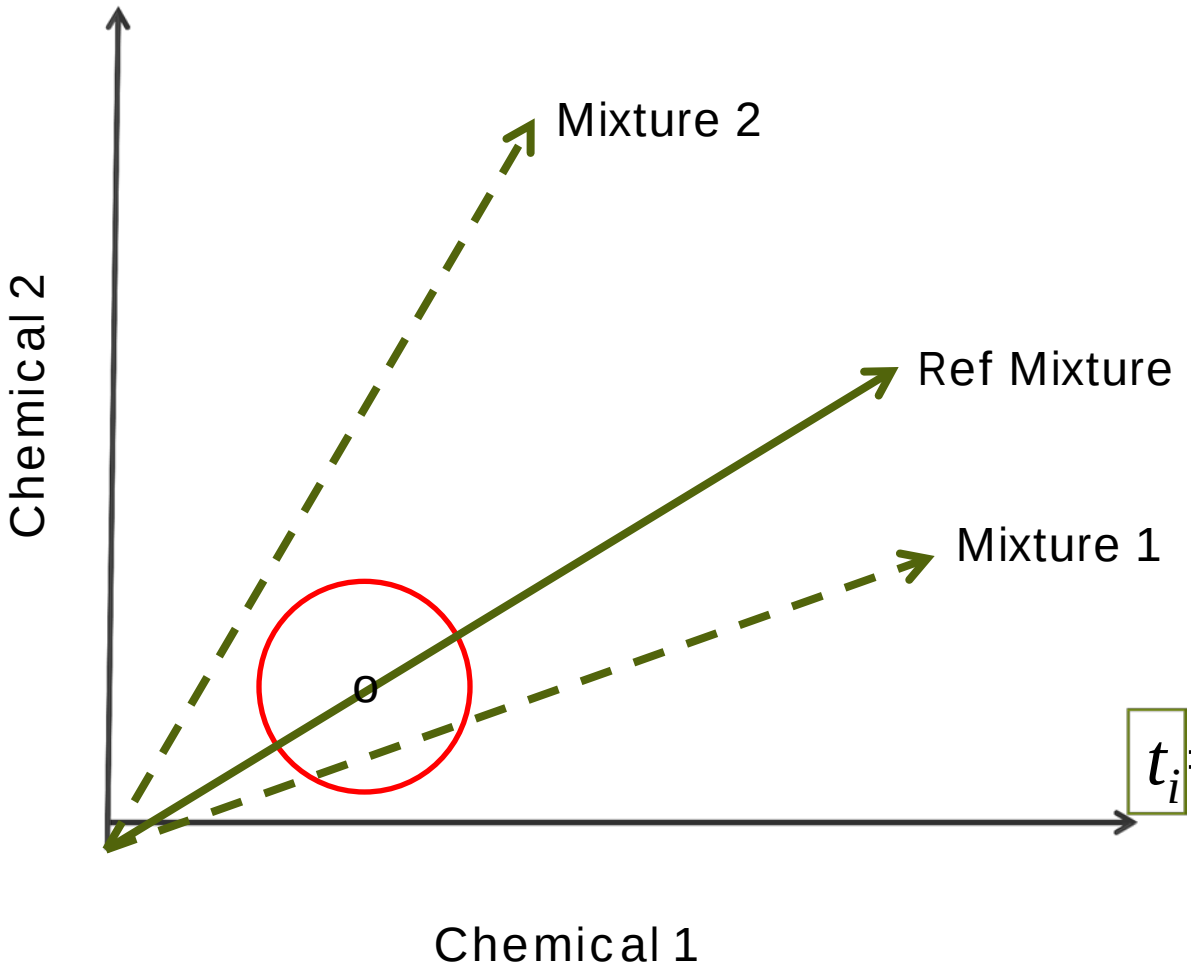
- Reject H_0 if upper confidence limit on $d < R$

