Human and Ecological Risk Assessment, 10: 619–629, 2004

ISSN: 1080-7039 print / 1549-7680 online
DOI: 10.1080/10807030490484192

PERSPECTIVE

Chemical Mixtures: An Unsolvable Riddle?

Christopher J. Borgert
Applied Pharmacology and Toxicology, Inc., Gainesville, FL, and Center for Environmental and Human Toxicology, Department of Physiological Sciences, University of Florida College of Veterinary Medicine, Gainesville, Florida, USA

ABSTRACT

It is difficult to overstate the complexity of assessing risks from chemical mixtures. For every valid reason to assess risks from mixtures, there appears an equally valid question as to whether it is possible to do so in a scientifically rigorous and relevant manner. Because so few data exist for mixtures, current mixture assessment methods must rely on untested assumptions and simplifications. That the accuracy of risk estimates improve with the number of chemicals assessed together as mixtures is a valid assumption *only* if assessment methods for mixtures are better than those based on individual chemicals. On the other hand, arbitrarily truncating a mixture assessment to make it manageable may lead to irrelevant risk estimates. Ideally, mixture assessments should be as broad as necessary to improve accuracy and reduce uncertainty over assessments that only use toxicity data for single chemicals. Further broadening the scope may be ill advised because of the tendency to increase rather than decrease uncertainty. Risk assessment methods that seek to be comprehensive at the expense of increased uncertainty can hardly be viewed as improvements. It would be prudent to verify that uncertainty can be reduced before burdening the risk assessment process with more complexity.

Key Words: chemical mixtures, drug interactions, chemical interactions, health risk assessment, data evaluation.

INTRODUCTION

Predicting the effects of chemical or drug mixtures has perplexed pharmacologists and toxicologists for decades. In pharmacology, one of the challenges has been to develop methods that predict whether combinations of medicines will interact adversely—either to increase toxicity or decrease efficacy. In toxicology, the primary challenge has been to develop methods for predicting the toxicity resulting from the

Address correspondence to Christopher J. Borgert, Ph.D., 2250 NW 24th Avenue, Gainesville, Florida 32605, USA. E-mail: cjborgert@APT-PHARMATOX.com

combined exposure to large numbers of chemicals present in diverse environmental media ranging from ambient air and water to food, drugs, and consumer products. These challenges are formidable from biological, chemical, and statistical perspectives and present significant obstacles to those who would formulate methodologies for assessing risks from exposure to mixtures.

Perhaps the most intractable aspect of mixture toxicity assessment is the sheer magnitude of the task. Not only is the number of unique mixtures in the environment practically infinite, mixtures are constantly changing in composition and concentration due to transformation and transport processes within organisms and environmental media. Risk assessment methods cannot possibly account for the complexity of these ever-changing mixtures; hence, regulatory agencies have found it necessary to allow vast simplifications in mixtures risk assessment methods (Hertzberg and Teuschler 2002), including simplifications that allow risk assessors to use toxicity data for single chemicals rather than mixtures (ATSDR 2001a, 2001b; USEPA 1999, 2000).

The extensive use of simplifications in mixture risk assessment has received sharp criticism and led to legislative mandates that require an increased level of sophistication. For example, provisions in the 1996 U.S. Food Quality Protection Act require the USEPA to assess aggregate exposures from multiple pesticide uses and cumulative toxicity that occur by common mechanisms of toxicity. In many respects, the requirement to increase sophistication has led to replacing simplifications with mere assumptions, few of which have been scrutinized experimentally. Many assumptions about mixtures seem rational enough, but without clear experimental data to support them, it is impossible to know whether their adoption increases or decreases uncertainty in risk assessments. The overarching theme of this commentary is that both the simplifications and the assumptions made in mixture risk assessment should be judged, not on the basis of their necessity, but rather, by the degree to which they reduce uncertainty and lead to more scientifically defensible risk assessments.

ARBITRARILY DEFINING MIXTURES

One of the most significant challenges in mixture risk assessment is defining and delimiting the mixture of concern. Environmental chemical mixtures can be defined on the basis of the source of the chemicals that comprise them, the medium in which they are found, or the biological receptor(s) that may become exposed. This is not only an analytical chemistry problem; it is largely a conceptual issue fundamental to the purpose of the risk assessment. Regardless of the basis for the definition, implementing it usually requires assumptions and simplifications.

