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EURL ECVAM strategy for achieving 3Rs impact in the assessment of toxicokinetics and systemic toxicity

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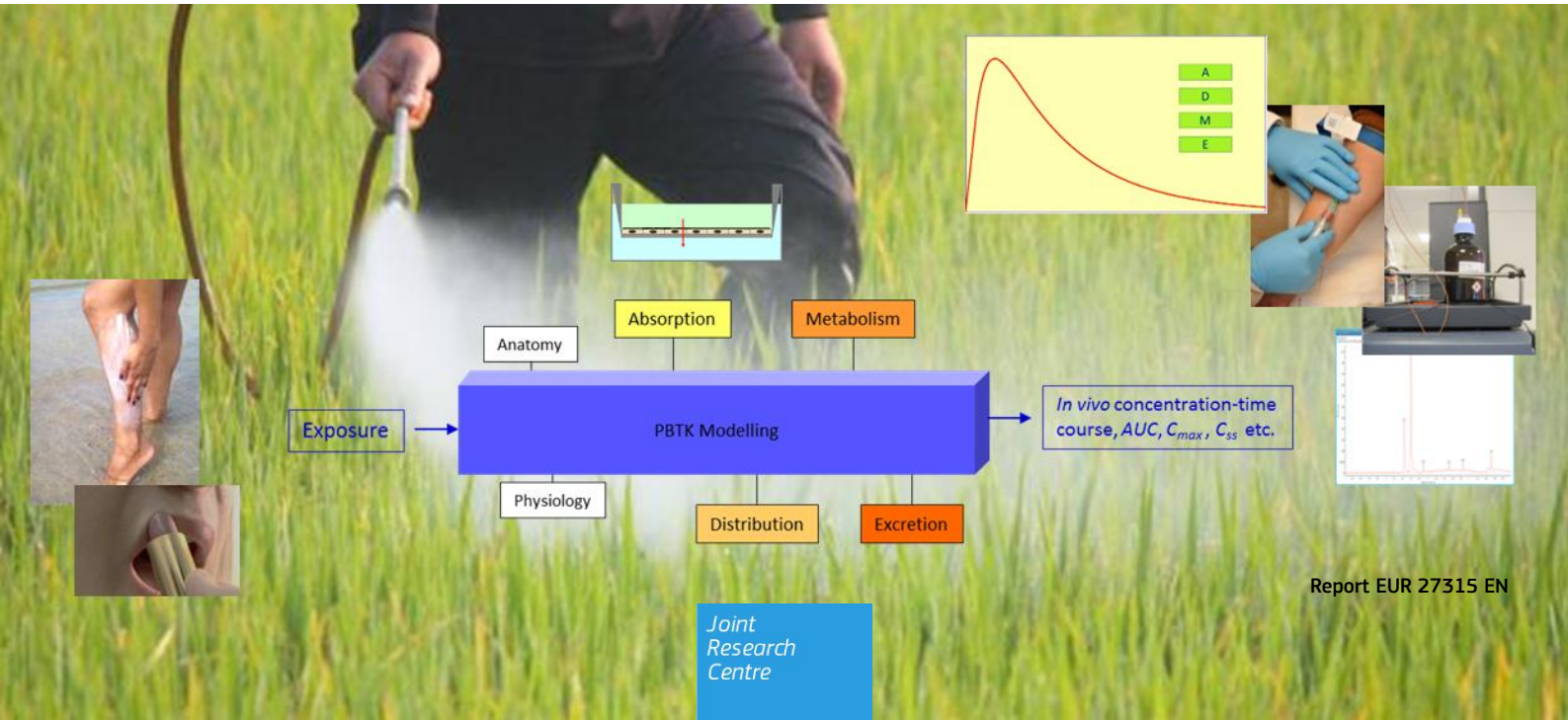
2015



