Food for Thought ...

Evidence-Based Absorption, Distribution, Metabolism, Excretion (ADME) and its Interplay with Alternative Toxicity Methods

Katya Tsaioun [1], Bas J. Blaauboer [2] and Thomas Hartung [1,3]

[1]Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT), Baltimore, USA; [2]Institute for Risk Assessment Sciences, Division of Toxicology, Utrecht University, Utrecht, The Netherlands; [3]University of Konstanz, CAAT-Europe, Konstanz, Germany

Summary

ADME (absorption, distribution, metabolism, elimination) has rapidly evolved over the past two decades, creating a unique interdisciplinary interface between medicinal chemists, biologists, formulators, toxicologists, clinicians, and regulators across industries, but has advanced most rapidly in the pharmaceutical industry. The implementation of ADME profiling of drug candidates, in conjunction with biological efficacy and safety optimization, has dramatically reduced pharmacokinetic drug failures in clinical trials and has become a *lingua franca* between disciplines that are involved in drug development. This article briefly reviews the basics and current state-of-the-art of ADME and the major lessons from the pharmaceutical industry on its efficient use, points out the importance of defining ADME properties leading to toxicity across industries for safety and toxicity prediction of chemicals, and raises the issues of quality, reliability, and reproducibility of tests and inclusion of ADME under the umbrella of evidence-based toxicology. Increasingly, *in vitro* results are used to inform ADME assessments and computer modeling. The aspects of kinetics of substances in cellular models themselves, however, are still too often neglected. ADME information will play a critical role in establishing quantitative *in vitro* to *in vivo* extrapolations (QIVIVE), integrated testing strategies, and systems toxicology approaches.

Keywords: ADME, pharmacokinetics (PK), physiologically-based pharmacokinetic (PBPK) model, systems toxicology, evidence-based toxicology

1 Introduction

Paracelsus, hailed as the founding father of toxicology, is known for the quote: "*All things are poison and nothing is without poison; only the dose makes a thing not a poison*." Today, we have refined this notion: The portion of the dose that interacts with the target in the body – the biologically active internal dose – is critical. Also, we know that this rule has exceptions, i.e., the dose-response relationship does not explain some phenomena in toxicology such as idiosyncratic toxicity, which is frequently driven by individual immune responses. These insights have revolutionized *in vivo* toxicology, especially drug development, but other areas that have not yet embraced them and still base their assessments on hazard only, such as the ingredients of industrial chemicals and food ingredients, are straggling.

Pharmacokinetics (PK, also most recently termed biokinetics), i.e., the assessment of absorption, distribution, metabolism, and excretion (ADME) of chemicals (xenobiotics), has transformed our understanding of *in vivo* pharmacology. Toxicokinetics (TK) is defined as the quantitation of the time course of toxicants in the body during the processes of ADME or clearance of toxicants. In other words, toxicokinetics is a reflection of how the body handles toxicants as indicated by the plasma concentration of that xenobiotic at various time points. When the toxicokinetic processes lead to a "biologically effective" dose of toxicant in the system, an adverse event is produced (Fig. 1).

Astonishingly, some areas of regulatory toxicology still do not require information on kinetics, although this significantly improves our understanding of the human relevance of toxicology findings. Kinetic phenomena, i.e., how the chemical becomes bioavailable to the cells as redistributions and transformations occur, are also of importance in *in vitro* models. However, although computational models of xenobiotic kinetics, such as physiologically-based pharmacokinetic (PBPK) models, rely on

Received October 10, 2016;
https://doi.org/10.14573/altex.1610101

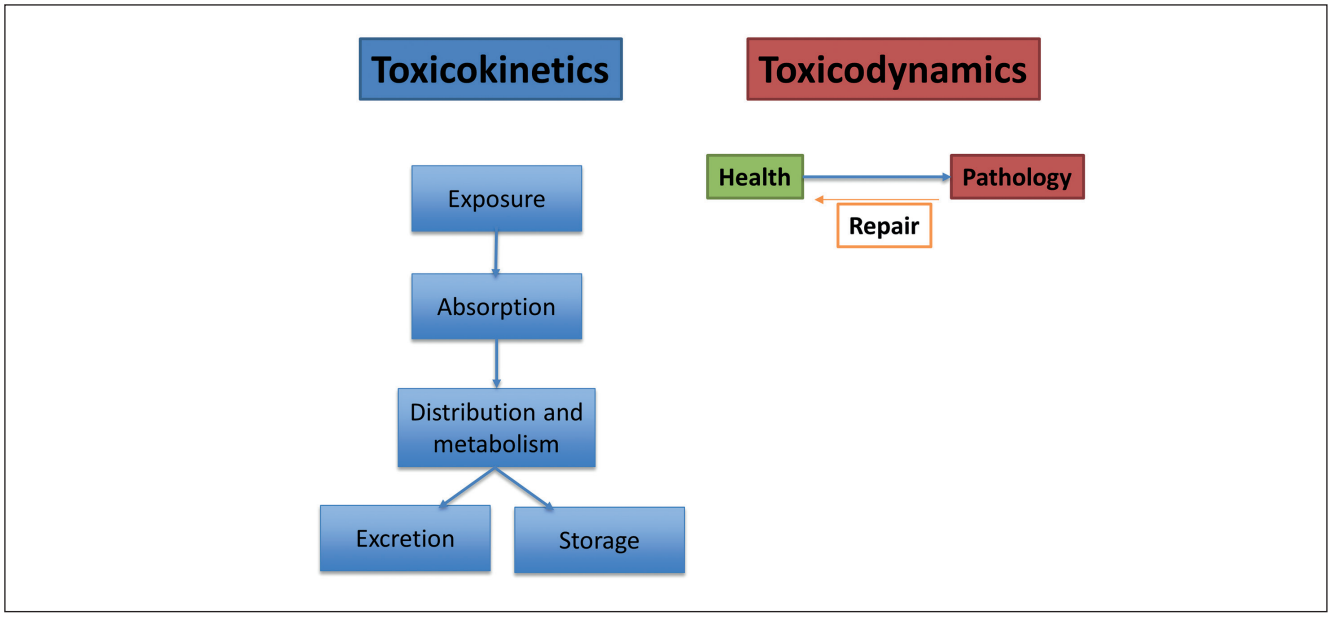

Fig. 1: The toxicological process

input from *in vitro* models, the *in vitro* kinetics are sometimes overlooked when the models are built and when the results are interpreted. Another area that has been left behind is *in vitro* toxicology. While *in vitro* models of metabolism and biological barriers have existed for close to two decades, until recently most *in vitro* toxicology neglected metabolism and for the most part did not routinely trace or understand the fate of substances in the culture dish, i.e., the *in vitro* PK or biokinetics (Fig. 2). However, since kinetic phenomena can skew the *in vitro* results, they need to be considered to make any useful *in vivo* predictions.

Computational models are increasingly playing the role of a bridge between *in vitro* data and *in vivo* predictions, increasing the predictive abilities of the *in vitro* results. There were significant advances in recent years in the area of structure-based *in silico* modeling of ADME properties (Moroy et al., 2011; van de Waterbeemd and Gifford, 2003). This resulted in a multitude of commercial and free *in silico* ADME prediction tools[1]. While hazard generally is a yes/no attribution of a property, it should be linked to some dose/response-relationship, because the kinetics of a substance are highly dynamic. This prompts the need for dynamic modeling of toxicokinetics, which is regularly used to explain adverse events in preclinical regulatory toxicology, but is also increasingly used to support predictive toxicology. Reverse biokinetics, i.e., quantitative *in vitro* to *in vivo* extrapolation (QIVIVE), is critically important for predicting *in vivo* exposures corresponding to active concentrations on a cell and tissue level (and, thus, for making predictive use of *in vitro* findings). This information represents the complement to hazard identification and characterization by cellular models in developing a systems toxicology approach. The ultimate goal of QIVIVE (Blaauboer, 2010) or building a systems toxicology approach (Hartung et al., 2012) clearly requires integration of *in silico* and *in vitro* tools that combine hazard assessments with ADME property predictions.

In the context of developing a roadmap for animal-free systemic toxicity testing, an expert workgroup discussed the contribution of kinetics (Basketter et al., 2012), which was further considered at two stakeholder forums in Brussels and Washington (Leist et al., 2014). Some key conclusions were:

- Kinetics is not a stand-alone test, but a necessary complement to *in vitro* tests.
- Mainly *in silico* approaches are in use, but they need further optimization.
- There is a need for more quality control in data collection and the incorporation of *in vitro* data into *in silico* models.
- There is a need for bioavailability and urinary excretion models.
- Overall, the reliable and predictive *in vitro* and *in silico* tools are within reach, with reasonable investment of time and resources.

This Food for Thought … article details the state-of-the-art of ADME in toxicology as well as the challenges and opportunities.

2 What is ADME?

When developing new chemicals, scientists, clinicians, toxicologists and regulators aim to alleviate a disease (pharmaceuticals), increase crop production (pesticides) or improve physical appearance (cosmetics), thus expecting a new chemical substance (xenobiotic) to change the physiological state of an organ or a pathway. This ability of the new chemical to alter biology normally abides by dose-response rules and is termed pharmacody-

[1] See https://www.click2drug.org/ is a comprehensive database o available computational ADME/QSAR/docking tools, maintained by the Swiss Institute of Bioinformatics.

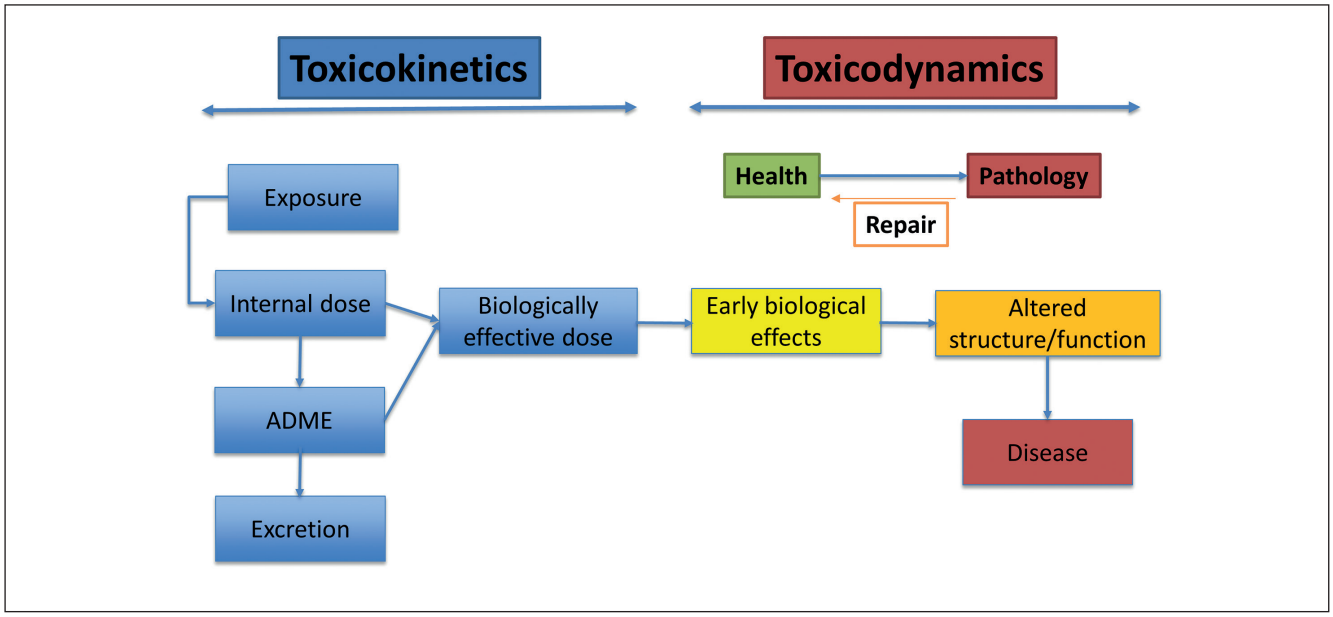

Fig. 2: The toxicological paradigm

namics (PD). When a new chemical enters the body, however, the body identifies it as foreign and as a potential toxicant and, thus, engages systems in many organs to resist its absorption, limit its distribution, and metabolize/transform it into a form that is easily eliminated/excreted from the body in urine or feces. All this is done by a human, animal, or plant in order to avoid potential toxicity – hence, ADMET, or ADME-Tox. These insights have been a part of chemical development for over a century. The associated research is frequently referred to as pharmacodynamic (PD), pharmacokinetic (PK) and toxicokinetic (TK) studies (Fig. 2).