Specifying R using Expert Judgment



Define R as the **distance** between A and B

DATA Poor Scenario Schematic

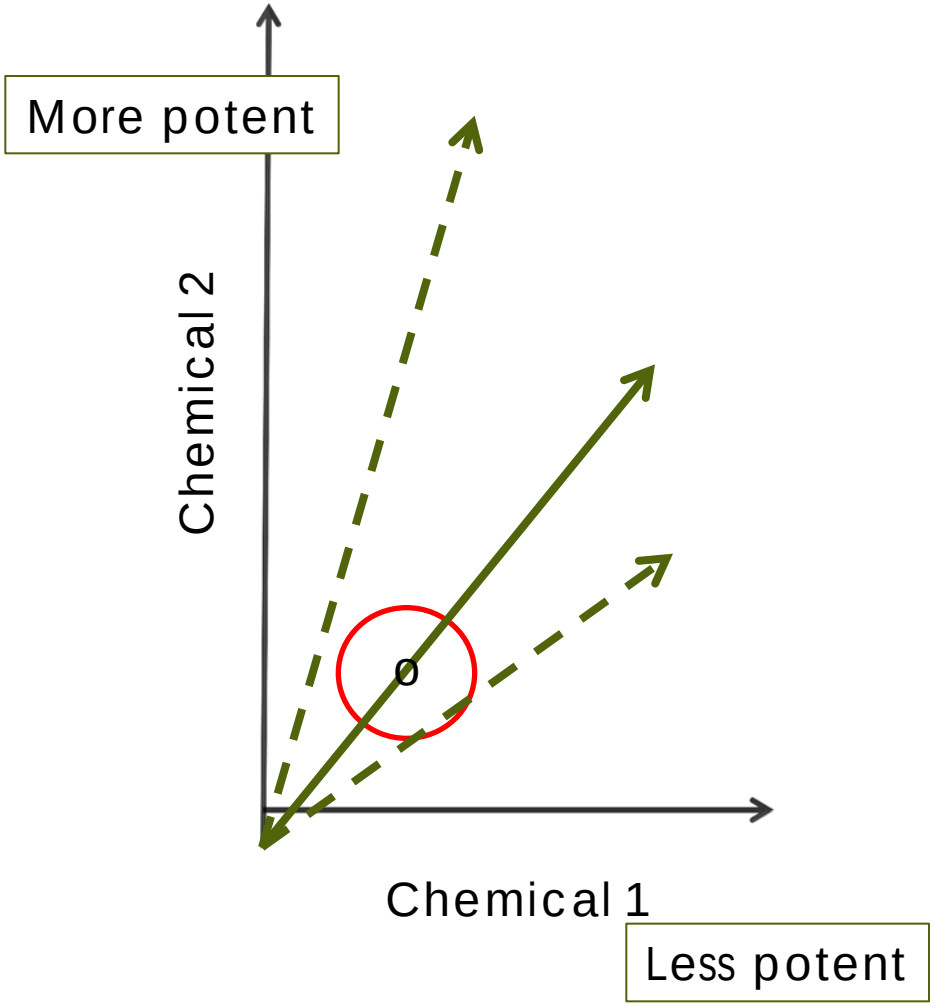


$$\theta_r = t_r \, \boldsymbol{a}_r$$

$t_i = m t_r$ for m close to 1

$$\theta_i^{adj} = t_r \, \boldsymbol{a}_i$$

DATA Poor Scenario Schematic adjusted by weights

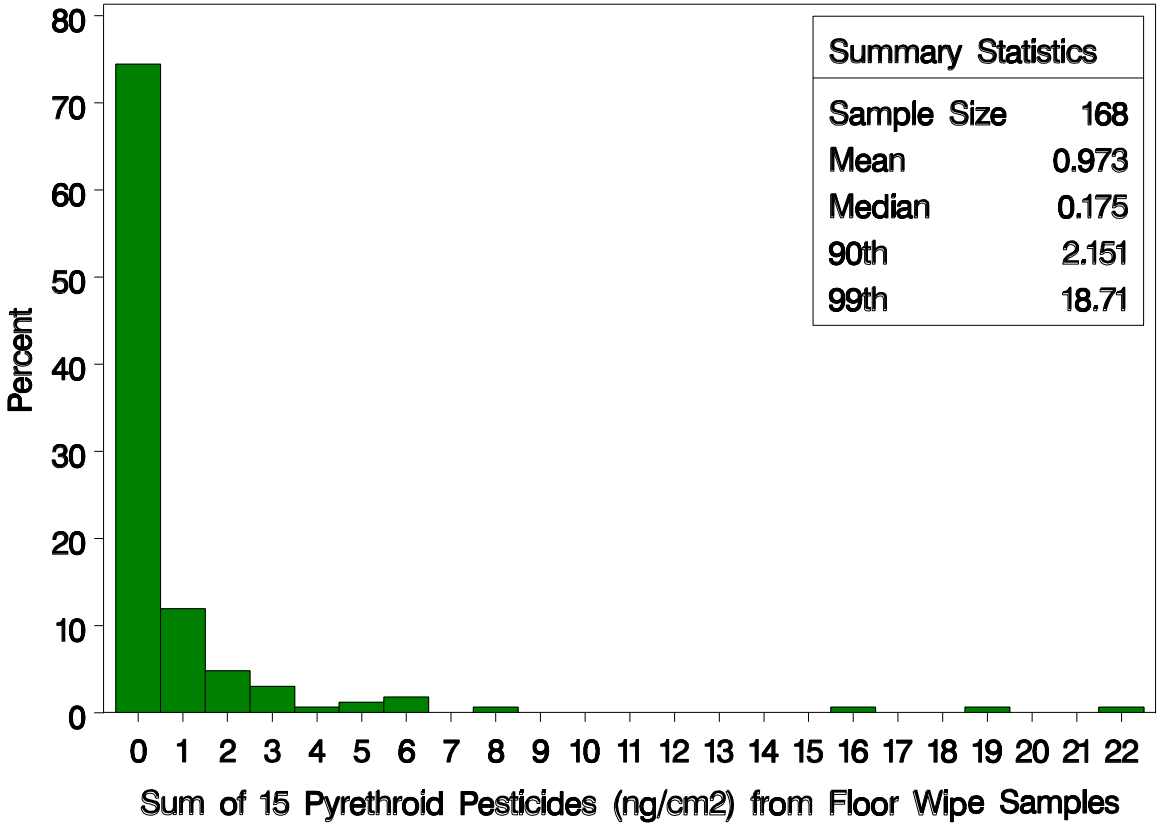


Weights are determined using relative potencies

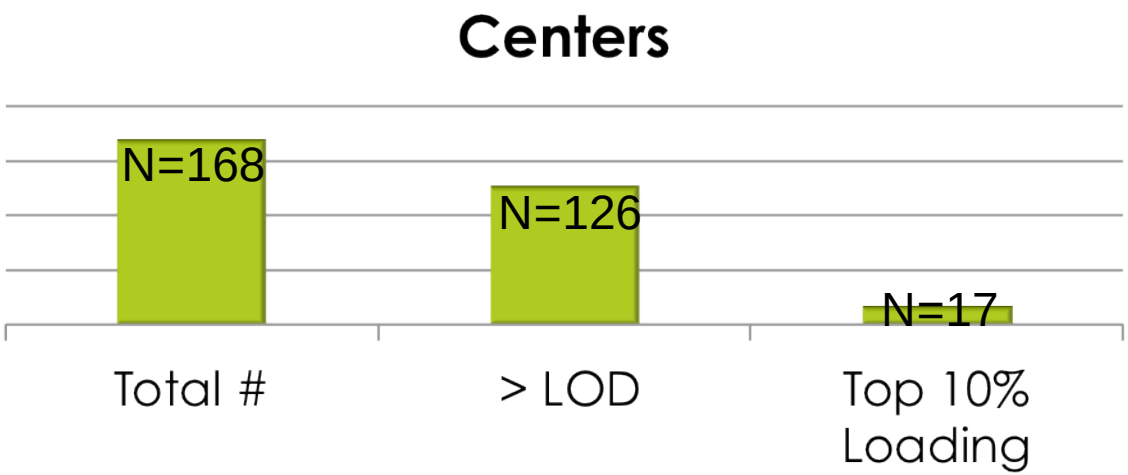
Motivating Case Study (Tulve et al, 2006)

- The First National Environmental Health Survey of Child Care Centers (CCC Study) was a probability-based national study of child care centers with a focus on aggregate exposure of children to pesticides.
