3.0 *IN VITRO* METHODS FOR ASSESSING ACUTE TOXICITY: BIOKINETIC DETERMINATIONS

3.1 Introduction

The biokinetics determinations Breakout Group (Breakout Group 2) was given the task of discussing and evaluating the capabilities of *in vitro* methods for providing biokinetic information (i.e., on absorption, distribution, metabolism, and excretion) that can be used to estimate target-organ dosimetry for acute toxicity testing. The Breakout Group was asked to identify future research needs in the area of biokinetics that will enable *in vitro* methods to more accurately predict acute toxicity *in vivo*. The role of quantitative structure-activity relationships (QSAR) and quantitative structure-property relationships (QSPR) in biokinetic determinations was also to be considered.

The Breakout Group was asked to answer a number of questions in three areas:

(1) The identification of the need for specific knowledge in the field of biokinetics;
(2) The current status of knowledge and technology in the field;
(3) Future directions for research.

The group discussions followed general lectures given in the Workshop's opening plenary session. A presentation to the Breakout Group entitled "An integrated approach for predicting systemic toxicity" was particularly relevant to the Breakout Group's responsibilities, demonstrating the central role of biokinetic modeling in the prediction of systemic toxicity using *in vitro* data (Blaauboer et al., 2000).

3.1.1 *General Discussion*

The goals for the Workshop were presented and the following specific questions were posed:

(1) What *in vitro* systems are available and how can these systems be applied and/or improved?
(2) What research requirements can be formulated?
(3) Which priorities can be set for research?

The discussions of the Breakout Group centered on the role of the kinetics of a chemical *in vivo* in its acute systemic toxicity. The following summary was developed as a point of departure for the Breakout Group's deliberations:

> Results obtained from *in vitro* studies in general are often not directly applicable to the *in vivo* situation. One of the most obvious differences between the situation *in vitro* and *in vivo* is the absence of processes regarding absorption, distribution, metabolism and excretion (i.e., biokinetics) that govern the exposure of the target tissue in the intact organism. The concentrations to which *in vitro* systems are exposed may not correspond to the actual situation at the target tissue after *in vivo* exposure. In addition, the occurrence of metabolic activation and/or saturation of specific metabolic pathways or absorption and elimination mechanisms may also become relevant for the toxicity of a compound *in vivo*. This may lead to misinterpretation of *in vitro* data if such information is not taken into account. Therefore, predictive studies on biological activity of compounds require the integration of data on the mechanisms of action with data on biokinetic behavior. Over the last decade, the feasibility of using mathematical models for interpretation of *in vivo* biokinetics has grown substantially. This development has been facilitated by the increasing availability of computer-based techniques for numerical solution of differential equation sets that characterize biokinetic processes (Blaauboer et al., 2000).

The Breakout Group also reached consensus on some terminology: the word "toxicokinetics" should be replaced by "kinetics" or "biokinetics". Problem areas in predicting kinetics of chemicals were noted in: (a) biotransformation (value of *in vitro* systems for determining biotransformation,

interpretation of *in vitro* data, scaling up to the *in vivo* situation); and (b) the passage across special barrier systems (e.g., in the gastrointestinal [GI] tract, the blood-brain barrier [BBB], and the kidney).

Short presentations on the following were provided as a focal point for Breakout Group discussions:

- Biokinetic modeling of acute exposure;
- QSAR/QSPR;
- BBB;
- Kidney barrier systems;
- Intestinal barrier;
- Metabolic activation, including different systems available for the liver (and extrahepatic tissue);
- Skin as a barrier;
- Microarray alternatives;
- Information from NIEHS Microarray Center;
- Expert systems for making predictions of a compound's partitioning and toxicity.

After the presentation on the use of Physiologically-Based Biokinetic (PBBK) models, the Breakout Group concluded that kinetics play a crucial role in estimating a compound's acute systemic toxicity. The use of these physiologically determined models has proven to be very useful in many aspects. Over the last ten years, the feasibility of this modeling approach has been greatly enhanced due to the availability of computer techniques that allow for the simultaneous numerical solution of differential equations. While species-specific anatomical and physiological data are generally available from the literature (e.g., Arms and Travis, 1988; Brown et al., 1997), compound-specific parameters for PBBK models (e.g., tissue-blood partition coefficients and the Michaelis-Menten constants Vmax and Km) are often still obtained by fitting these parameters to experimental data obtained *in vivo*. Proper use of PBBK models in itself can contribute to reduction and refinement of animal studies by optimization of study design through identification of critical parameters and time frames in kinetic behavior. In addition, incorporation of *in vitro*-derived parameters will lead to a further reduction of large-scale animal studies for quantitative assessment of the biological activity of xenobiotics.