Formerly, all these studies, aimed at understanding what a body would do to a new chemical, or xenobiotic, were performed in experimental animals, since at the time these were the only PK and toxicity models available. Reliance on animal models to make predictions about the human response requires species extrapolation, and that has been proven to be rather complex and unreliable (Chu et al., 2013; Hatton et al., 2015; Smirnova et al., 2014). In the last decade it was widely recognized that experimental animal models need to be improved upon, not only for reasons of ethics and predictive value, but also for economic reasons. As pharmaceutical industry profits fell, industry and academic researchers analyzing the research and development process realized that deficiencies in the low predictive abilities of tests prior to clinical trials in humans are a serious problem.

However, thanks to molecular and cell biology as well as high-throughput screening technologies, which made human proteins and tissues available and *in vitro* testing feasible, scientists, beginning at the end of the last century, began to develop *in vitro* technologies for evaluating human ADME properties of chemicals. The pharmaceutical industry led the way, quickly realizing that validating and adopting early ADME assays may provide a competitive advantage and mitigate costly risks of failure in late clinical development. And it appears that this strategy has paid off: Changing the drug development strategy by employing early *in vitro* ADME screens (Kassel, 2004) has played a key role in reducing failures in clinical trials for PK reasons by allowing researchers to optimize ADME properties early, thus reducing the probability of PK surprises such as a lack of efficacy or unexpected and sometimes fatal toxicity in the clinic (Arrowsmith and Miller, 2013). In the last decade, attention has focused on preventing adverse events and improving predictive ability and reproducibility of efficacy models – the major reasons for drug failure in the clinic (Kaitin and DiMasi, 2011). A multitude of mechanistic *in vitro* assays have been developed that address mechanisms of human toxicity[2]. However, we would like to return our attention to ADME and ask if we have indeed "solved" the ADME problem and can now return to addressing toxicity.

3 ADME properties leading to toxicity: Lessons from the pharmaceutical industry

The high attrition rate of drugs entering human trials has concerned the pharmaceutical field for many years (Hartung et al., 2013). By 1997 it was reported (Kennedy, 1997) that poor ADME properties and toxicity accounted for 60% of failures of chemicals in the drug development process. It turned out that many failures could be attributed to differences in kinetics between animal models and humans (Hann and Simpson, 2014; Lin, 1995; Martignoni et al., 2006; Lin, 1995; Musther et al.,

[2] In Vitro Toxicity Testing: Technologies and Global Markets – PHM017E. Retrieved February 15, 2015, from http://www.bccresearch.com/market-research/pharmaceuticals/in-vitro-toxicity-phm017e.html

2014; Shanks et al., 2009). Tackling these issues, it should be noted, has reduced the number of failures caused by ADME problems over the years (Kola and Landis, 2004): in 1991, adverse pharmacokinetic and bioavailability results were the most significant cause of attrition (about 40%) (Kubinyi, 2003), dropping to less than 10% in 2000. The availability of high-throughput and *in silico* techniques in drug metabolism and pharmacokinetics were critical in causing the reduction (van de Waterbeemd, 2002; van de Waterbeemd and Gifford, 2003; Dearden, 2007).

The cost of drug approvals has crossed the $1 billion per drug mark, and the cost of advancing a compound to Phase 1 trials can reach $100 million, according to the Tufts Center for the Study of Drug Development (DiMasi et al., 2003). Given these huge expenditures, substantial savings can accrue from early recognition of problems that would alert to a compound's potential to cause adverse effects leading to attrition (Caldwell et al., 2009; Kola and Landis, 2004). The costs associated with withdrawing a drug from the market after approval are even greater. Terfenadine, for example, is a potent hERG cardiac channel ligand, but it is metabolized by the liver enzyme CYP 3A4. If terfenadine is administered as a mono-therapy, it is metabolized by CYP 3A4 into fexofenadine, which is not a hERG inhibitor. In real clinical situations, however, terfenadine was frequently co-administered with the CYP 3A4 inhibitors ketoconazole or erythromycin (Honig et al., 1993). Blocking the metabolism of terfenadine caused over-exposure of patients, leading to increases in plasma terfenadine to levels that caused cardiac toxicity (Honig et al., 1992). The resulting withdrawal of the drug from the market (FDA, 2009) cost an estimated $6 billion. Another example is the broad-spectrum antibiotic trovafloxacin, which was introduced in 1997 and soon became Pfizer's top seller. The drug was metabolically activated *in vivo* and formed a highly reactive metabolite that caused severe hepatotoxicity (Ball et al., 1999). Trovafloxacin received a black box warning from the FDA in 1998 (Mandell and Tillotson, 2002) costing Pfizer $8.5 billion in lawsuits. With the development of technologies to measure the impact of new molecules on cardiac ion channels such as hERG and other important ADME parameters early in the discovery and development process, such liabilities are now recognized earlier, allowing for safer analogs to be advanced to more expensive preclinical and clinical stages.

The drug discovery industry is experiencing dramatic structural change and is no longer just the domain of traditional large pharmaceutical companies. Venture capital-funded startups, governments, venture philanthropy, and other nonprofit and academic organizations are important participants in the search for new drug targets, pathways, and molecules. These organizations frequently form partnerships, sharing resources, capabilities, risks, and rewards of drug discovery. Thus, it is becoming increasingly important to ensure that the money from investors, donors, and taxpayers is used efficiently to develop safe drugs for unmet medical needs. ADME profiling has been proven to play a crucial role and has demonstrated effectiveness in accelerating the discovery process and preventing poor drug candidates from entering clinical development.

4 ADME: Where basic and regulatory science meet

Regulatory authorities have relied on *in vivo* testing to predict the behavior of new molecules in the human body since the 1950s. Bioavailability, tissue distribution, pharmacokinetics, metabolism, and toxicity are typically assessed in one rodent and one non-rodent species (dog or nonhuman primate) prior to administering a drug to a human to evaluate pharmacokinetics and exposure in a clinical trial (Phase 1). The standard required methodology for biodistribution assessment uses radioactively labeled compounds. This is time- and resource-intensive both in terms of synthesizing sufficient amounts of radioactively labeled compound and of performing the animal experiments (Oldendorf, 1970). Therefore, these assays are implemented rather late in the preclinical development process when more resources are released to study the few molecules that have advanced to that stage.

With advances in cell and molecular biology, high-throughput screening, and miniaturization technologies in the 1990s, as well as stem cell-derived models at the beginning of this century, early *in vitro* ADME studies have been developed to predict *in vivo* animal and human results at a level of speed and cost-effectiveness appropriate for the early discovery stage. This progress in the science of ADME has created a new paradigm for advancing compounds from hit to lead, from lead to advanced lead, and on to nominated clinical candidates. Drug discovery programs using human enzymes and human-origin cells early in the discovery phase provide highly actionable information about the drug-likeness of new molecules, the potential to reach target organ, and indications of known human mechanisms of toxicities. ADME assessment of varying complexity is routinely performed on compounds that have shown *in vitro* efficacy in conjunction with, or just prior to, demonstrating early proof of principle *in vivo* (Tsaioun et al., 2009).

The efficacy of test compounds is now routinely assessed using a battery of *in vitro* models such as target binding or phenotypic screening followed by confirmation through *in vivo* efficacy models in an appropriate animal model. The predictive abilities of these tests depend largely on the therapeutic area and the animal model (Veazey, 2013; Tan et al., 2013). Understanding the relationship between drug plasma and tissue concentrations (PK) and efficacy (PD), termed PK/PD relationship, is crucial in supporting efficacy results and defining therapeutic and safety windows.

In vivo PK studies in a variety of animal models are routinely used for lead optimization to assess drug metabolism and absorption. It is important to note that there are significant differences in absorption and metabolism among species that cause conflicting predictions of degradation pathways of new chemical entities (NCEs). It is a standard practice in the pharmaceutical industry to use primary hepatocytes (Shih et al., 1999) and other models (Khetani et al., 2013) from different species to understand species differences in metabolism, identify metabolites that are uniquely human, and select species for preclinical development that are most relevant to the target organism. Table 1 lists the major assays used for prediction of ADME properties that underlie safety evaluation.

Tab. 1: ADME assays that inform toxicity at different stages of drug discovery and development

Stage of the Program	Absorption	Distribution	Metabolism
Hit-to-lead	Physicochemical properties (solubility, LogP) parallel artificial membrane permeability assay (PAMPA)	Plasma protein binding	Efficacy species and human liver microsomal stability, the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) trans-activation assay
Lead optimization	CaCo-2 colon epithelial permeability test Madin-Darby canine kidney cells transfected with the human MDR1 gene (MDCK-MDR1) permeability assay *In vivo* PK (rodent) Major transporters (MDR-1, BCEP)	*In vivo* tissue distribution (rodent)	Efficacy species, toxicology species and human primary hepatocyte stability and metabolite identification, Cytochrome P450 (CYP) inhibition, CYP induction
IND-enabling studies	*In vivo* PK (rodent and non-rodent) Comprehensive transporter panel	*In vivo* tissue distribution (rodent and non-rodent)	CYP inhibition, CYP induction

It is standard practice for pharmaceutical companies to include the drug-drug interactions (DDI) information in investigational new drug (IND) submissions, which help agencies evaluate human metabolism and potential safety of drug candidates. For example, *in vitro* DDI studies may now be conducted under the guidance from FDA[3, 4] and EMA[5]. The guidance documents outline methods for conducting CYP-450 inhibition and induction and P-glycoprotein (P-gp) and other transporter interaction studies.

5 Chemical safety testing and ADME

While ADME assays and principles remain the same, the application of these principles is unique to each industry and influenced by its regulatory environment, target market, route of exposure, type of exposure, safety margins, commercial factors, and other parameters. Correspondingly, the importance and implementation strategy of the various ADME assays are based upon the specifics of the industry. ADME assays can also be categorized into those that are routine and those reserved for more advanced profiling. This division is also a function of cost effectiveness and the need for specific information in development of a particular chemical. For instance, the prohibitive cost of some *in vitro* ADME assays, such as those using primary hepatocytes, are forcing the chemical and cosmetic industries to develop *in silico* prediction tools that allow them to model ADME properties based on cost-effective physico-chemical inputs.

