THE RISK ASSESSMENT OF CHEMICAL MIXTURES: A CONCEPTUAL APPROACH

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ABSTRACT

The U.S. Department of Energy is responsible for a large volume of contaminated material that resulted from past research and weapons production activities at a number of sites across the country. Many chemical and radioactive contaminants are present in areas that were previously used to discharge or dispose of wastes during the operational periods of these facilities, which extended through the end of the Cold War. The presence of multiple contaminants at these sites and the possibility for people to be exposed to different media by a variety of routes over time make the potential risks from exposures to chemical mixtures difficult to characterize. Further complicating this task is the fact that little information is available on the combined effects of multiple chemicals from exposures to environmental contamination. Data from laboratory experiments on single chemicals are available, and several databases on the toxicity of individual chemicals have been compiled, but relatively limited information is available on binary and more complicated mixtures. Moreover, most toxicity data are from animal studies and thus have the potential to inaccurately predict interactions that may occur in humans. These interactions may be manifested as antagonism (decreased effect) or synergism (increased effect).

Recognizing that humans are typically exposed to mixtures rather than single chemicals, a number of approaches and models have been developed to predict the toxicity of mixtures. These models are often simplistic and may fail to capture the mechanistically significant aspects of the toxicological interaction; conversely, they may be highly detailed and require too much information for practical use. There is a clear need to evaluate and refine the methodologies that have been proposed, to facilitate their use in risk assessments for mixtures at contaminated sites. The potential for significant interactions among specific contaminants creates the need for selecting not only priority chemicals but also priority mixtures from the typically large list of contaminants that can be present at these sites. This paper explores various factors that affect the selection of priority mixtures, assessment of mixture toxicity, and modeling of chemical interactions based on information found in the scientific literature and in assessment guidelines that have been recommended for single chemicals and mixtures. Combining this information, a conceptual approach is then proposed for evaluating risks of chemical mixtures.

INTRODUCTION

An important step in health risk assessment is the determination or prediction of the toxicity of chemicals to which humans may be exposed. Exposure scenarios typically involve more than one chemical. This paper presents a conceptual approach to predicting mixtures toxicity and associated health risks from exposures to multiple chemicals. This introductory section describes why chemical mixtures are a concern and what complex chemical interactions can arise from joint exposure. It also examines the relation of chemical interaction to risk assessment and the difficulties in predicting mixture toxicity. The second section (Methods) describes approaches taken by other researchers to address this issue of mixture toxicity and discusses some of the complexities involved. The third section (Recommendations) proposes a conceptual model that can help dissect and examine exposures to a hypothetical chemical mixture. The last section (Discussion) briefly outlines the way this model is being used to frame a focused pilot study for a mixtures risk assessment at a large U.S. Department of Energy (DOE) site.

Background

Human exposures to environmental toxins seldom involve one chemical exclusively (1,2). We are often exposed either simultaneously or sequentially to different concentrations of multiple chemicals with different toxicities. In conducting risk assessments for DOE sites, it may be important to consider the potential for increased or decreased health effects associated with exposures to chemical mixtures. Many hazardous waste sites track inventories of wastes and contaminated environmental media that contain more than 100 chemicals combined (3,4). At DOE sites, these inventories typically include

radionuclides and both organic and inorganic chemicals. These contaminants are commonly co-located, and the co-location patterns can change over time due to characteristic properties of the contaminants and hydrogeological and biological conditions at a given site. There is a need to consider the toxicity of the individual chemical components of a mixture separately as well as in combination with the other components. Determining both potential exposures to mixtures and associated health risks poses a major challenge for today's risk assessor.

Chemical Interactions

Chemical toxicity is typically characterized by using a dose-response relationship. Exposure of an organism results in the intake of a specific amount of a potentially toxic chemical or mixture. The dose is the amount of toxin effectively introduced through that exposure to the organism as a whole. A portion of the exposure dose may not be retained in the body, but rather it can be cleared through excretion or respiration. The dose is usually reported in terms of mass of chemical per mass of body weight of the organism per exposure time, e.g., mg/kg-day.

Exposure to a specific amount of toxin (or mixture) can cause a change in the biochemical, physiological, or histopathological conditions that existed within the body prior to the exposure. This is defined as the response of that organism to the given chemical dose. By measuring the doses of a chemical or mixture and the respective responses observed to result from those doses, one can derive a relationship between dose and response that is typically shown by a dose-response curve. The observed response does not necessarily yield information relevant to the biochemical mechanism by which the toxic components cause toxicity (5). However, lack of data on the underlying biochemical mechanism does not exclude observation of a relationship between physiological response and dose.

