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Physiologically based pharmacokinetic/pharmacodynamic modeling of chemical mixtures and possible applications in risk assessment

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Abstract

Human exposure to chemicals, be it environmental or occupational, 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 method is imperative. 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 exploitation of recent advances in computational technology; (2) the utilization of mathematical/statistical modeling; (3) coupling computer modeling with very focused, mechanistically based, and short-term toxicology studies. Our approach is, therefore, the utilization of physiologically based pharmacokinetic/pharmacodynamic (PB-PK/PD) modeling, coupled with very focused, model-directed toxicology experiments as well as other statistical/mathematical modeling such as isobolographic analysis and response surface methodology. Tissue dosimetry at the pharmacokinetic and pharmacodynamic levels is achievable with simple and complex, but chemically defined, mixtures. Our long-term goal is to formulate innovative risk assessment methodologies for chemical mixtures. In this presentation, one of our specific research projects is described: PB-PK/PD modeling of toxicologic interactions between Kepone and carbon tetrachloride (CCl_4) and the coupling of Monte Carlo simulation for the prediction of acute toxicity.

Keywords: Chemical mixtures; PB-PK/PD modeling; Risk assessment

1. Introduction

What is a chemical mixture? The correct answer is that almost everything around us in the envi-

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ronment is a chemical mixture. Other than those which we deal with in toxicology such as contaminated drinking water, gasoline vapors, etc., the foods we eat, the clothes we wear, the cosmetics, toiletries, and medicines we use, even our own body, are chemical mixtures. There is really no such a thing as 'single chemical exposure' in our life.

Contrary to this environmental reality, however, a majority of the toxicology studies conducted to date has been with single chemicals. Such an uneven distribution of research resources has been discussed in detail elsewhere (Yang, 1994; Yang et al., 1995). Of the number of conferences and workshops held in the 1980s, Calabrese (1991) insightfully concluded:

> "... 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..."
>
> "... medical-pharmaceutical communities ... have dealt with the severe challenges of drug-drug interactions for decades, with an extremely high level of academic, federal government, and private sector involvement. Without question, the medical-pharmaceutical professionals are well over a decade ahead of where their environmental colleagues are. This advanced state involves not only a better understanding of the pharmacokinetic and pharmacodynamic mechanisms of interactions, but also the organization, simplification, and dissemination of a large amount of information for use by researchers, governmental employees in agencies such as the U.S. Food and Drug Administration (FDA), physicians who manage patients, and pharmacists who dispense drugs within society. Yet the major proponents of the environmentally oriented activity noted above have not made adequate use of the impressive achievements of the medical-pharmaceutical communities..."
>
> "...the word 'environmental' encompasses both community and work environments. Even though society may be concerned with exposure to low levels of large numbers of chemical agents, many workers are exposed to high levels of multiple agents. The greatest potential for interactions of clinical significance is likely to result from industrial exposures..."

1.1. The immensity of the problem dealing with chemical mixtures

Systematic toxicity testing of chemical mixtures in the environment or workplace is highly impractical because of the immense number of mixtures involved as illustrated in the following two examples. For instance, 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.) and this is just for one concentration per chemical. As a further illustration, using a very conservative estimate of $100 000 for a 13-week subchronic toxicity study with a single species of rodent according to the NTP protocol, this alone translates into more than 3 trillion dollars for all the combinations, at one concentration per chemical, in the above example of a 25-chemical mixture (Yang, 1994; Yang et al., 1995). From a different perspective, there are nearly 100 000 chemicals being used in commerce (Huff et al., 1991). This means that there could be $100\,000 \times 99\,999/2 = 4\,999\,950\,000$ binary chemical mixtures. Assuming that 1% of these pairs of chemicals act synergistically or have other toxicologic interactions, there would still be 49 999 500 pairs. This is still an astronomically large number with respect to conventional toxicity testing.