- The study included 168 child care centers from 30 sampling units and measured pesticide occurrence.
- Samples were collected from July - October 2001 at multiple locations within a center by means of floor, tabletop, and desk wipes.
- Here, we consider only floor wipe data for pyrethroid pesticides.

Figure 1: Histogram of the sum of 15 pyrethroids/pyrethrins measured from floor wipes in 168 child care centers in the CCC Study.



Motivating Case Study (Tulve et al, 2006)



The average residues from the top five chemicals in the 17 “top” centers accounted for 96% of the sum.

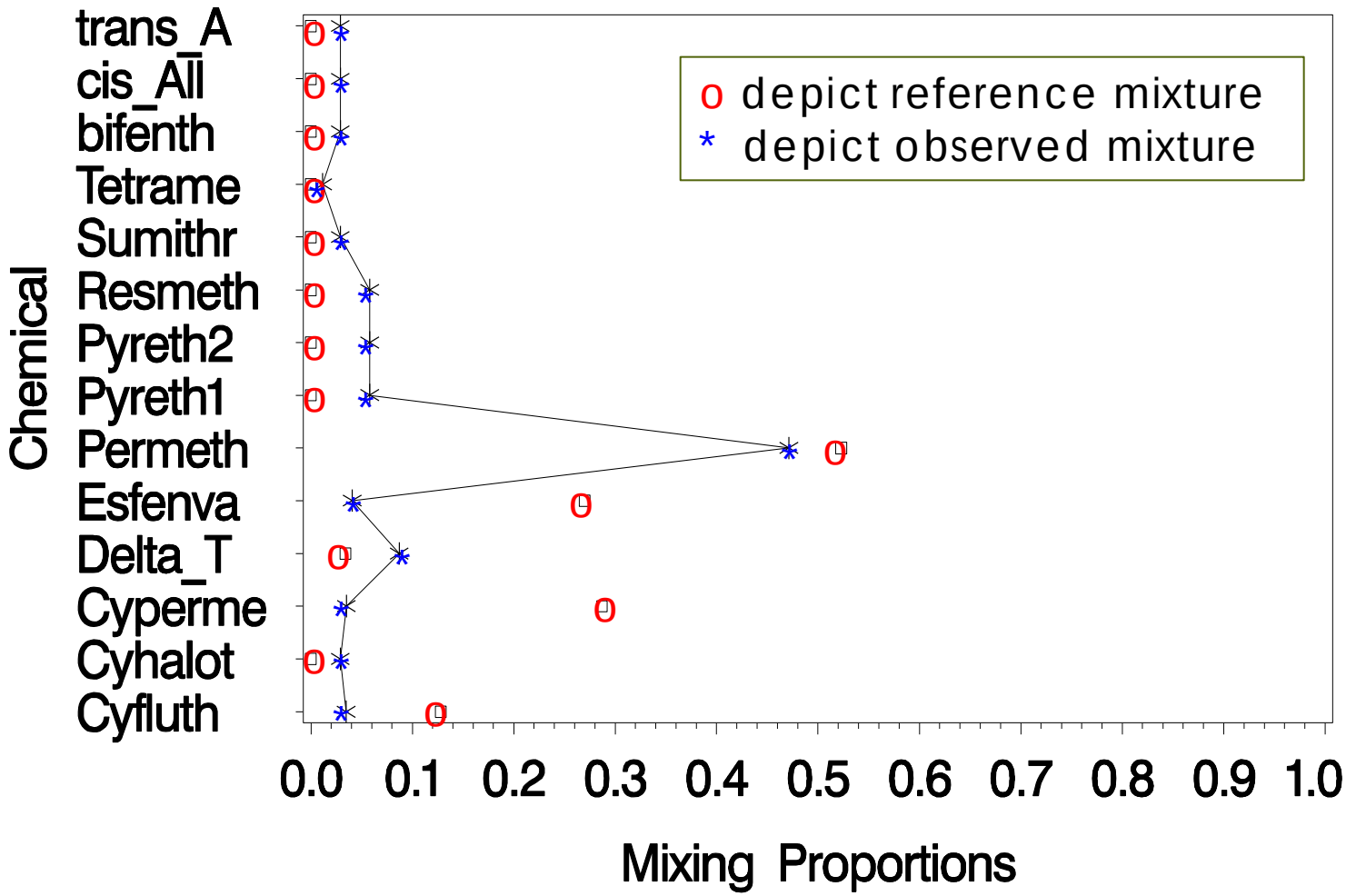
This average was the mixing ratio used in a neurotoxicity study of five pyrethroids (Wolansky et al).