The observed dose-response can be measured in many ways. One measurable response is population susceptibility, where the percentage of a population of test subjects affected by the mixture is reported as a function of the applied dose. The response can also be reported as a quantity such as the average variation in body weight of the affected test organisms or the amount of enzyme response elicited by a chemical exposure. The former is an end physiological response and the latter is an underlying biochemical response. Often the dose-response relationship of a chemical can be characterized by using a fitted mathematical relationship between the applied dose and the elicited response (6). The dose-response character of a chemical or a mixture may be interpreted as its toxicity. Chemicals can elicit multiple and varied toxic responses within the body, each potentially subject to complex interactions with both primary biochemical responses and secondary physiological responses of other chemicals in the mixture (7).

When an organism is exposed to more than one chemical, generally two types of response phenomena can observed. In the first type, the mixture toxicity results in the same effect that would be elicited if each of the components were operating independently. This type of mixture toxicity is termed additivity (5,6,8). There are two forms of additivity: dose addition and response addition (6,9,10). With dose addition, the chemicals behave similarly both physiologically and toxicologically, and each can be scaled to an index chemical. With response addition, the chemicals act independently.

In cases where the response is not additive, the chemical mixture interacts within the body such that the toxicity of one or more chemicals in the mixture is altered compared to what it would have been if the chemical(s) were introduced alone. The two general types of interaction that occur when additivity is not applicable are termed antagonism (infra-additivity) and synergism (supra-additivity) (6). For example, if the response is less than that predicted by a dose-response model for additivity, the interaction is said to be antagonistic; the response caused by one or more components is effectively reduced through interaction with another component. The converse is true for synergism, i.e., the response is effectively increased.

Risk Assessment

The human body is a complex system comprised of chemicals that form cells, organs, and tissues whose functions are interdependent. Introduction of a xenobiotic into the body can cause a cascade of

events that can affect multiple systems and ultimately result in illness or disease (6,10,11). Correlations between the health risk of a given toxin and the dose of the toxin have been characterized and made available in resource documents and databases through several government departments and agencies – including the U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) and Health Effects Assessment Summary Tables (HEAST) (12,13) and Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profiles. The ATSDR HazDat database also provides information on individual chemicals, and an initiative is underway to incorporate information on the toxicity of mixtures that will aid in conducting risk assessments (4).

In an attempt to examine the risk assessment of mixtures directly, the EPA compiled an extensive database of binary interaction studies (14). This database illustrates the breadth of mixtures toxicology and also shows the large diversity of data and assessment approaches that typify the field. For a given mixture of concern, it is unlikely that the dose ranges or toxic effects that have been examined in laboratory studies will encompass the many circumstances that arise in the environment – a fact that further complicates current mixtures toxicology and risk assessment. Durkin et al. (*1992)* (1) show that in addition to the lack of depth in existing interaction studies, the research seldom utilizes rigorous statistical analyses to examine the data obtained in an experimental setting. The EPA 1990 guidance document on chemical mixtures includes a more rigorous evaluation of the data in this mixtures database. The human body's response to exposure to multiple environmental chemicals that are concurrently or sequentially introduced remains only minimally characterized, and only in very specific cases that do not yet provide a sound basis for making decisions regarding potential mixtures risks. Because current data are insufficient to complete a model that represents a comprehensive, unifying theory of mixtures risk assessment, interim approaches must be constructed that will be able to accommodate new information as it is developed.

Difficulties of Predicting Chemical Interaction

The end goal of toxicology and health risk assessments is to predict the effect that chemicals or mixtures of chemicals could have on exposed individuals and populations. Because of the inability of current approaches to accurately predict health effects from exposures to environmental mixtures, risk analysts continue to pursue a better understanding of the individual elements of the assessment process. This is achieved by studying the physical properties of the chemicals and mixtures themselves, as well as studying the toxicities of chemicals and mixtures. Many limitations exist in our ability to obtain comprehensive toxicity data from *in vivo* or *in vitro* laboratory studies in controlled animal systems. Time and cost restrictions inhibit the thorough examination of even a small number of possible chemical combinations (15, 16, 17), especially considering the need for studies that address cross-species, age, and exposure route differences.

Additional specific limitations that affect the ability to develop mixtures toxicity data include the following. Laboratory experiments often use high doses of the toxin(s), which introduces uncertainty in extrapolating to the low doses typically relevant for environmental exposures (18). Also, realistic human exposure routes may be not be reflected in the toxicological study, as chemicals are often introduced via injection instead of ingestion (e.g., of drinking water) or as a pure vapor mist instead of as attached to particulate matter (as would be the case in an actual exposure scenario). These non-representative exposures can also alter the bioavailability of the chemical, which in turn can alter the effective dose of the toxin. Further, extrapolating toxicity data from nonhuman to human systems can introduce considerable uncertainty (19). Thus, while it is important to make the best use of existing data, it is also important to assess the applicability of those data to the hazard or risk problem being studied, so that the approximations will be educated and the uncertainties will be reported and quantified where possible.