1.2. Possibly workable approaches for chemical mixtures

Given the above discussion on the immensity of

the problems related to chemical mixtures, it is obviously impossible to rely on conventional toxicity testing. To have a reasonable chance of successfully dealing with the issues of toxicology of chemical mixtures, we must utilize and integrate: (1) Computational technology; (2) Focused, mechanistically-based, short-term toxicology studies; (3) Mathematical/statistical modeling.

In this paper, therefore, we present an example of the ongoing research activities in our laboratories. This example is an illustration of a first step toward the development of 'Predictive and Alternative Toxicology.' The importance of model-directed, focused experimental work is particularly emphasized. Finally, we present our strategy/approach toward the eventual establishment of innovative risk assessment methodologies for chemical mixtures.

2. Experimental approach and results

2.1. An example: PB-PK/PD modeling/Monte Carlo simulation of toxicologic interaction between Kepone and carbon tetrachloride and the initial development of predictive toxicology

This example illustrates the initial step of what we consider a 'Bottom-Up Approach' (El-Masri et al., 1995a; Yang et al., 1995). In this approach, PB-PK/PD modeling of simple (i.e. binary) chemical mixtures is carried out based on the mechanisms of toxicity of the binary interactions. Once a PB-PK/PD model is constructed and validated for a given toxicologic interaction for a binary chemical mixture, addition of a third, a fourth,... chemical(s) with similar or dissimilar toxic mechanism(s) may be effected and the PB-PK/PD modeling may go through an iterative process to develop more predictive power.

In the present example, the toxicologic interactions with respect to impairment of liver regeneration by Kepone in the hepatotoxicity of carbon tetrachloride (CCl_4) were studied by coupling experimental toxicology, PB-PK/PD modeling and Monte Carlo simulation (Phillips et al., 1994; El-Masri et al., 1995b). Given below is a discussion on: (1) some background information on Kepone and CCl_4 individually and in combination; (2) our approach for PB-PK/PD modeling and its validation using literature information; (3) our strategy for the coupling of a PB-PK/PD model with Monte Carlo simulation and the prediction of lethality, based on the pharmacodynamics of hepatotoxicity, in CCl_4-dosed rats with or without pretreatment of dietary Kepone: (4) comparison of model predicted results and experimental data gathered in our laboratory.

CCl_4 is a well-known hepatotoxicant (Plaa, 1991); its acute toxicity includes an accumulation of lipids (steatosis, fatty liver) and degenerative processes leading to cell death (necrosis). The mechanism of toxic action is believed to be principally due to lipid peroxidation as a result of trichloromethyl free radical formation from CCl_4 via enzymatic catalysis by the cytochrome P450 system (Plaa, 1991). The general morphological feature of CCl_4-induced liver toxicity involves fatty degeneration and necrosis in the centrilobular region and this is not generally seen unless a high dose of CCl_4 is given.

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 (Menzer, 1991). At relatively low levels (e.g. 10 ppm in the diet), even repeated dosing of Kepone in the diet for 15 days caused no apparent toxicity to the liver (Lockard et al., 1983a). However, Kepone was known to be a more potent uncoupler for oxidative phosphorylation than dinitrophenol (Desiah et al., 1977; Carmines et al., 1979), and it was shown to decrease enzyme activities such as glucose-6-phosphatase and ATPases in the intermediary metabolism (Curtis et al., 1979; Curtis and Mehendale, 1981).

The toxicologic interaction between Kepone and CCl_4 was uncovered by Curtis et al. (1979). These investigators 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 a subsequent intraperitoneal (i.p.) injection of a single, marginally toxic dose of CCl_4 (100 μl/kg). This toxicologic interaction is somewhat 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) CCl_4 is also dosed at a marginally toxic level; (3) the magnitude of toxicologic interaction is very large. Based on administered dose, the enhancement of CCl_4 lethality is about 67-fold. The mechanism of this toxicologic interaction was elucidated, following a series of studies (Mehendale, 1984, 1991, 1994), to be the obstruction by Kepone of the liver's regeneration process. Under the experimental conditions (i.e. 10 ppm dietary Kepone pretreatment for 15 days, followed by a single i.p. dose, 100 μl/kg, of CCl_4) of these investigators, the hepatocellular necrosis appeared to be pan-lobular without any site specificity.