It is often assumed that a risk assessment will be more accurate (or more conservative and thus, protective) if it evaluates in some collective fashion all chemicals to which a receptor is exposed rather than only a subset of those chemicals. This may seem intuitively reasonable, but in practice, most mixture assessments are spatially and temporally constrained. Typically, the chemicals evaluated as a mixture are only those that enter the environment from a particular source or that might be encountered at a particular site, and include only the set of chemicals measured at a single point in time rather than the sequence of chemicals that actually exist over time. Presumably, these simplifications constrain the scope of the assessment exercise to

make it manageable and to limit the range of risk mitigation strategies to those for which a responsible party may be held liable.

However practical this situation may appear, it seems reasonable, nonetheless, to ask whether the most toxicologically significant mixture effects are likely to occur between the chemicals encountered in the same place or between combinations of chemicals encountered in different places. For pharmaceuticals, one might posit that the mixture of concern should be delimited to the suite of prescription or over-the-counter (OTC) medications typically taken in combination, based on market survey or prescribing frequency data. However, delimiting the mixture so narrowly would not account for important pharmacological interactions that may occur with foods and dietary supplements. For example, grapefruit juice has been shown to enhance the pharmacological effect of a number of drugs due to inhibition of gastrointestinal drug metabolism and consequent increased GI absorption (Kane and Lipsky 2000) and several interactions are suspected with St. John's Wort due to cytochrome P450 inhibition (Moore *et al.* 2000).

In similar fashion, environmental toxicology must ask whether it is sufficient to assess the milieu of chemicals present at a particular site or within a particular medium rather than to evaluate the entire suite of chemicals to which a potential receptor might be exposed. For example, risk assessments to support occupational safety and health decisions might focus on the chemicals used in manufacturing in a particular work environment, ignoring the possibility that the majority of a worker's chemical exposures might occur outside of work from food, drugs, consumer products, and chemicals used in residential and other non-occupational environments. Not only does delimiting the mixture temporally and spatially reduce the number of mixture components arbitrarily, this practice ignores the importance of sequence of exposure and past toxic insults in determining the toxicity of many chemical combinations. The USEPA (2000) has acknowledged these issues by defining chemical mixtures irrespective of the spatial or temporal characteristics of their components.

Thus, on one hand, it is important to include all toxicologically significant chemicals in the mixture assessment, but on the other hand, it is usually necessary to limit the assessment to a manageable number of mixture constituents. There is a critical need to balance these contravening goals, but currently, no broadly applicable scientific method exists for doing so. Ideally, the mixture assessment should be only as broad as necessary to improve accuracy and reduce uncertainty over an assessment that considers only the toxicity of individual chemicals. Further broadening the scope of mixtures assessment would be ill advised because of the tendency to increase rather than decrease uncertainty. Assuming that the accuracy of mixture risk assessments improves with the number of mixture components assessed is valid *only* if the methods for predicting combined toxicity are better than methods based on toxicity for individual chemicals alone. If the primary reason for assessing mixtures were merely to increase conservatism in the risk estimate rather than improve accuracy, there are much simpler ways of achieving that end than attempting to predict combined toxicity. For some particularly important risk assessment goals, such as protecting infant health, conservatism may not be synonymous with health protection (Borgert *et al.* 2003).