The European Chemicals Agency (ECHA) manages the technical, scientific, and administrative aspects of the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation. REACH came into effect in June 2007 and was designed to regulate the manufacture, import, marketing, and use of industrial chemicals, including ingredients used for formulations otherwise regulated, such as pesticides and cosmetics. Manufacturers, importers and downstream users must demonstrate that the manufacture, import, and use of a substance does not adversely affect human health and that risks are adequately controlled (Hartung, 2010b; Sacco and Vezzoni, 2004). The need for determining the toxicokinetics (TK) profile is listed in Annex I (Section 1.0.2) of the legislation, but in Annexes VII-X it is not specifically required and its consideration is needed only if these data are available (Annex VIII-X). However, REACH does provide guidance (Guidance on information requirements and chemical safety assessment, Chapters R.7C and R.8) on the use of TK for selection of dose, route of administration, and test species, as well as on route-to-route extrapolation in the derivation of a no-effect level (DNEL). Each chemical should be registered with ECHA, along with information on properties, uses, and safe handling practices. While REACH regulation challenges the chemical industry to develop rapid, relevant, cost-effective *in vitro* assays to reliably predict human toxicity, it does not require ADME data, nor does it provide guidance on efficient new methods validation or acceptance by the agency. This is causing delays in the successful implementation of these assays, as chemical manufacturers want to use the validated assays that will get their products to the market faster (Hartung and Rovida, 2009). Thus, European REACH legislation has no requirement for ADMET data, and European cosmetics registration guidelines also do not mandate its use (although kinetics data are required).

In June 2016, President Obama signed into law the Frank R. Lautenberg Chemical Safety for the 21st Century Act[6], which

3 http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf

4 FDA (2006). Guidance for industry. Drug interaction studies – study design, data analysis, and implications for dosing and labeling. Draft Guidance. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf

5 EMA (2012). Guideline on the Investigation of Drug Interactions, CPMP/EWP/560/95/Rev. 1. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf

6 https://www.epa.gov/sites/production/files/2016-06/documents/bills-114hr2576eah.pdf

amends the Toxic Substance Control Act (TSCA) of 1976. The new law includes much needed improvements, including a mandatory requirement for EPA to evaluate existing chemicals with clear and enforceable deadlines; new risk-based safety standards; increased public transparency for chemical information; and consistent sources of funding for EPA to carry out its responsibilities under the new law. It requires that decisions for testing and assessing chemicals should be based on the weight of the scientific evidence. However, the exact role ADMET data will play in the new TSCA is not yet clear. Although ADME is not specifically mentioned in the document, it is implied throughout when *in vitro*, alternative, computational, and toxicity prediction methods are mentioned. While most toxicologists would agree that ADME needs to be thoroughly understood to predict safety and kinetics of chemicals in target organisms, there are concerns that if it is not mentioned in the regulatory document, industries will de-prioritize it in their safety testing. In line with the NAS 2007 Report "Toxicity Testing in the 21st Century: A Vision and a Strategy"[7] a range of papers appeared within the ILSI/-HESI program "RISK 21" (Beaumont and Smith, 2009; Doe et al., 2016; Embry et al., 2014; Pastoor et al., 2014), whereby an integral part of schemes predicting toxicity of compounds is the study of ADME.

The area in which kinetics is particularly important is nanotoxicology (Hartung, 2010a). The size of the particle, its composition and the kinetics of release of the active molecules are adding to the complexity of studying the ADME and toxicity of nanomaterials. Subsequently, we are only starting to understand the dramatically altered kinetics of nanomaterials and their biological consequences *in vivo* and *in vitro* (Hartung and Sabbioni, 2011).

6 Food, feed and food additives safety testing and ADME

The European Food Safety Authority (EFSA) is an agency whose role is to provide independent scientific advice and information in the form of opinions and technical reports to support community legislation and policies and to collect and analyze data allowing assessment and monitoring of risks in the food and feed sectors. The work of EFSA is mainly carried out in expert panels dealing with food additives, genetically modified organisms, food contaminants, transmissible animal diseases, and pesticides and their residues. In Directive 2010/63/EU[8], the EU Commission recommended that alternative models should include *in vitro* and *in silico* methods, as well as reduction and refinement of *in vivo* tests (Hartung, 2010a). Specifically, for ADME determination, the EU Commission favored the use of *in vitro* models from the same species as those used in pivotal studies and in human materials (liver microsomes and intact cell systems). In the European Union, risk assessment and authorization of plant protection products (PPPs) is carried out according to Regulation (EC) 1107/2009[9]. PPPs that are designed to control pests are toxic by definition and are normally actively introduced into the environment. Therefore, extensive testing before any decision on authorization is mandatory. Testing requirements for the assessment of active substances with respect to possible human health effects include a battery of *in vivo* tests (acute, subchronic, and chronic tests, and reproductive toxicity) in Annex II to Directive 91/414/EEC, while Annex III lists testing requirements for the final plant protection product.

Recently, a roadmap to integrate *in silico* and *in vitro* approaches in the area of safety assessment of food and food ingredients was published (Blaauboer et al., 2016) in which the emphasis was also on the kinetic behavior of the compounds under study.

7 The critical role of ADME for the future of *in vitro* toxicology

7.1 Well-established uses of ADME in *in vitro* toxicology

Prediction of metabolites

A broad variety of cellular and subcellular models of metabolism of xenobiotics are available (Costa et al., 2014; Donato et al., 2008; Gómez-Lechón et al. 2003; Gordon et al., 2015; Vermeir et al., 2005; Williams, 2014). Many focus on liver metabolism because of the extensive metabolic capacity of that organ, its central role in blood circulation, and as an important elimination/detoxification organ. Moreover, marked differences between species in liver metabolism confound the *in vivo* extrapolations.

There are still some challenges for predicting *in vivo* metabolism from *in vitro* data (Anderson et al., 2009), though at least hepatic clearance can be predicted well by scaling *in vitro* data (Barter et al., 2007; Brown et al., 2005; Carlile et al., 1999; Griffin and Houston, 2005; Houston and Kenworthy, 2000). Recent developments with hepatic models include the emergence of stem cell-derived liver cell models (Szkolnicka and Hay, 2016), broad access to primary human cells via commercial vendors, and cell lines with some metabolic capacity, such as HepaRG cells, derived from human hepatocellular carcinoma (Andersson et al., 2012; Guillouzo et al., 2007). Hence, increasingly, the *in vitro* data is relied upon for prediction of the *in vivo* metabolism of xenobiotics (Rostami-Hodjegan and Tucker, 2007).

Biological barrier models

Barrier models have advanced significantly since the first description of the intestinal barrier CaCo-2 cell model by Artursson (1990). Models of the outer epithelia of the human body – the skin, the intestine and the lung – have found applications in both research and industrial settings as solid alternatives to animal testing (Sexton et al., 2011; Gordon et al., 2015). A variety of approaches to modeling these barriers are currently employed in fields ranging from the utilization of *ex vivo* tissue to

7 https://www.nap.edu/read/11970/chapter/1

8 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

9 http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1107&from=EN

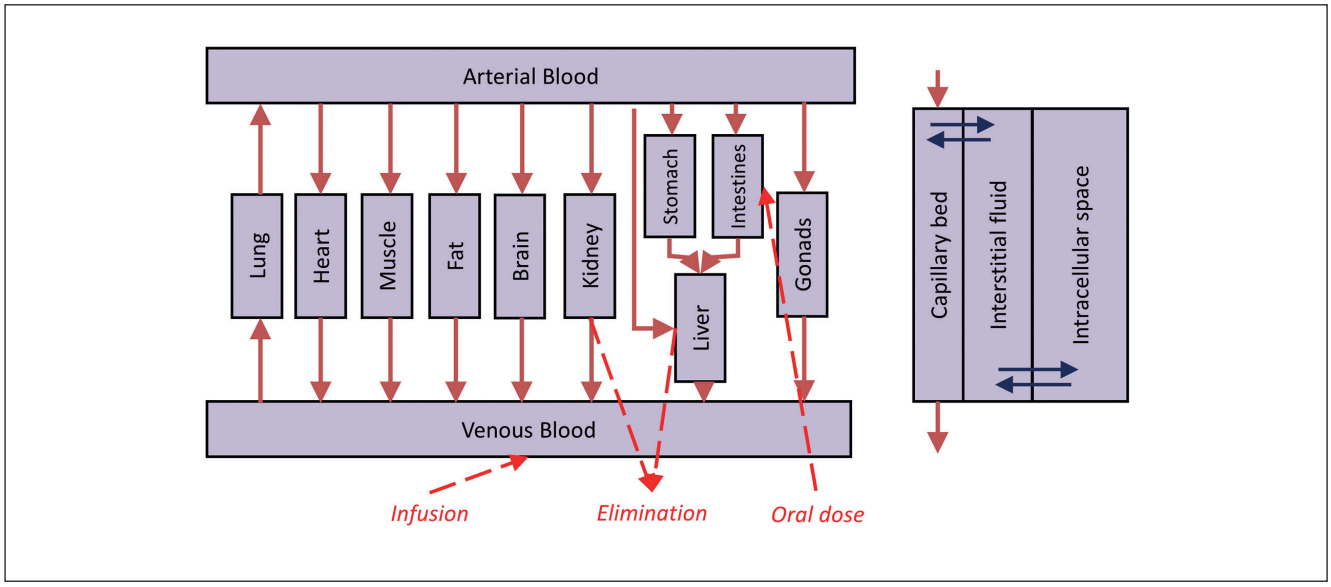

Fig. 3: A conceptual PBPK model used to predict somatic distribution and elimination

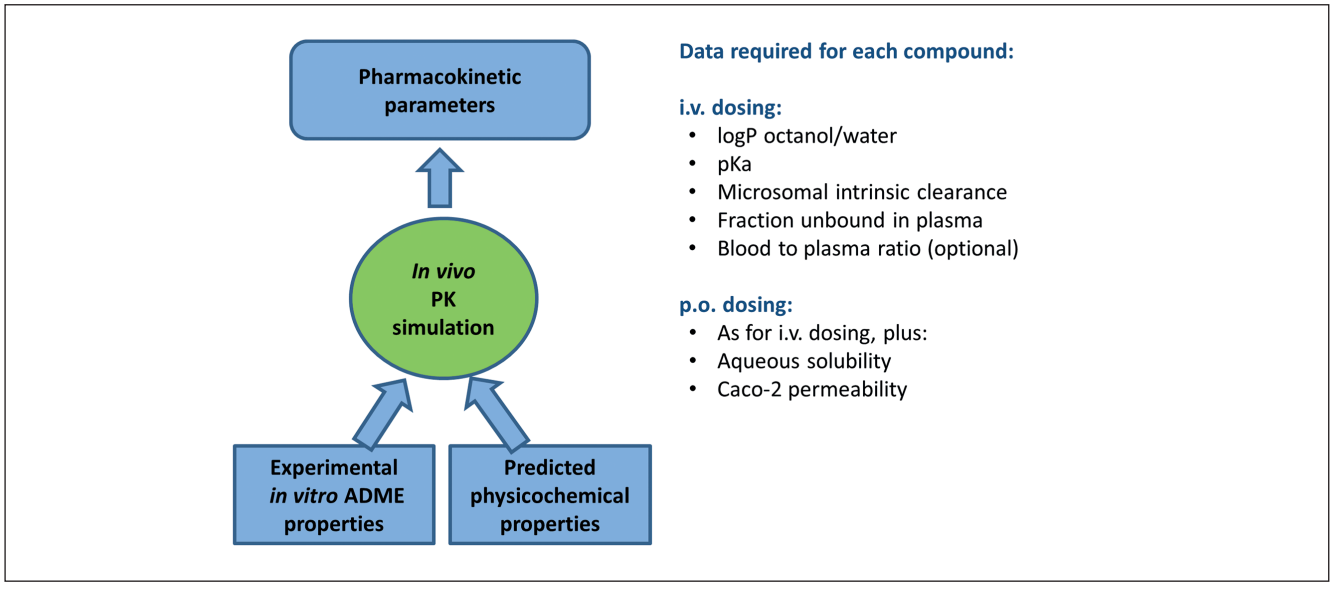

Fig. 4: Pharmakokinetic prediction

reconstructed *in vitro* models (Muoth et al., 2016) to chip-based technologies (Picollet-D'hahan et al., 2016), new generation of parallel artificial membrane permeability assays (PAMPA) (Chen et al, 2008) and *in silico* modeling of gastrointestinal absorption approaches with validated commercial models (Gozalbes et al., 2011; Sjögren et al., 2016). An international group of experts in the field of epithelial barriers was convened from academia, industry, and regulatory bodies to present both the state-of-the-art of non-animal models of the skin, intestinal, and pulmonary barriers and to discuss research-based, industry-driven, and regulatory-relevant directions for both the development of new models and the refinement of existing test methods. Issues of model relevance and preference, validation and standardization, acceptance, and the need for simplicity *versus* complexity were focuses of the discussions. These advances in barrier models were covered in one of our recent workshop report (Gordon et al., 2015).