The Breakout Group concluded that a distinction can be made between the goals to be achieved:

- Short-term: improvement of the interpretation of *in vitro* toxicity data for estimating rodent LD50 values;
- Long-term: using *in vitro* data for estimating/predicting sublethal acute toxic effects caused by chemicals in humans (e.g., represented by a TD10 value, i.e., the dose at which mild toxicity could be expected in no more than 10% of the exposed humans).

It will be obvious that the latter goal is of greater interest for the risk evaluation of chemicals, where the protection of humans with regard to toxic effects is the highest priority.

These different goals need different scientific activities; different groups of chemicals will need different approaches for modeling the kinetics. In some cases, a great deal of information is available (e.g., on low molecular weight; volatile lipophilic compounds). For these compounds, reasonable estimates can be obtained for their partitioning in the organism based on their physico-chemical properties. Many kinetic parameters (e.g., Vd and k_e) are also determined by the size of the dose (i.e., the amount of compound available for systemic circulation) because of capacity-limited processes in metabolism and transport.

3.1.2 *Subjects of Discussion*

The intestinal barriers, the role of the gut flora, first pass metabolism, and (counter) transport systems were discussed. A number of cell lines are available to estimate absorption through the gut barrier. BBB and skin absorption models were also addressed. *In vitro* methods for these systems exist, but none reflects the full metabolic and transport capacity seen *in vivo*.

The current status of systems to estimate the kidney epithelia as a barrier was discussed. These

systems include the use of renal cell lines, such as LLC-PK_1 cells and MDCK cells. The former cells form low resistance epithelial monolayers when grown on permeable supports; the latter form extremely high resistance. However, these cell lines do not express all the relevant transporters found *in vivo*. The lack of the organic anion transporter is particularly problematic and cell lines transfected with these transporters may be more appropriate. Currently, an ECVAM prevalidation study is under way of trans-epithelial resistance and inulin permeability as endpoints in *in vitro* nephrotoxicity testing.

The ability to estimate biotransformation reactions of chemicals is of particular interest since acute toxicity may be mediated through the bioactivation or deactivation of chemicals. *In vitro* systems designed to address this possibility include:

- Liver homogenates;
- Microsomal preparations;
- Isolated cells;
- Primary monolayer cultures;
- More complicated cell cultures (co-cultures, 3D cultures);
- Transgenic cell lines.

QSAR systems have also been proposed for modeling the metabolic biotransformation of chemicals. The use of QSAR/QSPR and the development of software systems to predict "chemical functionalities" of compounds which may be used to estimate kinetic behavior (including protein binding) and the toxicodynamics were also discussed.

3.2 Identifying Needs

3.2.1 *In Vitro Methods for Evaluating Chemical Kinetics*

As mentioned above, the Breakout Group recognized a short-term and a longer-term goal for using *in vitro* or other non-animal techniques for predicting acute systemic toxicity. First, one focuses on the longer-term goal: how to use these techniques for the evaluation of a chemical's kinetics and the ultimate prediction of sublethal acute toxic effects in humans. Section 3.4.4 concentrates on the short-term (interim) goal: how to improve the prediction of acute lethal effects in rodents. *In vitro* methods, in combination with knowledge of a chemical's structural properties, can be used to predict/determine the chemical's absorption, distribution, metabolism, and elimination in an intact organism. However, it will be a major challenge for the field of *in vitro* toxicology to identify the particular target tissue(s) or cells and the time course of clinical toxicity in the absence of *in vivo* observations.

In the short-term, physico-chemical properties can be used to predict/determine partition. QSAR (or QPPR) can be helpful for this determination (DeJongh et al., 1997). *In vitro* determinations of rates of metabolism and of passage of a chemical across membrane barriers (e.g., GI $\Rightarrow$ blood; blood $\Rightarrow$ brain) will improve the kinetic modeling. Taken together, these may be able to be used to calculate an LD50 value (as administered to an intact organism) from the LC50 value in a basal cytotoxicity test. Presentation of any such predicted LD50 value also requires concurrent presentation of the quantitative uncertainties attendant to that value. In the long-term, knowledge of a chemical's kinetics will need to include a comparison of the kinetic and the toxicodynamic time-profiles. Moreover, knowledge of kinetics assists in determining the mode of toxic action and vice versa (Ekwall et al., 2000; Liebsch et al., 2000). [see MEIC evaluation of acute systemic toxicity, Appendix E].

3.2.2 *Biokinetics in the Overall Toxicological Evaluation*

Biokinetics is essential for relating administered dose of toxicant to concentration at the target tissue(s). Tissue-specific concentration of the toxicant is one of the mechanisms that can result in organ-selective toxicity. In addition, biokinetics can establish whether metabolism plays a role in modulating the toxicity. Such modulation can either attenuate or enhance the toxicity.