Table 1: Summary percentage of pyrethroids measured from floor wipes in the CCC Study in the top 10% of the centers (in terms of total loading).

Chemical	Average percent in top 10% of CCC Study Centers	Normalized for top 6 to sum to 100*	Relative Potency Estimates**
Cyfluthrin	12.4	12.8	1.14
Cypermethrin	27.8	28.7	0.24
Delta/tralomethrin	3.2	3.4	1.00
Esfenvalerate	2.6	2.7	2.09
Cis-permethrin	19.1	52.3	0.06
trans-permethrin	31.3		
cis-allothrin	0.6	0	Set at 1.0
trans-allothrin	<1	0	Set at 1.0
Bifenthrin	<1	0	0.78
Cyhalothrin	1.9	0	1.90
Pyrethrin I	<1	0	Set at 1.0
Pyrethrin II	<1	0	Set at 1.0
Resmethrin	<1	0	0.01
Sumimethrin	<1	0	Set at 1.0
Tetramethrin	<1	0	Set at 1.0

* used by Wolansky et al in mixture dose-response study of neurotoxicity;

** Relative potency estimates from Wolansky et al (Wolansky *et al.* 2006; Wolansky *et al.* 2009); chemicals not evaluated by Wolansky et al have relative potency estimates set at 1.0



Upper 95% Conf Int on d = 4.48

Results from Case Study

- A weighted analysis was performed using relative potency estimates from Wolansky et al
- There were 114 centers from which the observed mixture is considered sufficiently similar to the reference mixture.
- That is, the upper confidence limit on the distance between the estimated reference BMD and the estimated BMD from the center's observed mixture was below the similarity boundary.
- 90% of the centers with at least some residue concentrations > LOD (N=126) were determined to be sufficiently similar to the reference mixture identified by the toxicology study.

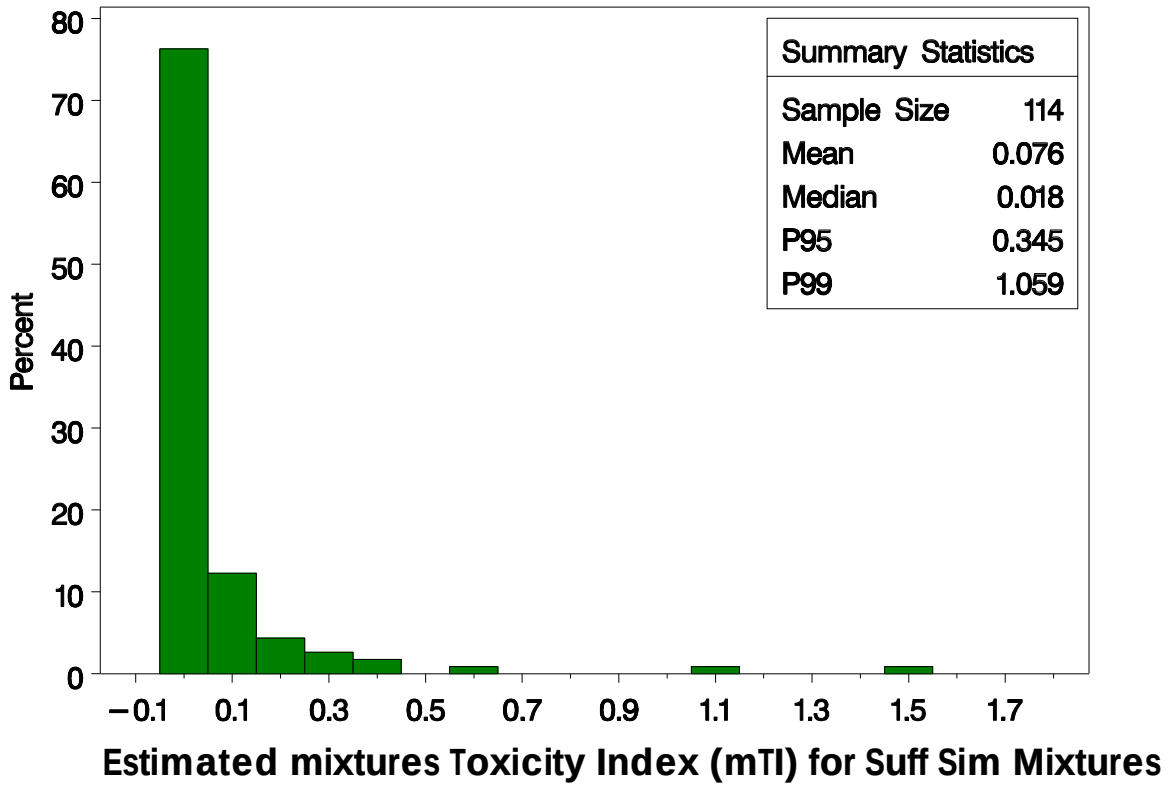
Define a mixture Toxicity Index (mTI)