METHODS

The field of chemical mixtures toxicity is very diverse and has undergone considerable innovation in recent years. While many methods have been developed and proposed for assessing the risks of chemical mixtures, these models are often simplistic and may fail to capture the mechanistically

significant aspects of the toxicological interaction. Conversely, they may be highly detailed and require too much information for practical use.

The simpler approaches provide reasonable initial estimations, and accompanied by thorough uncertainty analyses they can also yield insights into what aspects of the model would benefit from further enhancement. More complex approaches offer a more comprehensive framework, which although describing more closely the true complexity of the human system demands a substantial amount of information. These models may be simplified with sound assumptions; however, this typically introduces uncertainties that must be described and if possible quantified. The advantages and disadvantages of several approaches are discussed below.

Whatever methodology is chosen as an initial approach to conducting a mixtures risk assessment, it should take into account the very limited amount of information, and the even smaller amount of quantitative information, that is available for mixtures in real systems (6,10,20). Models are often developed with these information limitations in mind.

Recommended Guidelines for the Risk Assessment

The EPA has published several documents that specifically address the issue of health risk from exposures to chemical mixtures. These documents provide standardized definitions of mixture terminology, reviews of the literature, and recommended methods for predicting the toxicity of a chemical mixture. The documents also suggest methods that can be used to assess health risks. The complexities introduced in exposure pathway and contaminant fate and transport assessments for mixtures are not explicitly addressed by these documents, as other Agency guidance and resource documents have been developed to address those elements of the risk assessment process. Similarly, the approach for selecting priority mixtures from the full suite of contaminants found at a site is not explicitly discussed in the mixtures guidance documents. An overview of key mixtures documents is provided below.

In 1986, EPA published a guidance document on mixtures risk assessment (10). This document discusses some of the key issues, explicitly differentiating between a mixture and a single chemical, and introduces additional concerns that arise from mixture toxicology.

In 1990, a follow-up document was released to address the concern over technological capacity. This document further defines what a chemical mixture is, provides technical guidance regarding the tools used to quantify health risks, and supplies details for a mixtures risk assessment (6). An important recommendation made by the document is that these risk assessments be completed on a case-by-case basis. This recommendation acknowledges the large gaps in existing information for mixture toxicity and the potentially different character of any one mixture from another. It is unlikely that a single method can be exclusively used for risk assessment. Thus, it is important that available methodologies be evaluated and their applicability to various circumstances be assessed in order to gain insight into what characteristics a comprehensive and generally applicable mixtures methodology ultimately should possess.

The 1990 EPA guidance document goes on to suggest that each assessment be presented in full transparency. In other words, the risk assessor is encouraged to portray up-to-date scientific information, the most appropriate scientific interpretation of that information, and the approaches that might be used to conduct the risk assessment. The choice of the appropriate method is to be justified by citing the strengths and weaknesses of the approach taken. Discussions should cover the uncertainties resulting from system variability, assumptions made to allow the data to be manipulated, and limitations of the methods chosen. *In toto,* the assessment must be discussed inclusively, intact with the scientific rationale and basis for conclusions drawn or recommendations made.

The basic process for assessing risks at Superfund sites is discussed in the 1989 EPA document, Risk Assessment Guidance for Superfund (RAGS) Volume I (20). Although this document does not provide a detailed approach specifically for chemical mixture hazard identification or toxicology, it does clearly outline the overall risk assessment process. Particular considerations that co-located chemicals introduce to the system and their assessment are discussed.

Mixture Hazard Identification and Exposure Assessment

The first step of the risk assessment process for a contaminated site involves identifying the environmental hazards, notably the chemicals that could be harmful to humans if exposures were to occur. This includes consideration of what contaminants could be bioavailable to humans. A toxicity-concentration screen can then be conducted to define the contaminants of potential concern to be carried forward for more detailed analyses. This same consideration can be applied to the selection of mixtures of potential concern. Co-located chemicals have the potential to alter each other's behaviors not only within an organism but also in the various environmental media through which transport and environmental transformation occur. A mixture should be examined for its intrinsic properties that may cause it to behave differently from its individual components. Altered speciation or transport characteristics can change the exposure point concentrations and amounts taken into the body in the same manner that toxicological interactions can alter the effects co-exposed chemicals may have on the body following intake. Chemical co-location together with exposures to multiple media via multiple routes should be considered when identifying chemicals of concern for a mixtures risk assessment.

As the number of mixture components increases, the complexity of the risk assessment also increases. Hazard identification for single-chemical exposures is typically accomplished by assessing the frequency of occurrence, toxicity, and likelihood of exposure; screening-level risk assessments are then commonly conducted to focus the more detailed analyses (4). However, this information alone can be inadequate to characterize the potential health risks associated with exposures to a chemical mixture.