3.2.3 *Biokinetic Techniques as In Vitro Assays*

The following are techniques that need further development:

(1) *In vitro* determination of partition coefficients, metabolism, protein binding, and stability;
(2) Characterization of biotransformation enzymology;
(3) Structural knowledge and its translation into "chemical functionalities"; estimation of partition coefficients, metabolism, etc. ("*in silico*", including QSAR/QSPR);
(4) Biokinetic modeling, including the integration of toxicodynamic and biokinetic modeling in predicting systemic toxicity.

3.3 Current Status

3.3.1 *Prediction of Biotransformation*

Biotransformation can be carried out using human or animal hepatic subcellular fractions, human or animal primary hepatocytes, or human or animal hepatic precision-cut slices. The use of primary human hepatocytes in suspensions or culture requires specific expertise and may not be appropriate for use in all laboratories. Human or animal hepatic subcellular fractions can be cryopreserved and used at a later time to provide qualitative kinetic data, but these fractions may not reflect the integrated routes (activation and detoxification) of metabolism of a compound.

The selective use of cofactors can aid the determination of routes of metabolism. There is a need for standardization of the conditions for the preparation and incubation of rat hepatocytes. Rat hepatocyte incubations may overestimate the metabolic clearance of a compound. It is essential to quantify the rate of disappearance of the parent compound and desirable to quantify the rate of metabolite formation.

3.3.2 *Systems for Estimating Gastrointestinal Absorption*

Apparent membrane permeability and aqueous solubility are reasonably predictive of the fraction of a dose that will be absorbed through the GI tract. Several *in vitro* systems for measuring intestinal absorption include measuring apparent permeability constants in either intestinal tissue segments or cell monolayers that have been grown on a porous support. Cell lines used for this purpose include the human colon carcinoma cell line Caco-2, the canine kidney cell line MDCK, and the porcine kidney cell line LLC-PK_1. All systems are widely used in the pharmaceutical industry in the oral drug discovery process. Each system has advantages and disadvantages which may or may not be relevant depending on the chemical under study.

Cell lines do not require the use of animals. However, they often lack or have non-physiological levels of uptake and efflux transporters that are present *in vivo*. These transporters can dramatically affect the extent of bioavailability at low doses. The nature and extent of species differences in transporter activity/affinity is presently unknown. The Breakout Group consensus was that in the absence of data to the contrary, it would be appropriate to assume that an administered dose would be completely absorbed. This is a public health conservative approach. For those compounds where such an assumption is not appropriate, the above-mentioned *in vitro* systems can be used to provide experimental data on the fraction absorbed.

3.3.3 *Prediction of Renal Clearance/Accumulation*

Glomerular filtration and reabsorption in the proximal tubule determine the renal excretion of most compounds. These parameters can be predicted from the physico-chemical properties of the compound and its plasma protein (albumin) binding. These parameters are less predictable where active secretion or reabsorption and saturation kinetics are involved. Many of the currently available renal cell lines or renal cell primary cultures lack specific transporters (in particular, the organic anion transporter) which are implicated in the accumulation of several nephrotoxic compounds. The substrate specificity of other proximal tubular transporters is poorly defined.

3.4 Future Directions

3.4.1 *Proposed Approach for Consideration of Kinetics in the Estimation of Acute Oral Toxicity*

The diagram presented in Figure 3.1 illustrates a conceptual structure for the use of kinetic information in the estimation of acute oral toxicity. Under this scheme, available *in vitro* data on the absorption, tissue partitioning, metabolism, and excretion of a test material would be used to parameterize a chemical-specific biokinetic model (Clewell, 1993). In many cases, currently available QSPR/QSAR techniques could be used to estimate chemical properties and kinetics when the specific data for that chemical is lacking. For example, simple empirical correlations have been developed for estimating the tissue partitioning of a chemical from its water solubility, vapor pressure, and octanol/water partitioning (Paterson and Mackay, 1989; DeJongh et al., 1997). Emerging QSAR techniques (e.g., knowledge-based systems) may eventually prove useful in predicting potential target tissues for toxicity so that the appropriate assays of *in vitro* dynamics (response) could be selected. These target tissue assays would, in turn, provide information on the nature and location of the toxicity produced by the chemical (DeJongh et al., 1999).