As shown in Fig. 1, the pharmacokinetic portion of the PB-PK/PD model was an adaptation

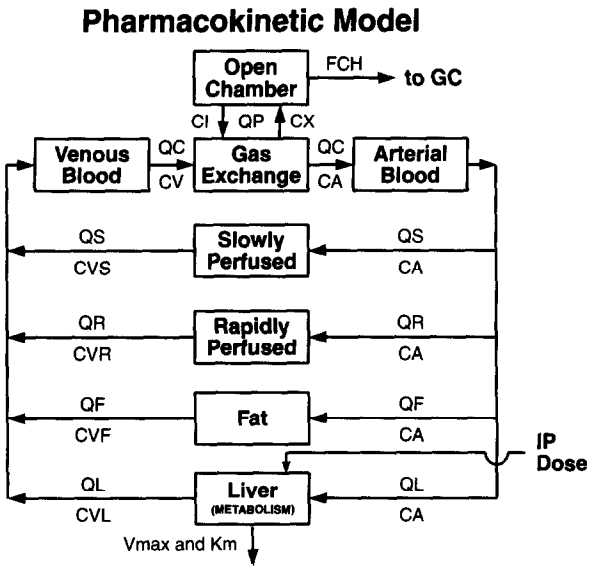

Fig. 1. A PB-PK model for CCl_4 adapted from Paustenbach et al. (1988). In this model CI is the inhaled chamber concentration of CCl_4, CX is the exhaled concentration of CCl_4, CA is the arterial blood concentration, QP is the lung ventilation rate, QC is the cardiac output, FCH is the air flow through the chamber, QI is the blood flow into each compartment I, and CVI is the venous blood concentration of CCl_4 leaving compartment I, where I = S, R, F, L for the slowly perfused, rapidly perfused, fat and liver compartments, respectively. Metabolic rate constants, V_{max} and K_m, are maximum velocity and affinity constant for saturation enzymatic reactions.

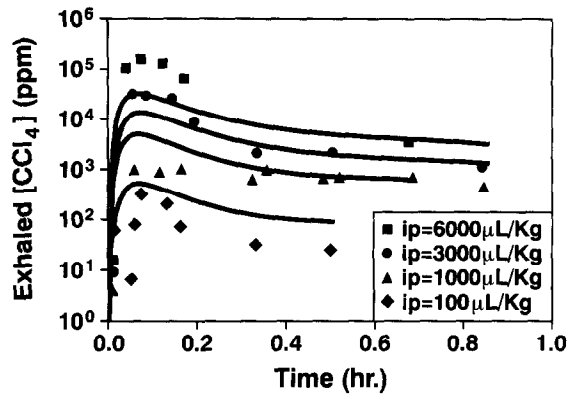

Fig. 2. The PB-PK/PD model predictions of the concentration of CCl_4 in the exhaled breath (symbols) of CCl_4-treated rats for different i.p. injections. The lines are the model predictions. The exhalation data were used for the estimation of the i.p. absorption first order rate constant (KA).

of the PB-PK model of Paustenbach et al. (1988). As shown in Fig. 2, initial verification of this PB-PK model was carried out by using data from exhaled breath analyses from CCl_4-treated rats in our laboratory (El-Masri et al., 1995b). This PB-PK model was then linked with a PB-PD model

