Commentary

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General Principles in the Assessment of Toxicity of Chemical Mixtures

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A toxicological interaction may be defined as a condition in which exposure to two or more chemicals results in a qualitatively or quantitatively altered biological response relative to that predicted from the action of a single chemical. Such multiple-chemical exposures may be simultaneous or sequential in time and the altered response may be greater or smaller in magnitude (*1,2*). The question of how the risk to health of exposure to a combination of chemicals compares to the estimated risk from exposure to each alone may be approached in several ways. The existing literature may be searched for laboratory, clinical or epidemiological studies that deal specifically with exposures to the combinations of chemicals in question. Laboratory and/or epidemiological studies may be initiated to specifically test for interactive effects of specific combinations of chemicals when and if concern for a specific joint exposure arises. Knowledge of the toxicokinetic and toxicodynamic characteristics of individual chemicals may be used to make judgements of the potential for altered health risk arising from specific combined exposure situations. All three of these have several limitations in any problem area dealing with potential exposures to numerous and diverse chemicals. The number of possible combinations of exposures is multiplicative as the list of individual chemicals of concern grows. Furthermore, the permutation of time considerations (that is; simultaneous, separate but close in time or far in time, repeated or single exposures to multiple chemicals) greatly complicates the design of studies for assessment of interactions.

As for the first approach, that of literature search for interaction data, there are few reports of laboratory or epidemiological studies that have addressed the question of toxic interaction of complex mixtures of uncertain and rather unpredictable constitution, as might arise from a chemical waste dump.

With regard to the second approach, chemical technology has advanced more rapidly than our abilities or resources to adequately assess the toxic hazards of the products of that technology as even single-chemical exposures. Comprehensive toxicological testing of the myriad combinations of chemicals to which humans may conceivably be exposed under conditions which may result in toxicological interactions would be a task of Herculean proportions.

The third of the above three approaches may be useful, provided that it is possible to identify the toxicokinetic and toxicodynamic factors that might contribute to altered organismic responses due to multiple-chemical exposures as contrasted with single-chemical exposures. In other words it is necessary to consider the basic principles underlying the mechanisms of toxic interactions in terms of these toxicokinetic and dynamic factors. This is a somewhat idealized approach to the problem, and one in which the reality of limited existing data for the individual constituents of a complex mixture will likely not permit quantitative assessments, nor, perhaps, even conclusive qualitative assessments. However, the identification of the sites and mechanisms at which and through which toxic interactions can occur may lead to other useful actions. A systematic approach to search of the literature on individual chemicals that may be involved in interactions could be developed. There may be more data on individual compounds that are specifically related to one or more of the sites and mechanisms of interactions than is apparent if data specifically relating to actual interactions are the

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determinants of the literature search. A set of data points could be identified which could be systematically pursued experimentally, if literature review indicated the need. Thus, the design of toxicological research in general may be aided by these guidelines or concepts whether directed toward individual chemicals or toward studies of multiple-chemical exposures. The essential features of a model could be identified which could be tested experimentally. This information might form the basis for mathematical analyses or predictive modeling.

In short, in order to attempt a broadly applicable assessment of the potential for toxic interactions among environmental pollutants it is essential to develop a systematic approach to the problem, even if that approach is theoretical with respect to the current knowledge base.

General Mechanisms of Toxicological Interactions

The injury produced by a chemical in a living organism is proportional to the quantity of the biologically active form of that chemical available to react with critical, responsive cellular macromolecules.

We may view the mechanisms of interactions from the biological sites at which they might rationally be expected to occur, and from the standpoint of how each of these critical sites may be altered by one chemical to cause an altered responsiveness to another chemical. Many chemicals are activated as well as detoxified or otherwise removed from the organisms. Therefore, we can think of three primary biological sites that are critical to the toxicity of the chemical. These are: (1) the actual macromolecular receptor sites at which the chemical, or its metabolite, acts to produce injury, i.e., sites of action; (2) the sites of loss at which the compound is effectively removed from the sites of action or its injury-producing potential is destroyed (these include enzymatic detoxification by binding or storage, and transport sites in organs of excretion); and (3) the enzymatic sites at which chemicals are converted to more biologically active substances, i.e., sites of activation.

Generally one can consider that the toxicity of one compound may be altered if another compound alters the number of one or more of these sites, for example by either induction or inhibition of enzymes that catalyze either activation or loss of a chemical; alters the affinity of any of the critical sites for the compound in question; or alters the intrinsic activity of the chemical at any of these critical sites.

Temporal Relationships

When an organism is subjected to multiple-chemical exposures, the nature and degree of toxicological interaction will be dependent in part, on the temporal relationships between or among exposures.

Sequence

Although, classically, considerations of toxicological interactions have dealt with simultaneous exposures to combinations of two or more chemicals, an equally likely situation would be that exposures to multiple chemicals would be sequential. The order in which these exposures occur and the length of time between them will be determinants of the likelihood of toxicological interaction.

When exposures occur simultaneously or very close in time, the likelihood that toxicological interactions will occur, is very likely to be dependent upon competition between chemicals for sites of absorption, biotransformation, actions and excretion. The concentration of each potentially interacting compound within the organism and their relative binding affinities and/or intrinsic activities will be most important.