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Abstract

Information on human toxicokinetics plays an important role in the safety assessment of chemicals, even though there are few data requirements in the EU regulatory framework. While existing EU test methods and OECD test guidelines are mostly based on animal procedures, there are increasing opportunities to achieve a 3Rs impact in this area by exploiting modern developments. For example, whole-body toxicokinetic information can be obtained by using physiologically-based toxicokinetic (PBTK) models that integrate data generated by in vitro methods for absorption, distribution, metabolism and excretion (ADME). The development of an infrastructure providing access to such models and their underlying data needs to be accompanied by the establishment of standards for human in vitro ADME methods, the development of guidance on the development and application of such models and the creation of regulatory incentives. Taking these needs into account, this report describes the EURL ECVAM strategy to achieve a 3Rs impact in the area of toxicokinetics and systemic toxicity. The proposed activities are expected to lay the foundation for a risk assessment approach that is increasingly based on human data. Implementation of the strategy will rely on the coordinated efforts of multiple stakeholders.

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Executive summary

Information on the human toxicokinetics, or biological fate of a substance, plays an important role in human safety assessment. While there are few explicit requirements in EU chemicals legislation for the generation of human toxicokinetic data, such as human *in vitro* or *in vivo* measurements or computational predictions, the use of these data to support the assessment of systemic toxicity is widely recommended in regulatory guidance. For the generation of data, some EU test methods and OECD test guidelines are available, but these are mostly based on animal procedures, the traditional means of obtaining whole-body toxicokinetic parameters. Exploiting modern developments in predictive toxicology, there are increasing opportunities to generate human whole-body toxicokinetic information by using physiologically-based toxicokinetic (PBTK) models. These models provide a means of integrating human data generated by in silico and in vitro methods for absorption, distribution, metabolism and excretion (ADME), the four underlying processes driving toxicokinetic behaviour. In general however, the lack of standardisation of these methods is hampering their regulatory acceptance and use.

This report outlines the strategy proposed by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) for achieving a 3Rs (replacement, reduction and refinement of animal experiments) impact in the area of toxicokinetics. The EURL ECVAM strategy identifies opportunities for generating and making better use of toxicokinetic data. Apart from specifying strategic aims and associated objectives to progress this field, the strategy is also intended to provide a framework for the identification and prioritisation of alternative test methods for ADME.

Efforts in this area should be directed towards developing standards that will increase the development, harmonisation, validation and acceptance of human-relevant methods for ADME of substances, including nanomaterials. This will enable the generation of reliable data for toxicokinetic modelling in support of chemical safety assessment. Although ADME and toxicokinetics usually consider single substances, the information obtained may inform on risk assessment issues of mixtures and combined exposures as well. In parallel, in order to promote modelling efforts, an infrastructure needs to be established to make any human data, as well as existing animal data, readily available. To enhance the uptake of PBTK models, good modelling practice needs to be further developed and accepted at an international level. Finally, guidance is needed on how best to use human ADME and toxicokinetics data for decision making purposes. Regulatory anchoring might provide a boost in this respect. These efforts are expected to lay the foundation for a risk assessment approach that is increasingly based on human data, ultimately obviating the need for animal studies.

The implementation of this strategy will rely not only on the efforts of EURL ECVAM, but on the collective and coordinated contribution of a wide range of stakeholders.

Glossary[1]