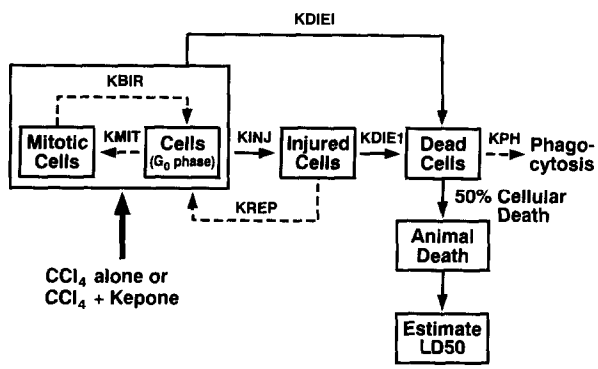

Fig. 3. A PB-PD model for toxicologic interactions between Kepone and CCl_4. This depicts the schematic of pharmacodynamic effects of CCl_4 on the cellular injury and death. The dashed lines depicts the processes that are affected by the presence of Kepone. When cells are exposed to the reactive metabolites of CCl_4, their inherent death rate is influenced by two mechanisms. Some cells die others are injured. 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 PB-PD model considers the effects of CCl_4, alone or in combination with Kepone, on cellular mitotic and birth rates. (After El-Masri et al., 1995b).

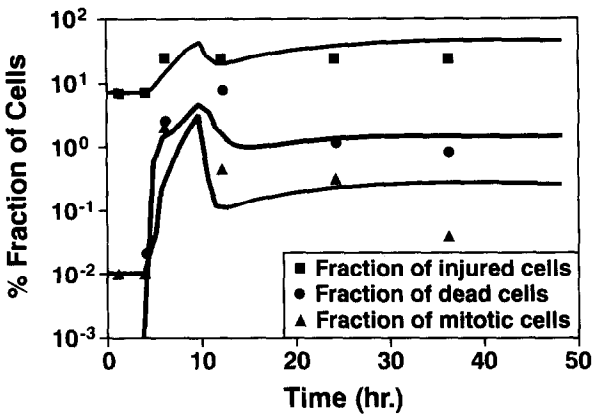

Fig. 4. The PB-PK/PD model predictions of the pyknotic, injured and mitotic cells from rats exposed to CCl_4 only. The experimental data were obtained from Lockard et al. (1983). The model predictions are given by the solid lines. (After El-Masri et al., 1995b).

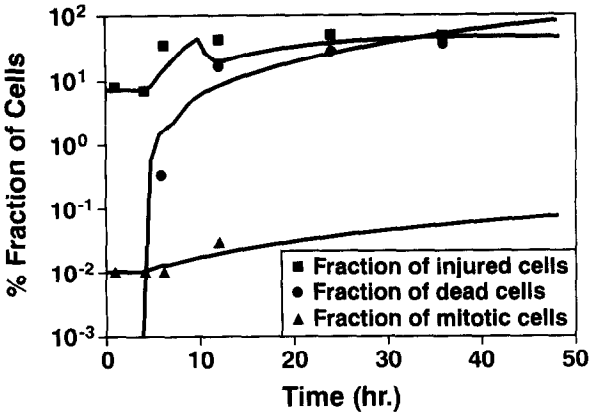

Fig. 5. The PB-PK/PD model description of the pyknotic, injured and mitotic cells from rats exposed to CCl_4 and Kepone pretreatment. The experimental data were obtained form Lockard et al. (1983). The model predictions are given by the solid lines. (After El-Masri et al., 1995b).

(Fig. 3), constructed in our laboratory based on the mechanistic information for Kepone and CCl_4. By incorporating cell birth/death processes as well as the pharmacokinetics and metabolism of Kepone and CCl_4 into the PB-PK/PD model, time course simulation of mitotic, injured and pyknotic cells following treatment with CCl_4 alone and Kepone (10 ppm in the diet for 15-day pretreatment) and CCl_4 combination could be carried out. Initial verification of the PB-PK/PD model was carried out by comparing simulation results with existing time course data in the literature (Lockard et al., 1983b) as shown in Fig. 4 and 5. The entire process mentioned above is being reported in detail elsewhere (El-Masri et al., 1995b).