$$mTI = \sum_{j=1}^{c} \frac{DI_{\mathrm{j}}\ (\mu\mathrm{g/kg/day})}{m\mathrm{RfD}_{\mathrm{j}}\ (\mu\mathrm{g/kg/day})}$$

where

- The $mRfD_j$s are defined from the component proportions of the mixture BMDL with adjustments for uncertainty
- The DI_js are calculated assuming a dermal exposure model (Morgan et al, 2005)

Figure: Histogram for the distribution of the mTI estimates using exposure data considered sufficiently similar to the reference mixture (defined by a weighted distance between the BMDs of less than 2.1 mg/kg).



The index is calculated assuming a 3 year old boy, 14.2 kg and 96 cm tall.

But which mixtures should be experimentally evaluated as the reference mixture(s)?

Selecting Mixtures to Evaluate

- Tornero-Valez et al, 2011 used biogeographic approaches used by ecologists to describe the formation of communities of animal species
  - Showed the co-occurrence pattern of pesticides in CCC is not random but resulted in specified combinations
- Maximum cumulative ratio (MCR) for screening mixtures (Price and Han, 2011)
- Principal components analyses have also been suggested (e.g., Smith et al) to identify relationships among components of mixtures

Selecting Mixtures to Evaluate: Case Study

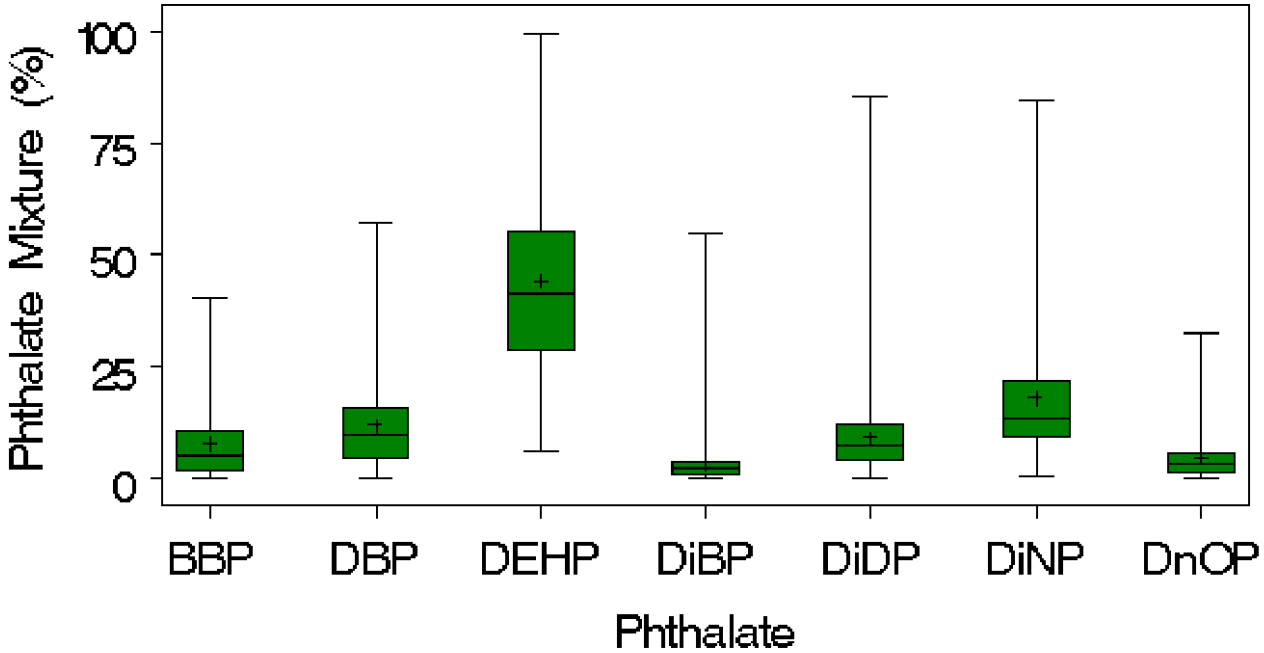
Phthalate monoesters	Parent diesters
Low molecular weight	
Mono-n-butyl (MBP)	DnBP
Mono-iso-butyl (MIB)	DiBP
Mono-benzyl (MZP)	BBP
High molecular weight	
Mono-(2-ethyl-hexyl) (MHP) Mono-(2-ethyl-5-hydroxyhexyl) (MHH) Mono-(2-ethyl-5-oxohexyl) (MOH) Mono-(2-ethyl-5-carboxypentyl) (ECP)	DEHP
Mono-n-octyl (MOP)	DnOP
Mono-(carboxyisooctyl) (COP)	DiNP
Mono-(carboxyisononyl) (CNP)	DiDP

