Session 6.2
Biokinetic modelling *in silico*

Integration of PBPK and Reaction Network Modelling: Predictive Xenobiotic Metabolomics

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Summary
Our research group has aimed to integrate computational modelling with in vitro and in vivo experimentation towards the advancement of chemical mixture toxicology while minimising animal use. In the case of complex chemical mixtures and their interactions, the computer-assisted approach of Biochemical Reaction Network Modelling offers a ray of hope. The possible linkage between this novel computational methodology and physiologically-based pharmacokinetic (PBPK) modelling could result in a multi-scale computer simulation platform capable of predicting complex pathway interactions and metabolite concentrations at the molecular level up to tissue and organ concentrations at the whole organism level.

Keywords: PBPK modelling, biochemical reaction network modelling

Introduction

Computer simulation has been used extensively in the physical sciences and engineering and its wide applications touch every aspect of our lives. One of the best examples to illustrate the maturity and utility of computer simulation is the modern jetliner. The Boeing 777 is the first jetliner to be 100 percent digitally designed using three-dimensional CAD/CAM (computer-aided design/computer-aided manufacturing) technology (http://www.boeing.com/commercial/777family/background.html). Throughout the design process, the airplane was "pre-assembled" on the computer, eliminating the need for a costly, full-scale mock-up. In contrast, the utilisation of computational technology in biology lags far behind that in physical sciences and engineering. This deficiency, as well as the importance of integrating computer technology into biology, has been recognised by other investigators, as reflected by the following quote from Craig Venter (Butler, 1999): "…If we hope to understand biology, instead of looking at one little protein at a time, which is not how biology works, we will need to understand the integration of thou-sands of proteins in a dynamically changing environment. A computer will be the biologist's number one tool…"

Computer simulation of biological processes is a realistic and workable method to either replace animal studies, or at least, reduce animal usage in experimentation. Ethical reasons aside, the reality is that experimental toxicology cannot keep pace with the number of chemicals in commerce (about 80,000), plus the new synthetic organic chemicals coming on stream (about 2,000/year). For instance, the U.S. National Toxicology Program with its predecessor, the National Cancer Institute, has conducted chronic toxicity/carcinogenicity studies on about 600 chemicals in their combined effort over the last 43 years. This represents a minuscule portion of the chemicals that are being used today. It is quite clear that we will never be able to catch up under the present mode of operation. Considering further the potential presence of numerous chemical mixtures, it is certain that alternative methods, such as computational tools, must be developed to handle the backlog more efficiently.

Our laboratory has been working on a possible solution to the above dilemma. We advocate the integration of physiologically

based pharmacokinetic (PBPK) modelling with biochemical reaction network (BRN) modelling to create multi-scale computer simulations. These models would provide predictions of the fate of a chemical or chemical mixture from the level of the whole organism down to molecular interactions (Klein et al., 2002; Liao et al., 2002; Reisfeld and Yang, 2004; Yang et al., 2004a; Mayeno et al., 2005). This methodology is currently at the research and development stage. When completed, chemicals or chemical mixtures with little or no animal toxicity data can be fed into the computer simulation program and their potential adverse health effects deduced from the metabolic reaction networks generated. In the sections below we will first discuss PBPK and BRN modelling, then the integration of these two types of models, and finally the concept of predictive xenobiotic metabolomics.

Materials and methods

PBPK modelling
PBPK is a special type of pharmacokinetics where physiology and anatomy of the animal or human body, and the biochemistry of the chemical or chemicals of interest, are incorporated into a conceptual model for computer simulation. Unlike classical pharmacokinetics, PBPK modelling is a powerful tool for many types of extrapolation: inter-species, inter-routes, inter-doses, inter-life stages, etc.

The concept of PBPK had its embryonic development in the 1920s. PBPK modelling blossomed and flourished in the late 1960s and early 1970s in the chemotherapeutic area due mainly to the efforts of investigators with expertise in chemical engineering. In the mid 1980s, work on PBPK modelling of volatile solvents started yet another "revolution" in the toxicology and risk assessment arena. Today, there are more than 1000 publications directly related to PBPK modelling of industrial chemicals, drugs, environmental pollutants, and simple and complex chemical mixtures. A book on PBPK modelling has recently been published from our laboratory in collaboration with others (Reddy et al., 2005).

