Methods and Models Beyond Dose-Addition for Evaluating Chemical Mixtures

Presenters: JE Simmons, DW Herr

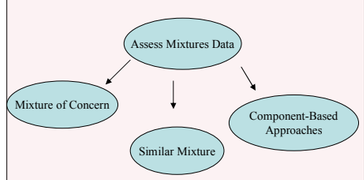
Contributing EPA Organizations: ORD: NHEERL, NCEA

LTG 2 Poster 03

Science Questions

- How can we develop the next generation of tools for assessing the toxicity of chemical mixtures?
- How can advanced methods and techniques, such as genomics and PBPK models, be used to inform future mixture risk assessments?

Mixtures Risk Assessments are based on the type and quality of available data. Whole mixture assessments are preferred, followed by assessments based on sufficiently similar mixtures. When suitable mixtures are not available, component approaches are used



Research Goals

The overall goal of this new research program is improvement in the three types of risk assessment processes (whole mixture, sufficiently similar mixture, component-based) by integrating toxicological and biostatistical research and applying new methods and technologies.

Goals for component-based methods are to develop user-friendly methods and models that can be applied to greater than binary mixtures, provide good fits to the single chemical data, predict mixture effects under clearly articulated definitions of additivity, and incorporate non-additive effects in the predictive model.

Goals for research on whole mixture assessments are improved power for whole mixtures studies in the low-dose region, and the development of methods to determine when two or more whole mixtures are 'sufficiently similar'.

Findings and Conclusions

Whole mixture assessments for the mixture of concern have been enhanced by the development of methods and procedures for power calculations (see Panel A), and methods for determination of those components of complex mixtures contributing to its toxicity (see Panel B).

Methods for sufficiently similar mixtures have been enhanced by the use of principal component techniques to evaluate the similarity of complex mixtures (Panel C).

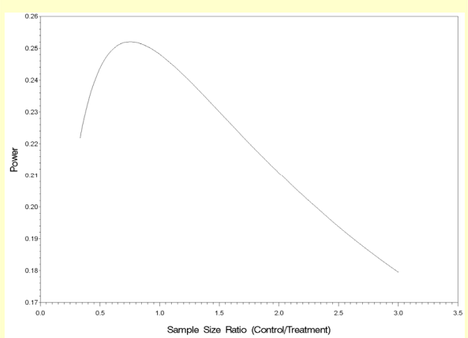
There has been rapid growth in the development of empirical, mathematical, and statistical models for the prediction of mixture effects by component-based dose-additive methods (see Poster 2-2). Defined mixtures have been analyzed by comparing dose-additive model predictions of expected mixture toxicity to experimental mixture data. Based on these studies, dose additivity generally holds in the low-dose region within an order of two-three fold.

The present suite of component-based models (except the LeBlanc-developed models [EPA STAR Grant]), are highly complex and not user friendly. We are developing a new generation of component-based methods. The proportional-response addition method is illustrated in Panel D.

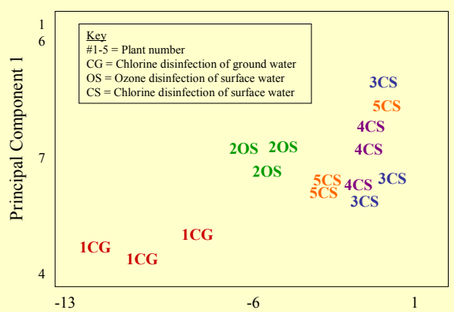
We will: examine established models for flexibility and ease of use; develop user-friendly models/techniques based on sound principles; use PBPK models to explore interactions on the basis of internal dose metrics; and use genomics tools to understand/predict the effect of mixtures and to identify sufficiently similar mixtures.

Methods/Approach

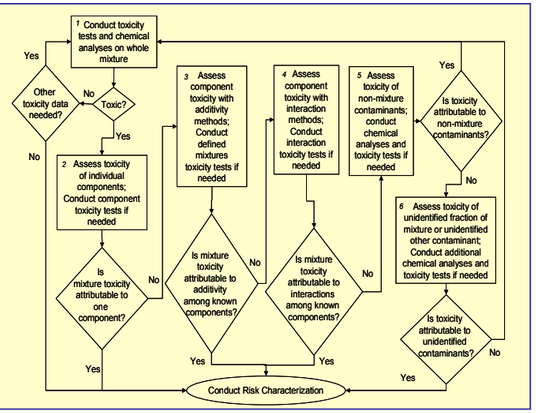
Panel A. Increased power for a multi-generational rodent study on environmentally relevant low doses of a drinking water disinfection by-product complex mixture was achieved by using unequal treatment group sizes. As is typical with complex environmental mixtures, available sample volume was rate limiting, so more dose groups could not be added. The graph below shows the impact of varying the ratio of control to treated animals on power to detect prenatal loss



Panel C illustrates the use of principal component analysis for determination of sufficient similarity. In the example below, chlorine disinfection of ground water is clearly separated from chlorine disinfection of surface water.