The individual routes of exposure are important, as they can affect the internal distribution and associated toxic effects of chemicals. While exposures can be assessed on a chemical-by-chemical basis (20), consideration of pre-exposure conditions and interactions resulting from transport and volatility may also be considered (3). In addressing exposures to mixtures, consideration should be given not only to combined exposures to multiple media but to how relative concentrations of contaminants in those media may change over time, for example due to differential transport caused by interactions among mixture components. Also, the exposure assessment should extend beyond the common assumption of an integrated chronic intake to consider intermittent and sequential exposures, as timing is an important factor for mixtures. For example, depending on the chemical-specific residence time, certain chemicals from an initial exposure may still be in the body when other chemicals are introduced by a subsequent exposure. This co-location in the body would lead to internal exposure to a mixture that did not exist at the time or location of the original exposure. That is, two chemicals separately introduced can still constitute a mixture if both are present (or residual effects are present) within the body at the same time, although this overlap may occur much later than the initial exposure. The chemicals to which an individual is assumed to be exposed, jointly or sequentially, can constitute the mixture of concern. In the next step, the mixture of concern must be assessed for its potential toxicity to humans, both as individual chemical components and as a whole mixture.

Approaches to Modeling Chemical Mixture Toxicity

A continuous toxicological model of the human body would offer the most complete representation of the responses predicted to be elicited by introduction of xenobiotics (7,11). The processes that describe the exposure, distribution, and excretion of the chemical mixture throughout the body are called toxicokinetic processes. The processes by which the chemical mixture interacts with the body in a way that changes the chemical or physical nature of its cells or tissues are called toxicodynamic processes (7,21). These generalized processes, which are modeled as functioning interdependently, are used to describe the functions of the human body and its response to given chemical insults. However, to model the entire human body as a continuous system requires a thorough and complete understanding of biological processes.

The simplest approach for predicting the toxicity of mixtures does not consider these complexities, as the default is to use the additive approach described earlier. The two types of additivity that can be used to model mixture toxicity are dose addition and response addition (6). Dose addition is recommended when assessing noncancer health effects. This approach is best justified when the mixture

components act by a similar toxicological action, with the contribution of each chemical to overall toxicity dependent on the relative toxicity of that component (5).

For individual contaminants, the application of additivity for most risk assessments requires the availability of allowable safe levels (for the noncarcinogenic endpoint) and dose-response relationships (for the carcinogenic endpoint) for chronic toxicity. IRIS (12) contains reference doses (RfDs) to assess the noncarcinogenic risk for ingestion exposures, reference concentrations (RfCs) to assess the noncarcinogenic risk for inhalation exposures, and cancer slope factors to assess carcinogenic risk for the appropriate exposure route; certain chemicals have both a slope factor and a reference dose or concentration (12). HEAST (13) also includes slope factors to estimate carcinogenic risks for radionuclides. These values for single chemical toxicity are derived from levels expected to have no adverse effect in animals, considering the chemical's lowest known adverse effect in a specific tissue or organ.

Though chemicals may share similar toxicological effects, the magnitude of their dose-response relationships may differ. Exposure levels can be scaled using the toxicity values given for chronic effects in IRIS and HEAST, e.g., by dividing the ingestion intake or exposure level by the RfD for oral exposure. When dose additivity applies, these scaled exposure levels of the mixture components can be added together to obtain a numerical representation of the mixture hazard, termed the hazard index (HI). If the HI exceeds unity, then the dose of that mixture is assumed to exceed the safe level for chronic exposure.

Response addition describes independent toxicological events elicited by mixture components that act independently, e.g., via different mechanisms (5). The sum of the predicted responses is the total of the potential deleterious effects of the components independently contributing to the excess health risk estimated for a receptor. Response addition is the default when assessing carcinogenic risk (6).

The values in these toxicity databases are based on the sensitivity of the most susceptible target organ (12). This allows the most toxic effect to be identified for the individual chemical. However, if there is a modification of the component toxicity in the mixture, the resultant effect on total health risk must be considered. One possible consequence of interaction is that the acute effects of a chemical may occur at lower doses, and possibly in a different tissue, than indicated in the single-chemical studies reflected in the database. Both types of additivity assume that the toxicological characteristics of the chemicals remain unaltered by the other mixture components. Thus, additivity as the default approach is inadequate to account for all interaction potential.

At the other end of the spectrum, one could attempt to parameterize the toxicology of the entire human body, but this would exceed our current level of understanding. The charge for risk assessors is to provide scientifically sound information to decision makers. For this reason, it is important to distill the most significant components from the complex, continuous model to create a simplified model that can address the key phenomena of mixture toxicity. This should be accomplished without neglecting those factors that could result in inaccurate estimates of risk.