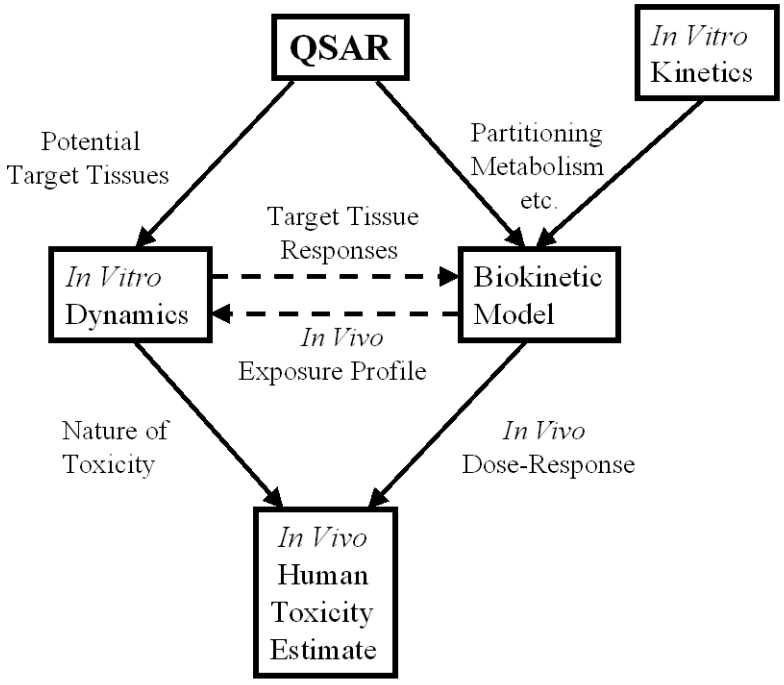

Figure 3.1. A recommended scheme for incorporation of QSAR (QSPR) data, *in vitro* data on kinetics and dynamics, and kinetic modeling in the estimation of human (or animal) toxicity

3.4.2 *Classification of Compounds Based on Their Physico-Chemical Properties*

The complexity of the biokinetic model would depend on the physico-chemical and biochemical characteristics of the chemical. In the specific case of acute toxicity, a simple one-compartment description of the administered chemical may suffice for many chemicals. The volume of distribution for such a model could be estimated

from the volume-weighted average of the estimated partitioning into various tissues, and estimates of fractional absorption and rate of clearance could be based on data for structurally similar compounds.

Each of these assumptions or predictions, however, introduces its own associated uncertainty into the result of the lethality risk estimate. Even with such a simple model, it may be possible to estimate the systemic concentrations that could be expected to result from an *in vivo* exposure to a given dose (DeJongh et al., 1999). Thus, the model could be used to relate the concentrations at which toxicity is observed in an *in vitro* toxicity assay to the equivalent dose that would be expected to be associated with toxicity for *in vivo* exposure. These models can also provide information on the temporal profile for tissue exposure *in vivo*, which can then be used in the design of the most appropriate *in vitro* experimental protocol (Blaauboer et al., 1999).

There are chemical classes for which a one-compartment description would not be expected to be adequate. However, the physiological mammalian structure (tissue volumes, blood flows, ventilation rate, glomerular filtration rate, etc.) is well characterized, and there is no difficulty in describing tissues separately. As mentioned above, techniques exist for estimating tissue-specific partitioning. Other data required would depend on the class of chemical. For volatile chemicals, ventilatory clearance can be estimated from the blood-air partition. For water-soluble chemicals, urinary clearance can be estimated from the glomerular filtration rate or the renal blood flow (for secreted compounds). For some classes of chemicals, it would also be necessary to determine the fractional binding of the chemical to plasma proteins or the partitioning of the chemical into red blood cells.

The greatest challenge in parameterizing the biokinetic model remains the estimation of metabolic clearance. The possibility is increasing to use *in vitro*-determined metabolic parameters (Vmax and Km) in order to accurately predict total body metabolic clearance (Houston and Carlile, 1997). Currently, it would be necessary to perform *in vitro* assays of the dose-response (capacity and affinity) for metabolic clearance (Kedderis, 1997; Kedderis and Held 1996; Kedderis et al., 1993). These assays are generally more expensive than the dynamic (toxicity) assays, since they necessarily involve the development of an analytical method for quantifying the concentration of the parent compound and its metabolite(s) in each tissue of interest over time. Quantification of the concentration of compound in the dynamic assays should also be preferred, but it is not absolutely necessary in that case. Eventually, as data accumulate for a large number of structurally-diverse materials, it might be possible to predict metabolism and disposition using knowledge-based systems.

An important underpinning of this process is that the kind of information necessary for a particular test material depends on its structure and physico-chemical properties. It seems reasonable to expect that chemicals could be categorized into classes based on their properties, and that this categorization would simplify the process of determining the data needed for a particular compound. This concept is illustrated in Figure 3.2. As noted above, the key physico-chemical properties of a test material involves its volatility (reflected in its blood-air partition, Hb/g), its water solubility (Sw), and its lipophilicity (reflected in its octanol-water partition, Ko/w). Compounds with similar properties can be grouped, and data from similar compounds can be used to fill gaps in the knowledge of a particular compound.