3Rs	replacement, reduction and refinement (of animal experiments)
ADME	absorption, distribution, metabolism and excretion
AUC	area under (the plasma concentration-time) curve
BPR	EU Regulation on Biocidal Products (EU, 2012); Regulation (EU) No 528/2012
CLP	EU Regulation on Classification, Labelling and Packaging of substances and mixtures (EC, 2008); Regulation (EC) No 1272/2008
C_{max}	maximum concentration (typically in plasma) following specified exposure/dose
C_{ss}	steady state concentration (typically in plasma) following specified exposure/dose
CTK	classical toxicokinetic (modelling)
CPR	EU Cosmetic Products Regulation (EC, 2009b); Regulation (EC) No 1223/2009
DB-ALM	EURL ECVAM DataBase service on ALternative Methods
EC	European Communities
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
F	fraction bioavailable unchanged (parent substance, i.e. non-metabolised)
human	based on a human *in vitro* method, a human *in vivo* measurement or on a prediction tool based on one of these data types (PBTK, QSAR)
IATA	integrated approaches to testing and assessment (to accommodate use of non-animal data)
KinCalTool	EURL ECVAM Kinetics Calculation Tool
KinParDB	EURL ECVAM Kinetic Parameters DataBase
OECD	Organisation for Economic Co-operation and Development
PBTK	physiologically-based toxicokinetic (modelling)
PPPR	EU Regulations on Plant Protection Products (EC, 2009a; EU, 2013); Regulation (EC) No 1107/2009 and Commission Regulation (EU) No 283/2013
QSAR	quantitative structure-activity relationship
REACH	EU Regulation on Registration, Evaluation, Authorisation and restriction of CHemicals (EC, 2006); Regulation (EC) No 1907/2006
SCCS	European Commission Scientific Committee on Consumer Safety
SEURAT-1	EC-FP7/Cosmetics Europe initiative on Safety Evaluation Ultimately Replacing Animal Testing - Towards the Replacement of *in vivo* Repeated Dose Systemic Toxicity Testing
TG	test guideline
TK	toxicokinetics – describes and models the time-dependent fate of a substance within the body in dependence of its ADME (rate and extent); synonymous to pharmacokinetics or PK
$t_{1/2}$	blood/plasma half-life
t_{max}	time to maximum concentration (typically in plasma) following specified exposure/dose
TWI	Tolerable Weekly Intake

[1] Coloured terms are clickable for direct internet links

Contents

1 – Introduction

Toxicokinetics (TK) describes the concentration and time-dependent fate of a substance within an organism whereas toxicodynamics describes the subsequent interaction with biological targets and how this may lead to adverse health effects. The time-course of the internal or systemic exposure is the combined outcome of four underlying processes: absorption, distribution, metabolism and excretion (ADME). Although they are strongly linked, it is important to distinguish between ADME and TK. *In vitro* methods can provide data on individual ADME parameters, but do not directly generate whole-body (animal or human) TK parameters such as the maximum concentration reached in blood/plasma. Traditionally, TK parameters have been obtained from *in vivo* experiments, but there are increasing opportunities to derive this information by physiologically-based toxicokinetic (PBTK) modelling (Figure 1). PBTK models provide a means of simulating TK profiles by integrating (chemical-independent) physiological and anatomical information with (chemical-dependent) ADME parameters. The latter can be generated by quantitative structure-activity relationship (QSAR) models and *in vitro* methods. PBTK models are increasingly being used in the chemical risk assessment process to take into account relevant *in vivo* differences (cross-species, cross-route and inter-individual) and to make better use of *in vitro* toxicity results.

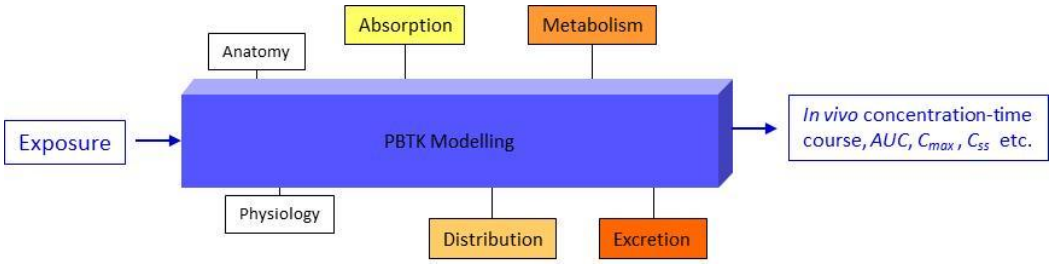

Figure 1: Physiologically-based toxicokinetic (PBTK) modelling integrating ADME parameters derived from *in silico* and *in vitro* methods to simulate the concentration-time course of a substance *in vivo*.

