The use of physiologically-based pharmacokinetic/pharmacodynamic dosimetry models for chemical mixtures


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Abstract

Human exposure to chemicals is rarely, if ever, limited to a single chemical. Therefore, it is essential that we consider multiple chemical effects and interactions in our risk assessment process. However, with the almost infinitely large number of chemical mixtures in the environment, systematic studies of the toxicology of these chemical mixtures with conventional methodologies and approaches are impossible because of the immense resources and unrealistically long durations required. Thus, the development of ‘Predictive and Alternative Toxicology’ is imperative. At Colorado State University (CSU), our research effort is entirely devoted to this challenge. In order to have a reasonable chance to deal with the complex issue of toxicology of chemical mixtures, we believe that the following concepts must be considered: (1) the utilization of computer; (2) the exploitation of mathematical/statistical methodologies; (3) developing very focused, mechanistically based, and short-term toxicology studies; (4) coupling computer/mathematical modeling with mechanistically-based toxicology. Our strategy is therefore the utilization of physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling, coupled with very focused, model-directed toxicology experiments as well as other statistical/mathematical methodologies such as Monte Carlo simulation, isobolographic analysis, and response surface methodology. We believe that ‘Predictive and Alternative Toxicology’ in terms of tissue dosimetry at the pharmacokinetic and pharmacodynamic levels is achievable with simple and complex but chemically defined mixtures. In this presentation, we describe two ongoing research projects as an illustration of our ‘Bottom-Up’ and ‘Top-Down’ approaches for handling the chemical mixtures: (1) PBPK/PD modeling of toxicologic interactions between Kepone and carbon tetrachloride (CCl₄) and the coupling of Monte Carlo simulation for the prediction of acute toxicity; (2) the conceptual development of PBPK/PD modeling for a more complex chemical mixture of seven groundwater contaminants from hazardous waste sites and the consideration of subfractionation of this chemical mixture.

Keywords: Chemical mixtures; PBPK/PD modeling; Pharmacokinetics; Pharmacodynamics

1. Introduction

What is a chemical mixture? The correct answer is that almost everything around us in the
environment is a chemical mixture: a breakfast with pancakes, raspberries, orange juice, and coffee; a hamburger with lettuce and tomato; a gourmet dinner of crawfish, asparagus and wine; the suits and dresses we wear; the cosmetics, toiletries and medicines we use, etc. Even our own body is a chemical mixture. Considering all the above then, there is really no such a thing as 'single chemical exposure' in our life.

In contrast with this reality, however, about 95% of the toxicology studies conducted to date have been with single chemicals [1,2]. This represents a very slanted distribution of research resources. From a different perspective, Calabrese [3] insightfully concluded from the outcomes of a number of conferences and workshops held in the 1980s, many with participation of Blue Ribbon Panels of Experts:

"... a careful reading of many of the proceedings from conferences, workshops, and reports of expert committees reveals a repetitious restatement of the obvious: for example, humans are not exposed to single agents; the environment provides exposure to a complex daily mixture of agents; health standards have long ignored the issue of multiple exposures; and this should be an area of high priority..."

"... predictive systems are desperately needed since it is impossible to study all interactions – elementary statistical analysis clearly illustrates the folly of such an exercise..."

Systematic toxicity testing of chemical mixtures in the environment or workplace using conventional toxicology methodologies is highly impractical because of the immense numbers of mixtures involved. For example, a chemical mixture with 25 component chemicals has $2^{25} - 1$ or 33,554,431 combinations (i.e. one chemical at a time, any two chemicals in combination, any three in combination, etc.) [1,2]. Furthermore, this huge number of combinations is just for one concentration per chemical. From a different perspective, there are about 600,000 chemicals being used in our society [4]. Just considering binary chemical mixtures, this means that there could be $600,000 \times 599,999/2 = 359,999,400,000$ pairs of chemicals. Assuming that only 0.001% of these pairs of chemicals act synergistically or have other toxicologic interactions, there would still be 3,599,994 binary chemical mixtures possessing toxicologic interactions. Further, toxicologic interactions undoubtedly exist among chemical mixtures with three or more component chemicals; the number of possible combinations for these latter mixtures is almost infinite. These are astronomically large numbers with respect to systematic toxicity testing.