The fundamentals of PBPK modelling are to identify the principal organs or tissues involved in the disposition of the chemical of interest and to correlate the chemical absorption, distribution, metabolism, and excretion (ADME) within and among these organs and tissues in an integrated and biologically plausible manner. A scheme is usually formed where the normal physiology is followed in a graphical manner (i.e., a conceptual model). Within the boundary of the identified compartment (e.g., an organ or tissue), whatever "comes in" must be accounted for via whatever "goes out" or whatever is transformed into something else. This mass balance is expressed as a mathematical equation with appropriate parameters carrying biological significance. A series of such mass balance equations representing all of the interlinked compartments is formulated to express a mathematical representation, or model, of the biological system. This model can then be used for computer simulation to predict the time course behaviour of any given parameter.

For more detailed information on PBPK modelling and its related methodologies, readers are referred to two recent publications (Yang et al., 2004b; Reddy et al., 2005).

Biochemical reaction network modelling
Biochemical reaction network (BRN) modelling has its origin in chemical and petroleum engineering. It was successfully employed in computer modelling and simulation of the complicated processes in oil refineries. A reaction network model is a tool that is used to predict the amounts of reactants, intermediates, and products as a function of time for a series of coupled chemical reactions (potentially numbering in the tens of thousands of reactions). Broadbelt et al. (1994, 1996) refined previous ideas and used concepts of graph theory to represent species connectivity. They also made use of computational quantum chemistry and linear free energy relationships (LFER) to automate the process of determining reaction rate constants. The essential idea is that the model takes, as input, specifications for the reactants (usually in terms of their chemical structures), as well as rules stipulating the nature of the relevant chemical reactions. Algorithms within the reaction network model develop the associations between species and create and solve the controlling kinetic equations in the reaction model. Thus, the outputs of the simulation are the connections between reaction species as well as the concentrations of these species over time. The idea of using reaction network modelling for biomedical applications was put forth by a joint effort between our laboratory and Rutgers University (Klein et al., 2002).

Over the last four years, a programme, BioTRaNS (**Bio**chemical **T**ool for **R**eaction **N**etwork **S**imulation), was created *de novo* in our laboratory based on an extensive review of the literature on molecular modelling of substrates of P450 enzyme systems. BioTRaNS integrates modules of our own creation, as well as existing software and database tools, such as CORINA (molecule structure prediction), MOPAC7 (quantum chemical calculations), GraphViz (mathematical graph visualisation), and Daylight and OpenEye toolkits (symbolic molecule manipulation and chemical/biochemical reaction transformations and prediction). As part of the BioTRaNS effort, other methods (quantitative structure activity relationships, decision trees) have also been developed for the prediction of the probabilities of cytochrome P450 binding of chemicals.

The novelty and advantages of BioTRaNS over previous and other frameworks are as follows:

a) It was written from the ground up to focus on biological applications rather than on petrochemical applications.

b) It allows user-friendly programme usage and interaction.

c) It specifically considers enzyme-substrate interactions.

d) It has "hooks" or interfaces to communicate and interact with PBPK or other modelling tools.

e) It uses a cheminformatics-industry standard means of representing molecules.

f) It is flexible and user-friendly in terms of specifying reactions and reaction feasibility.

g) It uses a dynamically-updated database of molecules and molecular properties.

h) It is well documented for the user.

i) It has a well-defined and documented application programming interface (API) for programmers to use when designing applications to interact with the present application.

