

Research Area: Mixtures

The environment of humans and other organisms contains complex chemical mixtures consisting predominantly of natural products (e.g., foodstuffs, atmospheric gases, minerals and organic matter in water and soil) and many synthetic chemicals (e.g., medicines, consumer products, industrial chemicals). The composition and relative concentrations of these mixtures vary widely, fluctuate rapidly, and present the possibility for toxicological interactions between the components. Since it is virtually impossible to obtain toxicity data for each unique mixture that may occur in the environment, the risk assessment of chemical mixtures is particularly challenging.

To simplify the task, one of two approaches is typically used to estimate mixture toxicity. Whole-mixture approaches attempt to predict the toxicity of unstudied mixtures from data for "similar," well-tested mixtures. Component approaches attempt to predict mixture toxicity from data on the individual components of a mixture and interactions between them. Both predictions are complicated by the influence that dose and relative component concentrations can have on potential interactions and toxicity. However, few whole mixtures have been assessed toxicologically and few relevant and rigorously conducted interaction studies are available. Furthermore, the available studies typically test concentrations where observable toxicity occurs rather than the lower concentrations typical of environmental exposures. Consequently, mixture risk assessments currently rely on conservative assumptions to account for the considerable uncertainties inherent in predicting mixture toxicity.

In response to questions and concerns about mixtures found in the environment, policy and decision-makers face pressures to revise mixture risk assessment methodologies in a more conservative direction as the public and various interest groups speculate about the human and ecosystem effects associated with exposure to mixtures of natural and synthetic chemicals found or presumed to be in the environment. Two fundamental policy assumptions currently drive a need for additional research to reduce uncertainty in risk assessments for chemical mixtures:

1. An increased concern for ecological and human health effects is warranted merely because chemicals are found in mixtures. Some go so far as to assert that the fact that chemicals are present in the environment as mixtures makes policies based on single chemical toxicology irrelevant.
2. Improved toxicological assessment methods & databases are needed to properly understand the potential effects of chemical mixtures.

Until some of these concerns are addressed by the scientific and policy communities, some people assert that risks posed by mixtures in the environment, including those mixtures containing chlorinated compounds, cannot be managed.

Not surprisingly, facing this type of concern, regulators will use conservative dose additivity assumptions for chemical mixtures present in the environment because they lack data and appropriate methods for assessing mixtures.

While these overarching policy assumptions are too broad to test with specific scientific research programs, they appear to have led to a series of more specific science policy assumptions used in mixture risk assessment, some of which can be evaluated and tested more directly. Both the US Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry have developed new guidance documents for assessing the toxicity of mixtures. This new guidance expands previous approaches by using toxicological information on "similar"

mixtures to infer the toxicity of untested mixtures, incorporates interaction data into the risk assessment, and uses mode of action information to infer the combined action of chemicals in mixtures (i.e., to predict synergism, antagonism, or non-interaction). Use of such information, however, requires assuming several premises that have either not been well investigated or not established.

One assumption is that published data on chemical interactions is sufficiently reliable for use in risk assessment. This assumption, however, may be at variance with EPA's own evaluation regarding the quality of statistical methods used in many toxicological interaction studies (EPA, 1990; 1999) and with a recent publication enumerating flaws in study design and data interpretation common in the interaction literature (Borgert et al. 2001). With specific regard to chlorinated chemistry, scientists have commented that the reliability of interaction studies on chlorinated compounds has not been systematically assessed or determined to be of sufficient quality that its use would reduce uncertainty in mixture risk assessments. Such an evaluation is needed to determine the strength and extent of the existing data as a basis for risk assessment of chlorinated mixtures, as well as to identify data gaps that should be addressed in future research projects on chlorinated compounds.

A second assumption is that using interaction information on only one or a few chemicals in a mixture will reduce uncertainty in the toxicological assessment of the entire mixture. This premise has not been systematically tested, but may be at variance with emerging information regarding the degree of interaction information needed to predict the toxicity of a mixture of many chemicals. Although information on higher-order interactions (3-way; 4-way, etc.) may be unnecessary to reduce uncertainty in the toxicological assessment of a mixture, reliable binary (two-way) interaction information for each

pair of chemicals present in a mixture may be necessary.¹ Without information on binary interactions between each pair of chemicals, using interaction data for only a few chemicals in a mixture could actually increase uncertainty rather than reduce it.

A third premise assumes that chemicals with similar modes of action display dose-additive behavior in mixtures. Dose addition is a non-interaction model based on the behavior of multiple doses of a single chemical. Dose addition treats different chemicals as if they were merely dilutions of the same chemical and can be related by potency values (an example is the use of Toxic Equivalency Factors for dioxins and related compounds). This means that chemicals with similar modes of action are assumed to be dose additive throughout their dose response ranges - i.e., both above and below their no-effect levels - just as dilutions of a single chemical would be additive. Under the assumption of dose addition, mixtures could produce adverse effects even when all constituents are present at concentrations below their no-adverse-effect level. This premise has not been systematically investigated for sets of chemicals known to have similar mechanisms of action, much less for groups of chemicals that are only assumed to have similar modes of action, such as chlorinated dioxins and dibenzofurans.

An alternative model of non-interaction — independence — cannot be ruled out on the basis of data. Independence would predict that mixtures of chemicals have no toxicological effects when the concentrations of all constituents are present below their individual no-adverse-effect levels, regardless of their

¹ This information was summarized by Dr. Kannan Krishnan of the University of Montreal during his presentation at a conference on chemical mixtures held at Colorado State University, January 16-19, 2001.

mechanisms of action. The assumption of dose addition versus independence for chemicals with similar modes of action is a broad scientific question not confined to chlorinated chemistry, but it may be a particularly critical question for the risk assessment of chlorinated chemicals whose concentrations decline relatively slowly in the environment (e.g., chlorinated dioxins) or to which exposures may be continuous (e.g., DBPs).

A related question is whether the mode of action for certain chlorinated chemicals is sufficiently similar to infer not only their combined action but also the similarity of mixtures composed of them. The accuracy of such assumptions will have a profound impact on the uncertainty of mixture risk assessment based on whole mixture approaches.

RESEARCH THEMES:

RFHEE intends to partner with governmental agencies and other organizations, to examine and understand the potential impacts from mixtures of chlorinated compounds, including TCDD and dioxin-related compounds and disinfection byproducts. Specifically, of concern to RFHEE is the sufficiency/insufficiency of the current databases for chlorinated compounds to properly inform public policy decisions about the potential effects of key chlorinated mixtures, such as TCDD and dioxin-related compounds, and disinfection byproducts (DBPs). Of particular interest to RFHEE is to focus its support on projects that:

Contribute to the understanding of the assumptions underlying:

- Component-based approach for mixtures of chlorinated compounds found in the environment.
- "Representative" whole mixtures, for testing and assessing mixtures of variable composition and concentrations of mixtures that might include chlorinated compounds (e.g., breast milk contaminants).

CANDIDATE PROJECT AREAS:

SEE SEPARATE LIST OF PROJECTS.

READINGS CITED:

Borgert, C.J., Price, B., Wells, C., and Simon, G.S. (2001). Evaluating interaction studies for mixture risk assessment. *Human and Ecological Risk Assessment* **7(2)**:259-306.

USEPA (U.S. Environmental Protection Agency). 1990. Risk Assessment Forum: Technical support document on risk assessment of chemical mixtures. U.S. Environmental Protection Agency, Washington, D.C.

U.S.EPA (United States Environmental Protection Agency). 1999 . Risk Assessment Forum. Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Washington, D.C.: U.S. Environmental Protection Agency. NCEA-C-0148.