2. Approaches for chemical mixtures

Given the above discussion, it is obviously impossible to rely on conventional toxicity testing methodologies to deal with chemical mixtures. Thus, we must use and integrate: (1) computational technology; (2) focused, mechanistically-based, short-term toxicology studies; (3) mathematical/statistical modeling.

Can 'Predictive and Alternative Toxicology' be developed for chemical mixtures using physiologically-based pharmacokinetics/pharmacodynamics (PBPK/PD) coupled with statistical/mathematical modeling? In our opinion, the answer to this question is yes. Because the toxicity produced by xenobiotics in the body is mediated by interactions between the chemicals and their metabolites and biological molecules or structures [5], understanding pharmacokinetics and pharmacodynamics of xenobiotics is therefore a necessity in toxicology. With the advent of PBPK/PD and computer modeling, correlation of quantitative and temporal descriptions of xenobiotic concentrations at target tissues or organs with specific toxicities becomes an attainable reality. By linking the interactive chemical components in a chemical mixture at the level of pharmacokinetic and/or pharmacodynamic modeling, we believe that it is possible to deal with the health effects, collectively, of the chemical mixture of interest.

We propose the 'Bottom-Up' and 'Top-Down' approaches for reaching the ultimate goal of predictive and alternative toxicology for chemical mixtures. Using examples, we explain, briefly, these two approaches below.
2.1. Bottom-up approach

The 'Bottom-Up' approach refers to systematic toxicologic interaction studies starting with binary chemical mixtures based on toxic mechanism(s). Using PBPK/PD modeling as a guide, a third, fourth, etc. chemical is then added based on mechanistic considerations. In this way, we build up the chemical mixture as well as the interlinkage of PBPK/PD modeling of all the components of the chemical mixture. Ultimately, the integrated PBPK/PD model would encompass all the toxicologic interactions in the chemical mixture and it would be able to predict toxicities for the entire mixture. For instance, we have already studied the toxicologic interactions with respect to impairment of liver regeneration by Kepone in the hepatotoxicity of CCl₄ by coupling experimental toxicology, PBPK/PD modeling, and Monte Carlo simulation [6,7]. Since Kepone pretreatment is a prerequisite for this toxicologic interaction, we must hold Kepone as a constant component in all mixtures. Thus, with the addition of two new, known hepatotoxins, 1,1,2,2-tetrachloroethane (1,1,2,2-TE) and hexachloro-1,3-butadiene (HCBD), two new binary chemical mixtures are formed (i.e. Kepone + 1,1,2,2-TE; Kepone + HCBD). Modeling and experimental toxicology results may be obtained on these binary chemical mixtures. Subsequently, we may form the three-component chemical mixtures (i.e. Kepone + CCl₄ + 1,1,2,2-TE; Kepone + CCl₄ + HCBD; Kepone + 1,1,2,2-TE + HCBD), and the four-component chemical mixture (i.e. Kepone + CCl₄ + 1,1,2,2-TE + HCBD). Of course, modeling and experimental toxicology results will be obtained on these chemical mixtures as well. In this manner, more and more complicated chemical mixtures are built up based on mechanisms of toxicity.

As a glimpse of the possible utility of this type of approach, we discuss the findings of PBPK/PD modeling of a binary chemical mixture (Kepone and CCl₄) based on mechanisms of toxicity of interactions and the application of computer technology in acute toxicity studies.