A simplified description and information flow is illustrated in figure 1 (see Mayeno et al., 2005 for a more detailed description). In brief, BioTRaNS takes a description of a set of chemicals and the enzymes believed to be involved in their metabolism and produces the detailed metabolic pathways, showing the interconnections between the metabolites as well as the concentrations of all of these chemical species over time.

a) First, the user inputs the concentration of a single chemical (or concentrations of individual components in a chemical mixture) as well as the types and amounts of enzymes that the user selects to act on the chemicals.

b) The reactants, along with data from the Molecule Property Database, are fed into the Feasibility Module, which computes the probability that each given reactant will be a substrate for each of the specified enzymes. If the probability of an interaction is below a user-defined threshold, the reactant does not undergo chemical transformation via that enzyme. Even though our initial development of BRN modelling has specifically focused on certain types of chemical mixtures, this feasibility module will be further developed to give the software the capability to predict substrate feasibility of any chemical that will be studied in the future.

c) The Transformation Module, with chemical transformation information from the Reaction Rules Database, performs the "virtual chemistry" of transforming the reactants to products. These products in turn become reactants that are checked for reaction feasibility.

d) The Pathway Module uses the metabolites generated from step "c" to establish the connections between all metabolites,

forming the basis for the reaction network. The network structure or topology can be used to give various "views" of the reaction interconnections.

e) Simultaneous to pathway creation, the Kinetics Module creates the reaction rate equations (ordinary differential equations) and retrieves the reaction rate constants for these reactions from the Rate Constant Database, or estimates them from related data, such as physicochemical, electronic, and quantum mechanical properties.

f) The total set of rate equations is then solved by the Solver Module to determine the concentrations of all species as a function of time.

From the information set produced by BioTRaNS an investigator can examine the nature and lifetimes of species of interest and, in the context of health risks, easily locate highly reactive species. Moreover, due to its design, information can be fed back and forth between BioTRaNS and lower level (e.g., enzyme induction) and higher level (organ/organism level) modelling tools to give a more complete picture of the risk.

Results

Biochemical reaction network modelling of a mixture of four volatile organic chemicals

As an example of the use of BRN modelling to examine interactions, we will use a mixture of four volatile organic chemicals (VOCs): trichloroethylene (TCE), chloroform (CF), tetrachloroethylene (Perc), and 1,1,1-trichloroethane (MC). All four
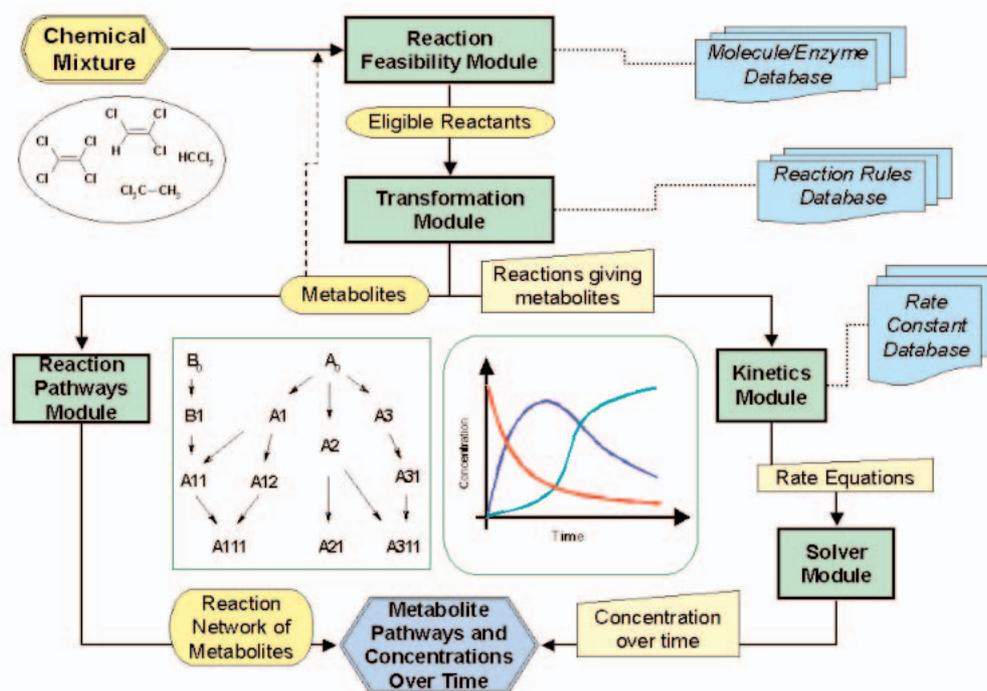

Fig. 1: Information flow through the BioTRaNS

are prevalent drinking water or ground water contaminants and they are likely to be present in such media together. This study was reported in a recent publication (Mayeno et al., 2005).