PREDICTING THE UNPREDICTABLE—INTERACTION, NON-INTERACTION, AND MODE OF ACTION

One of the primary reasons for assessing mixtures rather than simply adding risks for individual chemicals is to address the concern that risk estimates for single chemicals might grossly underestimate toxicity due to the potential for synergism between mixture components. Indeed, the specter of synergism has been used to raise concerns about a range of different chemical mixtures from prescription diet aids to environmental estrogens. Notoriety aside, it is important to consider objectively the likely public health and environmental consequences of synergistic interactions (Groten 2000). A synergistic interaction can be extremely useful and economically valuable when it confers therapeutic advantage or reduces the opportunity for acquired resistance in viruses, cancers, or among microbial or insect pests. For this reason, countless resources have been spent by the pharmaceutical and pesticide industries to develop useful synergistic combinations for medicine and agriculture. With a few exceptions, these efforts have been generally unsuccessful. Although many therapeutically advantageous drug combinations have been identified, this should not be taken as evidence of drug synergism; pharmacologic addition, toxicologic antagonism, and a reduced chance of tolerance or resistance developing in target organisms are more common means of conferring therapeutic advantage (Berenbaum 1988). Furthermore, it can be shown mathematically that interactions, including synergism, are more likely to occur in the mid-range of the dose-response curve than at either high or low extreme (Berenbaum 1989). Consequently, synergism should be easier to identify among pharmaceuticals, which are used in biologically active concentrations, than among environmental contaminants present at concentrations below the observable effect range. The fact that pharmacologists and toxicologists have found so few biologically significant synergistic interactions, despite great scientific, professional and financial motivation to do so, suggests that these interactions are probably also rare in the environment.

Regardless of the probability that synergistic interactions are causing significant environmental or clinical problems, a satisfactory method to evaluate synergism in risk assessments would be a welcome advancement. A methodology that can identify the chemical combinations most likely to be synergistic would limit the scope of a mixtures assessment to a manageable number of chemicals and simultaneously focus the assessment on the components of greatest concern. Such methods have been proposed (Durkin *et al.* 1995). However, due to the complexities discussed in this commentary, such elegant methods are much more easily conceived than implemented for most chemicals.

The first challenge for addressing synergism is to define it. Within the field of interaction pharmacology and toxicology, there has been considerable debate over the proper definitions of terms such as *synergism*, *antagonism*, *potentiation*, and *additivity*, and as a result, there appears to be widespread confusion over terminology outside this narrow field. The confusion is responsible, in part, for the limited amount of interaction data useful for risk assessment (Hertzberg and McDonnell 2002). Synergism can be defined broadly as a type of "interaction" in which chemicals produce more toxicity as a combination than would be predicted by their actions separately. Another way of stating this concept is that lower concentrations of chemicals are

required to produce a particular magnitude of effect when the chemicals are administered together than would be predicted from their actions separately. Antagonism is the converse; *i.e.*, less effect produced, or more chemical required, than predicted.

The word "predicted" is of critical importance in these definitions. It is important to appreciate that synergism cannot be inferred whenever a mixture of chemicals produces an effect greater than the same chemicals administered separately. Rather, the determination requires a comparison of the observed effect with the effect *predicted* based on the concentration-effect relationships of the individual components of the mixture. The *predicted* effect can be derived by applying either concentration addition or independence, both of which are widely accepted as valid models of non-interaction (Berenbaum 1981). These concepts have been reviewed extensively elsewhere (Berenbaum 1989; Greco *et al.* 1995; Cassee *et al.* 1998; USEPA 2000; Borgert *et al.* 2001; Tallarida 2001). For risk assessment, the critical issue is that interactions—synergism and antagonism—cannot be directly tested; rather, these interactions are inferred from experimental results that deviate from a model of non-interaction based on the concentration-effect characteristics of the individual mixture components. Thus, with regard to predicting synergism (or antagonism), toxicologists and risk assessors face the conundrum of predicting dose-response phenomena that are, by definition, not readily predicted by any simple model.