However, when exposure to different chemicals are separate in time, the biological half-life of each chemical or its metabolites, the duration of binding to tissue macromolecules and the rate of repair of injury may all assume greater importance as mechanisms of toxicological interaction, than affinity and intrinsic activity.

Frequency

The frequency of exposure can also be a determinant of whether or not toxicological interactions will occur. Obviously, the more often exposure to a chemical occurs, the greater the statistical probability it will occur in the presence or close in time to exposure to another, possibly interacting, chemical. But beyond merely statistical considerations are the influence of frequency of exposure on the accumulation of a body burden of the chemical, the accumulation of cellular injury with or without accumulated body burden, and opportunity for reversal of action or repair of injury. These are all factors that influence the opportunity for toxicological interactions, of different mechanism types, to occur.

Toxicokinetics

Clearly the kinetic relationships between or among two or more biologically active chemicals controls

the likelihood of toxicological interactions. The type of information that must be available for each chemical in order to accurately predict its potential for interaction with another must extend beyond the usual kinetics of disposition, biotransformation and receptor binding. Information on the kinetics of reversibility of injury, such as kinetics of cell repair, or resynthesis of irreversibly inhibited enzymes, must also be available.

Predicting Interactions

Basic principles concerning the kinetics of reaction of a chemical with primary sites of action (tissue receptor sites) and with secondary tissue sites of reaction are important to a consideration of the joint toxic action of chemicals. Most of these were succinctly reviewed by Veldstra (*3*) 25 years ago. With respect to the joint action of chemicals, three factors were identified by Veldstra as being most important: (1) relative affinities (taken as the net of the association and dissociation rate constants) of the individual chemicals for sites of action (e.g., target enzymes, neuro-effector sites or other vital target sites), (2) relative affinities for sites of loss (e.g., detoxifying enzymes, nonvital tissue binding sites, pathways of excretion and storage sites), and (3) the intrinsic activity of the compounds at their sites of actions. It is also important to keep in mind the concept that there are a limited number of sites of action or sites of loss within any organism. Therefore, there will be a limiting dosage range within which synergism or antagonism can be demonstrated. Although when Veldstra published his review there were few known examples of biological activation of chemicals, this phenomenon is now well established and therefore sites of activation, as well as sites of loss and sites of action, are identified as reaction loci for toxic interactions.

Toxicological interactions can be perceived, in general, as taking two forms: the quantity of an active form of one or more chemicals available to the pool of critical cellular macromolecules is altered by the presence (or past presence) of one or more other chemicals, or the reactivity of the critical macromolecules with the active form(s) of exogenous chemicals is altered by the presence (or past presence) of one or more other chemicals which may or may not themselves be capable of eliciting a response. The first case can be said to involve primarily sites of loss or sites of activation of a chemical and case two represents interaction at sites of action. In the latter case either affinity for or intrinsic activity at the site of action may be altered.

One compound may alter the number or the activity of either sites of activation or sites of loss of a second compound. But the extent that this will be manifest in an altered susceptibility of the total organism to toxic action of the second compound will depend upon whether the altered sites and pathways are rate-limiting with respect to the toxic action. If alternative and unaffected pathways or sites of activation or loss are present, the predicted interaction may not develop; or it may be modulated in its intensity.

The proximity of sites of action to the critical sites of activation or loss may be an important determinant of toxic interactions. Thus, the induction of an activation enzyme in liver cells may have unexpectedly little effect on acute toxicity that is mediated through a target enzyme (i.e., the site of action) located in the brain, if the active metabolite produced by the liver is so reactive that it irreversibly binds to noncritical molecules in liver cells (where it is produced) or if it is detoxified as rapidly as it is produced. Thus, with multiple pathways of metabolism which both activate and detoxify organic chemicals, and with multiple sites of action which may vary widely in their criticality to life, it is often necessary to consider the so-called opportunity factors in predicting or understanding the mechanisms of toxic interactions. Opportunity for a certain critical reaction of one compound to occur in the face of other competing macromolecular reactions can be dramatically altered by other chemicals. That is, if one compound, such as a mixed function oxidase inducer, increases the concentration of microsomal sites of detoxification as well as microsomal sites of activation, will the induction of the detoxifying system effectively reduce the opportunity for the parent compound to be activated to a tissue-reactive form? Other time-related opportunity factors relate to the rate of reversal of the injury, or the binding of an active metabolite to a primary site of action in relation to the net rate at which the active metabolite can accumulate at the site of action.

Conclusion

The prediction of the likelihood of increased risk due to multichemical exposures will require detailed information concerning each of the components. Investigators studying single chemicals should be encouraged to collect the kind of data that will allow a biomathematical/kinetic approach for obtaining a first approximation of the likelihood of one compound interacting toxicologically with another. Considerable validation of this approach will be required before it can be used for definitive decisions regard-

ing increased risk of multichemical exposure. However, increased emphasis on understanding cellular mechanisms and chemical disposition of single chemicals should provide the basic data necessary to test the hypothesis that toxicological interactions are predictable with reasonable accuracy from appropriate quantitative data on each component of a mixture. Thus, creative application of data obtained in the molecular toxicology laboratory may enable us to examine the "laundry list" of chemicals from varied dump sites and make reasonable predictions as to whether there is increased health risk because of likelihood of toxicological interactions.

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