The interconnected biotransformation pathways of all four VOCs (fig. 2) illustrate the close relationship among the metabolic pathways of these chemicals, their shared enzyme systems, the potential for generating the same reactive species from different parent compounds, and the dynamic interactions among the linked pathways influencing the possible outcome of toxicities. Our laboratory has completed the qualitative aspects of the biochemical reaction network modelling of the four volatile organic chemicals based on biochemical reaction mech-

anisms of the relevant CYP and related enzymes. Moreover, we have incorporated enzyme-reaction mechanisms to help predict metabolite formation. For instance, the mechanism-based biotransformation of TCE, as generated by BioTRaNS, is shown in figure 3. The first step of CYP2E1-catalysed oxidation involves the formation of an intermediate between the high-valent iron-oxo complex, $[(FeO)^{3+}]$ of the CYP haeme and the alkene, as postulated by Miller and Guengerich (1982). This intermediate has been linked mechanistically to the 1,2-shifts of Cl (or H), leading to the formation of an aldehyde (or acid chloride), as shown in figure 3. Details of these (bio)chemical processes and subsequent step-wise reaction mechanisms, with their respective
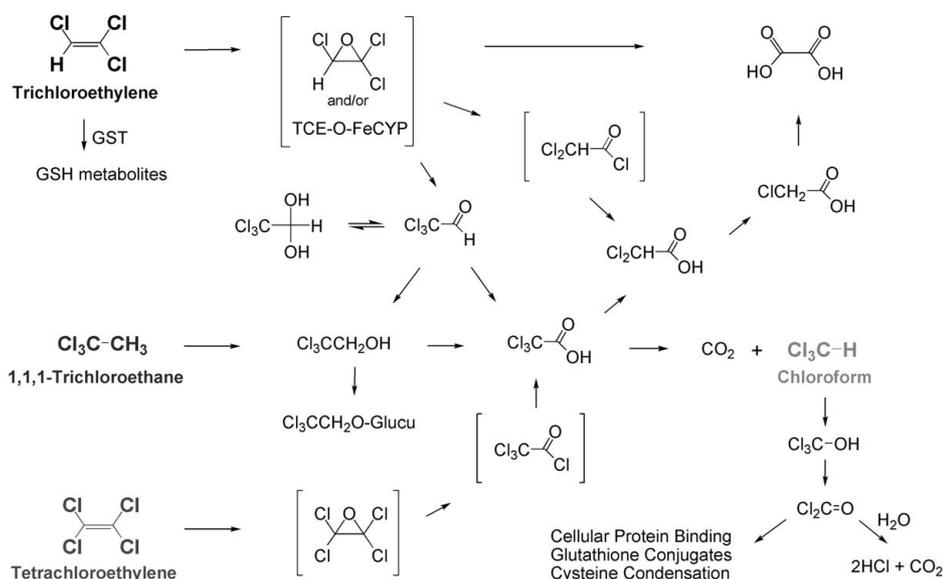