One way to avoid the need to predict interactions is to assume that in mixtures containing many chemicals, synergism and antagonism will essentially cancel one another. Predicting the toxicity of the mixture is then a matter of predicting which chemicals will be non-interactive according to independence (response addition) and which will be non-interactive according to concentration addition (dose addition). In other words, rather than attempt to predict interactions, the focus is on how to add the toxicity of chemicals in a mixture. Concentration addition (or dose addition) is based on the concept that a single chemical does not interact with itself, and thus, multiple doses of one chemical are non-interactive (Loewe and Muischnek 1926). Predicting that two 325 mg aspirin tablets will produce the same analgesic effect as a single 650 mg tablet is a simple example of dose addition. Current risk assessment methodologies typically extend dose addition to groups of chemicals with similar modes of action based on the assumption that one chemical can be replaced by an equi-effective concentration of any similarly acting chemical. Continuing with the analogy, the analgesic effect of 325 mg aspirin tablet and 200 mg ibuprofen could be predicted by summing the ratio of dose to relative analgesic potency for each drug. In risk assessment, this has become known as the toxic equivalency, or TEQ approach. The TEQ approach was developed for true chemical congeners that share pharmacokinetic and pharmacodynamic behavior, molecular targets, and have parallel dose-response curves, but is probably inappropriate for chemicals that deviate significantly from these requirements (Safe 1998). The TEQ approach was initially applied to assess risks posed by mixtures of dioxins and dibenzofurans (Safe 1990), but recent data and conceptual concerns call into question its applicability for these (Toyoshiba *et al.* 2004) and other groups of chemicals (Safe 1998; Borgert *et al.* 2003). The hazard index calculation used in CERCLA-style risk assessments is another example of concentration addition, wherein the mixture is assessed by summing the ratio of received dose to reference dose (RfD) for each component.

In contrast to concentration (dose) addition, independent action (also called response addition) is based on a model of probabilistic independence and assumes that the toxicity of chemical A is unaffected by the presence of chemical B in a mixture (Bliss 1939). Independence is the non-interaction model recommended for mixtures of chemicals that act by different modes of action (ATSDR 2001a, 2001b; USEPA 2000). Cancer risk calculations are an example of response addition, wherein the overall risk for cancer from a mixture is calculated from the risks posed by each individual component. Current risk assessment guidance for chemical mixtures also recommends the use of response addition for chemicals that act dissimilarly in producing toxic endpoints other than cancer. Independence and concentration addition can be expected to give the same prediction only when applied to linear dose-response curves that intersect the origin of the dose-response plot. Under all other conditions, independence and concentration addition models are likely to yield different results (Greco *et al.* 1995). A special case of independent action, called effect summation, predicts mixture toxicity by summing the effects of the individual components. In some circumstances, effect summation can yield the paradoxical result that a chemical is synergistic with itself (Berenbaum 1989). For chemicals that exhibit a toxicity threshold, effect summation would predict a mixture effect of zero when all mixture constituents are present below the threshold concentration, whereas concentration addition could predict a supra-threshold response. To illustrate, consider a mixture of three nephrotoxic chemicals, each present at one-half its threshold concentration for producing tubular acidosis. Effect summation would predict a sub-threshold effect for the mixture (*i.e.*, $0 + 0 + 0 = 0$) whereas concentration addition would predict measurable tubular acidosis ($0.5 + 0.5 + 0.5 = 1.5$). Risk assessors should be aware that the important differences between various non-interaction models are often ignored in the published literature.

Predicting mixture toxicity for risk assessment has thus become an exercise in choosing between models of non-interaction based on the presumed mode of action of mixture components. This is an interesting and somewhat paradoxical development because an empirical test for interactions has often been used to differentiate chemicals that act by similar versus dissimilar mechanisms (Dawson and Poch 1997; Borgert *et al.* 2004a). From a practical perspective, it would seem that interaction studies are potentially much more informative about mechanisms than mechanistic studies are about interactions (Tallarida 2001; Borgert *et al.* 2004b). Although concentration addition has been verified at the molecular and cellular level for some chemicals with similar molecular targets (Silva *et al.* 2002) and for some nephrotoxicants, the general case remains to be established at the level of organisms or populations (Groten 2000).

Discerning the mode of action for all components of a mixture might appear to be more tractable than predicting synergism or antagonism, but it is a complex issue in practice. A mode of action can be viewed as a category of mechanisms that share particular key features or steps. Although several sets of criteria have been set forth for identifying the key mechanistic features that define a mode of action (ATSDR 2001a, 2001b; USEPA 1999, 2000, 2003; Mileson *et al.* 1998), the degree to which those key features must be understood in order to predict combined toxicity has not been established on the basis of data (Borgert *et al.* 2004b). Complexities that may need to be experimentally explored include interaction thresholds

(el-Masri *et al.* 1996a,b), causal relationships between various mechanistic steps, pharmacokinetic behavior (Haddad *et al.* 2000), mechanisms of interaction, and the dose-dependence of various toxicity mechanisms (Borgert *et al.* 2004b).

