

Physiologically-based Kinetic Modelling (PBK Modelling): Meeting the 3Rs Agenda

The Report and Recommendations of ECVAM Workshop 63^a

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Preface

This is the report of the 63rd of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). ECVAM's main objective, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which are relevant to the biosciences and which *reduce, refine* or *replace* the use of laboratory animals. One of the first priorities set by ECVAM was the implementation of procedures that would permit the acquisition of expertise about the state-of-the-art of non-animal test development and validation, and the potential for incorporating such alternative tests into regulatory procedures. It was decided that this would be best achieved through a programme of ECVAM workshops, each addressing a specific topic, and at which selected groups of independent international experts would review the current status of various types of *in vitro* tests and their potential uses, and make recommendations about the best ways forward.

The workshop entitled *Physiologically-based Kinetic Modelling (PBK Modelling): Meeting the 3Rs Agenda* was held at ECVAM (Ispra, Italy) on 10–12 October 2005, building upon a programming meeting held at Ispra on 31 May and 1 June 2005. The participants included international experts in PBK modelling and in the risk assessment of chemical products, from academia, regulatory or risk assessment advisory bodies, and industry. The objectives of the workshop were:

- to better define the potential role of PBK modelling, as a set of techniques capable of contributing to the *reduction, refinement* and *replacement* of the use of laboratory animals in the risk assessment process of potentially toxic chemicals;
- to discuss the need for technical improvement in PBK modelling and its applications; and
- to identify the need to increase understanding and, potentially, acceptance by the regulatory

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authorities, of the capabilities and limitations of PBK modelling techniques in toxicological risk assessment.

This consensus report describes the strategy proposed by the participants to satisfy the identified needs, and provides recommendations to promote the use of PBK modelling techniques, as a means of supporting non-animal-based toxicology testing strategies. It is also intended to provide the non-expert reader with an overview of the field in relation to the Three Rs, with some key references.

Terminology

Kinetics is the study of the absorption, distribution, metabolism and excretion (ADME) of compounds in organisms. The science of kinetics was mainly developed in relation to pharmacology, hence the term *pharmacokinetics*, which comes from two Greek words, *pharmaco* — meaning drug or poison, and *kinesis* — meaning movement. Because the use of *pharmacokinetics* has been understood, in many cases, to restrict this definition to drugs, and particularly to drugs used at doses within or close to the therapeutic range, other terms have been created to cover the concept of ADME in other, different contexts. Thus, *toxicokinetics* is often used to describe the kinetics of a substance in the context of toxicological studies, and *biokinetics* has been proposed as a more general term to describe the kinetics of substances in the body.

The modelling techniques constituting the theme of this workshop have been usually called physiologically-based pharmacokinetic modelling (PBPK), although other combinations of terms are possible, such as physiologically-based biokinetic modelling (PBBK). In this report, it has been chosen to use the terms *kinetics* and *physiologically-based kinetic modelling* (PBK modelling). The task of choosing more-complex terms has therefore been left to readers, based on their own preferences and on the context. It should be noted that, when literature or Internet searches are performed, different terminologies and/or spellings will extract different publications. The use of “modelling” is more common in European publications, whereas the use of “modeling” is more common in the United States.

Data-based and PBK Models

When studying the fate and effects of a xenobiotic (exogenous chemical), such as a drug, or an environmental or occupational pollutant, one of the objectives is to relate, directly or more often indirectly, the target tissue or organ response to the concentration *versus* time profile of the xenobiotic in that tissue. Various types of mathematical mod-

els have been used to describe the kinetic behaviour of xenobiotics. All these models simplify the biological complexity by subdividing the body into discrete elements, referred to as compartments. Two general types of compartmental models have been used for kinetic modelling: data-based (or empirical) models and physiologically-based kinetic models.