The amount of research that has been conducted on chemical mixture toxicity is extensive. Thousands of studies have been performed in the areas of toxicokinetics, toxicodynamics, animal-to-human extrapolation of toxicity data, experimental determination of dose-response data, estimation of low-dose effects from-high dose experiments, statistical methods for planning experiments efficiently, quantitative models of structure-activity relationships (QSARs), determination of toxicological mechanisms, and statistical approaches to examining toxicity data. Most of these topics are discussed in a 1995 special edition of the journal Toxicology, which contains papers from a conference on the state of mixtures toxicology (22).

Dose Measurement or Prediction

The simplest mixture models will only define the toxicity of a very specific mixture. Consider as an example a mixture M that contains chemicals A, B, and C in the following proportions by weight: 20% A, 45% B, and 35% C. For simplicity, it is assumed that someone is exposed to this mixture by only one pathway, ingestion of water contaminated with M. Further assuming a standard drinking water ingestion rate and hypothetical exposure point concentration, it is estimated that the individual's chronic

intake of M is 0.1 mg/kg-d. This information can be used to assess the toxicological response to the mixture as follows.

The dose to the biological system is 20% A, 45% B, and 35% C, but this does not necessarily indicate that the dose reaching different tissue types and organs can be described with the same percentages. When chemicals affect more than one organ or tissue type, the dose to each organ must be determined. The way that a chemical partitions or distributes itself in the human body can be quantified with toxicokinetic principles, given appropriate data. If A, B, and C do not distribute proportionally to one another, the relative levels of toxin affecting each organ or tissue type will be different from those to which the person was originally exposed.

Assume further that the chemicals in the mixture do not partition proportionally but are represented by the following distributions: chemical A distributes 50% to the liver, 40% to the kidney, and 10% to the pancreas; B distributes as 10%, 60%, and 30%; and C distributes as 30%, 20%, and 50%, respectively. The total dose and the breakdown by mixture components are given in Table 1. These data illustrate that the effective dose to the entire system is different from that observed at the target organs.

Table 1. Hypothetical exposure to a mixture of chemicals A, B, and C results in the following doses to the indicated organs (in mg/kg-d; may not sum exactly due to rounding).

	Dose	**A**	**B**	**C**
Total	0.100	0.020	0.045	0.035
Liver	0.025	0.010	0.005	0.011
Kidney	0.042	0.008	0.027	0.007
Pancreas	0.034	0.002	0.014	0.018

If the exposure dose were used as the effective dose in calculating health risk, the calculation would be inaccurate in two ways pertaining to the toxicokinetics of the system. The first is that the total dose to the system would be overestimated. While the total dose to the entire system is 0.100 mg/kg-d, the largest effective dose to any target organ is 0.042 mg/kg-d (to the kidney). Noting that the effective dose of A to the pancreas is only 0.002 mg/kg-d may provide impetus for focusing on components B and C as chemicals of primary concern for risk to the pancreas, depending on additional toxicity information.

The second lesson to be learned from this hypothetical scenario is that the ratios of the mixture are different at each target tissue and also different than the makeup of the original dose. This possibility has very important implications when considering kinetically limited toxic mechanisms. For example, if chemical A interacted with chemical B in the kidney and liver via the same mechanism, one would expect very different end results at the two target tissues. The ratio of A:B in the liver is 2 and in the kidney is 0.3, but the exposed doses would suggest a higher ratio. This disparity in component concentration affects the kinetics and thus the toxicity of the components at each target tissue. These kinds of variations should be considered when attempting to predict mixture toxicity.

It is important to recognize that the scenario above treated the chemicals implicitly as distributing independently of each other. Each component was partitioned on the basis of individual contaminant data. For the sake of simplicity this assumption was not stated. However, in a real system it is possible that the mixture components could impact each other's partitioning. This would further complicate the system, in that the distribution may not be readily predicted from individual component data. Knowledge of internal distribution, as discussed above, can have important implications regarding the dose of the mixture and the corresponding toxicity computation.

The area of study that examines the physical distribution of chemicals in a system is called toxicokinetics or pharmacokinetics (7). Data on the toxicokinetic parameters and distribution mechanisms of individual chemicals that make up mixtures need to be collected to fully understand mixtures behavior. When these data are unavailable and information for like chemicals is used instead, the associated uncertainty should be addressed in the risk assessment.

Target-Tissue Toxicity and Interaction

Once the mixture has arrived at the target organs or tissues, it can induce its toxicity. The representation of the toxic action of a chemical at a target organ is termed toxicodynamics or pharmacodynamics (7). This area of study includes the examination of any interaction in which the toxin alters the chemical or physical (and biological) nature of the organ or tissue. Toxicodynamic interactions can alter the dose-response character of the chemicals, resulting in an "actual" dose versus an effective dose. The actual dose is the amount of mixture, with its respective component contributions, that reaches the target organ. The effective dose is the dose needed to elicit the observed response. An example of exposure to a hypothetical binary mixture should help illustrate this concept.