- Phthalates are considered to be anti-androgens; concerns for fetal development
- Biomonitoring data (NHANES, 2005-06) includes phthalate monoesters in pregnant women (N=130)
- LMW phthalates found in medicines, adhesives, cosmetics, etc
- HMW phthalates found in soft plastic (toys, food containers, etc)

Case Study: Phthalate Mixtures

- Concentrations of phthalate monoesters were used to estimate the corresponding daily intake of the corresponding diesters
- Human metabolism studies provide excretion factor estimates linking urinary concentrations of monoesters to a parent compound (David, 2000; Koch et al, 2007)

Case Study: Phthalate Mixtures



Sample Size	130	130	130	130	130	130	130
25th	2.1	4.7	29	1.1	4.3	9.2	1.6
Median	5.3	9.9	42	2.5	7.5	13	3.2
75th	11	16	56	3.9	12	22	5.6
Maximum	41	57	99	55	85	84	33

Case Study: Phthalate Mixtures

Table: Pearson correlation coefficient estimates between estimated daily intakes (DI) of the seven phthalate diesters (log10 scale) for the 130 pregnant women in NHANES

Estimate (p value)	DiBP	BBzP	DEHP	DiNP	DiDP	DnOP
DBP	0.50 (p<0.001)	0.43 (p<0.001)	0.12 (p=0.174)	0.12 (p=0.172)	-0.03 (p=0.750)	-0.16 (p=0.062)
DiBP		0.23 (p=0.010)	0.20 (p=0.023)	0.12 (p=0.191)	0.10 (p=0.248)	-0.12 (p=0.182)
BBzP			0.12 (p=0.162)	0.13 (p=0.131)	0.09 (p=0.301)	-0.17 (p=0.048)
DEHP				0.53 (p<0.001)	0.39 (p<0.001)	0.05 (p=0.548)
DiNP					0.52 (p<0.001)	0.08 (p=0.394)
DiDP						0.10 (p=0.271)

Case Study: Phthalate Mixtures

- Conducted a principal components analysis (PCA) to evaluate exposure patterns in pregnant women.
- Analysis was conducted on log-transformed values due to severe skewness.
- Using the Rule of 1 (i.e., select PCs based on eigenvalues>1), only 2 PCs were selected
- These 2 PCs accounted for 56% of the variability in the exposure pattern of the estimated DI for the 7 phthalates

Case Study: Phthalate Mixtures

	PC1	PC2
DBP	**0.55**	**-0.64**
DiBP	**0.56**	**-0.45**
BBzP	**0.5**	**-0.47**
DEHP	**0.69**	**0.36**
DiNP	**0.72**	**0.45**
DnOP	**-0.08**	**0.50**
DiDP	**0.59**	**0.52**