Data-based kinetic models

A typical data-based compartmental model attempts to relate the *in vivo* observed blood or tissue concentration–time profile of the parent chemical or metabolite to the administered dose of, or to the exposure to, the parent chemical, by using a set of mathematical equations. The parameters for these equations are determined from experimental data following the time-course of the chemical in body fluids and, occasionally, in specific tissues. A typical model might consist of only one or two compartments, sometimes three, but rarely more than three, within each of which chemicals are assumed to be homogeneously distributed, i.e. intra-compartmental diffusion is instantaneous. A both widely used and very useful model includes a central compartment, in rapid equilibrium with the blood, and a peripheral compartment, where concentration can be related to that of the central compartment by rate constants describing uptake from, and return to, the blood. The choice of the model structure rests upon the goodness-of-fit to the experimental data, which can be assessed both visually, via appropriate graphical displays, and numerically, by using appropriate statistical methods. The numerical values of the model parameters, i.e. volumes of the compartments and rate constants, are derived from the experimental data by numerically fitting the model to the data. Because both the model structure and the parameter values are essentially determined by the experimental data, these models are commonly called data-based compartmental models. The data-based models can be used for interpolation, although they are not well-suited to extrapolation beyond the range of doses, dose routes, species and populations included in the studies used to construct them. However, these models can make useful predictions, e.g. related to the impairment of excretory organs, in particular, in renal insufficiency.

PBK models

Some good general reviews on PBK modelling have been published (1–3). In contrast to empirical models, the compartments of PBK models refer to actual organs and tissues connected by the arterial and venous blood flows. Instead of being defined by

the experimental data, the relevant organ and tissue groups are described by weight or volume and by blood perfusion rates. Most of these physiological descriptors can be obtained from the literature (4–8). When a physiological parameter is not available in the species of interest, data can be taken from another species, and allometric scaling can be used to estimate the desired value (9).

Transfers between compartments, as well as metabolism and excretion processes, are described by a set of differential equations, incorporating the physiological parameters as well as chemical-specific parameters, used to quantify:

- the relative affinities of the compound for blood, tissues and air: these are termed partition coefficients;
- the permeability of the tissues to the compound, when permeability-limited distribution, as opposed to blood flow-limited distribution, has to be incorporated into the model for some organs or tissues; and
- the ability of the tissues to transport, metabolise or excrete the compound.

The chemical-specific parameters are mostly determined *a priori*, by using a range of techniques, from *in silico* approaches to *in vitro*, *ex vivo* or *in vivo* experiments (10–12).

PBK models can be described as consisting of three major elements:

- a model structure, describing, in a more-or-less simplified manner, the biological system of interest;
- a set of physiological parameters used to quantitatively describe this structure (volumes, flows); and
- a set of substance-specific parameters used to quantitatively describe the interactions between the system and the chemical of interest (partition coefficients, permeability parameters, clearance parameters).

Model input requirements include a mathematical description of:

- the route (or routes) of administration or exposure: typically, in chemical risk assessment, the major routes are the oral, dermal and inhalation routes; and
- the pattern of dosing or of exposure: description of the time periods when the body is exposed to a particular compound, and to what amounts or concentrations.

A quantitative description of bioavailability, i.e. the rate and extent of absorption of the substance from the site of administration/exposure into the circulation, has to be incorporated into the model. This can be either very simple, by using a single zero-order or first-order absorption input function, e.g. into the portal or systemic blood, or more complex, by describing in more detail the absorption processes (e.g. through successive skin layers). The absorption process can, and in many cases has to be, incorporated as a sub-model within the PBK model, by using relevant inputs such as physico-chemical and permeability properties.

The resulting model can yield a prediction of the kinetic behaviour of the chemical within the biological system, without being based on actual *in vivo* experiments, this being in contrast with the above-mentioned data-based modelling approaches. The model can then be refined to incorporate additional insights gained from comparisons with experimental data. In this way, a well-constructed model is generated, that can be used for quantitative extrapolations well beyond the range of experimental conditions. Indeed, the predictive power of these models has led to the increasing use of PBK-based chemical risk assessments.