The mechanisms by which chemicals interact and the dose-dependence of those mechanisms may prove to be the most critical of all issues to address, particularly the dose-dependence of biological effects and interactions in the sub-threshold range for observable toxicity. The work of Hermanns and colleagues (see review in McCarty and McKay 1993) is perhaps the most innovative attempt at predicting the toxicity of complex mixtures containing components below their individual threshold concentrations for observable toxicity. Using standard aquatic toxicity models, these researchers showed that at sub-threshold concentrations, mixtures of organic chemicals fail to exhibit the toxicities of the individual components, but instead, conform to concentration addition for general narcosis. The narcotic potency of the mixture can be estimated quite accurately from the concentrations and the octanol-water partition coefficients of the mixture components.

Rather than attempting to predict interaction or non-interaction, it would seem that computing mixture toxicity from empirical interaction data would be a more direct means of estimating mixture toxicity and would reduce uncertainty in mixture risk assessments. Indeed, computational methods have been devised to predict the toxicity of complex mixtures based on pair-wise interaction data for mixture components (Haddad *et al.* 2000), and these methods have been validated for small sets of test mixtures (Haddad *et al.* 2001). A weight of evidence procedure (Mumtaz and Durkin 1992) that modifies the traditional hazard index calculation based on published interaction data is currently used to develop interaction profiles for various chemical mixtures (ATSDR 2001a,b). Unfortunately, published interaction data are lacking for the vast majority of drugs and environmental contaminants (Hertzberg and Teuschler 2002), and so the weight of evidence procedure is limited in its application. Moreover, many published interaction studies suffer serious methodological deficiencies that limit their use in risk assessment (Borgert *et al.* 2001). Some of the more common problems stem from an apparent misunderstanding about the nature of interaction and non-interaction (Berenbaum 1989) and misunderstanding of the statistical methods required for testing and interpreting interactions (USEPA 1990). The fundamental criteria for designing and interpreting interaction studies include the need to adequately assess dose response curves of the component chemicals individually and in combination, the need to test a specific no-interaction hypothesis using appropriate statistical tests, and the need to evaluate the interaction at relevant levels of biological organization (Borgert *et al.* 2001).

JUST TEST THE MIXTURE

One great advantage of predicting mixture toxicity from data on individual chemicals or combinations of a few chemicals is that theoretically, the data can be applied to many different mixtures containing different chemicals in different ratios and proportions. However, considering the difficulty of estimating mixture toxicity from data on individual components, their mechanisms of action, and data on their interactions, one might ask why not simply perform toxicity testing on the mixture of

concern, treating it as a unique single substance? This approach, sometimes called the "whole mixture approach," obviates any need to identify toxicity interactions produced by mixture components because these will be reflected in the toxicity of the mixture itself. The toxic effects and potency of the mixture can be assessed as is routinely done for single chemicals. This would simplify the determination of LOECs (lowest observed effect concentrations) and NOECs (no observed effect concentrations) if the data are more directly applicable to the mixture of concern and thus more readily interpretable than data on individual components or mixtures of only a few chemicals.

Of course, the whole mixture approach is not without significant limitations. In order to conduct toxicity tests, many mixtures would have to be extracted from the environmental medium in which they occur, and then concentrated (or diluted) to conduct toxicity tests. Because the identity, the concentration, and the relative proportions of constituents can affect mixture toxicity, any of these manipulations could introduce differences between the mixture tested and the mixture found in the environment. Such differences could reduce the relevance of the results. Finally, the sheer number of unique mixtures that exist in the environment precludes testing each, and indeed, it would be nearly impossible to completely identify and quantify every last component of even one mixture, let alone characterize the changes in mixture composition that occur with time.