Fig. 2: Combined metabolic pathways of trichloroethylene, chloroform, tetracholoroethylene, and 1,1,1-trichloroethane (only major pathways shown)
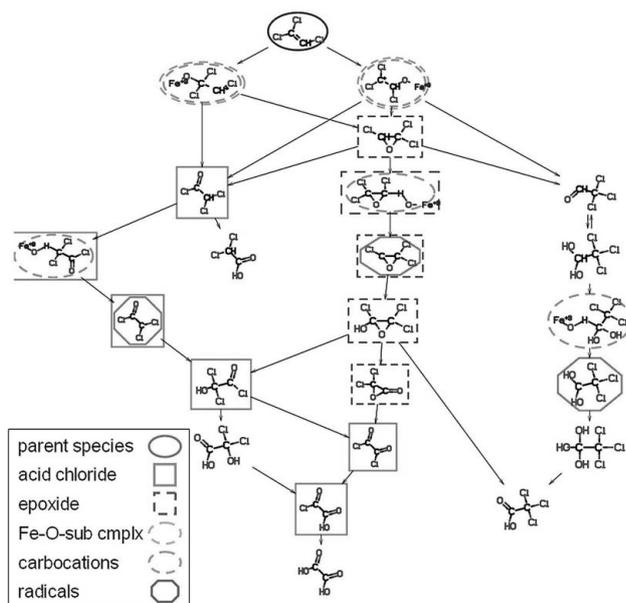
Fig. 3: A portion of the BioTRaNS-generated biotransformation pathways using a postulated mechanism for CYP-mediated TCE oxidation (Miller and Guengerich, 1982). Reactive metabolites are highlighted as follows: epoxides (brown, box, dashed); acid chlorides (orange, box, solid); and starting chemicals (blue, ellipse, solid). Please see website www.altex.ch for figure 3 in colour. (Reprinted with permission from Mayeno et al., 2005. Copyright (2005) American Chemical Society).
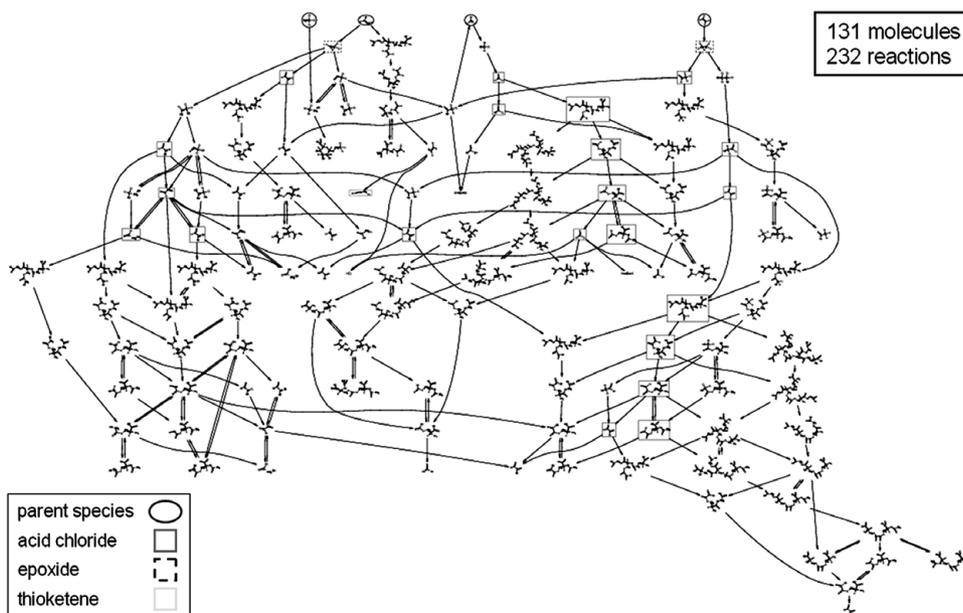
Fig. 4: BioTRaNS-generated biotransformation reaction network for four volatile organic chemicals: trichloroethylene, tetrachloroethylene, methyl chloroform, and chloroform. The software generated this figure based on reaction rules and interconnected the pathways via metabolites in common. Reactive species were highlighted in red boxes, after substructures (SMARTS) of these species were input by the user. Please see website www.altex.ch for figure 4 in colour. (Reprinted with permission from Mayeno et al. 2005. Copyright (2005) American Chemical Society)
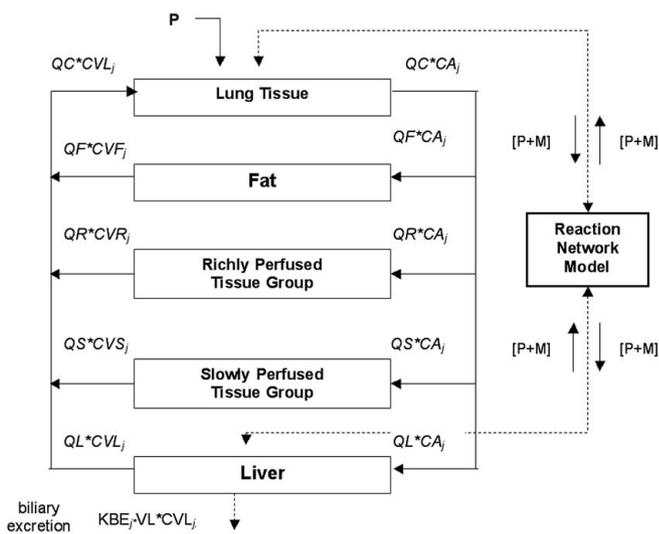
Fig. 5: A graphical representation of a preliminary conceptual integrated physiologically-based pharmacokinetic/biochemical reaction network model for benzo[a]pyrene.
P = parent compound (BaP); M = metabolites; Q = flow rate; CA = arterial blood; CV = venous blood.