Pharmacokinetic modeling of the in vivo situation

Pharmacokinetic models can be divided into two general groups: data-based experimental (classical) models and physiologically-based models (Andersen, 1991; Filser et al., 1995). Physiologically based pharmacokinetic (PBPK) models (Fig. 3), traditionally used in clinical trial design, are now being developed and used in earlier stages of discovery and development across industries. PBPK models require *in vitro* ADME data in addition to information about the physical and chemical properties of a compound

(see example in Fig. 4) to predict the internal dose that will be distributed to target organs, and they are increasingly being used in risk assessment across industries (McLanahan et al., 2014). They can be used for *in vitro* to *in vivo*, route to route, and animal to human extrapolations (Basketter et al., 2012; Espié et al., 2009; Rietjens et al., 2011). PBPK modeling allows the prediction of the time course of a compound's concentration in blood and target tissues. It also has the potential to address repeated dose toxicity testing (Pfaller et al., 2015; Hamon et al., 2015; Kramer et al., 2015). Information from other models, such as those predicting metabolite formation, are increasingly included in PBPK models, rendering results that are more predictive (Lock et al., 2012). Some experts argue that PBPK models incorporating data from quantitative structure-activity relationship (QSAR) models, coupled with *in vitro* assays of tissue/organ toxicity, have the potential to replace *in vivo* animal studies for quantitative assessment of the biological activity of xenobiotics (Blaauboer, 2010). An ECVAM workshop (Bouvier d'Yvoire et al., 2007) addressed the state of physiologically-based pharmacokinetic (and toxicokinetic) modeling, highlighting needs and opportunities to meet the 3Rs agenda. The report was instrumental in establishing the EU consortium Predict-IV, which combined PBPK modeling with long-term exposures and omics technologies to address repeated dose toxicity. The results of the project are summarized in a special issue of *Toxicology In Vitro*, December 2015 (Pfaller et al., 2015).

7.2 Newer ADMET prediction tools

Some of the newer ADMET tools that can aid toxicologists in both the pharmaceutical and chemical safety areas capitalize on advances in our growing understanding of mechanisms underlying human toxicity and the development of tests to detect it.

Mitochondrial toxicity

One specific example of how the chemical industry can directly benefit from the experience of the pharmaceutical industry is via knowledge of the mitochondrial function and toxicity and the simple cost-effective assay that is now routinely performed to predict this mitochondrial toxicity in drugs.

Over the past decade, enormous strides have been made in our understanding of the role that drug and environmental toxicity can play in causing mitochondrial dysfunction. In addition to generating ATP and playing a role in apoptosis, mitochondria play critical roles in other key processes including calcium, copper, and iron homeostasis; heme and iron-sulfur cluster assembly; synthesis of pyrimidines and steroids; thermogenesis and fever response; and calcium signaling (Meyer et al., 2013). Mitochondrial toxicants injure mitochondria by inhibiting respiratory complexes of the electron chain, inhibiting or uncoupling oxidative phosphorylation, inducing mitochondrial oxidative stress, or inhibiting DNA replication, transcription, or translation (Rodríguez-Enríquez et al., 2001).

Many drugs that have been withdrawn from the market due to organ toxicity have been found to be mitochondrial toxicants, which caused the pharmaceutical industry to put resources into understanding the mechanism and developing tools to predict mitochondrial toxicity at early stages in drug development (Dykens and Will, 2007). Traditionally, mitochondrial toxicity testing is performed in immortalized cell lines that have been adapted for rapid growth in a reduced-oxygen atmosphere. Their metabolism is often anaerobic (glycolysis) despite having functional mitochondria and an adequate oxygen supply. Because cells normally generate ATP for energy consumption aerobically by mitochondrial oxidative phosphorylation, the anaerobic metabolism of transformed cell lines is less sensitive to mitochondrial toxicants, causing systematical underreporting in toxicity testing (Marroquin et al., 2007; Rodríguez-Enríquez et al., 2001). To address this issue, HepG2 and NIH/3T3 cell models that can be grown in media in which glucose is replaced by galactose were developed at Pfizer (Dykens and Will, 2007). The change in sugar results in the metabolism of the cell possessing a respiratory substrate that is both more similar to normal cells and sensitive to mitochondrial toxicants without reducing sensitivity to non-mitochondrial toxicants.

Environmental chemicals that accumulate in mitochondria include polycyclic aromatic hydrocarbons (PAHs) (Backer and Weinstein, 1982); some alkylating agents (Wunderlich et al., 1972); cationic metals, such as lead, cadmium, mercury, and manganese (Atchison and Hare, 1994; Castellino and Aloj, 1969; Gavin et al., 1992); and some organic chemicals, including ethidium bromide, paraquat, and 1-methyl-4-phenylpyridinium (MPP+) (Mehta et al., 2008). The presence of cytochrome P450s in mitochondria (Omura, 2006) can activate chemicals that are relatively nonreactive prior to metabolism, such as PAHs and mycotoxins (Dong and Lee, 2009; Genter et al., 2006). With the simple reproducible glucose/galactose assay in transformed cell lines, chemicals now can be easily screened for mitochondrial toxicity.

7.3 The emerging role of ADME in *in vitro* toxicology

In vitro toxicology is increasingly developing methods that satisfy the information needs of regulatory toxicology as summarized on behalf of the European Commission (Adler et al., 2011) and confirmed by independent expert review (Hartung et al., 2011). Going a step further, a roadmap was developed by expert consensus on achieving animal-free systemic toxicity testing, including toxicokinetics (Basketter et al., 2012; Leist et al., 2014).

In vitro pharmacokinetics

It should be self-evident that *in vitro* pharmacokinetics should be given the same attention as *in vivo* (Heringa et al., 2004). *In vitro* behavior of an added substance, however, is usually neglected in favor of nominal concentrations (Kramer et al., 2007). Setting aside the fact that the compounds' purity is rarely verified experimentally, therefore, making even nominal concentrations inaccurate, many other factors affect the effective amount of substance *in vitro* (Fig. 5).

These parameters will change the concentration of free substance, which is typically considered available to act on its targets (Groothuis et al., 2015). We should keep in mind that it is not necessarily the concentration (as peak concentration or area under the curve) that matters, but that some lipophilic

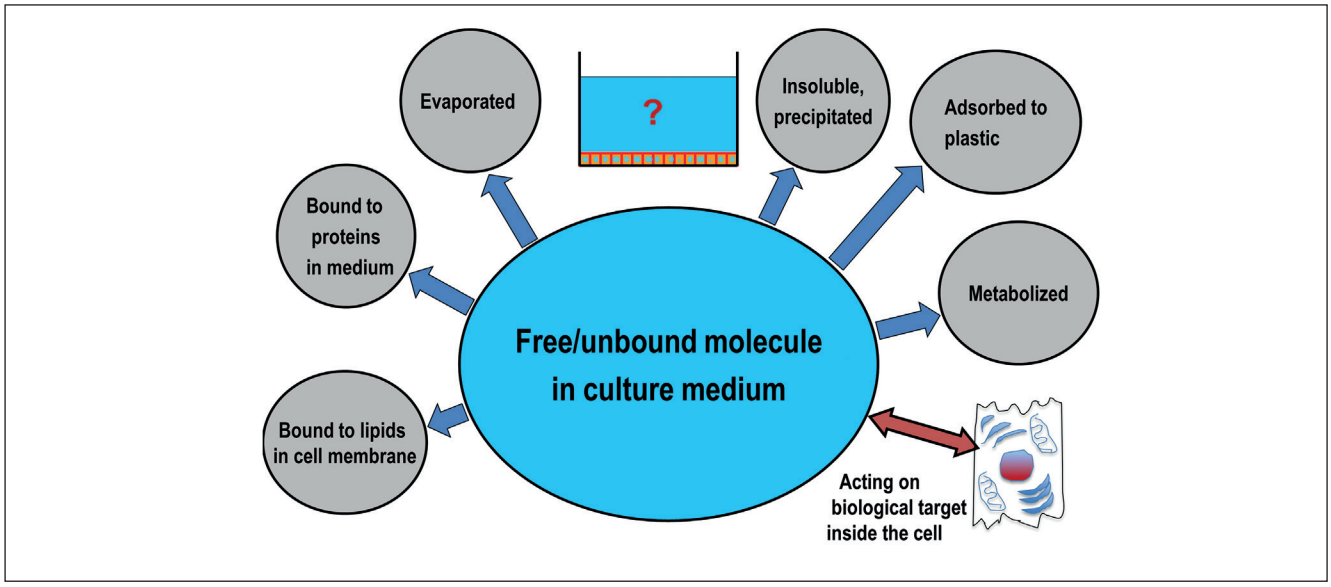

Fig. 5: *In vitro* biokinetics, or possible fate of a molecule

substances can accumulate in biological systems, making the absolute amount (and thus the proportion of cell culture media versus cell mass) a determinant of a substance's effects.

Another aspect that has received much attention in recent years is the difference in the kinetic considerations between 2-dimensional traditional *in vitro* systems and *in vivo* systems. *In vivo* kinetics using any route of administration normally follow the mathematical Bateman function (Garrett, 1994) with the xenobiotic plasma concentration time course increasing to a peak (C_{max}) and then decreasing as a function of distribution to the tissues, metabolism and elimination. In this mathematical model, the necessary simplifications of body processes and mathematical principles are applied to the various processes. A basic type of model used in pharmacokinetics is the compartmental model. Compartmental models are categorized by the number of compartments needed to describe the chemical's behavior in the body. There are one-compartment, two-compartment, and multicompartment models. The compartments do not represent a specific tissue or fluid but may represent a group of similar tissues or fluids. These models can be used to predict the time course of drug concentrations in the body. In contrast to *in vivo* kinetics, *in vitro* the substance typically is added instantaneously, and remains in the test tube or multi-well plate for the duration of the experiment. The consequences of this are many, e.g., the long exposure of the cells to the same concentration of the chemical may be responsible for a false-positive toxicity result, as in an *in vivo* situation the realistic exposure would be much shorter and to lower concentrations due to distribution and elimination processes or induce defense mechanisms that make the cell more resilient, as recently hypothesized (Smirnova et al., 2015). In response to this problem, multiple 3-dimensional and "organ-on-a-chip" technologies are being developed that address this issue of kinetics, introduce shear stress and otherwise mimic the blood flow to the tissue (Jonczyk et al., 2016; Marx et al., 2016; Oomen et al., 2016; Visone et al., 2016)

8 Assessing human metabolism *in vitro*

Most *in vitro* models used to lack metabolic capacities (Coecke et al., 2006). One might argue, though, that models with no metabolic capacity may be at least as informative as models with a metabolism that is substantially different from that of the target species (human), as in the case of animal models. How often might metabolism by animal species give false assurances of human safety due to differences in metabolism? At the same time, lack of defense mechanisms might be as important as lack of activation by metabolism to bias *in vitro* effects and thus model predictivity (Smirnova et al., 2015).