The Use of PBK Modelling in Toxicological Risk Assessment

The work of toxicologists can be viewed as the study of the ways in which xenobiotic chemicals can cause adverse effects in biological systems, e.g. in an organism. This is usually done by breaking down complex biological structures into simpler systems for study, from the macroscopic down to the molecular level: tissues, cells, sub-cellular elements, enzymes from sub-cellular compartments, or DNA from the nucleus. Inevitably, tissue derivatives are studied under conditions in which they are divorced from the complex, interacting, milieu from which they came — and this must affect the results obtained. As a consequence, the interpretation of such data has many caveats. An ability to re-introduce the knowledge gathered from simpler systems into a more complex, biologically-realistic system is essential (13, 14). An important strength of PBK models is that they can provide this greater complexity, as well as a platform for integrating the disparate biological information described previously. The importance of the various anatomical, physiological, biochemical and physico-chemical parameters in a model can be studied individually or interactively, in order to investigate the magnitude and significance of any impact they might have on the behaviour of a chemical.

The use of the scientific information generated on the potential toxicity of a compound needs to be integrated into the overall assessment of the risk to

the environment and to human health of that particular compound, also taking into account the exposure scenarios determined by its use. Toxicological evaluations are conducted by using animals or, as much as possible, alternatives to animals. The data are then extrapolated into humans, by using various assumptions in order to establish exposure limits that will protect people. A good understanding of the kinetics of the compound contributes significantly to the risk assessment, because it relates external exposure to the relevant tissue concentrations. PBK modelling is an essential tool for providing the means of achieving the necessary integration and potentially decreasing uncertainty.

The search for alternatives to the use of animals for toxicity is focused on the use of tissues and cells from various organs, preferably of human origin. The use of these *in vitro* techniques, is hampered by an extrapolation problem similar to that described above: studying simple systems isolated from a realistic, complex biological context. The interpretation of data from these alternative systems by using kinetic information is crucial, because concentrations and their time-courses in the target tissues provide the necessary link for *in vitro* to *in vivo* extrapolation. Because PBK modelling, coupled to kinetic-dynamic modelling, is the best way to integrate all the necessary information, it is an essential adjunct technique, which has the potential to significantly reduce the numbers of animals needed in toxicological evaluations.

Developments since the 1995 ECVAM Workshop on Biokinetics

An ECVAM workshop, entitled *The Use of Biokinetics and In Vitro Methods in Toxicological Risk Evaluation*, was held in 1995 (10). A substantial part of this report deals with PBK modelling, and a number of conclusions and recommendations were made. Ten years later, many of these recommendations have been followed, and the PBK modelling approach has been increasingly incorporated into the development of alternative testing strategies (14–17).

Recent legislative developments, especially in relation to cosmetic products (18) and industrial chemicals (19), have greatly increased the need for PBK modelling as a tool for integrating data, and in particular, data which do not rely on *in vivo* experiments.

The new European REACH (Registration, Evaluation and Authorisation of Chemicals) regulation on chemical products was adopted on 18 December 2006 (19). Although no formal requirement for kinetic data is indicated in the REACH legislation, there is a need for such information on chemicals, in support of other toxicological evidence. PBK modelling is a way of generating such information

as early as the initial stages of risk assessment, and all along the risk assessment process. At each step of risk assessment, there should be an evaluation of whether the degree of uncertainty on, or confidence in, the information generated is appropriate to meet the objective, i.e. to answer the biological questions asked with sufficient strength for making a decision.

Uses of PBK Modelling in Relation to the Three Rs

PBK modelling can potentially make considerable contributions to the Reduction, Refinement and Replacement of *in vivo* animal studies, via several direct and indirect approaches. These include the improved design of *in vivo* studies, better interpretation of *in vitro* results, and more-accurate extrapolation procedures.

PBK modelling as a tool for improving the design of *in vivo* studies

PBK modelling can generate useful information on the kinetics of test compounds in situations different from the ones already tested, or even before any *in vivo* testing. The degree of precision and certainty of the information generated will naturally depend on the problem considered and on the stage of development of the PBK model.

PBK modelling can contribute to the Reduction of animal experiments, by permitting the extrapolation of results to groups of individuals, species, or exposure conditions other than the ones tested. At the other end of the spectrum, PBK modelling can be seen as a technique for allowing better extrapolation of kinetic or toxicological results to humans, thus increasing confidence in risk assessment decisions. This, in turn, can indirectly reduce the number of animal experiments needed.

PBK modelling can also support a Refinement approach in *in vivo* studies, by allowing the remaining necessary *in vivo* experiments to be performed at lower doses and the extrapolation of the results to higher doses.