For this latter reason, guidance documents for mixture risk assessment (USEPA 2000; ATSDR 2001a,b) recommend using data on a similar mixture as a surrogate for the mixture of concern. Indeed, the ability to conduct toxicity tests on a surrogate mixture and apply the data to many different environmental mixtures is appealing from both scientific and practical perspectives, but again, limitations and challenges abound. Foremost is the challenge of defining the level of similarity necessary to extrapolate toxicity data from one mixture to another. It seems reasonable that some degree of both toxicological and chemical similarity would be important for such extrapolations, but currently, there is no consensus on what chemical and toxicological features are essential. Nonetheless, extrapolating data from surrogate mixtures to environmental mixtures of concern is likely to be an important tool for mixtures risk assessment. Computerized methods that "lump" chemicals into groups based on physical chemical properties, structure activity relationships, and pharmacokinetic modeling may be applicable for some types of complex mixtures (Verhaar *et al.* 1997). For mixtures generally, it will be important not only to articulate clear guidelines for determining when two mixtures are sufficiently similar to justify using one as a surrogate for another, but also to formulate a method to verify that the guidelines are reliable.

CONCLUSIONS

In summary, it seems that for every valid reason to assess risks posed by chemical mixtures, there remains an equally valid question as to whether it is possible to do so in a scientifically rigorous and relevant manner. Until the scientific and technical challenges are overcome, it is incumbent on risk assessors to evaluate the uncertainties inherent in various approaches to mixture risk assessment and

to clearly communicate those uncertainties. Risk assessment methods that seek to be comprehensive at the expense of increased uncertainty can hardly be viewed as improvements. We might do better to verify that we can reduce uncertainty before burdening the risk assessment process with more complexity.

One way of reducing uncertainty might be to focus future research on identifying the mechanisms by which chemicals are most likely to interact in toxicologically significant ways, and on developing rapid assays to identify the chemicals that can participate in those mechanisms. Similar types of approaches have been explored for use in drug development (Hori 1997). For environmental risk assessment, it would seem most productive to focus on mechanisms of interaction that can occur at environmentally relevant concentrations, and to identify dose-dependent transitions in those mechanisms. Pharmacokinetic rather than pharmacodynamic mechanisms would seem to be more likely sources of toxicologically significant interactions, based on published literature (Krishnan and Brodeur 1991). Statistical optimization techniques may hold promise for determining the degree of mixture complexity at which various assessment methods contribute more uncertainty to the risk estimate than alternative methods for single chemicals. Ultimately, improving mixture risk estimates depends on developing clear hypotheses that allow us to test, refine, and validate the underlying assumptions.

REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry). 2001a. Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures. Atlanta, GA, USA

ATSDR (Agency for Toxic Substances and Disease Registry). 2001b. Guidance for the Preparation of an Interaction Profile. Atlanta, GA, USA

Berenbaum MC. 1981. Criteria for analyzing interactions between biologically active agents. Adv Cancer Res 35:269–335

Berenbaum MC. 1988. Synergy and antagonism are not synonymous with therapeutic advantage and disadvantage. J Antimicrob Chemother 21:497–500

Berenbaum MC. 1989. What is synergy? [published erratum appears in Pharmacol Rev 1990, 41:422]. Pharmacol Rev 41:93–141

Bliss CI. 1939. The toxicity of poisons applied jointly. Ann Appl Biol 26:585–615

Borgert CJ, Price B, Wells C, *et al.* 2001. Evaluating interaction studies for mixture risk assessment. Hum Ecol Risk Assess 7:259–306

Borgert CJ, LaKind JS, and Witorsch RJ. 2003. A critical review of methods for comparing estrogenic activity of endogenous and exogenous chemicals in human milk and infant formula. Environ Health Perspect 111:1020–36

Borgert CJ, Gross TS, Guiney PD, *et al.* 2004a. Interactive effects of DDE and methoxychlor on hormone production in bass gonadal cultures. Environ Toxicol Chem (*in press*)

Borgert CJ, Quill TF, McCarty LS, and Mason AM. 2004b. Can mode of action predict mixtures toxicity for risk assessment? Toxicol Appl Pharmacol (*in press*)

Cassee FR, Groten JP, van Bladeren PJ, *et al.* 1998. Toxicological evaluation and risk assessment of chemical mixtures. Crit Rev Toxicol 28:73–101

Dawson DA and Poch G. 1997. A method for evaluating mechanistic aspects of chemical mixture toxicity. Toxicol Methods 7:267–78