Taking as an example a binary mixture of chemicals A and B, these two chemicals are known to distribute independently of one another to the kidney and the liver in fractions of 0.75 and 0.25, and 0.65 and 0.35, respectively. The response elicited by the chemicals independently is also well known, as are the doses at which effects begin to occur in each organ. These first-effect levels for the two chemicals acting separately are 1 and 2 mg/kg-d of chemical A for the kidney and liver, respectively, and 0.75 and 0.5 mg/kg-d for chemical B, respectively. If the dose reaches or exceeds these amounts, some effect would be expected.

Table 2 shows the partitioning of this hypothetical mixture of A and B that is expected to distribute as $D_{a,i}$ – where D_a represents the "actual" dose of the chemical distributed to a given target organ and i represents that chemical, here A or B. $D_{e,i}$ is the effective dose of A and B as would be determined if the observed response were back-calculated to estimate the dose to the target organ by using the known dose-response relationship. The data in this table suggest that different responses mechanisms are at play.

Table 2. Hypothetical distribution and toxicological interaction of a binary mixture. $D_{a,i}$ is the actual dose of chemical i at the respective organ, and $D_{e,i}$ is the effective dose. The level at which effects have been observed in the system (from animal studies) is shown for the target organs. The assumed dose of the total mixture is 2 mg/kg-d, with 0.8 mg/kg-d of A and 1.2 mg/kg-d of B.

Organ	$D_{a,i}$	$D_{e,i}$	Dose Threshold for Target Organ
A-Kidney	0.6	0.64	1
B-Kidney	0.78	0.6	0.75
A-Liver	0.2	0.16	2
B-Liver	0.42	0.6	0.5

For each chemical, the magnitude of the actual dose, the effective dose, and the effect level are displayed comparatively for both organs. The actual doses for the chemical-target organ interactions are below the threshold level with the exception of chemical B in the kidney. This indicates that without consideration of toxicodynamic interactions, chemical B could be implicated as potentially inducing a kidney effect. However, the actual responses to the chemical exposure would yield different results. Calculating the effective dose of each chemical on the basis of the known dose-response relationship would show that chemical B was present at an effectively lower dose in the kidney than expected. The toxic effect is effectively elicited in the liver from chemical B.

These data indicate that the interaction in the kidney is presumably antagonistic toward B and in the liver synergistic toward B. The actual and effective doses of A change relatively little between the two organs. This would suggest that A has the capacity to toxicodynamically interact with B to alter the toxicological nature of B.

RECOMMENDATIONS

The following section describes a proposed conceptual model for an organism exposed to a mixture of chemicals. Within this conceptual model, a framework for conducting a risk assessment of a chemical mixture is being developed. This framework is briefly described in the subsequent discussion.

Conceptual Model

The intricacies described in previous examples should be systematically addressed in each unique mixture risk assessment that is carried out. It is important to have a framework that will identify both potential interactions and assist in setting research priorities. The simple conceptual model described here represents an organism with two organ/tissue types. The organism is assumed to be exposed to one contaminated medium. The chemicals taken in may distribute disproportionately to the component fractions in the exposure dose (7,11).

The internal compartments can physically or chemically change the mixture components of the original exposure. The first compartment can pass the altered or unaltered mixture to the other compartment through fluid exchange, and it can also pass the mixture to the excretion compartment of the organism. The excretion compartment can function comparably to the other compartments with the exception that clearing the mixture from this compartment also clears it from the entire system. The excretion compartment can also reintroduce toxins to the organism through reabsorption.

The conceptual model can be extrapolated to a human system in which there are many more compartments and potential pathways for the mixture to follow. The simple nature of the conceptual picture allows a simple definition of variables and system components.

Figure 1 shows one type of medium, j (shown here as A), to which exposure might occur. The chemicals present in the medium are labeled as $C^{b}_{i,j}$ – where i = 1,2,..., n are the chemical species present at initial concentration C in medium A, and b is a tracking index for chemical i. As chemical i is taken in and undergoes transformation, it is important to monitor it through the organism. Therefore, each chemical i is labeled with a tracking index, b, that is initially set at 0 and remains so if the chemical is unchanged via biological processes (e.g., unaltered speciation, oxidation state, conformation, or chemical structure). Each time the chemical changes, the index b is increased by one. For example, if copper is introduced in the +1 oxidation state, b = 0. However, if copper were oxidized to the divalent state upon absorption into the blood, the index b of the absorbed species would be 1. The dose of chemical i resulting from exposure to medium j can then be represented as $E^{b}_{i,j}$. In the simple example where b is assumed to always be zero, this simplifies to $E_{i,j}$. The exposure dose in the simple example is $E_{i,A}$.