PBK modelling as a tool for supporting the interpretation of *in vitro* toxicology data

The concentrations of a parent chemical and its relevant metabolites, and their time-courses, provide the physical link needed to extrapolate from *in vitro* toxicodynamic effects to an *in vivo* prediction of toxicity. The modelling of *in vivo* concentrations, coupled to kinetic/dynamic modelling of both the *in vitro* and the *in vivo* situations, is indispensable in this process. PBK modelling is the best possible

approach, because it permits a realistic simulation of a number of relevant *in vivo* situations, whilst only requiring limited experimental evidence. The proof of concept of this type of approach has already been established (13–15, 17). However, there is a trade-off between the amount of relevant experimental evidence available and the uncertainty of parameter values, of model structures and, finally, of the quantitative output of the models. This uncertainty has an impact on the level of confidence in the predictions made by using the models that have been developed.

PBK modelling and the potential for extrapolation in risk assessment

It is neither feasible nor desirable to test experimentally all relevant toxicological situations, whether in animals or in humans. This is due to the great variety of potential exposure conditions, usually quite different between experimental studies and typical human occupational or environmental exposures, and to intra-individual and inter-individual variability, which can rarely be sufficiently well captured in experimental studies. Therefore, a number of extrapolations from a limited set of experimental situations are necessary for the assessment of human health risks. Coupled to pharmacokinetic/pharmacodynamic (PKPD) modelling, PBK modelling is best suited to simulate toxicity outcomes by using the information obtained in limited toxicology testing. Subject to a requirement that, in each case, care must be taken to ensure that the domain of validity of the model spans the set of conditions for the range of extrapolation, these powerful tools can be used to perform:

Inter-species extrapolations. The requirement for these is that the anatomical–physiological structure described by the PBK model is common to a group of species (e.g. mammals). Species-specific information is then only carried by parameter values (e.g. organ volumes or tissue blood flows). Extrapolation is achieved by simply changing parameters to values specific to the species of interest.

Inter-individual or intra-individual extrapolations. These refer to the fact that a given exposure may induce different effects in the individuals of a population, and that the same individual may respond differently to the same exposure at different times in its life. These extrapolations are performed by setting parameter values to those of the sub-population or individual of interest, and are mainly used to predict the differential effects of chemicals on sensitive populations (such as children, pregnant women, the elderly, the obese, and the sick, taking into account genetic variation of key metabolic enzymes, etc.). The toxicokinetic behaviour of a

compound can also be studied under special conditions, such as physical activity.

Inter-dose extrapolations. These can be partly achieved with a PBK model (or totally achieved, in as much as the same internal dose induces quantitatively the same effect). Models used for inter-dose extrapolation should correctly capture both the linear and non-linear steps of the biological processes involved, e.g. in the transport and metabolism of the chemical studied.

Inter-route of exposure extrapolations. A model may describe several routes of exposure. For example, ingestion can be modelled as a direct infusion into the liver compartment or via the gastro-intestinal tract; dermal absorption may also be modelled via a skin compartment. It is then possible to explore and simulate different exposure scenarios.

Technical Steps Involved in PBK Modelling

Compound specification. PBK modelling addresses individual, well-defined compounds, for which the structure and properties can be assigned a single value, albeit with some attached uncertainty or variability. Building a model covering several compounds is accomplished by assigning a complete model to each of them, e.g. for a compound and its metabolites. Each metabolite has to be described in the model in terms of input (formation rate), distribution, metabolism and excretion. The same principle applies to mixtures. Also, possible interactions between mixture components or between parent compound and metabolites, need to be explicitly described, which is only possible if some quantitative information on the processes involved is available (20, 21).

Model structure. Initially, when very little information is available, the model structure is unavoidably minimal (i.e. a *de minimis* model). Usually, a “standard” animal or human will be initially modelled. Such an initial model could be, for example, a so-called “generic” model as described by Brightman *et al.* (22, 23), or something similar. The initial model will include the major kinetic steps, i.e. absorption, distribution, metabolism and excretion (ADME). Such early-stage PBK modelling has been shown to successfully support drug development decisions (24–27).