Durkin P, Hertzberg R, Stiteler W, *et al.* 1995. The identification and testing of interaction patterns. Toxicol Lett 79:251–64

el-Masri HA, Constan AA, Ramsdell HS, *et al.* 1996a. Physiologically based pharmacodynamic modeling of an interaction threshold between trichloroethylene and 1,1-dichloroethylene in Fischer 344 rats. Toxicol Appl Pharmacol 141:124–32

el-Masri HA, Tessari JD, and Yang RS. 1996b. Exploration of an interaction threshold for the joint toxicity of trichloroethylene and 1,1-dichloroethylene: Utilization of a PBPK model. Arch Toxicol 70:527–39

Greco WR, Bravo G, and Parsons JC. 1995. The search for synergy: A critical review from a response surface perspective. Pharmacol Rev 47:331–85

Groten JP. 2000. Mixtures and interactions. Food Chem Toxicol 38:S65–71

Haddad S, Charest-Tardif G, Tardif R, *et al.* 2000. Validation of a physiological modeling framework for simulating the toxicokinetics of chemicals in mixtures. Toxicol Appl Pharmacol 167:199–209

Haddad S, Beliveau M, Tardif R, *et al.* 2001. A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. Toxicol Sci 63:125–31

Hertzberg RC and MacDonell MM. 2002. Synergy and other ineffective mixture risk definitions. Sci Total Environ 288:31–42

Hertzberg RC and Teuschler LK. 2002. Evaluating quantitative formulas for dose-response assessment of chemical mixtures. Environ Health Perspect 110 (Suppl 6):965–70

Hori W. (ed.) 1997. Drug-drug interactions: Analyzing in vitro-in vivo correlations. Transcript of IBC's International conference "Drug-drug interactions: Analyzing in vitro-in vivo correlations," December 10–11, 1996. Washington, DC, USA

Kane GC and Lipsky JJ. 2000. Drug-grapefruit juice interactions. Mayo Clin Proc 75:933–42

Krishnan K and Brodeur J. 1991. Toxicological consequences of combined exposure to environmental pollutants. Arch Complex Environ Studies 3:1–106

Loewe S and Muischnek H. 1926. Effect of combinations: Mathematical basis of problem. Arch Exp Pathol Pharmakol 114:313–26

McCarty LS and MacKay D. 1993. Enhancing ecotoxicological modeling and assessment: Body residues and modes of toxic action. Environ Sci Technol 27:1719–28

Mileson BE, Chambers JE, Chen WL, *et al.* 1998. Common mechanism of toxicity: A case study of organophosphorus pesticides. Toxicol Sci 41:8–20

Moore LB, Goodwin B, Jones SA, *et al.* 2000. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc Natl Acad Sci USA 97:7500–02

Mumtaz MM and Durkin PR 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 8:377–406

Safe SH. 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21:51–88

Safe SH. 1998. Hazard and risk assessment of chemical mixtures using the toxic equivalency factor approach. Environ Health Perspect 106:1051–8

Silva E, Rajapakse N, and Kortenkamp A. 2002. Something from "nothing"—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ Sci Technol 36:1751–6

Tallarida RJ. 2001. Drug synergism: Its detection and applications. J Pharmacol Exp Ther 298:865–72

Toyoshiba H, Walker NJ, Bailer AJ, *et al.* 2004. Evaluation of toxic equivalency factors for induction of cytochromes P450 CYP1A1 and CYP1A2 enzyme activity by dioxin-like compounds. Toxicol Appl Pharmacol 194:156–68

USEPA (United States Environmental Protection Agency). 1990. Technical Support Document on Risk Assessment of Chemical Mixtures. EPA/600/8-90/064. Washington, DC, USA

USEPA (United States Environmental Protection Agency). 1999. Guidance for Identifying Pesticide Chemicals That Have a Common Mechanism of Toxicity. Washington, DC, USA

USEPA (United States Environmental Protection Agency). 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Washington, DC, USA

USEPA (United States Environmental Protection Agency). 2003. Draft Final Guidelines for Carcinogen Risk Assessment. Washington, DC, USA

Verhaar HJ, Morroni JR, Reardon KF, *et al.* 1997. A proposed approach to study the toxicology of complex mixtures of petroleum products: The integrated use of QSAR, lumping analysis and PBPK/PD modeling. Environ Health Perspect 105(Suppl 1):179–95