This bottleneck is being actively addressed by a number of academic and industry groups with liver models such as HepaRG cells (Guillouzo et al., 2007), micropatterned co-cultured human hepatocytes (Khetani et al., 2013) and others. These models not only have the potential to provide information about human metabolites, leap-frogging the uncertainties of extrapolation from animal models, but, moreover, can provide information about individual differences in metabolism, test chronic dosing and allow the detection of individual differences in metabolism, entering the realm of personalized safety prediction (Skardal et al., 2016).

9 Quantitative *in vitro* to *in vivo* extrapolation (QIVIVE)

In vitro toxicology ultimately desires to predict the effects of a xenobiotic on a whole organism level, and that requires extrapolation (QIVIVE). One of the authors (B.B.) and colleagues long ago spearheaded a discussion of integrated testing of *in vitro*, *in silico*, and ADME information (Blaauboer et al., 1998; Blaauboer, 2010; DeJongh et al., 1999; Forsby and Blaauboer, 2007). Over the years, they and others have developed a number of successful examples, including neurotoxicity (DeJongh

et al., 1999; Forsby and Blaauboer, 2007), acute oral toxicity and repeated dose toxicity (Gubbels-van Hal et al., 2005), developmental toxicity (Louisse et al., 2010; Verwei et al., 2006) and genotoxicity (Paini et al., 2010). Within the framework of the European integrated project Predict-IV (Pfaller et al., 2015) work was devoted to pharmacokinetic modeling of *in vitro* experiments, physiologically based pharmacokinetic (PBPK) modeling, mechanistic models of toxicity for the kidney and brain, large scale dose-response analysis methods, and biomarker discovery tools (Hamon et al., 2015). The value of this emphasis on biokinetics for evaluating the toxicity of compounds after repeated exposure of cellular systems *in vitro* was clearly shown in this project (Kramer et al., 2015; Wilmes et al., 2013).

10 ADME contributions to integrated testing strategies

We are increasingly forced to acknowledge that our information about the safety of substances cannot be satisfied by single assays. At the same time, a simple battery of tests bears the risk of accumulating false positives if it is built on a static, limited modeling data set. A more intelligent "integrated" combination of different tests with *in silico* tools with machine-learning capabilities promises to optimize resources and achieve better predictivity. This has been termed integrated testing strategies (ITS) or, more recently, integrated approaches to testing and assessment (IATA) by OECD (Hartung et al., 2013; Tollefsen et al., 2014; Kleensang, 2013). It is clear that an ideal ITS includes information about ADME to improve the prediction of *in vivo* results.

The need for knowledge about the PK behavior of compounds becomes clear in an example of dermal exposure to cosmetics, where the abovementioned shift in toxicology is evident. This shift from apical endpoints in intact animals after exposure to a certain external dose to mechanistically based descriptions of the toxic processes makes it evident that emphasis should be put on the estimation of the internal dose in different tissues and cells. For these processes, the application of PBPK models is essential (Yoon et al., 2015). Thus, there is a need to describe the processes of dermal uptake and the consequent distribution of compounds to arrive at internal exposure estimates. This will also aid the design of *in vitro* testing, since it will inform the appropriate choice of cell and tissue culture systems as well as the relevant range of concentrations applied to them. Once the toxicity of the compound (and its relevant metabolites) is quantified in the relevant *in vitro* systems, the application of biokinetic modelling in the process of reverse dosimetry is just as important (Louisse et al., 2010).

11 The advent of systems ADME-Tox

The components of the body affected by a toxicant represent a network. Systems biology uses the relationships between all elements rather than approaching them separately, and attempts to unite biological fields (Hood and Galas, 2003). Systems biology is defined as (Duffus et al., 2007; Ferrario et al., 2014):

> *Study of the mechanisms underlying complex biological processes as integrated systems of many diverse, interacting components. It involves (1) collection of large sets of experimental data (by high-throughput technologies and/or by mining the literature of reductionist molecular biology and biochemistry);*
> *(2) proposal of mathematical models that might account for at least some significant aspects of this data set;*
> *(3) accurate computer solution of the mathematical equations to obtain numerical predictions; and*
> *(4) assessment of the quality of the model by comparing numerical simulations with the experimental data.*

This concept is being applied to toxicology (Ekins et al., 2005). The move towards systems toxicology and massively parallel techniques opens new opportunities while also creating problems in deriving meaningful information out of the wealth of generated data (Hartung et al., 2012). Such data is increasingly represented as networks in which the vertices (e.g., transcripts, proteins, or metabolites) are linked by edges (correlations, interactions, or reactions, respectively). Networks can vary in function. Some are undirected graphs that enable only the study of structure whereas others, like biochemical networks, are characterized by interactions of varying strengths, strongly nonlinear dynamics, and saturating response to inputs (Wagner, 1996).

Network analysis has evolved into a very active, interdisciplinary area of research encompassing biology, computer science, and social and information sciences. Many studies are highly theoretical, but they may hold a lot of promise to identify signatures (SoT) and pathways of toxicity (PoT) (Adeleye et al., 2015; Kleensang et al., 2014). Network research has three primary goals. First, it aims to understand statistical properties that characterize the network structure in order to suggest appropriate ways to measure these properties. This is very relevant to SoT identification. Second, it aims to create models of networks for understanding the meaning of these properties and how they interact. Third, it aims to predict the behavior of networked systems on the basis of measured structural properties, and this can be very useful in elucidating PoT. Given the dose-response nature of toxicity and, thus, dependence of hazard assessment on exposure, it is natural to start thinking about development of the concept of systems ADME-Tox. ADME is moving hazard data to a systems level. At the same time, *in silico* tools such as the combination of PBPK and QIVIVE in systems biology has improved the prediction of ADME (Ekins, 2016; Rostami-Hodjegan, 2012), making it feasible to make predictions of human toxicity using ITS approaches exclusively using *in vitro* and *in silico* tools.

Ultimately, systems toxicology aims to model the effects of toxicants in metabolic networks (Bugrim et al., 2004; Tamaddoni-Nezhad et al., 2007). Integrating cellular metabolism into a multi-scale, whole-body model is being actively investigated (Krauss et al., 2012) and drug effects on these networks, termed systems pharmacology, have been proposed (Li et al., 2012). This depends critically on combining dynamic and kinetic information. Thus, the avenues toward systems toxicology require the development of components for ADME prediction.

12 Evidence-based methodologies and ADME

While ADME studies play an important role in shedding light on differences between species, as shown here, understanding the kinetics and exposure that underlie toxicity and finding reliable ADME data in the literature can be daunting. ADME results are scattered among hundreds of papers describing experiments performed according to a number of protocols that are not always shared in the publications. Finding reproducible results that were obtained using the same methods with the same materials on the same species on the same compound is frequently a time-consuming and disappointing exercise. Finding reliable data in the literature on the differences between rat and human liver metabolism for a particular chemical, for example, can be even more challenging. To make useful comparisons, methodological details, such as the source of a testing material, how it is handled, and testing conditions (incubation temperature, controls used, stock, and working concentrations of all reagents, and testing time points) need to be required by publishers and mentioned explicitly in publications. Differences in these methodologies and equipment used by different laboratories contribute to the many redundant experiments, leaving us with no easy way to compare the results.

This situation regarding ADME test quality and reliability is comparable to how reviews were handled in clinical research before the evidence-based medicine/health care (EBM/EBHC) movement established systematic reviews as the best approach to summarizing all available evidence relating to a research question. The need for reproducible, transparent, and comprehensive syntheses of the ever-growing volume of medical evidence triggered the development of rigorous approaches to review the literature: question formulation, literature search, evidence selection, and evidence integration. The Cochrane Collaboration[10] played a key role in fostering this process over the past two decades. The systematic reviews that are at the heart of evidence-based approaches have become the lifeblood of medicine by evaluating dozens to hundreds of studies involving thousands of patients in different clinical settings to reveal the larger trends that smaller analyses may conceal. Incorporating systematic reviews and other aspects of evidence-based approaches into toxicology and ADME can help us ensure that our evaluation of drugs and chemicals is objective and transparent.

The Evidence-based Toxicology Collaboration (EBTC)[11] was established in 2011 (Zurlo, 2011) following a conference in Como in 2007 (Griesinger et al., 2008) to bring evidence-based (EB) methodologies into toxicology (Guzelian et al., 2005; Hoffmann and Hartung, 2006; Hartung, 2009; Hoffmann et al., 2014) and safety sciences. It has a number of multi-stakeholder working groups focusing on specific tasks, such as transfer of EB methods from medicine to toxicology, literature-based test methods comparison and validation, *in vitro* high-throughput methods for comparison and validation, and development of risk of bias tools for *in vitro* and *in silico*/modeling studies. All of these tools will be applicable to *in vitro* and *in vivo* ADME study assessment and should be a part of any systematic chemical safety assessment. EBTC and its methods working group will shortly publish Guidance on Evidence-based Toxicology, which will describe the process for evaluating *in vitro* and *in vivo* toxicity assays using evidence-based methods. The guidance will define how evidence-based methods can be used to assess toxicological studies transparently.

Evidence-based methodologies can help improve ADME data rigor and validity. Incorporating systematic reviews (Stephens et al., 2016) and other aspects of evidence-based approaches such as quality scoring (Samuel et al., 2016) into toxicology and ADME can help us ensure that our use of these sciences to evaluate drugs and chemicals is objective and transparent. In summary, ADME data need to be a part of any systematic review of chemical safety, and should be part of the process of evidence-based toxicology.

13 Conclusions

The critical role of ADME for interpreting *in vivo* data has been shown by the lowering of attrition of drugs in human trials following industry-wide standardization and implementation of high-throuput ADME protocols. However, while drug development has embraced ADME for predicting and analyzing substance effects (Singh, 2006; Tsaioun and Jacewicz, 2009; Zhang et al., 2012), other fields are not using the ADME information to its full potential. For example, the largest regulatory industrial chemical program, the European REACH legislation, has no explicit requirement for ADME. Similarly, cosmetics are typically registered in Europe without such data (Hartung, 2008), though the ban on animal testing does name kinetics as an information requirement under the 2013 legislation. In both cases, kinetics information – if incorporated at all – is used for weight of evidence evaluations and to adjust the impact of risk assessment hazard. However, as with exposure considerations (which are often similarly neglected), the knowledge about bioavailability and kinetics of the xenobiotics, could help standardize information requirements and adjust safety factors for risk assessments.