Absorption. The modelling of absorption is adapted to the route of exposure (usually oral, dermal or by inhalation). When it is by the oral or dermal routes, as a minimum, the extent and rate of absorption will be specified directly. More-sophisticated oral absorption models, which take into account the sol-

ubility, the expected barrier permeability and the expected ionisation of the compound, can often be applied. When the exposure is by inhalation, the simple absorption models rely on the blood–air partition coefficients of volatile compounds. More-complex inhalation models are also possible, for volatile compounds, aerosols or particulate matter. If indicated by past experience with similar compounds or initial knowledge on metabolism, a liver first-pass (oral route) step or a metabolism at the site of entry (all routes) step can also be included.

Distribution. The initial modelling of distribution should take into account blood partitioning, protein binding and organ distribution, as reflected by organ partition coefficients. The relevant organs can be specified separately or grouped (“lumped”) together, depending on physiological considerations and on expectations linked to the physico-chemical characteristics of the compound. The conditions under which organs with sufficiently similar kinetic behaviour with respect to the compound studied can be considered as a single physiological compartment, grouped (“lumped”) together, or considered separately (“split”), have been reviewed by Nestorov *et al.* (28). One major reason for developing lumping techniques and using them whenever possible, was to reduce the calculation burden. However, with the rapid increase of computing power, this is less and less necessary, and the interest becomes more conceptual. At this initial modelling stage, distribution to organs is assumed to be blood-flow limited, unless there are pre-existing data which indicate that a permeability-limited model is more appropriate for some organs.

Metabolism. Metabolism is initially modelled via the appropriate scaling up of the estimated total intrinsic metabolic clearance of the organ, contributed to by all the metabolic enzymes involved. A decision has to be made as to whether or not protein binding or blood cell partitioning restricts the extraction of the compound to its free plasmatic fraction. In addition, active uptake and saturable (non-linear) metabolism should also be considered.

Excretion. The modelling of excretion is often, at least initially, restricted to the glomerular filtration of the free plasma concentration of the compounds, although some more-sophisticated general models of renal excretion have been described.

Linearity. In the initial PBK modelling exercise, because no information is available to the contrary, it is usually assumed that distribution and metabolism are linear processes, i.e. essentially without saturation, or metabolism induction or inhibition. Only the simplest absorption and excretion models are intrinsically linear, and more-sophisticated (albeit “generic”) models have the potential to

introduce some degree of non-linearity, if the exposure conditions are such as to make non-linearity apparent (e.g. if the doses are large enough, and sustained for long enough).

Parameter values. The generation of compound-specific parameter values to be used as input into the models, and the uncertainty associated with them, is an important aspect of PBK modelling, and is described elsewhere (10, 11). This aspect is not developed in the present report, because its consideration was not one of the objectives of the workshop.

Model evolution. The complexity of the models used should increase progressively, as more data on the kinetics of the compound accumulate, thereby increasing knowledge of the kinetic processes involved and of the parameter values. This should only occur when such increased knowledge is useful to the ongoing risk assessment process. This process of increasing model refinement is described as a proposed “tiered approach to PBK modelling” in the following section, which reports on the output of one of the working groups of the workshop.

A Proposed Tiered Approach to PBK Modelling

It should be understood that the tiers outlined here must be seen as steps in an iterative process, starting with available data and knowledge and a generic PBK modelling approach. Sensitivity Analysis (SA) has the overall objective of determining how sensitive the model output is to changes in the assumptions (parameter values, model structure). It is used to assess whether the model is fit for purpose, in which case it can be used to help the toxicologist to make the decision as to whether or not further testing should be conducted. If the model appears not to be fit for purpose, the initial modelling, coupled with SA and the consideration of external information, is used to define the requirements for further experimental data. Additional experimental work will generate data of a kinetic or dynamic nature, which will be used to refine the model, as well as to direct further toxicology testing, as necessary. The refined model will then undergo the same process of SA and, if necessary, further refinement and data generation, until the output of the process is deemed to be fit for purpose, i.e. for toxicological evaluation. The proposed approach distinguishes three tiers, as follows:

- *Tier 1:* Preliminary estimation of systemic exposure to determine the strategy for further testing;
- *Tier 2:* Design of the toxicity testing on the basis of the output of the initial PBK modelling; and

— *Tier 3*: More-refined PBK modelling to prepare the final risk assessment.