This PB-PK/PD model was then coupled with Monte Carlo simulation for the purpose of predicting the acute lethality of CCl_4 alone and in combination with Kepone (10 ppm in the diet for 15-day pretreatment). This coupling enabled us to 'conduct' (i.e. simulate) acute toxicity studies electronically on 1000 rats/dose with normal range of variabilities with respect to body and liver weights as well as other physiological parameters (for details, see El-Masri et al., 1995b). As shown in Table 1, these a priori predictions of lethality were in agreement with experimentally derived values except at a very high CCl_4 dose (i.e. 6000 μl/kg). In this latter case, the under-prediction of lethality was due to extra-hepatic toxicity, most likely neurotoxic effects on the central nervous system. Histomorphometric analyses supported this explanation (Fig. 6). The extent and prevalence of hepatocellular necrosis at 6000 μl CCl_4/kg was disproportionally small because some of the rats died of CNS effects before hepatotoxicity could develop (Fig. 6).

Table 1
Kepone/carbon tetrachloride mortality by PB-PK/PD modeling coupled with Monte Carlo simulation vs. experimentally observed

Dose given		Model predictions[a]		Observed[b]	
Kepone (ppm)	CCl_4 (μl kg^{-1})	Dead rats	% Dead	Dead rats	% Dead
0	100	0	0.0	0	0.0
0	1000	1–2	13.2	1	11.1
0	3000	3	32.8	4	44.4
0	6000	4–5	47.8	8	88.8
10	10	0	0.0	0	0.0
10	50	4–5	47.5	4	44.4
10	100	8–9	84.0	8	88.8

[a]Mortalities in 48 h, $n = 9$; Monte Carlo simulation, $n = 1000$.
[b]Actual lethality studies ($n = 9$).
After El-Masri et al., 1995b.

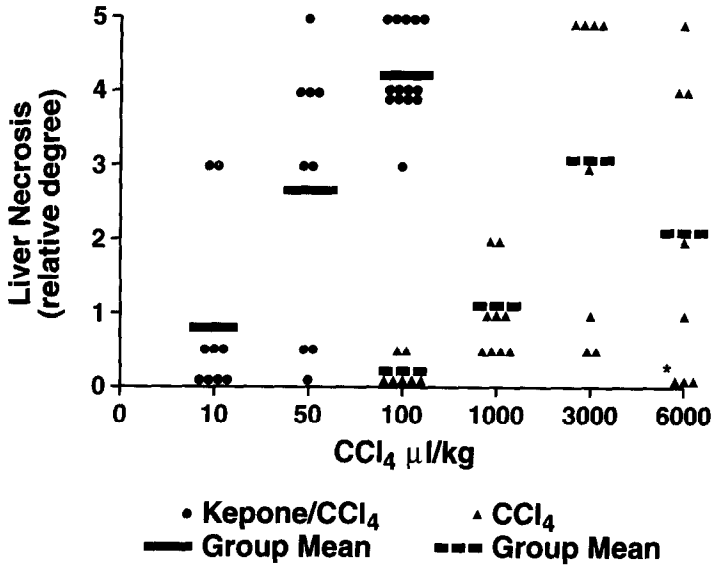

Fig. 6. Histomorphometric analyses of liver necrosis of rats treated with CCl_4 alone or in combination with Kepone.

2.2. Future directions and refinement of the PB-PK/PD model for Kepone and CCl_4

As we stated earlier, the above experiments and approach represent the first step in our development of predictive and alternative toxicology. There is definitely room for improvement. As a continuation of our effort described above, two aspects are being explored. First, to improve the above PB-PK/PD model which is principally based on a CCl_4 model, we are developing a PB-PK model for Kepone which will be linked with the CCl_4 model as shown in Fig. 7. In doing so, the pharmacokinetics of Kepone may be incorporated into the overall modeling of toxicologic interactions between Kepone and CCl_4.

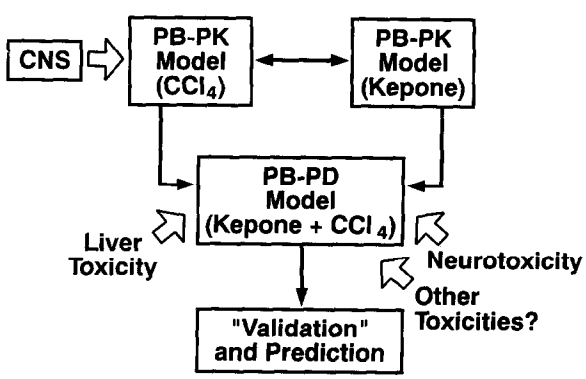

Fig. 7. Further refinement of the PB-PK/PD model for Kepone and CCl_4; addition of PB-PK model for Kepone and other toxicity endpoints to form an 'Integrated Toxicology Model.'

