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Predicting interactions from mechanistic information: Can omic data validate theories?

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Abstract

To address the most pressing and relevant issues for improving mixture risk assessment, researchers must first recognize that risk assessment is driven by both regulatory requirements and scientific research, and that regulatory concerns may expand beyond the purely scientific interests of researchers. Concepts of "mode of action" and "mechanism of action" are used in particular ways within the regulatory arena, depending on the specific assessment goals. The data requirements for delineating a mode of action and predicting interactive toxicity in mixtures are not well defined from a scientific standpoint due largely to inherent difficulties in testing certain underlying assumptions. Understanding the regulatory perspective on mechanistic concepts will be important for designing experiments that can be interpreted clearly and applied in risk assessments without undue reliance on extrapolation and assumption. In like fashion, regulators and risk assessors can be better equipped to apply mechanistic data if the concepts underlying mechanistic research and the limitations that must be placed on interpretation of mechanistic data are understood. This will be critically important for applying new technologies to risk assessment, such as functional genomics, proteomics, and metabolomics. It will be essential not only for risk assessors to become conversant with the language and concepts of mechanistic research, including new omic technologies, but also, for researchers to become more intimately familiar with the challenges and needs of risk assessment.

Keywords: Mechanism of action; Mode of action; Drug interaction; Chemical mixtures; Toxicogenomics

Introduction

Toxicity data are lacking for most drug and chemical mixtures, and so risk estimation typically relies on model predictions. Predicting mixture toxicity has traditionally been approached in one of two ways. The first is to predict toxicity of the mixture of interest based on a similar mixture for which toxicity data are available. The challenges presented by this approach have been summarized (Borgert, 2004) and will not be expanded here. The second approach is to predict the toxicity of the mixture from toxicity data on individual components of the mixture, including the pharmacological and toxicological interactions that may be produced by combined action of the components. This paper addresses the second approach. Throughout this paper, "chemical" is used in the general sense and may include drugs, pesticides, food ingredients, etc.

While toxicity data on individual chemicals are often available, reliable and relevant data on interactions are lacking for most chemical combinations (Borgert et al., 2001; EPA, 1988; Hertzberg and MacDonell, 2002; Hertzberg and Teuschler, 2002). For this reason, mixture risk assessments must often predict the combined action of chemicals in mixtures in the absence of clear empirical data on which to do so. Here again, at least two fundamentally different approaches are possible. One approach would be to make mathematical predictions about combined action based purely on the shape of the dose–response curves of the individual components of the mixture; in other words, to mathematically predict the aggregate dose–response curve from knowledge about the individual dose–response curves. This approach has received little attention, probably because a clear biological basis for it has never been developed. The more widely used approach predicts which of

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two mathematical models of combined action best applies based on mechanistic presumptions about the individual chemicals in the mixture. The rationale behind using mechanistic understanding to predict the combined action of chemicals in mixtures relies implicitly on the terminology and concepts used to define interaction, non-interaction, and mode or mechanism of action. This paper explores these concepts and the assumptions and limitations inherent in each, and suggests ways of integrating research on interactions more tightly with the needs of risk assessment.

Interaction and mode of action

Interactions are of two fundamental types: synergistic or antagonistic. Synergism means that when chemicals are administered together, more of a particular response is produced than was expected, or, that a given level of a particular response is produced with a lower dose of chemicals than expected. Antagonism means the converse: that when chemicals are administered together, less of a particular response is produced than expected, or that a higher dose than expected is required to produce a given level of the particular response. These definitions create a logical conundrum if the goal is to predict interactions, which are defined as the unexpected. Furthermore, interactions with significant biological consequences may be relatively rare and synergisms may be opposed by antagonisms. Together, these factors may justify, at least in part, the implicit assumption used in mixture risk assessment that non-interaction is expected.

The concept of non-interaction has both pharmacologic and mathematical implications, but these implications are not necessarily related to one another. A true test of pharmacological non-interaction would require a comparison of biological outcome between conditions that allow and disallow interactions. Since few experimental systems provide the opportunity to conduct such comparisons, non-interaction has been defined on the basis of mathematical models. Two classical models for non-interaction have gained acceptance and widespread use in pharmacology and toxicology. These are Loewe additivity (Loewe and Muischneck, 1926), also called dose addition, and Bliss independence (Bliss, 1939), also called response addition. Both models involve simple addition of parameters related to the components of the mixture, but as implied by their common names, Bliss adds responses whereas Loewe adds doses. Although neither model has a clear biological basis, deductive reasoning has been used to align the parameters added by each model with notions about the mechanistic relatedness of the components of the mixture.

Bliss independence assumes that a non-interacting chemical produces a certain level of response as if the other chemicals in the mixture are not present. Predicting the response of the mixture then becomes an exercise in summing the level of response produced by each individual component of the mixture. If the level of response produced by the individual components of a mixture is independent and can be combined by simple summation, inductive reasoning leads to the assumption that those levels of response must have been produced by independent pharmacologic/toxicologic mechanisms. Thus, Bliss independence (response addition) is used in mixture risk assessments to predict the combined response to a mixture of chemicals believed to produce that response by different (independent) mechanisms of action.

Loewe additivity assumes that chemicals producing a particular response can be treated as simple dilutions of one another, differing only in potency, and thus, Loewe additivity (dose addition) has been aligned with the combined response to chemicals that share a similar mechanism of action. This alignment is a logical extension of Berenbaum's assertion (Berenbaum, 1981) that the pharmacologic response to multiple doses of a single chemical must always be additive, by mathematical definition. In other words, two 325 mg aspirin tablets must produce the same level of response as one 650 mg aspirin tablet, by simple mathematical definition. He argued that this must be true regardless of whether the chemical shows "self-interaction," and thus, that dose addition serves as a clear sham model of non-interaction for interaction studies.[1] Although Berenbaum argued that the choice of a non-interaction model for chemical combination studies should not be based on presumptions about mechanisms of action (Berenbaum, 1989), his argument that multiple doses of a single chemical serve as the proof-of-principle for dose addition appears to has led to the assumption that since multiple doses of the same chemical have the same mechanism of action and are dose additive, mixtures of different chemicals with similar mechanisms will also behave by dose addition. It is important to appreciate the logical impediment to testing this assumption empirically, and why caution must be exercised when interpreting studies that purport to have empirically tested the correlation of similar mechanism and dose addition; confirmation (or refutation) of the correlation depends entirely on the accuracy and level of mechanistic understanding of the chemicals used to test the correlation (Borgert et al., 2004; Greco, 2001; Berenbaum, 1989).

Regardless of the strength of the empirical basis for correlating similar and dissimilar mechanisms with dose and response addition, predicting the toxicity of mixtures for risk assessment has become, *de facto*, an exercise in predicting the mechanistic similarity and dissimilarity of mixture constituents. Mechanistic similarity, however, is a vague concept that has yet to be clarified scientifically (Borgert et al., 2004). Imprecise use of terminology is largely to blame for at least some of the vagary, so it is important to clarify a few definitions[2]. A mechanism of action is the detailed, step-wise sequence of events proceeding from absorption of an effective dose of a substance to the manifestation of a specific biological effect. A "mode of action" is a category of mechanisms that share common key features. Many more chemicals will share modes of action than have the same mechanism.

[1] In this context, "self-interaction" would mean that there is not a constant proportionality between changes in response relative to changes in dose.

[2] The definitions of mode and mechanism of action provided here are consistent with those in the recent published literature (Dellarco and Wiltse, 1998; Schlosser and Bogdanffy, 1999; Sonich-Mullin et al., 2001; Borgert et al., 2004).