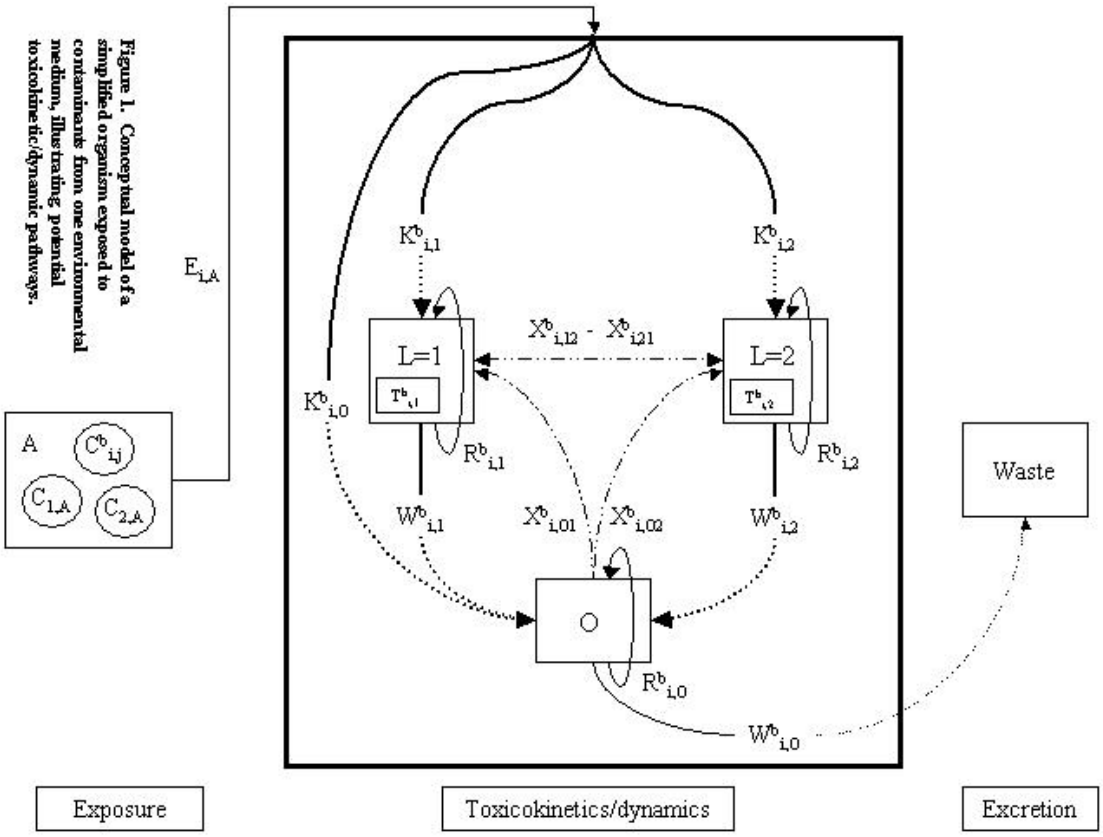

Figure 1. Conceptual model of a simplified organism exposed to contaminants from one environmental medium, illustrating potential toxicokinetic/dynamic pathways.

Toxicokinetics

After exposure, each chemical dose, $E_{i,A}$, is absorbed into the fluids that transport material among the compartments, which represent various organs and tissues inside the body. A fraction of the mixture that is introduced will not be incorporated into these fluids and will pass via other fluids to the excretion or output compartment of the system, O, along pathway K_O. The remaining fraction of the mixture will be transported along pathways K_L to compartments L, where L = 1 or 2. K represents a particular toxicokinetic path in the system that is the input of a chemical from the original exposure dose, $E_{i,A}$, into compartment L.

Toxic effects in a real system will occur along toxicodynamic pathways. However, this does not preclude alteration of the chemical along a toxicokinetic pathway. Each toxicokinetic pathway must include mechanistic consideration of the possible biotransformation of the chemical as it moves through the body. This is represented in Figure 1 by a change in the line representing the path from solid to dotted. The tracking index, b, will also change accordingly. Thus, a chemical may appear as $K^b_{i,L}$ when entering a toxicokinetic path K_L, and may finish as $K^{b+1}_{i,L}$. Toxic response is restricted to toxicodynamic paths within the compartments.

Outputs or waste flows from the other compartments to the excretion compartment are labeled $W^b_{i,L}$, where W is an output pathway of chemical i from compartment L. The tracking index, b, reflects any changes in chemical i. Excretion from compartment O results in removal of the chemical from the system along a path designated $W^b_{i,O}$.