Secondly, because of the obvious limitation of a PB-PK/PD model which centers exclusively on pharmacodynamics of liver toxicity, we should consider the concept of integrated toxicology in our PB-PK/PD modeling to account for different toxicologic endpoints under the conditions of a wider dosing regimen. Fig. 7 is also a graphical illustration of such a concept for Kepone and CCl_4. As PB-PK/PD modeling progresses to a higher and higher level of sophistication in toxicology, it is probably inevitable that multiple toxic endpoints are incorporated into the model. As we illustrated in the above example of toxicologic interaction between Kepone and CCl_4, it is essential to take into consideration other toxicities in higher dose ranges in order for the PB-PK/PD model to have adequate predictive capacity.

3. Discussion and perspectives

The greatest utility of PB-PK/PD modeling in risk assessment, particularly in chemical mixtures, is its potential for predictive and alternative toxicology. By 'Predictive Toxicology,' we are refering to tissue dosimetry at the pharmacokinetic and

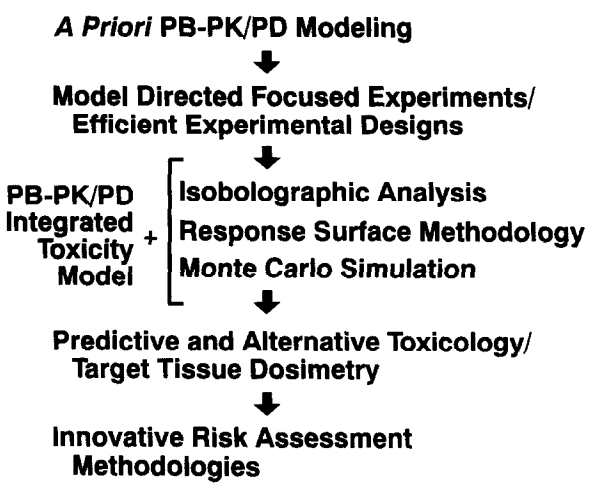

Fig. 8. Our proposed strategy/approach to develop 'Predictive and Alternative Toxicology' and formulate innovative risk assessment methodology' for chemical mixtures.

pharmacodynamic levels. By 'Alternative Toxicology,' we are aiming at minimizing animal experimentation, as illustrated in the example given (Monte Carlo simulation coupled with PB-PK/PD modeling). Thus, the application of PB-PK/PD to risk assessment of chemical mixtures may have several advantages: (1) The incorporation of toxicologic interactions based on mechanistic considerations; (2) In the Hazard Identification step, it is far less resource intensive and it will reduce animal killing; (3) Reducing the necessity of using large uncertainty factors. Accordingly, PB-PK/PD modeling will provide more realism into the risk assessment process. Of course, one must be aware of the fact that PB-PK/PD modeling has its own intrinsic 'uncertainties'; thus, as much as practicable, any PB-PK/PD model must be rigorously validated with experimental results before being used.

The linkage of two of the most challenging areas in toxicology today, (a) PB-PK/PD and statistical/mathematical modeling and (b) experimental toxicology of chemical mixtures, will have immense potential in application to risk assessment for chemical mixtures. Fig. 8 represents the possible application of combined PB-PK/PD modeling to chemical mixtures and the development of innovative risk assessment methodologies for chemical mixtures. We are attempting to couple PB-PK/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 technique such as Monte Carlo simulation, we may then predict tissue dosimetry at the pharmacokinetic and pharmacodynamic levels. Using such values as benchmark doses, human risk assessment of chemical mixtures may be carried out with less uncertainty.

Acknowledgement

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