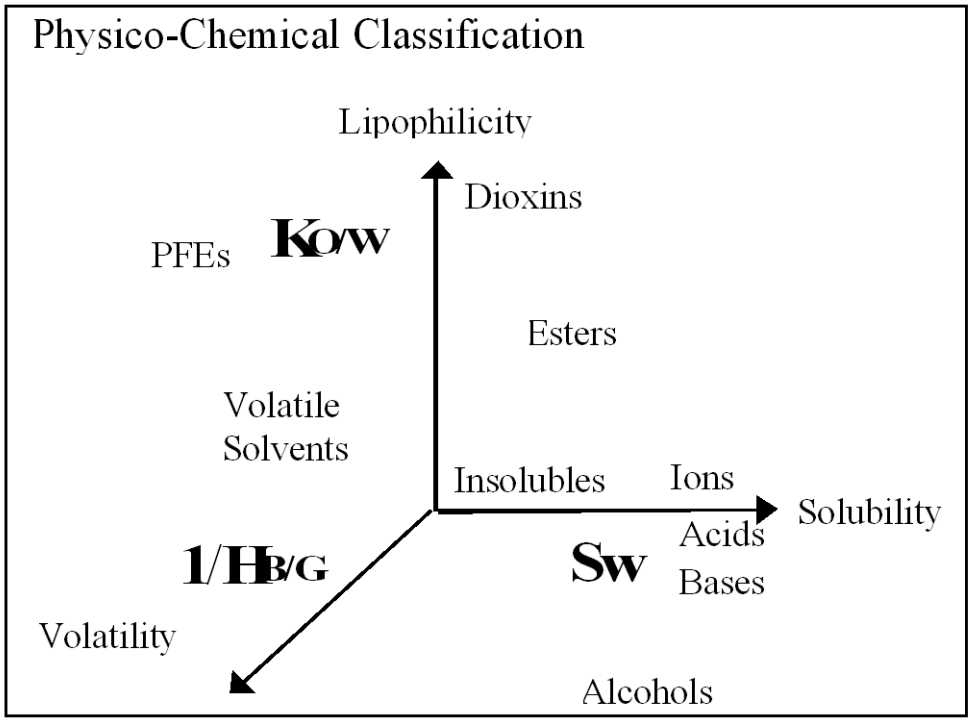

Figure 3.2. Classification of compounds based on their physico-chemical properties

There are two advantages of this *in vitro*/modeling approach over the traditional *in vivo* LD50 test. First, the *in vitro*/modeling approach can provide more extensive information than a traditional oral LD50 value provides. As information accumulates across chemicals, QSAR techniques could play a correspondingly greater role in the prediction of both kinetic and dynamic information. It is likely that QSAR techniques would be more successful for these fundamental processes and simple *in vitro* assays than they have been for the prediction of the *in vivo* assay. Secondly, all of these assays should be performed using human cell systems. The Breakout Group consensus was that *in vitro* testing should, when possible, be performed with human cells rather than rodent cells. This obviates the need, inherent in the rodent LD50 test, to extrapolate from rodents to humans. The uncertainties with the current approach of extrapolating *in vitro* derived data employing human cell cultures to the situation in the intact situation in humans will generally be smaller than those uncertainties for extrapolating data from animal cell experiments to humans.

Classification of chemicals according to their physico-chemical properties has been done extensively in the past. This approach has proven to be useful to predict effects, particularly within closely related classes of chemicals. However, this approach has limitations; it should not be used outside the boundaries of the prediction model used (i.e., the effects that can be predicted should be within the scope of the model assumptions).

If the focus is on the use of *in vitro*-derived data, then the importance of using specific cell systems becomes more important if one is looking at more specific forms of toxicity. Then the biological properties of the cells used become more important. Ultimately, there are two questions that coexist all the time: What does the chemical do to the cell?; and what does the cell do to the chemical? From this conceptual point of departure, the rate-determining step and more

often the rate-limiting steps need to be identified for mathematical modeling.

This problem and part of its solution can be illustrated based on central nervous system (CNS) vs. liver effects of solvents (limit it to small molecular weight chlorinated aliphatics). It is known from the Meyer-Overton rule (Meyer, 1937) that these anesthetic chemicals are very predictive of one another's CNS effects *in vivo*. However, these predictions do not hold for chronic liver effects and vice versa. This is understandable since the two effects have nothing in common, kinetics being the rate-determining step for anesthesia (wake-up driven by elimination of the chemical) vs. dynamics being the rate-determining step for liver cancer (slow reversibility of preneoplastic foci after complete elimination of the solvent). However, an acute endpoint such as reduced flicker fusion reflex is a much more sensitive endpoint of impairment than is chronic liver cancer. Therefore, people will be protected from cancer if regulation is based on the acute effect without the need for elaborate PBBK models based on metabolism in the liver.