originally published sources, have been described in our recent paper (Mayeno et al., 2005). Our computer-generated interconnected metabolic pathways for these four VOCs (fig. 4) demonstrate the metabolite inventory and interconnections ("predictive xenobiotic metabolomics"). The predicted pathways (fig. 4) match the known metabolic pathways (fig. 2) very well.

Linking physiologically-based pharmacokinetic models and biochemical reaction network models

Since PBPK and BRN models address different aspects of predictive toxicology, linking them will yield a powerful tool. To this end, the development and validation of an integrated BRN/PBPK model of benzo[a]pyrene (BaP) was studied (Liao, 2004). In this case, the BRN model used was a predecessor to the BioTRaNS model described earlier. figure 5 illustrates the interconnection between the PBPK and BRN models. The linkage between the two models, in this case, occurs in the liver compartment (the major organ responsible for metabolism) via the venous circulation.

The PBPK model predicts the ADME of BaP and metabolite(s) of interest circulated to the organs, while the BRN model calculates the amounts and rates of metabolites formed and unmetabolised parent chemical(s) that can be distributed back to organs/tissues via PBPK modelling. A general equation, for any tissue or organ, is:

$$Vi\frac{dC_{ij}}{dt} = Q_i\left(CA_j - CV_{ij}\right) - \text{Metab}_{ij} - \text{Elim}_{ij} + \text{Absorp}_{ij} - \text{Pr}\,Binding_{ij}$$

where V_i represents the volume of tissue group i, Q_i is the blood flow rate to tissue group i, CA_j is the concentration of chemical j in arterial blood, and C_{ij} and CV_{ij} are the concentrations of

chemical j in tissue group i and in the effluent venous blood from tissue i, respectively. $Metab_{ij}$ is the rate of metabolism for chemical j in tissue group i; it can be predicted by BRN models in the liver and other metabolising organs and is equal to zero in other tissue groups. $Elim_{ij}$ represents the rate of elimination from tissue group i (e.g., biliary excretion from the liver), $Absorp_{ij}$ represents uptake of the chemical from dosing (e.g., oral dosing), and $PrBinding_{ij}$ represents protein binding of the chemical in the tissue.

Figures 6 and 7 are representative simulations of the integrated model against published data in the literature. The high quality of the simulations, which were obtained by specifying reaction rules, is remarkable. It is noteworthy that our BRN modelling indicated that the biotransformation of BaP, a single compound, involves 246 possible reactions and 150 possible metabolic products (Liao, 2004). These results demonstrate the potential for complexity, the capability of BRN modelling, and the promise of linked PBPK-BRN models.

Discussion and conclusions

Metabolomics is the study of the metabolites contained in a human or animal cell, tissue or organ and involved in primary and intermediary metabolism. It is an emerging "–omics" technology that has already shown great promise in providing important and relevant health-related information (Brindle et al., 2002).