The discussion follows the order of: (1) background toxicology information on Kepone and CCl₄ singly and in combination; (2) our effort on PBPK/PD modeling and model validation using published data; (3) the coupling of PBPK/PD model with Monte Carlo simulation and the prediction of acute toxicity (i.e. mortality), based on pharmacodynamics of hepatotoxicity, in CCl₄-dosed rats with or without pretreatment of dietary Kepone; (4) comparison of computer-predicted results and observed data from experiments conducted in our laboratory.

CCl₄ is a well-known hepatotoxin [8]. Following free radical formation through the P450 enzyme system, the toxicity of CCl₄ can be an accumulation of lipids (steatosis, fatty liver) and degenerative processes leading to cell death (necrosis) [8]. Kepone (also known as chlordecone) is found in the environment as a result of photolytic oxidation of Mirex, a pesticide used for the control of fire ants, or as a pollutant from careless and irresponsible discharge [9]. At relatively low levels (e.g. 10 ppm in the diet), even repeated dosing of Kepone in the diet up to 15 days caused no apparent toxicity to the liver [10].

The toxicologic interaction between Kepone and CCl₄ was reported by Curtis et al. [11]. They illustrated that a 15-day dietary exposure of male rats to Kepone at 10 ppm, an environmentally realistic level of contamination, markedly enhanced liver toxicity produced by an intraperitoneal (i.p.) injection of a marginally toxic dose of CCl₄ (100 μl/kg). This toxicologic interaction is unique in that: (1) unlike many other toxicologic interaction studies which were usually dealing with acute toxicity at very high doses, Kepone in this instance is administered at a very low environmental level; (2) CCl₄ is also dosed at a marginally toxic level; (3) the magnitude of toxicologic interaction is very large. Based on administered dose, the enhancement of CCl₄ lethality is about 67-fold. The mechanism of this toxicologic interaction was elucidated to be the obstruction by Kepone of the liver's regeneration process [12-14].

We chose this binary interaction as a model system to develop our 'Bottom-Up' approach for chemical mixtures. As shown in Fig. 1, the pharmacokinetic portion of the PBPK/PD model was an adaptation of the PBPK model of Paus-
Fig. 1. A PBPK model for CCl₄ adapted from Paustenbach et al. [15]. CI and CX are concentrations of CCl₄, in the inhaled (thus chamber concentration) and exhaled breath; CV and CA represent venous and arterial blood concentrations of CCl₄; Q depicts blood flow rate. S, R, F, L refer to slowly perfused, rapidly perfused, fat, and liver compartments, respectively; Vmax and Km are in vivo hybrid constants representing maximal velocity and affinity constants for enzyme systems involved in the metabolism of CCl₄. (After El-Masri et al. [6].)

Fig. 2. The PBPK/PD model predictions of the concentrations of CCl₄ in the exhale breath (symbols) of CCl₄-treated rats for different i.p. injections. The lines are the model predictions. (After El-Masri et al. [6].)

Fig. 3. A PBPD model for toxicologic interactions between Kepone and CCl₄. This depicts the schematic of pharmacodynamic effects of CCl₄ on cellular injury and death. The dashed lines depicts the processes that are affected by the presence of Kepone. When cells are exposed to the reactive metabolites of CCl₄, their inherent death rate is influenced by two mechanisms. A major mechanism of cellular injury leading to death is through lipid accumulation which is illustrated here as the formation of injured cells and dead cells via two rate constants KINJ and KDIE1. For simplicity, all other causes of cell death including natural cell death and other CCl₄-related toxicities are lumped together into a hybrid constant KDIE1 as a second mechanism. The injured cells can either be repaired back to viable cells or continue to die. All dead cells, whether induced to die or injured to death, are removed from the liver by phagocytosis. Additionally, the PBPD model considers the effects of CCl₄ alone or in combination with Kepone, on cellular mitotic and birth rates. (After El-Masri et al. [6].)

carried out by comparing simulation results with existing time course data in the literature [16] as shown in Figs. 4 and 5.
A. Fraction of injured cells

B. Fraction of dead cells

C. Fraction of mitotic cells

Fig. 4. The PBPK/PD model predictions of the pyknotic, injured and mitotic cells from rats exposed to CCl₄ only. The experimental data were obtained from Lockard et al. [16]. The model predictions are given by the solid lines. (After El-Masri et al. [6].)