The acute toxicity of all these solvents consists of CNS depression leading to respiratory failure without regard to the route of administration. These considerations will become more important when one moves away from the prediction of acute lethal toxicity towards predicting more subtile sublethal (acute) effects. However, these points are essential for modeling (sub)-chronic toxicity.

3.4.3 *Kinetic Support of Interim Rat LD50 Estimate*

In developing the approach just described, the focus of the Breakout Group was on the prediction of human TD10 values (i.e., the dose at which mild toxicity could be expected in no more than 10% of exposed humans). However, the Breakout Group acknowledged that there will be a need in the short-term for the estimation of rodent LD50 values under the HPV chemical program. The following discussion describes the application of the approach described above for this latter need.

3.4.3.1 ***Research and Development Needs***

In the first step, estimates of key kinetic parameters can be obtained either from data available on the chemical or from the use of QSPR techniques (which are based on physico-chemical properties of the compound). QSPR techniques can be used as a first approximation of key kinetic parameters such as absorption, partition, etc. If one can use kinetic data that are actually measured, then these data will prevail.

- Octanol/water partition coefficient;
- Water solubility;
- Saturation vapor pressure or blood-air partition;
- PKa;
- Molecular weight/volume (for estimating gastrointestinal absorption);
- Hydrogen bond donors/acceptors (for estimating gastrointestinal absorption).

This prior knowledge on kinetic parameters or the estimation on the basis of QSPR data can then be used to evaluate the *in vitro* LC50 values for a chemical. The assumption is that this LC50 value is equal to the concentration in the intact organism at which cells die *in vivo*. Depending on the chemical's physico-chemical properties, the kinetic model to be used for this estimation may be simple or more complex. For many (e.g., water-soluble compounds) a simple one-compartment model can be used to estimate the oral dose that would result in an average systemic exposure equivalent to the *in vitro* LC50 value over the time period of interest. The key factors needed for the model would be estimates of the oral bioavailability, tissue partitioning (to obtain the volume of distribution), and total clearance. Depending on the properties of the compound, the clearance could be dominated by metabolism, urinary excretion, or pulmonary ventilation. In most cases, metabolic clearance will have to be determined empirically.

A key problem for this near-term application is that many HPV chemicals may not have adequate analytical methods yet developed. Therefore, metabolism assays may be too expensive and time-consuming for high-throughput LD50

estimation. However, a simple, conservative estimate for the oral dose resulting in systemic exposure equivalent to an *in vitro* LC50 value could be obtained by assuming 100% bioavailability, ignoring metabolic clearance, and simply estimating tissue partitioning to obtain the volume of distribution (Vd). For example, a commonly used default for the volume of distribution for water-soluble chemicals as a function of body weight (b.w.) is:

$$Vd = 0.65 * b.w.$$

In this simple approximation, the relationship between the *in vivo* and *in vitro* assays could be described by the formula:

$$LD50 = LC50 * Vd / b.w..$$

Other adjustments could be made to this approach for chemicals where ventilatory or urinary clearance would be important, as described in the previous section. In addition, if data on bioavailability are available, such information could be factored in to obtain a more accurate LD50 estimate. An additional benefit of this approach is that similar calculations could be used to convert the *in vitro* LC50 value to an *in vivo* LC50 value for acute inhalation. These assumptions, however, introduce inherent uncertainties into the resulting calculation of the oral LD50 value and depending upon the material of concern, may result in substantial inaccuracies.

It is not certain that the approach described here is actually viable; in particular, it needs to be determined whether sufficient information is available on the compounds of interest to support the necessary calculations. A first step would be to characterize the HPV chemicals in terms of their physico-chemical properties and determining the range and most frequent combinations of physico-chemical properties. This would provide a basis for the selection of "proof of concept" chemicals (not necessarily HPV chemicals) that could be used to evaluate the kinetic parameter estimation paradigm described here.

Another useful exercise would be to identify the compounds that represent the outliers in the RC correlations of *in vitro* basal cytotoxicity assays with LD50 values. By determining the physico-chemical properties of these compounds, and knowing their target tissues, it might be possible to identify factors that could improve the correlation (e.g., consideration of BBB penetration) between predicted oral LD50 values in rodents and empirical values. In this way it might be possible to define a "predictive range" for various chemical properties over which the *in vitro* assay might be expected to provide reasonable LD50 estimates. Also, exclusion rules for identifying compounds for which the results of the *in vitro* assay should not be relied upon might be defined.