There are very few legal requirements in the EU chemicals legislation for the generation of human ADME and TK data and the requirements for ADME and TK data are not consistent (Table 1). However, the use of ADME/TK data when available to support the assessment of systemic toxicity is highly recommended in regulatory guidance and scientific opinions (Table 2). For the generation of new data, only three ADME/TK test methods are available in the EU test methods regulation (EU, 2012) and in the OECD (Organisation for Economic Co-operation and Development) guidelines for the testing of chemicals. With the exception of OECD TG 428 for *in vitro* dermal absorption, guideline methods such as OECD TGs 417 and 427 are based on animal tests (OECD, 2004a; OECD, 2004b; OECD, 2010). OECD TG 417 studies typically provide rather isolated species-, dose-, and route-specific (mostly oral) data on absorption, tissue distribution or metabolism. In rare cases, OECD TG 417 is used to give the integrated TK profile of a substance, i.e. the concentration-time course of the parent compound and its metabolites. Although many *in silico* and *in vitro* methods with varying stages of maturity are available and used for integrated PBTK modelling to predict the concentration-time course, these methods are not generally sufficiently standardised (Bessems *et*

al., 2014) and scrutinised for relevance and reliability which is hampering their regulatory acceptance and widespread use.

The need for human TK information on one hand, combined with the paucity of legal requirements for animal TK data on the other, provides an opportunity to develop a risk assessment approach that is increasingly based on human ADME and TK data. A fundamental transition is needed in the toxicological testing and risk assessment methodology away from the widely used default approach using external animal dose levels and external human exposures. At best this only accommodates the use of species- and route-specific information on absorption, instead of taking the systemic exposure (*AUC*, C_{max} etc.) into account. A scientifically more advanced and toxicologically relevant approach based on internal concentrations is highly recommended. In addition to regulatory drivers (e.g. EU ban on animal testing of cosmetic ingredients), such a transition is motivated by scientific considerations. Animals are often poor models for humans due to sometimes well-known qualitative and also quantitative differences in their physiology and metabolism (Coecke *et al.*, 2005; Pelkonen *et al.*, 2009; Greek and Menache, 2013; Coecke *et al*, 2014). In order to use human *in vitro* toxicity data for human risk assessment, a stronger focus on internal exposure (e.g. AUC and C_{max} of the putative toxicant) is warranted. In this approach, *in vitro* free (unbound to protein) concentrations (points of departure) would be compared to simulated or measured (e.g. by biomonitoring) human *in vivo* systemic exposure free concentrations. The resulting margin of internal exposure would then be used to characterise the risk (Bessems *et al.*, manuscript in preparation).

Until a full replacement of animal testing for systemic toxicity is reached, a more intelligent and systematic generation and use of ADME/TK information will provide a means of reduction and refinement. For example, the reliable prediction of extremely low absorption could support the waiving of animal toxicity test requirements based on the low internal exposure expected. The prediction and the measuring of internal exposure can also support dose range finding, e.g. avoiding irrelevant high-dose testing if high doses are predicted to result in kinetic non-linearity. Measuring systemic exposure across dose levels, sexes, study durations, species, strains and life stages in ongoing animal toxicity studies, i.e. without using satellite animals (preferably using microsampling), will significantly reduce uncertainties involved in various extrapolations needed in the risk characterisation phase (Bessems and Geraets, 2013; Terry *et al.*, 2014). Moreover, it may reduce the chance that the results of one animal study will unnecessarily trigger another. This could happen for example when non-linearity of the dose-response is caused by non-linearity of the internal exposure. Lastly, ADME properties form an important source of information in integrated approaches to testing and assessment (IATA) that are designed to optimally exploit various streams of non-animal data (OECD, 2015).

The purpose of this document is to present the EURL ECVAM strategy to avoid, reduce and refine animal testing in the assessment of toxicokinetics and systemic toxicity of substances. This strategy is also applicable to nanomaterials, although specific technical provisions are needed in places due to their particular physicochemical properties. The intention was to focus on pragmatic options that could be expected to have a significant short to mid-term 3Rs impact, while at the same time laying the foundation for a risk assessment approach that is increasingly based on human ADME/TK data. The implementation of this strategy will rely not only on the efforts of EURL ECVAM, but on the collective and coordinated contribution of a wide range of stakeholders.