The combination of *in vivo*, *in vitro* and *in silico* tools is more common here than in many other fields of toxicology. There is an enormous need to incorporate *in vitro* ADME tools in toxicology to improve prediction of human adverse events and spare animal lives. To make *in vitro* data more predictive of the whole organism response, we need to address both the kinetics of substances in *in vitro* models and their modeling to extrapolate to *in vivo* predictions. PBPK methods lend themselves to such reverse kinetics, but examples of their use for QIVIVE, to our knowledge, are not yet generalizable to multiple chemical series. The overall premise of PBPK models with their requirement for *in vitro* barrier and metabolism input and their superiority to purely *in silico* models holds promise for ITS (or IATA)

[10] http://www.cochrane.org/

[11] http://www.ebtox.org

to be developed and validated in the near future, with evidence-based ADME data being an integral part of such predictions.

References

Adeleye, Y., Andersen, M., Clewell, R. et al. (2015). Implementing toxicity testing in the 21st century (TT21C): Making safety decisions using toxicity pathways, and progress in a prototype risk assessment. *Toxicology 332*, 102-111. http://dx.doi.org/10.1016/j.tox.2014.02.007

Adler, S., Basketter, D., Creton, S. et al. (2011). Alternative (non-animal) methods for cosmetics testing: Current status and future prospects – 2010. *Arch Toxicol 85*, 367-485. http://dx.doi.org/10.1007/s00204-011-0693-2

Andersen, M. E. (1991). Physiological modelling of organic compounds. *Ann Occup Hyg 35*, 309-321. http://dx.doi.org/10.1093/annhyg/35.3.309

Anderson, S., Luffer-Atlas, D. and Knadler, M. P. (2009). Predicting circulating human metabolites: How good are we? *Chem Res Toxicol 22*, 243-256. http://dx.doi.org/10.1021/tx8004086

Andersson, T. B., Kanebratt, K. P. and Kenna, J. G. (2012). The HepaRG cell line: A unique in vitro tool for understanding drug metabolism and toxicology in human. *Expert Opin Drug Metab Toxicol 8*, 909-920. http://dx.doi.org/10.1517/17425255.2012.685159

Arrowsmith, J. and Miller, P. (2013). Trial watch: Phase II and phase III attrition rates 2011-2012. *Nat Rev Drug Discov 12*, 569. http://dx.doi.org/10.1038/nrd4090

Artursson, P. (1990). Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J Pharm Sci 79*, 476-482. http://dx.doi.org/10.1002/jps.2600790604

Atchison, W. D. and Hare, M. F. (1994). Mechanisms of methylmercury-induced neurotoxicity. *FASEB 8*, 622-629.

Backer, J. M. and Weinstein, I. B. (1982). Interaction of benzo(a) pyrene and its dihydrodiol-epoxide derivative with nuclear and mitochondrial DNA in C3H10T 1/2 cell cultures. *Cancer Res 42*, 2764-2769.

Ball, P., Mandell, L., Niki, Y. et al. (1999). Comparative tolerability of the newer fluoroquinolone antibacterials. *Drug Saf 21*, 407-421. http://dx.doi.org/10.2165/00002018-199921050-00005

Barter, Z. E., Bayliss, M. K., Beaune, P. H. et al. (2007). Scaling factors for the extrapolation of in vivo metabolic drug clearance from in vitro data: Reaching a consensus on values of human microsomal protein and hepatocellularity per gram of liver. *Curr Drug Metab 8*, 33-45. http://dx.doi.org/10.2174/138920007779315053

Basketter, D. A., Clewell, H., Kimber, I. et al. (2012). A roadmap for the development of alternative (non-animal) methods for systemic toxicity testing – t4 report. *ALTEX 29*, 3-91. http://dx.doi.org/10.14573/altex.2012.1.003

Beaumont, K. and Smith, D. A. (2009). Does human pharmacokinetic prediction add significant value to compound selection in drug discovery research? *Curr Opin Drug Discov Devel 12*, 61-71.

Blaauboer, B. J., Barratt, M. D. and Houston, J. B. (1998). The integrated use of alternative methods in toxicological risk evaluation – ECVAM integrated testing strategies task force report 1. *Altern Lab Anim 27*, 229-237.

Blaauboer, B. J. (2010). Biokinetic modeling and in vitro – in vivo extrapolations. *J Toxicol Environ Health B Crit Rev 13*, 242-252. http://dx.doi.org/10.1080/10937404.2010.483940

Blaauboer, B. J., Boobis, A. R., Bradford, B. et al. (2016). Considering new methodologies in strategies for safety assessment of foods and food ingredients. *Food Chem Toxicol 91*, 19-35. http://dx.doi.org/10.1016/j.fct.2016.02.019

Bouvier d'Yvoire, M., Prieto, P., Blaauboer, B. J. et al. (2007). Physiologically-based kinetic modelling (PBK Modelling): Meeting the 3Rs agenda. The report and recommendations of ECVAM Workshop 63. *Altern Lab Anim 35*, 661-671.

Brown, H. S., Ito, K., Galetin, A. et al. (2005). Prediction of in vivo drug-drug interactions from in vitro data: Impact of incorporating parallel pathways of drug elimination and inhibitor absorption rate constant. *Br J Clin Pharmacol 60*, 508-518. http://dx.doi.org/10.1111/j.1365-2125.2005.02483.x

Bugrim, A., Nikolskaya, T. and Nikolsky, Y. (2004). Early prediction of drug metabolism and toxicity: Systems biology approach and modeling. *Drug Discov Today 9*, 127-135. http://dx.doi.org/10.1016/S1359-6446(03)02971-4

Caldwell, G. W., Yan, Z., Tang, W. et al. (2009). ADME optimization and toxicity assessment in early- and late-phase drug discovery. *Curr Top Med Chem 9*, 965-980. http://dx.doi.org/10.2174/156802609789630929

Carlile, D. J., Hakooz, N. and Houston, J. B. (1999). Kinetics of drug metabolism in rat liver slices: IV. Comparison of ethoxycoumarin clearance by liver slices, isolated hepatocytes, and hepatic microsomes from rats pretreated with known modifiers of cytochrome P-450 activity. *Drug Metab Dispos 27*, 526-532.

Castellino, N. and Aloj, S. (1969). Intracellular distribution of lead in the liver and kidney of the rat. *Br J Ind Med 26*, 139-143. http://dx.doi.org/10.1136/oem.26.2.139

Chen, X., Murawski, A., Patel, K. et al. (2008). A novel design of artificial membrane for improving the PAMPA model. *Pharm Res 25*, 1511-1520. http://dx.doi.org/10.1007/s11095-007-9517-8

Chu, X., Bleasby, K. and Evers, R. (2013). Species differences in drug transporters and implications for translating preclinical findings to humans. *Expert Opin Drug Metab Toxicol 9*, 237-252. http://dx.doi.org/10.1517/17425255.2013.741589

Coecke, S., Ahr, H., Blaauboer, B. J. et al. (2006). Metabolism: A bottleneck in in vitro toxicological test development. The report and recommendations of ECVAM workshop 54. *Altern Lab Anim 34*, 49-84.

Costa, A., Sarmento, B. and Seabra, V. (2014). An evaluation of the latest in vitro tools for drug metabolism studies. *Expert Opin Drug Metab Toxicol 10*, 103-119. http://dx.doi.org/10.1517/17425255.2014.857402

Dearden, J. C. (2007). In silico prediction of ADMET properties: How far have we come? *Expert Opin Drug Metab Toxicol 3*, 635-639. http://dx.doi.org/10.1517/17425255.3.5.635

DeJongh, J., Nordin-Andersson, M., Ploeger, B. A. et al. (1999).

Estimation of systemic toxicity of acrylamide by integration of in vitro toxicity data with kinetic simulations. *Toxicol Appl Pharmacol 158*, 261-268. http://dx.doi.org/10.1006/taap.1999.8670

DiMasi, J. A., Hansen, R. W. and Grabowski, H. G. (2003). The price of innovation: New estimates of drug development costs. *J Health Econ 22*, 151-185. http://dx.doi.org/10.1016/S0167-6296(02)00126-1

Doe, J. E., Lander, D. R., Doerrer, N. G. et al. (2016). Use of the RISK21 roadmap and matrix: Human health risk assessment of the use of a pyrethroid in bed netting. *Crit Rev Toxicol 46*, 54-73. http://dx.doi.org/10.3109/10408444.2015.1082974

Donato, M. T., Lahoz, A., Castell, J. V et al. (2008). Cell lines: A tool for in vitro drug metabolism studies. *Curr Drug Metab 9*, 1-11. http://dx.doi.org/10.2174/138920008783331086

Dong, T. T. T. and Lee, B.-K. (2009). Characteristics, toxicity, and source apportionment of polycylic aromatic hydrocarbons (PAHs) in road dust of Ulsan, Korea. *Chemosphere 74*, 1245-1253. http://dx.doi.org/10.1016/j.chemosphere.2008.11.035

Duffus, J. H., Nordberg, M. and Templeton, D. M. (2007). Glossary of terms used in toxicology, 2nd edition (IUPAC Recommendations 2007). *Pure Appl Chem 79*, 1153-1344. http://dx.doi.org/10.1351/pac200779071153

Dykens, J. A. and Will, Y. (2007). The significance of mitochondrial toxicity testing in drug development. *Drug Discov Today 12*, 777-785. http://dx.doi.org/10.1016/j.drudis.2007.07.013

Ekins, S., Nikolsky, Y. and Nikolskaya, T. (2005). Techniques: Application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends Pharmacol Sci 26*, 202-209. http://dx.doi.org/10.1016/j.tips.2005.02.006

Ekins, S. (2016). Systems-ADME/Tox: Resources and network approaches. *J Pharmacol Toxicol Methods 53*, 38-66. http://dx.doi.org/10.1016/j.tips.2005.02.006

Embry, M. R., Bachman, A. N., Bell, D. R. et al. (2014). Risk assessment in the 21st century: Roadmap and matrix. *Crit Rev Toxicol 44, Suppl 3*, 6-16. http://dx.doi.org/10.3109/10408444.2014.931924

Espié, P., Tytgat, D., Sargentini-Maier, M.-L. et al. (2009). Physiologically based pharmacokinetics (PBPK). *Drug Metab Rev 41*, 391-407. http://dx.doi.org/10.1080/10837450902891360

FDA (2009). FDA terfenadine withdrawal. 2009. http://www.fda.gov/safety/safetyofspecificproducts/ucm180605.htm

Ferrario, D., Brustio, R. and Hartung, T. (2014). Glossary of reference terms for alternative test methods and their validation. *ALTEX 31*, 319-335. http://dx.doi.org/10.14573/altex.1403311

Filser, J. G., Csanády, G. A., Kreuzer, P. E. et al. (1995). Toxicokinetic models for volatile industrial chemicals and reactive metabolites. *Toxicol Lett 82-83*, 357-366. http://dx.doi.org/10.1016/0378-4274(95)03566-4

Forsby, A. and Blaauboer, B. (2007). Integration of in vitro neurotoxicity data with biokinetic modelling for the estimation of in vivo neurotoxicity. *Hum Exp Toxicol 26*, 333-338. http://dx.doi.org/10.1177/0960327106072994

Garrett, E. R. (1994). The Bateman function revisited: A critical reevaluation of the quantitative expressions to characterize concentrations in the one compartment body model as a function of time with first-order invasion and first-order elimination. *J Pharmacokinet Biopharm 22*, 103-128. http://dx.doi.org/10.1007/BF02353538

Gavin, C. E., Gunter, K. K. and Gunter, T. E. (1992). Mn^{2+} sequestration by mitochondria and inhibition of oxidative phosphorylation. *Toxicol Appl Pharmacol 115*, 1-5. http://dx.doi.org/10.1016/0041-008X(92)90360-5

Genter, M. B., Marlowe, J., Kevin Kerzee, J. et al. (2006). Naphthalene toxicity in mice and aryl hydrocarbon receptor-mediated CYPs. *Biochem Biophys Res Commun 348*, 120-123. http://dx.doi.org/10.1016/j.bbrc.2006.07.025

Gómez-Lechón, M. J., Donato, T., Ponsoda, X. et al. (2003). Human hepatic cell cultures: In vitro and in vivo drug metabolism. *Altern Lab Anim 31*, 257-265.