Fig. 5. The PBPK/PD model description of the pyknotic, injured and mitotic cells from rats exposed to CCl₄ and Kepone pretreatment. The experimental data were obtained from Lockard et al. [16]. The model predictions are given by the solid lines. (After El-Masri et al. [6].)

To work toward the goal of ‘Predictive and Alternative Toxicology,’ this PBPK/PD model was coupled with Monte Carlo simulation to predict the acute lethality of CCl₄ alone and in combination with Kepone (10 ppm in the diet for 15-day pretreatment). In doing so, we were able to conduct acute toxicity studies on computer with a very large sample (i.e. 1000 rats/dose) [7]. As shown in Table 1, these a priori predictions of lethality were in very good agreement with experimentally-derived values except at very high CCl₄ dose levels. In this latter case, the under-prediction of lethality was due to toxicity other than the liver, most likely neurotoxic effects on the central nervous system. Histomorphometric analyses of liver supported this explanation [6,7]. The extent and prevalence of hepatocellular necrosis at 6000 µl/kg was disproportionately small because some of the rats died of CNS effects of CCl₄ before hepatotoxicity could be developed.

2.2. Top-down approach

The ‘Top-Down’ approach, as the name implies, will start out with a more complex chemical mixture of several to many component chemicals. We use below a chemical mixture of seven groundwater contaminants (arsenic, benzene, chloroform, chromium, lead, phenol, and trichloroethylene) to illustrate the essence of the ‘Top-Down’ approach. From earlier studies [17,18], we have already obtained interesting preliminary findings on the complete mixture and some of its submixtures. Since there are \(2^7 - 1 = 127\) combinations for seven chemicals at only one concentration, systematic toxicity testing on all the combinations is a prohibitively expensive effort. Thus, we tried to minimize experimentation by using educated guesses to set study priorities on those submixtures to be tested. For instance, the initial fractionation into a metal submixture and organic chemical submixture appeared to be a reasonable first step. Because we were interested in finding out the potential promoter activities of this chemical mixture, its submixtures, and components, further subfractionations according to the known carcinogenicity of these chemicals seem to be a reasonable approach as well. In this manner, we conduct experiments on finer and finer submixtures until we get to individual chemicals. As a representative scenario, Fig. 6 illustrates this approach graphically.

Using the ‘Top-Down’ approach, we will study a total of five chemical mixtures and seven individual chemicals. Although the overall number of combinations for seven chemicals is 127, we believe that a simplified top-down scheme (Fig. 6) based on our knowledge and experience for the seven chemicals would be sufficient to
Table 1
Kepone/carbon tetrachloride-induced mortality by PBPK/PD modeling coupled with Monte Carlo simulation vs. experimentally observed (after El-Masri et al. [6])

<table>
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<tr>
<th>Dose given</th>
<th>Model predictions*</th>
<th>Observedb</th>
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<tr>
<td>Kepone (ppm)</td>
<td>CCl₄ (µl kg⁻¹)</td>
<td>Dead rats</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>1000</td>
<td>1–2</td>
</tr>
<tr>
<td>0</td>
<td>3000</td>
<td>3</td>
</tr>
<tr>
<td>0</td>
<td>6000</td>
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<tr>
<td>10</td>
<td>10</td>
<td>0</td>
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<tr>
<td>10</td>
<td>50</td>
<td>4–5</td>
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<tr>
<td>10</td>
<td>100</td>
<td>8–9</td>
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* Mortalities in 48 h, n = 9; Monte Carlo simulation, n = 1000.