3.4.3.2 ***Tiered Approach for Evaluating Acute Toxicity***

A particular problem area in terms of the predictive value of the currently available *in vitro* toxicity assays is where toxicity is secondary to metabolic activation. In particular, it is possible that rapid oxidative or reductive metabolism could result in acute liver toxicity from oral exposure. Examples of such toxicity is the production of phosgene by the oxidative metabolism of chloroform and the acute liver necrosis seen after carbon tetrachloride exposure. Such toxicity would not be observed in *in vitro* assays using basal cells with little or no metabolic competence.

One possible approach for dealing with this problem is illustrated in Figure 3.3. The first step would be to estimate hepatocyte metabolism at a relatively low concentration (e.g., 10 micromolar). If the rate of metabolism (Vmax/Km) observed is low, then the basal cell LC50 value could be relied upon. If, however, the rate is high, then it would be necessary to identify the responsible enzyme system. This identification could be performed, for example, by using a microsomal (S9) fraction with selective addition of cofactors or inhibitors. If these studies indicate that the primary enzyme system is oxidative or reductive, then the possibility of toxicity associated with metabolic activation exists. In this case it would be necessary to perform a hepatocyte cytotoxicity assay. If the LC50 value for the hepatocytes was much lower than for the basal cells, it would be necessary to characterize the concentration-response for metabolism in order to predict the *in vivo* doses that might be associated with toxicity. On the other hand, if the primary metabolism represents detoxication (conjugation, sulfation, etc.), then the (acute) toxicity of the metabolites

will generally be much lower and, therefore, the basal cell assay results for the parent compound could be used with some confidence to calculate the LD50 value.

An alternative approach, suggested by Breakout Group 3, would be to begin with a basal cell cytotoxicity assay (to screen out highly toxic compounds) and then perform a toxicity assay with a hepatocyte primary culture. If similar LC50 values were obtained in both assays, the concern for toxicity secondary to metabolic activation could be effectively ruled out. In such cases, a much less extensive characterization of metabolism would be needed to support an estimate of clearance. On the other hand, if the toxicity in the hepatocyte assay was strikingly greater than that for the basal cells, the more complete characterization of metabolism discussed above would be justified.

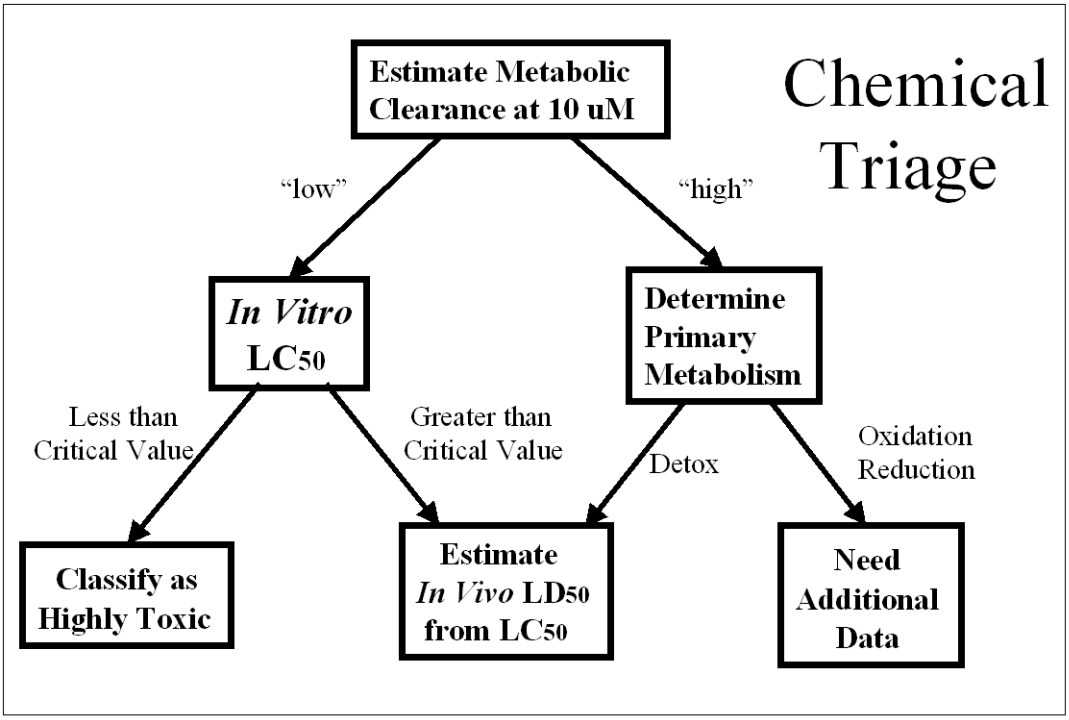

Figure 3.3: Tiered approach for evaluating acute toxicity

3.5 Recommendations

Table 3.1 (Section 3.5.2) lists a number of specific research areas in the area of biokinetics that the Breakout Group felt would improve the ability to use *in vitro* information in the prediction of acute toxicity. The following discussion highlights some of these research areas and illuminates some concerns emphasized by the Breakout Group.