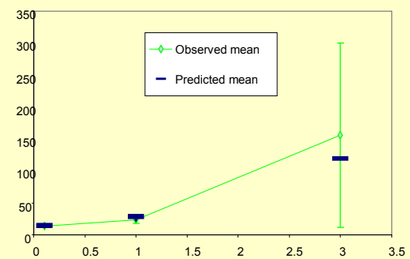


Panel B. A new strategy has been developed for identifying the individual components or fractions of a complex mixture that are associated with its toxicity that uses available chemical composition and individual chemical toxicity data.



Panel D. Under proportional response addition (PRA), the response (R) to the whole mixture at the total dose, D, is a weighted average of the responses to the individual components at D, where the weights for the individual responses are equal to the proportions of the components in the mixture: $R_{mix}(D) = D_1/D[R_1(D)] + D_2/D[R_2(D)] + ... + Dn/D[Pn(D)]$.

Panel D2 shows, for the binary combination of CHC3 and BDCM (1:1 mixing ratio), the means predicted by PRA under an assumption of additivity and the experimental observed means (SDs)



Binary Combination	Mixing Ratio	Results of PRA Analysis
CHCl3:BDCM	1:1	ADD: Deviation from Additivity not detected – All endpoints, All dosages
	2.7:1	ADD: Deviation from Additivity not detected – All endpoints, Both dosages
CHCl3:CDBM	1:1	ADD: Deviation only for AST at 0.1 mmol/kg/day – Greater than Additive
	6.5:1	ADD: Deviation from Additivity not detected – All endpoints, Both dosages
CHCl3:CHBR3	1:1	ADD: Deviation from Additivity not detected – All endpoints, All dosages
	65:1	ADD: Deviation from Additivity not detected – All endpoints, Both dosages
BDCM:CDBM	1:1	ADD: Deviation from Additivity not detected – All endpoints, Both dosages
	2.4:1	ADD: Deviation only for Rel liver wt at 3 mmol/kg/day – Greater than Additive
BDCM:CHBr3	1:1	ADD: Deviation from Additivity not detected – All endpoints, All dosages
	24:1	ADD: Deviation from Additivity not detected – All endpoints, Both dosages
CDBM:CHBr3	1:1	ADD: Deviation from Additivity not detected – All endpoints, All dosages
	10:1	ADD: Deviation from Additivity not detected – All endpoints, Both dosages

Table D1 shows the 6 binary combinations of the 4 regulated trihalomethanes (THMs) evaluated by PRA. PRA does not require an assumption of similarity of mode of action or similarly shaped dose-response curves. PRA is computationally simpler but the results of the THM mixtures are consistent with the results of more complex models

Impact and Outcomes

The outcomes of this program will be :

- Readily accessible tools and techniques for understanding the joint toxic action and interaction of chemical mixtures and for assessment of the potential human health risk posed by exposure to mixtures
- Improved characterization of joint toxic action at various levels in mixture dose-response curves
- Improved attribution of mixture toxicity to the correct components or fractions of chemical mixtures

Future Directions

- Examine established models for flexibility and ease of use
- Continue to develop user-friendly models/ techniques based on sound principles
- Identify factors that influence the ability to detect interactions
- Use PBPK models to explore interactions on the basis of internal dose metrics
- Explore genomics as a tool for understanding and predicting the effect of mixtures and identifying sufficiently similar mixtures

Researchers Involved

NHEERL/ORD/U.S. EPA
A.B. DeAngelo
M.V. Evans
D.W. Herr
J. E. Simmons

NCEA/ORD/U.S. EPA
L.K. Teuschler
G. Rice

N.C. State University
G. LeBlanc.

Elon University
Karen Yokely

TERA
R. Hertzberg

Battelle
P.I. Feder
C. Dingus

Syracuse
J. Colman
W. Hartley

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Cumulative Risk