Case Study: Phthalate Mixtures

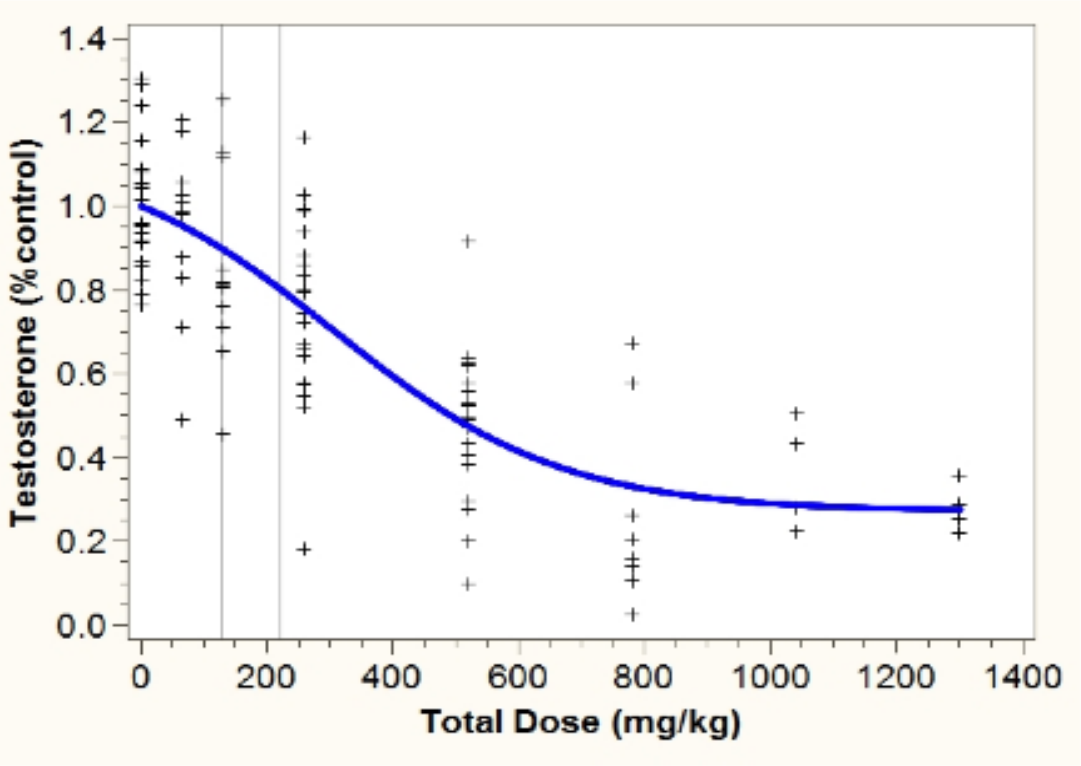
	PC1	proportions	PC2	PC2A	proportions	PC2B	proportions
DBP	**0.55**	0.15	**-0.64**	0.64	0.41	0	0
DiBP	**0.56**	0.16	**-0.45**	0.45	0.29	0	0
BBzP	**0.5**	0.14	**-0.47**	0.47	0.30	0	0
DEHP	**0.69**	0.19	**0.36**	0	0	0.36	0.20
DiNP	**0.72**	0.20	**0.45**	0	0	0.45	0.25
DnOP	**0**	0.00	**0.50**	0	0	0.5	0.27
DiDP	**0.59**	0.16	**0.52**	0	0	0.52	0.28
SUM	**3.61**	**1**		**1.56**	**1**	**1.83**	**1**

Case Study: Phthalate Mixtures

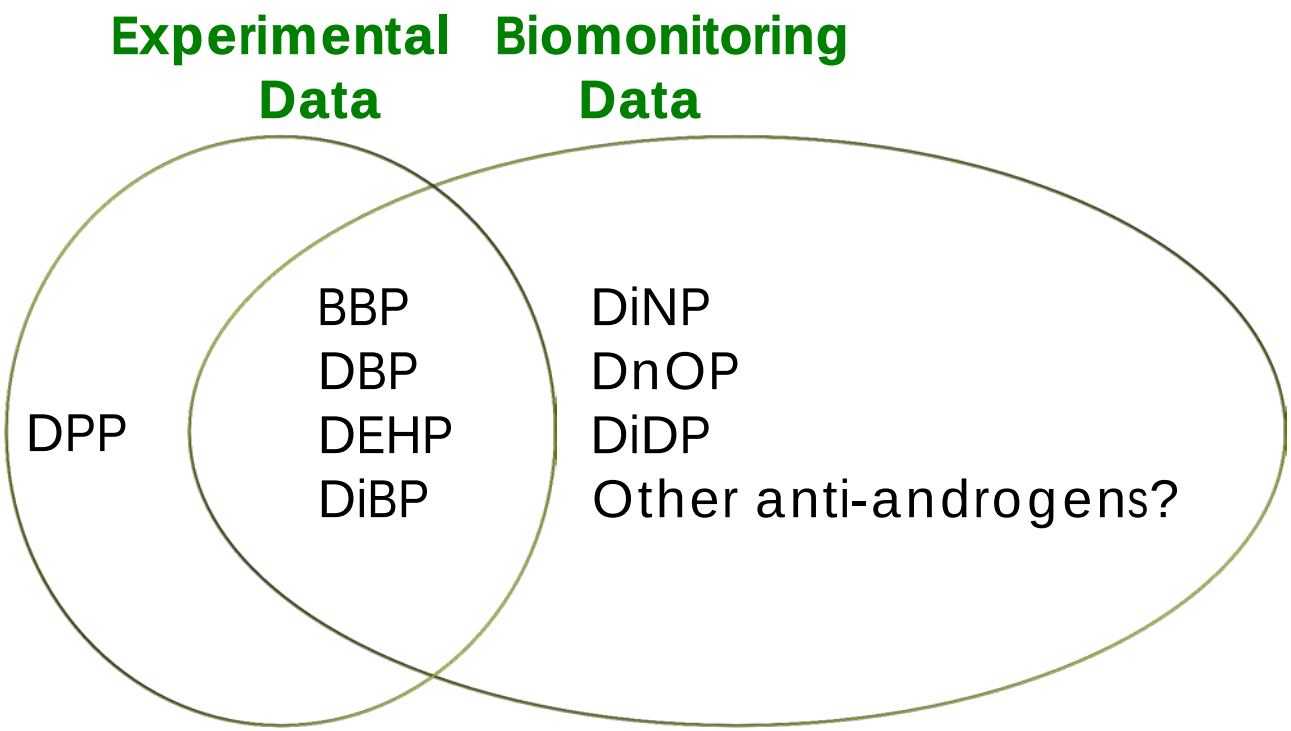
Howdeshell et al (2008)conducted a mixture study of 5 phthalates to test additivity where pregnant rats were dosed with the mixture based on their potency.