Tier 1: Preliminary estimation of systemic exposure to determine the strategy for further testing

This tier is schematically divided into four steps:

Step 1: Data on likely external exposure are collected or generated, together with quantitative estimates. In the absence of external exposure, PBK modelling is not relevant. In cases where the exposure-based waiving of testing is to be considered, this may be the only step.

Step 2: A base-set of chemical-specific data are collected, for the generation of input parameters by using the appropriate scaling equations. Depending on the context and stage of risk assessment, the numerical values for this base-set can be generated from *in silico* (QSAR/QSPR) and/or *in vitro* procedures. These parameter values will be used for input into a generic PBK model. For example, a “generic” PBK model as described by Brightman *et al.* (23) for oral exposure, requires quantitative values for solubility, intestinal barrier permeability, a measure of lipophilicity (such as log P or log D, for compounds potentially ionised at physiological pH values), an estimate of the fraction of the compound bound to plasma proteins, and an estimate of intrinsic hepatic clearance.

Step 3: If absorption by the relevant route(s) is predicted with adequate certainty to be below a given threshold, or thresholds to be defined, leading to a decision not to test for systemic effects, further modelling is unnecessary. Only local effects need be considered.

Step 4: By using exposure scenarios and initial chemical-specific data, a generic PBK model is used, in order to generate information on systemic exposure, e.g. the area under the curve (AUC) of the concentration of unbound active chemical in plasma, and information on potential accumulation (rate and extent).

Tier 2: Design of toxicity testing on the basis of the output of the initial PBK modelling

The concentration range used in target organ toxicity testing is chosen, taking into account the preliminary kinetic information generated in Tier 1, and in particular, predicted concentrations and accumulation potential in specific organs/tissues. Toxicodynamic testing will also be directed by infor-

mation coming from other sources, and in particular, structural similarities with compounds of known toxicity, e.g. QSARs or other knowledge-based systems. The result of this testing will be the establishment of relationships between concentration, *in vitro* exposure pattern and effects for the *in vitro* systems.

Tier 3: More-refined PBK modelling

The objective of this tier is to prepare the translation of the outcome of the toxicity data into the relevant *in vivo* situations. This should support the extrapolation of *in vitro* toxicity data to predicted toxicity *in vivo*, and the extrapolation of observed or predicted toxicity between exposure routes, between exposure intensities and patterns, and between species.

More-refined PBK modelling will be conducted at this stage. This involves further informing the modelling process by generating more data. In most cases, the comparison of the model output to *in vivo* kinetic data is necessary, both to evaluate the predictivity of the model and to inform the modelling process. The generation of additional information should be carefully planned, in order to save time, expenditure and animal consumption. The corresponding *in vitro* or *in vivo* experiments should be designed with a precise objective, i.e. to yield quantitative information on one or more of the processes of absorption/bioavailability, distribution, metabolism and excretion. Sensitivity analysis is a key tool at this stage, because it focuses the testing on critical parameters which influence the predicted concentrations and their time-courses. Knowledge of these critical parameters will also help to identify potentially more-susceptible subgroups at an early stage. Ultimately, the models should provide estimates of concentrations in potential target organs/tissues and their likely time-courses, with a degree of uncertainty compatible with the requirements of the risk assessment process, in the relevant populations.

Variability and Uncertainty

Variability and uncertainty are integral parts of potentially all PBK model components and predictions. It is necessary to assess both, in order to determine the confidence to place in these predictions, so that they can be useful for risk analysts and decision-makers.

Variability

Variability typically refers to differences in the values of model parameters among individuals (inter-

individual variability) or across time within a given individual (intra-individual variability). It is inherent in animal and human populations, and can stem from such factors as genetic differences, activity levels, lifestyles, physiological status, age, and sex.