Since xenobiotics, including drugs, affect our health in both positive and negative ways, it is reasonable to expand the domain of metabolomics to include xenobiotic reaction network pathways in our assessment of human health. With the linkage of PBPK and BRN modelling as described above and the possible linkage to other modelling tools (fig. 8), the field of "*Predictive Xenobiotic Metabolomics*" can be advanced. Using such an approach towards chemical and chemical mixture toxicology, reduction and even total avoidance of animal usage can be realised in the future.
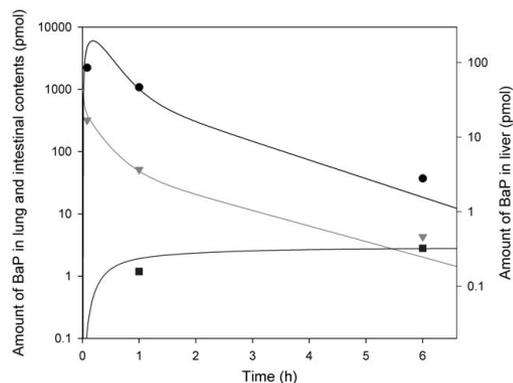

Fig. 6: BaP in liver (●), lung (▲), and intestinal contents (■) of rats after exposure to BaP intra-tracheally at 1 mg/kg body weight. (Data from Weyand and Bevan, 1986)
Solid lines represent biochemical reaction network (BRN)/ physiologically-based pharmacokinetic (PBPK) model simulations.
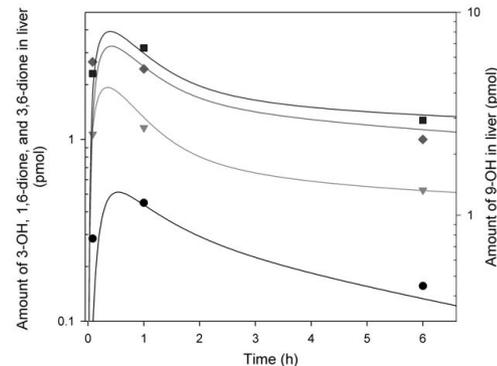
Fig. 7: Amount of BaP metabolites, 3-OH (▼), 9-OH (●), 1,6-dione (◆), and 3,6-dione (■), in liver of rats after exposure to BaP intra-tracheally at 1 mg/kg body weight. (Data from Weyand and Bevan, 1986)
Solid lines represent BRN/PBPK model simulations.
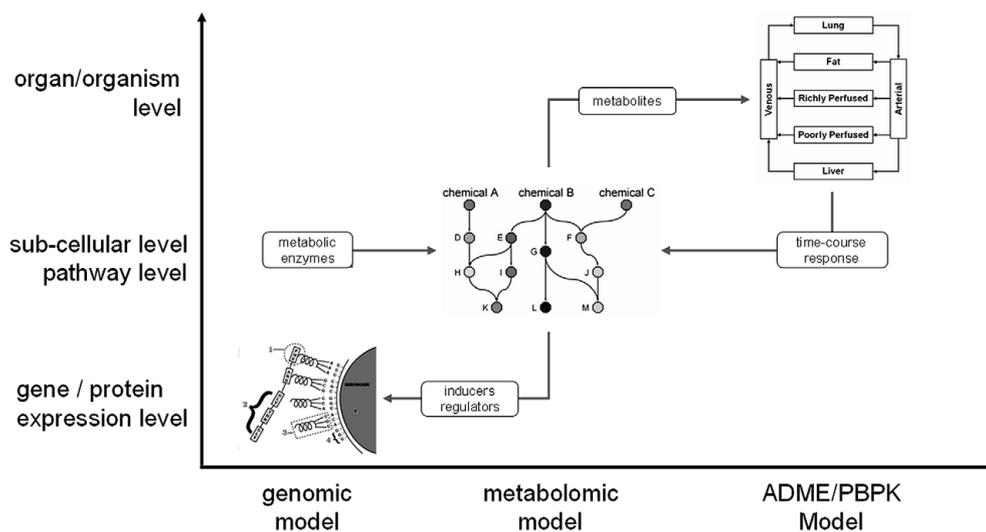
Fig. 8. Multi-scale modelling and systems biology approach towards global toxicological effects of a chemical or chemical mixtures.

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