Fetal testicular testosterone production was measured on GD18.

Observed NOAEL from this study was 130 mg/kg.
For a BMR=10% (i.e., 90% control), estimated BMD=127 and BMDL=63 mg/kg.

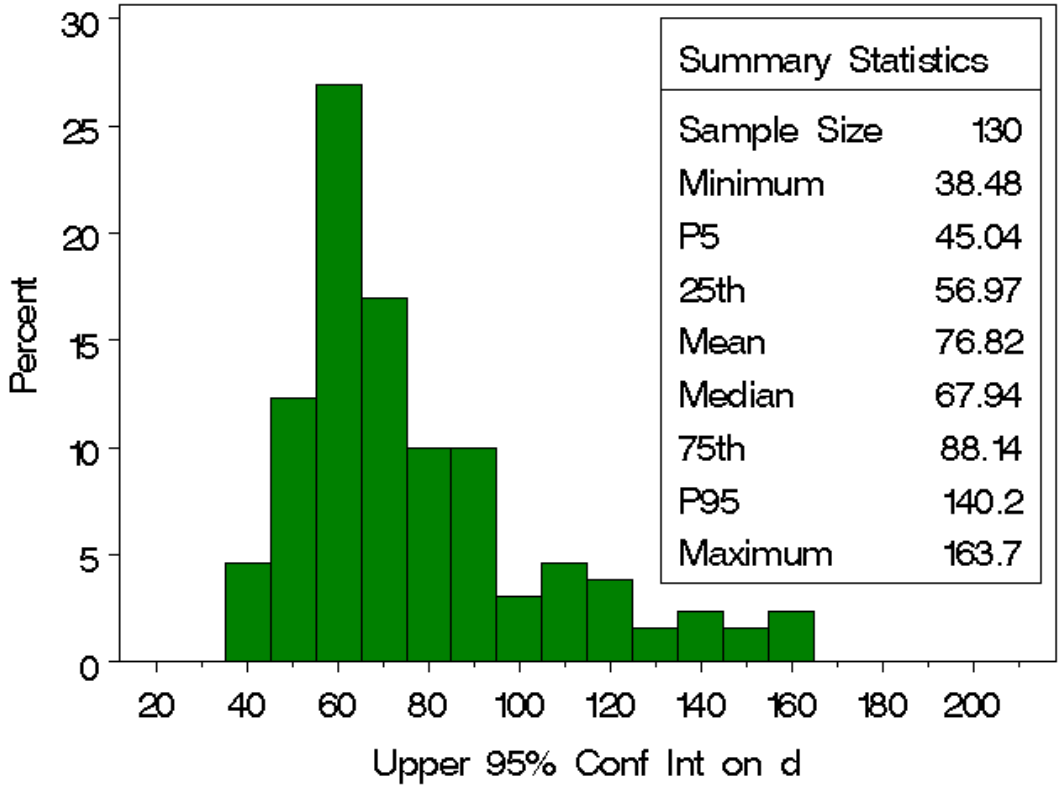


Case Study: Phthalate Mixtures



How similar is the reference mixture to mixtures estimated from biomonitoring data in pregnant women?

Case Study: Phthalate Mixtures



Data poor case with experimental data only at reference mixture.

Assuming a similarity region defined by the upper CL on the distance between the BMD10 and BMD20: R=52.

18 (14%) of 130 subjects have estimated DIs considered sufficiently similar to the reference mixture.

Discussion

- We have developed an objective approach for defining sufficiently similar mixtures from occurrence data and biomonitoring data without assuming additivity.
- The test FOR sufficient similarity has sound statistical properties.
- The mixture Toxicity Index (mTI) provides a comparison of the range for daily intake of mixtures to PODs for the mixtures considered sufficiently similar to the reference mixture.
- The mTI is different from the Hazard Index which assumes additivity.

Discussion

- The whole mixture approach provides a convenient strategy to evaluate non-chemical stressors.
- Consider a mixture study with groups of animals with:
  - "low stressors" (e.g., good nutrition)
  - "high stressors" (e.g., poor nutrition)
  - Study design allows for testing for differences in the BMDs in the two cases
- Limitations of the test FOR sufficient similarity includes the inability to make claims regarding the mixtures not considered sufficiently similar to the reference mixture.
- Methods for describing the mixtures "inside" the similarity region would be useful. For example, what proportion of mixtures identified through biomonitoring are included in the inference?

Discussion

- Ideally, biomonitoring data and other exposure/occurrence studies can be used to inform the selection of mixtures to be experimentally evaluated.
- In component-based approaches, *in vitro* data are useful in testing the default assumption of additivity.
- In whole mixture approaches, *in vitro* data may be useful as a screening tool for prioritizing mixtures for further *in vivo* testing. Seemingly, *in vivo* testing is necessary for defining PODs for environmental mixtures.
- Even at screening stages, early inclusion of human exposure assessment to identify relevant mixtures is important.

Acknowledgements

- **Sufficient Similarity**

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  - Scott Marshall, BiostatSolutions
  - LeAnna Stork, Monsanto Co
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- **Estimating DI in phthalates**

  - Holger Koch, Andreas Kortenkamp and other CHAP members

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