The most complicated, and thus most refined, way is to have a PBPK/PD model for each of the chemical components in the chemical mixture. These models are then linked at pharmacokinetic and/or pharmacodynamic level(s) to include all known toxicologic interactions. Even though this may require very complex modeling and computer simulation, the current capability of computational technology should permit us to do so. In between the above two ways is a compromise approach. Here the chemical engineering concept of ‘lumping analysis’ [21] may be applied to ‘lump’ certain chemicals into a group as an entity. Considering the successful application of this technique in chemical engineering processes, it is reasonable to assume that the application of ‘lumping analysis’ for PBPK/PD modeling of chemical mixture toxicology is possible.

3. Future directions and refinement of PBPK/PD modeling

The above experiments and approaches represent the first step in our development of ‘Predictive and Alternative Toxicology.’ There is definitely room for improvement. Presently, two aspects are being explored. First, to improve the PBPK/PD model for Kepone and CCl₄ interaction, we are incorporating: (1) a PBPK model for Kepone to account for the pharmacokinetics of Kepone which will be linked with the CCl₄...
4. Discussion and perspectives

Our research effort on PBPK/PD modeling with chemical mixtures aims at developing ‘Predictive and Alternative Toxicology.’ By ‘Predictive Toxicology,’ we are referring to tissue dosimetry at the pharmacokinetic and pharmacodynamic levels. By ‘Alternative Toxicology,’ we are working toward minimizing animal experimentation, as illustrated in the example given on Monte Carlo simulation coupled with PBPK/PD modeling of Kepone/CCl₄ interactions. The application of PBPK/PD to risk assessment of chemical mixtures may have several advantages: (1) The incorporation of mechanistic information on toxicologic interactions; (2) It conserves resources and it reduces animal killing and suffering in the Hazard Identification step; (3) Reducing the necessity of using large uncertainty factors. Thus, PBPK/PD modeling will provide more realism into the risk assessment process. Of course, one must be aware of the fact that PBPK/PD modeling has its own intrinsic ‘uncertainties’: therefore, as much as practicable, any PBPK/PD model must be rigorously validated with experimental results before ‘Predictive Toxicology’ so derived becomes meaningful.

The linkage of two of the most challenging areas in toxicology today: (a) PBPK/PD and statistical/mathematical modeling; and (b) experimental toxicology of chemical mixtures will have immense potential in application to risk assessment to chemical mixtures. Fig. 8 is our strategy for ‘Predictive and Alternative Toxicology’ for chemical mixtures and the development of ‘Innovative Risk Assessment Methodologies for Chemical Mixtures.’ We are attempting to couple PBPK/PD and other experimental toxicology with isobolographic analysis and/or response surface methodology for the modeling and analysis of toxicologic interactions. With the aid of techniques such as Monte Carlo simulation, we may better predict tissue dosimetry at the pharmacokinetic and pharmacodynamic levels. Using such values as benchmark doses,

<table>
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<th>A Priori PB-PK/PD Modeling</th>
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<tbody>
<tr>
<td>Model Directed Focused Experiments/ Efficient Experimental Designs (e.g., Central Composite, 2^K Factorial)</td>
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\text{PB-PK/PD and Integrated + Toxicity Model} \rightarrow \text{Isobolographic Analysis and Median Effect Principle} \rightarrow \text{Response Surface Methodology} \rightarrow \text{Monte Carlo Simulation} \rightarrow \text{Predictive and Alternative Toxicology/ Target Tissue Dosimetry} \rightarrow \text{Innovative Risk Assessment Methodologies}
\]

*Fig. 8. Our proposed strategy/approach to develop ‘Predictive and Alternative Toxicology’ and formulate ‘Innovative Risk Assessment Methodology’ for chemical mixtures.* (Modified from El-Masri et al. [20].)
human risk assessment of chemical mixtures may be carried out with less uncertainty.

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