The interplay of these factors influences the kinetics, and therefore the potential toxicity, of xenobiotics. PBK modelling based on mean data that give no estimates of inter-subject variability, has a limited ability to address the extremes of risk in real populations (29). A way forward is to incorporate the information on variability from *in vitro* studies and the demographic and epidemiological aspects of the target population, in order to model inter-individual variability in potential toxic response (30). Variability can be observed and registered as information about the model, but it cannot be reduced. An important feature of variability is that it does not tend to decrease when larger samples of a population are examined.

The use of standard values for the PBK model parameters tends to give a false impression of precision for physiological values and thus for model predictions. At best, such standard or default values are approximate values of the average for a human population. Variability in parameter values can be represented by probability distributions. How this variability influences model predictions can be evaluated by using Monte Carlo simulation methods. These methods consist of: specifying a probability distribution for each model parameter; sampling randomly each model parameter from its specified distribution; running the model by using the sampled parameter values; and computing various model predictions of interest. Instead of specifying independent distributions for parameters, a joint probability distribution can be assigned to a group of parameters to describe their correlation. Similar or more-refined techniques have been used for several years in the field of drug pharmacokinetics, and are collectively known as Population Pharmacokinetics.

Uncertainty

Uncertainty can be defined as the inability to make precise and unbiased statements. Unlike variability, uncertainty in the information may decrease with the size of the sample studied or the precision of the measurements performed, and can be further reduced by conducting additional optimised experiments or by a better understanding of the process under study. Uncertainty can have various sources:

Inherent errors in experimental data. There can be uncertainty about the underlying process by which data were obtained (coding and reading errors, systematic measurement errors, etc.). Experimental data are typically known to have a finite precision, dependent on the apparatus used. However, such

uncertainties can easily be assessed with quality measurement data, and can be modelled with probability distributions (e.g. the measured quantity is distributed normally, with a mean equal to the actual quantity and a given standard deviation).

High inherent variability of biological systems. In some cases, it is possible to fully know the variability, e.g. by exhaustive enumeration, with no uncertainty attached. However, variability may itself be a source of uncertainty in predictions, if it is not fully understood and is ascribed to randomness. In addition, describing physiological variability through probability distributions may be a large source of modelling errors and model uncertainty, if sufficiently adequate distributions are not known.

Complexity and unknown nature of the phenomena involved (model specification). The source of uncertainty in the model structure can be primarily a lack of the theoretical knowledge needed to correctly describe the phenomenon of interest on all scales. In this case, the world is not fully understood and therefore cannot be modelled exactly. The summing up, within a model, of a massive amount of information can, in itself, be a technical challenge. An organism can be viewed as an integrated system, with strong and multiple correlations among its components (e.g. a large liver volume might be expected to be associated with a large blood flow). Given the complexity of an organism, it is not feasible to integrate all the interactions between its components (most of them are not even fully known and quantified) in the development of a PBK model. Therefore, modellers have to simplify reality, in order to build a PBK model. Such assumptions will, however, introduce uncertainty. To begin to quantify model uncertainty by using a general statistical approach, the accuracy of the model in the prediction of some already-available datasets may be evaluated. Models based on different assumptions can be tested, and statistical criteria (such as the Akaike criterion) can be used to discriminate between models. The use of Bayesian model averaging methods to blend together the predictions of different models, can also be considered.

On this note, it is worthwhile mentioning that an international workshop, on *Uncertainty and Variability in Physiologically Based Pharmacokinetic Models* (31), was held in Research Triangle Park, North Carolina, USA, from 31 October to 2 November 2006. This event, which was coordinated under an initiative of the International Program for Chemical Safety (IPCS), was sponsored by the US Environmental Protection Agency (EPA), Office of Research and Development, the National Center for Environmental Assessment (NCEA), the US National Center for Computational Toxicology (NCCT), the US National Health Effects and

Environmental Research Laboratory (NHEERL), and the US National Institute of Environmental Health Sciences (NIEHS).