3.5.1 *Long-Term Research Needs*

3.5.1.1 ***Metabolites and Acute Toxicity***

In some cases, a circulating metabolite can be responsible for acute toxicity in a tissue remote from its generation. Kidney toxicity from some chlorinated alkenes has been shown to result from the production of a GST conjugate (in the liver) which is converted to the cysteine conjugate in the

kidney, and then activated to a toxic mercaptan by beta-lyase. Another example: the CNS effects of chloral hydrate result from the metabolite trichlorethanol, which is produced in the liver. In cases such as these, metabolite-specific kinetic data are necessary to estimate target tissue exposure, and *in vitro* toxicity assays would have to be conducted with the metabolite(s) responsible for the observed toxicities. The latter, requires structural identification and synthesis of the metabolite(s) of concern in sufficient quantities to conduct these studies.

Other important research areas include the development of validated, stable human hepatocyte systems, as well as *in vitro* systems for key transporters (renal, biliary, etc.). A long-range goal should be the development of template PBBK models for the various classes of chemicals. Target tissues evaluated by *in vitro* assays would be included explicitly in the physiological structure of these models. The models would provide a mechanistic description of barrier functions (gut, bile, kidney, blood-brain barrier, skin), so that the data obtained from transporter assays could be readily incorporated.

3.5.1.2 ***QSPR Applications***

At the same time, specific QSPR applications need to be developed to provide the kind of information required by PBBK models (metabolism constants, binding, etc.). Unfortunately, the principal limitation in the development of useful QSPR applications appears to be the dearth of suitable data available for training knowledge-based systems.

3.5.1.3 ***Kinetics and Dynamics***

The interaction between kinetics and dynamics needs to be explored. For example, the effect of toxicity on the metabolism and excretion of a chemical or, conversely, the effect of metabolism or reabsorption on the toxicity of a chemical must be taken into account. Rigorous analyses of the time dimension in the conduct of these assays to account for duration and frequency of exposure is also an area that needs to be addressed. Because of cell viability issues, it may not be possible to reproduce the time frame of *in vivo* tissue exposure using *in vitro* systems. Also, the time frame for the appearance of toxicity may be quite different from the time frame for exposure to the chemical (Soni et al., 1999).

It is important to recognize that the proposed schemes (Figures. 3.1 and 3.2), and the discussion above, concern only the approximation and prediction of acute oral toxicity. It was neither the intent nor the purpose of the Breakout Group that these conclusions could be extended in any way to other types of toxicity that are relevant to public health risk assessment (e.g., developmental toxicity, sensitization, carcinogenesis, etc.). In the final analysis, *in vivo* exposure captures the effects of many potentially complex interactions that may be difficult to reproduce with *in vitro* systems.

3.5.2 *Research Needs for the Application of In Vitro Methods to the Prediction of Acute Chemical Toxicity*

Table 3.1 Biokinetic Research Needs

Kinetics	Kinetics-Dynamics Interface (Feedback)	Dynamics	Extrapolation
Understand the relationship between molecular structure, physical-chemical properties, and kinetic behavior of chemicals in biological systems. Develop mathematical modeling techniques to describe complex kinetic systems. Develop mathematical modeling techniques for tissue modeling (anatomically correct models). Develop algorithms to determine the optimum kinetic model for a particular chemical. Conduct research on modeling of fundamental kinetic mechanisms. Develop an optimal battery of *in vitro* assays to evaluate chemical-specific kinetic parameters. Develop QSAR models to predict kinetic parameters. Develop a library of generic models that are acceptable for regulatory risk assessments. Establish a database of chemical-independent parameters (mouse, rat, human).	Understand and model the mechanisms regulating the expression of proteins involved in kinetic processes – (metabolizing enzymes, transport enzymes, metallothionein, membrane channels, etc.). Understand and model effects of changes in physiological processes on kinetics of chemicals.	Develop *in vitro* biological models that are equivalent to *in vivo* tissues (i.e., models that maintain specified differentiated functions that are important for the toxicological phenomena under study). Develop mathematical modeling techniques to describe individual variability (genetic background). Develop mathematical modeling techniques to describe complex dynamic systems and genetic networks at the cellular and at the systemic level. Establish lines of differentiated human cells (e.g., derived from stem cells). Understand and model mechanisms of multi-cellular interactions in development of toxic responses (co-cultures). Understand and model relationships between cellular responses and biomarkers of systemic responses.	Inter- and intra-species extrapolation; comparison of genomic differences, or species-specific expression differences between species and within one species (e.g. polymorphisms in biotransformation enzymes). High dose - low dose extrapolation

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