Exchange of circulating fluids between compartments is another mechanism of excretion that may not lead to removal of the chemicals from the system. The exchange pathways, X, are labeled $X^b_{i,LL'}$, where compartment L is the source of the pathway and L' is the recipient compartment. For example, in

figure 1, if L =1, then L' = 2 and vice versa. These paths are assumed to flow in both directions. The excretion compartment can also participate in exchange between compartments. The paths flowing out of the excretion paths to compartments 1 and 2 are $X^b_{i,O1}$ and $X^b_{i,O2}$ respectively. The reverse flows, from the compartments 1 and 2 to the excretion compartment, are incorporated in the waste flows, $W^b_{i,L}$.

Another phenomenon that is incorporated into the model is the possible reabsorption of chemicals into the tissue after output has occurred. This is represented by paths labeled $R^b_{i,L}$, where L again is the compartment from which the original chemical was excreted and to which reabsorption will occur. Reabsorption in the excretion compartment is also considered with designation of pathway $R^b_{i,O}$.

Toxicodynamics

Each chemical may have a toxic interaction inside each compartment. The toxicodynamics of the system are considered for each chemical within each compartment. Thus, the dose-response character of the chemicals will be represented as a response to the dose of each chemical, $C^b_{i,L}$, in the compartment. L indicates the compartment where the dose was applied, i is the chemical, and b is the tracking number of the chemical.

The toxic response is labeled for each compartment as $T^b_{i,L}$ and is shown in a box within the compartment. Boxed Ts are the toxic response to either chemical i or the progeny compound indicated by tracking index b. There are no arrows to or from the toxic responses to disallow mobility of the effect. The chemical species are mobile, but the effects are considered to be tissue-specific and, for purposes of simplification, do not migrate throughout the body.

DISCUSSION

Once the exposure dose is distributed within the human body, a toxicity assessment might be carried out by determining the effects caused by exposure to each chemical in the various compartments. Effects can be added for each compartment to consider all possible responses. This is the approach taken by Mumtaz et al. (23) to assess mixtures toxicity. However, the only factor considered in this work was the equivalent of $C^b_{i,L}$, which the authors called target-organ toxicity doses (TTDs).

This approach did not attempt to examine the mixtures for interactions between the chemicals or their metabolites. As noted earlier, the toxicokinetics of one chemical may affect or be affected by the toxicokinetics of another chemical. This leads to the necessity to treat all possible toxic effects of each chemical simultaneously. Another simplification made by the authors was the assumption that the exposure concentration was the same as the target-organ dose. Using the parameters developed for the conceptual model describe here, this would translate to $E^b_{i,A} = C^b_{i,L}$. One might conclude that this assumption is conservative and that assuming the largest possible dose to each sensitive organ would provide a very conservative risk assessment. This, however, is not always the case.

In some instances, the toxicity of a chemical is not directly proportional to the dose. For example, hormesis (internal regulation) affects a chemical's dose-response relationship and if not reflected can lead to incorrect predictions. In these instances, the ratios of the concentrations of the mixture components at the target organs can become more important than the absolute concentrations. The concentration and makeup of the mixture that reaches the target organ may be different from the mixture that was originally introduced to the human and should be considered in any risk assessment that addresses possible interactions.

The conceptual model illustrated here is being used in a preliminary pilot application to assess potential mixtures risks for a large U.S. Department of Energy (DOE) site contaminated by past processing and disposal associated with the weapons program. As part of this pilot study, the specifics of chemical transport and toxicity within an organism will be investigated. Chemical toxicity profiles will be developed using this conceptual model to maintain a consistent framework. Where available, information will be gathered to address each of the generalized processes described in the conceptual model. Each chemical will be investigated as in Mumtaz et al. (23), and toxicokinetic and toxicodynamic properties will also be assessed. These profiles will feed a database that can be used to investigate the overlap of partitioning, residence time, and potential for toxic effects among mixture components. This

overlap will be examined for factors that might cause variations from additive toxicity, the default assumption.

As the tailored toxicity profiles are developed, modifications necessary to the model will become apparent. The database and the conceptual model will be iteratively refined as the pilot study is pursued. The conceptual model will then be assessed using a well-characterized mixture. The example mixture for the DOE site will be examined component by component, as no mixture toxicity data are available for this specific study mixture. Results will be compared with the known toxic properties of the well-characterized mixture to provide a means for evaluating the model.

The conceptual model proposed here is intended to account for many key complexities inherent in mixture exposures and dose-response relationships, and it contains a number of parameters that should be considered in a comprehensive mixtures assessment. Although the model could be evaluated as a continuous system, this would be very difficult and sufficient data do not currently exist to support that effort. Thus, the approach offered here is to examine this continuous system as a series of discrete events designated by system pathways E, K, X, W, R, and T. This allows each chemical to be assessed for interactions that might affect distribution or toxicity in the context of the other compartments and other chemicals within the overall system. This conceptual approach has the benefit of simplifying a very complex system while highlighting specific factors important to mixtures assessment.

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