Gordon, S., Daneshian, M., Bouwstra, J. et al. (2015). Non-animal models of epithelial barriers (skin, intestine and lung) in research, industrial applications and regulatory toxicology. *ALTEX 32*, 327-378. http://dx.doi.org/10.14573/altex.1510051

Gozalbes, R., Jacewicz, M., Annand, R. et al. (2011). QSAR-based permeability model for drug-like compounds. *Bioorg Med Chem 19*, 2615-2624. http://dx.doi.org/10.1016/j.bmc.2011.03.011

Griesinger, C., Hoffmann, S., Kinsner, A. et al. (2008). The emerging concept of evidence-based toxicology (EBT) – results of the 1st international forum towards EBT. *ALTEX 25 Suppl*. http://www.altex.ch/resources/griesinger.pdf

Griffin, S. J. and Houston, J. B. (2005). Prediction of in vitro intrinsic clearance from hepatocytes: Comparison of suspensions and monolayer cultures. *Drug Metab Dispos 33*, 115-120.

Groothuis, F. A., Heringa, M. B., Nicol, B. et al. (2013). Dose metric considerations in in vitro assays to improve quantitative in vitro-in vivo dose extrapolations. *Toxicology 332*, 30-40. http://dx.doi.org/10.1016/j.tox.2013.08.012

Gubbels-van Hal, W. M. L. G., Blaauboer, B. J., Barentsen, H. M. et al. (2005). An alternative approach for the safety evaluation of new and existing chemicals, an exercise in integrated testing. *Regul Toxicol Pharmacol 42*, 284-295. http://dx.doi.org/10.1016/j.yrtph.2005.05.002

Guillouzo, A., Corlu, A., Aninat, C. et al. (2007). The human hepatoma HepaRG cells: A highly differentiated model for studies of liver metabolism and toxicity of xenobiotics. *Chem Biol Interact 168*, 66-73. http://dx.doi.org/10.1016/j.cbi.2006.12.003

Guzelian, P. S., Victoroff, M. S., Halmes, N. C. et al. (2005). Evidence-based toxicology: A comprehensive framework for causation. *Hum Exp Toxicol 24*, 161-201. http://dx.doi.org/10.1191/0960327105ht517oa

Hamon, J., Renner, M., Jamei, M. et al. (2015). Quantitative in vitro to in vivo extrapolation of tissues toxicity. *Toxicol In Vitro 30*, 203-216. http://dx.doi.org/10.1016/j.tiv.2015.01.011

Hann, M. M. and Simpson, G. L. (2014). Intracellular drug concentration and disposition – the missing link? *Methods 68*, 283-285. http://dx.doi.org/10.1016/j.ymeth.2014.05.009

Hartung, T. (2008). Food for thought ... on alternative methods for cosmetics safety testing. *ALTEX 25*, 147-162. http://www.altex.ch/All-issues/Issue.50.html?iid=101&aid=1

Hartung, T. (2009). Food for thought … on evidence-based toxicology. *ALTEX 26*, 75-82. http://www.altex.ch/Current-issue.50.html?iid=105&aid=1

Hartung, T. and Rovida, C. (2009). That which must not, can not be... A reply to the EChA and EDF responses to the REACH analysis of animal use and costs. *ALTEX 26*, 307-311. http://www.altex.ch/Current-issue.50.html?iid=111&aid=7

Hartung, T. (2010a). Comparative analysis of the revised Directive 2010/63/EU for the protection of laboratory animals with its predecessor 86/609/EEC – a t[4] report. *ALTEX 27*, 285-303. http://www.altex.ch/Current-issue.50.html?iid=121&aid=5

Hartung, T. (2010b). Food for thought ... on alternative methods for chemical safety testing. *ALTEX 27*, 3-14. http://www.altex.ch/Current-issue.50.html?iid=112&aid=1

Hartung, T. and Sabbioni, E. (2011). Alternative in vitro assays in nanomaterial toxicology. *Wiley Interdiscip Rev Nanomed Nanobiotechnol 3*, 545-573. http://dx.doi.org/10.1002/wnan.153

Hartung, T., Blaauboer, B. J., Bosgra, S. et al. (2011). An expert consortium review of the EC-commissioned report "alternative (non-animal) methods for cosmetics testing: Current status and future prospects – 2010." *ALTEX 28*, 183-209. http://dx.doi.org/10.14573/altex.2011.3.183

Hartung, T., van Vliet, E., Jaworska, J. et al. (2012). Food for thought ... Systems toxicology. *ALTEX 29*, 119-128. http://dx.doi.org/10.14573/altex.2012.2.119

Hartung, T. (2013). Food for thought … Look back in anger – What clinical studies tell us about preclinical work. *ALTEX 30*, 275-291. http://dx.doi.org/10.14573/altex.2013.3.275

Hartung, T., Luechtefeld, T., Maertens, A. et al. (2013). Food for thought … Integrated testing strategies for safety assessments. *ALTEX 30*, 3-18. http://dx.doi.org/10.14573/altex.2013.1.003

Hatton, G. B., Yadav, V., Basit, A. W. et al. (2015). Animal farm: Considerations in animal gastrointestinal physiology and relevance to drug delivery in humans. *J Pharm Sci 104*, 2747-2776. http://dx.doi.org/10.1002/jps.24365

Heringa, M. B., Schreurs, R. H., Busser, F. et al. (2004). Toward more useful in vitro toxicity data with measured free concentrations. *Environ Sci Technol 38*, 6263-6270. http://dx.doi.org/10.1021/es049285w

Hoffmann, S. and Hartung, T. (2006). Toward an evidence-based toxicology. *Hum Exp Toxicol 25*, 497-513. http://dx.doi.org/10.1191/0960327106het648oa

Honig, P. K., Woosley, R. L., Zamani, K. et al. (1992). Changes in the pharmacokinetics and electrocardiographic pharmacodynamics of terfenadine with concomitant administration of erythromycin. *Clin Pharmacol Ther 52*, 231-238. http://dx.doi.org/10.1038/clpt.1992.135

Honig, P. K., Wortham, D. C., Zamani, K. et al. (1993). Effect of concomitant administration of cimetidine and ranitidine on the pharmacokinetics and electrocardiographic effects of terfenadine. *Eur J Clin Pharmacol 45*, 41-46. http://dx.doi.org/10.1007/BF00315348

Hood, L. and Galas, D. (2003). The digital code of DNA. *Nature 421*, 444-448. http://dx.doi.org/10.1038/nature01410

Houston, J. B. and Kenworthy, K. E. (2000). In vitro-in vivo scaling of CYP kinetic data not consistent with the classical Michaelis-Menten model. *Drug Metab Dispos 28*, 246-254.

Jonczyk, R., Kurth, T., Lavrentieva, A. et al. (2016). Living cell microarrays: An overview of concepts. *Microarrays (Basel) 5*. http://dx.doi.org/10.3390/microarrays5020011

Kaitin, K. I. and DiMasi, J. A. (2011). Pharmaceutical innovation in the 21st century: New drug approvals in the first decade, 2000-2009. *Clin Pharmacol Ther 89*, 183-188. http://dx.doi.org/10.1038/clpt.2010.286

Kassel, D. B. (2004). Applications of high-throughput ADME in drug discovery. *Curr Opin Chem Biol 8*, 339-345. http://dx.doi.org/10.1016/j.cbpa.2004.04.015

Kennedy, T. (1997). Managing the drug discovery/development interface. *Drug Discov Today 2*, 436-444. http://dx.doi.org/10.1016/S1359-6446(97)01099-4

Khetani, S. R., Kanchagar, C., Ukairo, O. et al. (2013). Use of micropatterned cocultures to detect compounds that cause drug-induced liver injury in humans. *Toxicol Sci 132*, 107-117. http://dx.doi.org/10.1093/toxsci/kfs326

Kleensang, A., Maertens, A., Rosenberg, M. et al. (2014). t[4] workshop report: Pathways of toxicity. *ALTEX 31*, 53-61. http://dx.doi.org/10.14573/altex.1309261

Kola, I. and Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov 3*, 711-715. http://dx.doi.org/10.1038/nrd1470

Kramer, N. I., Di Consiglio, E., Blaauboer, B. J. et al. (2015). Biokinetics in repeated-dosing in vitro drug toxicity studies. *Toxicol In Vitro 30*, 217-224. http://dx.doi.org/10.1016/j.tiv.2015.09.005

Krauss, M., Schaller, S., Borchers, S. et al. (2012). Integrating cellular metabolism into a multiscale whole-body model. *PLoS Comput Biol 8*, e1002750. http://dx.doi.org/10.1371/journal.pcbi.1002750

Kubinyi, H. (2003). Drug research: Myths, hype and reality. *Nat Rev Drug Discov 2*, 665-668. http://dx.doi.org/10.1038/nrd1156

Leist, M., Hasiwa, N., Rovida, C. et al. (2014). Consensus report on the future of animal-free systemic toxicity testing. *ALTEX 31*, 341-356. http://dx.doi.org/10.14573/altex.1406091

Li, C., Shang, D., Wang, Y. et al. (2012). Characterizing the network of drugs and their affected metabolic subpathways. *PloS One 7*, e47326. http://dx.doi.org/10.1371/journal.pone.0047326

Lin, J. H. (1995). Species similarities and differences in pharmacokinetics. *Drug Metab Dispos 23*, 1008-1021.

Lock, E. F., Abdo, N., Huang, R. et al. (2012). Quantitative high-throughput screening for chemical toxicity in a population-based in vitro model. *Toxicol Sci 126*, 578-588. http://dx.doi.org/10.1093/toxsci/kfs023

Louisse, J., de Jong, E., van de Sandt, J. J. M. et al. (2010). The use of in vitro toxicity data and physiologically based kinetic modeling to predict dose-response curves for in vivo developmental toxicity of glycol ethers in rat and man. *Toxicol Sci 118*, 470-484. http://dx.doi.org/10.1093/toxsci/kfq270

Mandell, L. and Tillotson, G. (2002). Safety of fluoroquinolones: An update. *Can J Infect Dis 13*, 54-61. http://dx.doi.org/10.1155/2002/864789

Marroquin, L. D., Hynes, J., Dykens, J. A. et al. (2007).