Recent and Current Initiatives to Promote PBK Modelling in Research and in Risk Assessment

A number of recent and ongoing initiatives have the aim of promoting the use of PBK modelling in the research and development of drugs and chemicals, as well as in risk assessment. A non-comprehensive list includes:

- A two-day workshop on *Physiologically Based Pharmacokinetics in Drug Development and Regulatory Science*, which was organised by the Center for Drug Development Science, Georgetown University, Washington, DC, USA, on 30 May 2002, and attracted more than 120 participants from industrial, academic and regulatory backgrounds (3).
- An ongoing IPCS initiative, which has the objective “*to promote best practice in PBPK modelling, including transparency, to facilitate understanding, and sharing of, national and international risk assessment reports*”. This will be achieved by issuing a guidance document following the coordination and convening of *ad hoc* workshops, and also by drawing on previously-prepared documentation. A first workshop, centred on variability and uncertainty, was held in the USA in autumn 2006 (31), and another one, centred on good modelling practice and regulatory acceptance, was held in Greece in the spring of 2007 (32).
- The ongoing COST action B25 (33) on Physiologically-based Pharmacokinetics and Dynamics, which started in January 2006. Coordinated by Alan Boobis, its main objective is “*to improve the utility and interpretation of scientific information obtained either during pharmaceutical product development or, subsequently, through observations in humans, to predict the safe and effective use of drugs and other chemicals in the Medicine and Health field.*”
- Projects in the European Union 6th Framework Programme of Research and Development, that include PBK modelling as a prediction tool or as an integrative tool, e.g. project 2-FUN (<http://www.2-fun.org>), project ACute-Tox (<http://www.acutetox.org>), and project OSIRIS. The 7th Framework Programme also includes PBK modelling, both as a tool and as a topic for research. This reflects both the high interest in the application of PBK modelling and the need for further develop-

ments, in terms of both techniques and regulatory use. The PBK modelling workshop reported here, together with a former workshop on *in vitro* methods for predicting long-term toxicity (34), formed the basis for a large-scale collaborative project proposal currently under negotiation (Predict-IV).

- A number of US-based and Canada-based projects, that also involve PBK modelling, particularly in the environmental risk assessment field. Recent key outputs include, for example, a seminal paper on the methodology for evaluating PBK modelling at the regulatory level (35), a 2006 report by the EPA entitled *Approaches for the Application of Physiologically Based Pharmacokinetic Models and Supporting Data in Risk Assessment* (36), and a draft *Document on Good Modelling Practices for Pharmacokinetic Models in Risk Assessment*, prepared for the Existing Substances Division, Health Canada.

Recommendations

Although PBK modelling and its applications have gained ground in the past ten years, the recommendations formulated in the 1995 ECVAM workshop on *The Use of Biokinetics and In Vitro Methods in Toxicological Risk Evaluation* (10) are essentially still valid.

During the present workshop, the following recommendations were particularly emphasised, some of which support and add weight to the 1995 recommendations. They can be put into three categories: quality of PBK modelling; availability of reference data and models; and development of testing strategies.

Quality of PBK modelling

1. There is a need to develop consistency of models and a framework of reference for the evaluation of models at the regulatory level. This should increase confidence in the use of PBK modelling for making regulatory decisions.
2. In line with the first recommendation, the development of guidance on good modelling practice is essential. This should cover all relevant aspects of PBK modelling, and in particular, model structure, quality of input, methods for dealing with uncertainty and variability, and model documentation.
3. There is a need for more training and communication in kinetics and modelling, and specifically in PBK modelling, at all stages of risk assessment, from data generation to regulatory decision-making, for those both in industry and in regulatory agencies.

Availability of reference data and models

4. A database should be developed for models that have been evaluated against experimental and observational *in vivo* data.
5. There is a need for an overview of existing PBK model systems, with respect to the chemical space of the tested chemicals, taking into account the proprietary rights related to any useful information. This would permit an *a priori* estimation of the possibilities of using PBK modelling approaches for a particular chemical.

Development of testing strategies

6. There should be investment in the building of models for new chemicals, on the basis of non-animal data, for comparison with models established for compounds with sufficiently similar structures.
7. It is strongly recommended that PBK modelling be brought into the risk assessment process as early as is feasible, whenever it is likely that PK information will be beneficial. The tiered approach to PBK modelling described above, affords a framework for such a process.
8. The use of integrated strategies in risk assessment, including PBK modelling and comparisons with similar compounds for which data are available, should be encouraged.
9. There is a need for publicly-accessible decision support systems, into which PBK modelling is integrated.

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