Circumventing the Crabtree effect: Replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. *Toxicol Sci 97*, 539-547. http://dx.doi.org/10.1093/toxsci/kfm052

Martignoni, M., Groothuis, G. M. M. and de Kanter, R. (2006). Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol 2*, 875-894. http://dx.doi.org/10.1517/17425255.2.6.875

Marx, U., Andersson, T. B., Bahinski, A. et al. (2016). Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. *ALTEX 33*, 272-321. http://dx.doi.org/10.14573/altex.1603161

McLanahan, E. D., White, P., Flowers, L. et al. (2014). The use of PBPK models to inform human health risk assessment: Case study on perchlorate and radioiodide human lifestage models. *Risk Anal 34*, 356-366. http://dx.doi.org/10.1111/risa.12101

Mehta, R., Shangari, N. and O'Brien, P. J. (2008). Preventing cell death induced by carbonyl stress, oxidative stress or mitochondrial toxins with vitamin B anti-AGE agents. *Mol Nutr Food Res 52*, 379-385. http://dx.doi.org/10.1002/mnfr.200600190

Meyer, J. N., Leung, M. C. K., Rooney, J. P. et al. (2013). Mitochondria as a target of environmental toxicants. *Toxicol Sci 134*, 1-17. http://dx.doi.org/10.1093/toxsci/kft102

Moroy, G., Martiny, V. Y., Vayer, P. et al. (2011). Toward in silico structure-based ADMET prediction in drug discovery. *Drug Discov Today 17*, 44-55. http://dx.doi.org/10.1016/j.drudis.2011.10.023

Muoth, C., Wichser, A., Monopoli, M. et al. (2016). A 3D co-culture microtissue model of the human placenta for nanotoxicity assessment. *Nanoscale 8*, 17322-17332. http://dx.doi.org/10.1039/C6NR06749B

Musther, H., Olivares-Morales, A., Hatley, O. J. D. et al. (2014). Animal versus human oral drug bioavailability: Do they correlate? *Eur J Pharm Sci 57*, 280-291. http://dx.doi.org/10.1016/j.ejps.2013.08.018

Oldendorf, W. H. (1970). Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. *Brain Res 24*, 372-376. http://dx.doi.org/10.1016/0006-8993(70)90123-X

Omura, T. (2006). Mitochondrial P450s. *Chem Biol Interact 163*, 86-93. http://dx.doi.org/10.1016/j.cbi.2006.06.008

Oomen, P. E., Skolimowski, M. D. and Verpoorte, E. (2016). Implementing oxygen control in chip-based cell and tissue culture systems. *Lab Chip 16*, 3394-3414. http://dx.doi.org/10.1039/C6LC00772D

Paini, A., Punt, A., Viton, F. et al. (2010). A physiologically based biodynamic (PBBD) model for estragole DNA binding in rat liver based on in vitro kinetic data and estragole DNA adduct formation in primary hepatocytes. *Toxicol Appl Pharmacol 245*, 57-66. http://dx.doi.org/10.1016/j.taap.2010.01.016

Pastoor, T. P., Bachman, A. N., Bell, D. R. et al. (2014). A 21st century roadmap for human health risk assessment. *Crit Rev Toxicol 44, Suppl 3*, 1-5. http://dx.doi.org/10.3109/10408444.2014.931923

Pfaller, W., Prieto, P., Dekant, W. et al. (2015). The Predict-IV project: Towards predictive toxicology using in vitro techniques. *Toxicol In Vitro 30*, 1-3. http://dx.doi.org/10.1016/j.tiv.2015.10.006

Picollet-D'hahan, N., Dolega, M. E., Liguori, L. et al. (2016). A 3D toolbox to enhance physiological relevance of human tissue models. *Trends Biotechnol 34*, 757-769. http://dx.doi.org/10.1016/j.tibtech.2016.06.012

Rietjens, I. M. C. M., Louisse, J. and Punt, A. (2011). Tutorial on physiologically based kinetic modeling in molecular nutrition and food research. *Mol Nutr Food Res 55*, 941-956. http://dx.doi.org/10.1002/mnfr.201000655

Rodríguez-Enríquez, S., Juárez, O., Rodríguez-Zavala, J. S. et al. (2001). Multisite control of the Crabtree effect in ascites hepatoma cells. *Eur J Biochem 268*, 2512-2519. http://dx.doi.org/10.1046/j.1432-1327.2001.02140.x

Rostami-Hodjegan, A. and Tucker, G. T. (2007). Simulation and prediction of in vivo drug metabolism in human populations from in vitro data. *Nat Rev Drug Discov 6*, 140-148. http://dx.doi.org/10.1038/nrd2173

Rostami-Hodjegan, A. (2012). Physiologically based pharmacokinetics joined with in vitro-in vivo extrapolation of ADME: A marriage under the arch of systems pharmacology. *Clin Pharmacol Ther 92*, 50-61. http://dx.doi.org/10.1038/clpt.2012.65

Sacco, M. G., and Vezzoni, P. (2004). Response to Gribaldo and Hartung: New hepatocytes for toxicology? *Trends Biotechnol 22*, 615-616. http://dx.doi.org/10.1016/j.tibtech.2004.10.011

Samuel, G. O., Hoffmann, S., Wright, R. A. et al. (2016). Guidance on assessing the methodological and reporting quality of toxicologically relevant studies: A scoping review. *Environ Int 92-93*, 630-646. http://dx.doi.org/10.1016/j.envint.2016.03.010

Sexton, K., Balharry, D., Brennan, P. et al. (2011). Proteomic profiling of human respiratory epithelia by iTRAQ reveals biomarkers of exposure and harm by tobacco smoke components. *Biomarkers 16*, 567-576. http://dx.doi.org/10.3109/1354750X.2011.608855

Shanks, N., Greek, R. and Greek, J. (2009). Are animal models predictive for humans? *Philos Ethics Humanit Med 4*, 2. http://dx.doi.org/10.1186/1747-5341-4-2

Shih, H., Pickwell, G. V., Guenette, D. K. et al. (1999). Species differences in hepatocyte induction of CYP1A1 and CYP1A2 by omeprazole. *Hum Exp Toxicol 18*, 95-105. http://dx.doi.org/10.1191/096032799678839699

Singh, S. S. (2006). Preclinical pharmacokinetics: An approach towards safer and efficacious drugs. *Curr Drug Metab 7*, 165-182. http://dx.doi.org/10.2174/138920006775541552

Sjögren, E., Thörn, H. and Tannergren, C. (2016). In silico modeling of gastrointestinal drug absorption: predictive performance of three physiologically based absorption models. *Mol Pharm 13*, 1763-1778. http://dx.doi.org/10.1021/acs.molpharmaceut.5b00861

Skardal, A., Shupe, T. and Atala, A. (2016). Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. *Drug Discov Today 21*, 1399-1411. http://dx.doi.org/10.1016/j.drudis.2016.07.003

Smirnova, L., Hogberg, H. T., Leist, M. et al. (2014). Developmental neurotoxicity – challenges in the 21st century and in vitro opportunities. *ALTEX 31*, 129-156. http://dx.doi.org/10.14573/altex.1403271

Stephens, M. L., Betts, K., Beck, N. B. et al. (2016). The emergence of systematic review in toxicology. *Toxicol Sci 152*, 10-16. http://dx.doi.org/10.1093/toxsci/kfw059

Szkolnicka, D. and Hay, D. C. (2016). Concise review: Advances in generating hepatocytes from pluripotent stem cells for translational medicine. *Stem Cells 34*, 1421-1426. http://dx.doi.org/10.1002/stem.2368

Tamaddoni-Nezhad, A., Chaleil, R., Kakas, A. C. et al. (2007). Modeling the effects of toxins in metabolic networks. *IEEE Eng Med Biol Mag 26*, 37-46. http://dx.doi.org/10.1109/MEMB.2007.335590

Tan, S. M., Sharma, A., Yuen, D. Y. C. et al. (2013). The modified selenenyl amide, M-hydroxy ebselen, attenuates diabetic nephropathy and diabetes-associated atherosclerosis in ApoE/GPx1 double knockout mice. *PloS One 8*, e69193. http://dx.doi.org/10.1371/journal.pone.0069193

Tollefsen, K. E., Scholz, S., Cronin, M. T. et al. (2014). Applying adverse outcome pathways (AOPs) to support integrated approaches to testing and assessment (IATA). *Regul Toxicol Pharmacol 70*, 629-640. http://dx.doi.org/10.1016/j.yrtph.2014.09.009

Tsaioun, K. and Jacewicz, M. (2009). De-risking drug discovery with ADDME – avoiding drug development mistakes early. *Altern Lab Anim 37, Suppl 1*, 47-55.

Tsaioun, K., Bottlaender, M., Mabondzo, A. et al. (2009). ADDME – avoiding drug development mistakes early: Central nervous system drug discovery perspective. *BMC Neurol 9, Suppl 1*, S1. http://dx.doi.org/10.1186/1471-2377-9-S1-S1

van de Waterbeemd, H. (2002). High-throughput and in silico techniques in drug metabolism and pharmacokinetics. *Curr Opin Drug Discov Devel 5*, 33-43.

van de Waterbeemd, H. and Gifford, E. (2003). ADMET in silico modelling: Towards prediction paradise? *Nat Rev Drug Discov 2*, 192-204. http://dx.doi.org/10.1038/nrd1032

Veazey, R. S. (2013). Animal models for microbicide safety and efficacy testing. *Curr Opin HIV AIDS 8*, 295-303. http://dx.doi.org/10.1097/coh.0b013e328361d096

Vermeir, M., Annaert, P., Mamidi, R. N. V. S. et al. (2005). Cell-based models to study hepatic drug metabolism and enzyme induction in humans. *Expert Opin Drug Metab Toxicol 1*, 75-90. http://dx.doi.org/10.1517/17425255.1.1.75

Verwei, M., van Burgsteden, J. A., Krul, C. A. M. et al. (2006). Prediction of in vivo embryotoxic effect levels with a combination of in vitro studies and PBPK modelling. *Toxicol Lett 165*, 79-87. http://dx.doi.org/10.1016/j.toxlet.2006.01.017

Visone, R., Gilardi, M., Marsano, A. et al. (2016). Cardiac meets skeletal: What's new in microfluidic models for muscle tissue engineering. *Molecules 21*. http://dx.doi.org/10.3390/molecules21091128

Wagner, A. (1996). Does evolutionary plasticity evolve? *Evolution 50*, 1008. http://dx.doi.org/10.2307/2410642

Williams, D. P. (2014). Modeling hepatic drug metabolism and toxicity: Where are we heading? *Future Med Chem 6*, 725-727. http://dx.doi.org/10.4155/fmc.14.31

Wilmes, A., Limonciel, A., Aschauer, L. et al. (2013). Application of integrated transcriptomic, proteomic and metabolomic profiling for the delineation of mechanisms of drug induced cell stress. *J Proteomics 79*, 180-194. http://dx.doi.org/10.1016/j.jprot.2012.11.022

Wunderlich, V., Tetzlaff, I. and Graffi, A. (1972). Studies on nitrosodimethylamine: Preferential methylation of mitochondrial DNA in rats and hamsters. *Chem Biol Interact 4*, 81-89. http://dx.doi.org/10.1016/0009-2797(72)90001-4

Yoon, M., Kedderis, G. L., Yan, G. Z. et al. (2015). Use of in vitro data in developing a physiologically based pharmacokinetic model: Carbaryl as a case study. *Toxicology 332*, 52-66. http://dx.doi.org/10.1016/j.tox.2014.05.006

Zhang, D., Luo, G., Ding, X. et al. (2012). Preclinical experimental models of drug metabolism and disposition in drug discovery and development. *Acta Pharmaceutica Sinica B 2*, 549-561. http://dx.doi.org/10.1016/j.apsb.2012.10.004

Zurlo, J. (2011). Evidence-based toxicology collaboration kick-off meeting. *ALTEX 28*, 152. http://www.altex.ch/resources/altex_2011_2_149_155_ConfRep.pdf

Conflict of interest statement

The authors have no conflict of interest to declare.

Acknowledgements

The Evidence-based Toxicology Collaboration is funded by the Center for Alternatives to Animal Testing (CAAT).

Correspondence to

Thomas Hartung, MD PhD
Center for Alternatives to Animal Testing
Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Str.
Baltimore, MD, 21205, USA
e-mail: thartun1@jhu.edus