2 – Regulatory provisions on ADME and TK

Within EU regulatory frameworks, route-to-route extrapolation is probably the most important use-case for information on species- and route-specific ADME and, if available, systemic exposure (whole-body TK). However, such information is not consistently required. Table 1 provides an overview of data requirements and recommendations under the frameworks that are most relevant in this respect, i.e. CLP (classification and labelling), REACH (industrial chemicals), CPR (cosmetics), BPR (biocides) and PPPR (pesticides). It is noted that over time, legislative frameworks have placed increasing emphasis on the use of systemic exposure information for human risk assessment purposes. Except for the CPR however, these needs are being addressed for the most part using animal data.

Table 1: Requirements and recommendations for ADME/TK information[2] in EU legal frameworks[3].

Regulation	Required or recommended	What ADME and/or TK parameter?	Use
CLP Regulation (EC) No 1272/2008	**Not required** but use if available	Non-specific but numerous examples about use of species- and route-specific TK information	Shall and/or should be used as weight of evidence to classify, lower the classification or abstain from classification for a particular toxicodynamic endpoint.
REACH Regulation (EC) No 1907/2006	**Not required** but use if available	TK (A, D, M, E)	In REACH Guidance documents, many examples of recommendations that would replace default assessment factors (e.g. Sections R.7.12 and R.8.4 in Chapters R.7.C and R.8, respectively).
CPR Regulation (EC) No 1223/2009	**Recommended** by SCCS (2012)	**Human** systemic exposure **Human** dermal absorption Biotransformation	Route-to-route extrapolation
BPR Regulation (EU) No 528/2012	**Required**	A: rate and extent D: tissue M: pathway + degree E: routes and rate	When accumulation indicated, 90 d study preferred over 28 d. If no significant human exposure and no systemic absorption F = 0, reproduction toxicity study not needed.
PPPR Regulation (EC) No 1107/2009 Commission Regulation (EU) No 283/2013	**Required**	Oral A, D, M, E Oral F, AUC, C_{max}, T_{max} Bioaccumulation potential, $t_{1/2}$ Often dermal A (*in vitro* **human**), D, M, E and F Sometimes inhalation A *In vitro* comparative metabolism TK short-term toxicity studies	Study design (e.g. dose selection) Interspecies extrapolation Route-to-route extrapolation Residue definition (testing of metabolites)

[2] Except when stated otherwise, in this table all ADME and TK parameters refer to ***animal*** ADME and TK.

[3] See glossary for full titles of the regulations and explanation of the toxicokinetic terms, including A, D, M, E.

In addition to the legal requirements, more detailed recommendations are laid down in various guidance documents, developed for example by the EU Member States (e.g. REACH Guidance) or by Scientific Committees (e.g. Notes of Guidance: SCCS, 2012). Table 2 provides examples of how the required and/or recommended ADME/TK information can be used in regulatory decision making in the EU.

Table 2: Use cases for ADME and TK information suggested by various EU guidance.

Use cases	Examples	Source
Waiving[4] specific *in vivo* study	Reproductive study if no systemic absorption. Dermal acute toxicity if no dermal absorption.	BPR
	If somatic genotoxicant and germ cells reached, then *in vivo* germ cell genotoxicity can be skipped.	EURL ECVAM Strategy Genotoxicity (Corvi, 2013), EURL ECVAM Strategy Acute systemic toxicity (Prieto, 2014)
	If substance accumulates, skip 28 d study and do 90 d. Inclusion blood sampling one study may avoid another.	REACH, BPR, PPPR
Read across	Toxicokinetic studies, kinetic and metabolic factors.	ECHA report alternatives (ECHA, 2014)
IATA	ADME and TK models are regarded to be basic elements.	ECHA report alternatives (ECHA, 2014), OECD WS Report (OECD, 2015)
	Skin bioavailability critical event in adverse outcome pathway skin sensitisation.	EURL ECVAM Strategy Skin sensitisation (Casati, 2013)
	Metabolic stability/clearance + metabolite identification *in vitro*. Possibly preventing *in vivo* acute systemic tox. testing.	EURL ECVAM Strategy Acute systemic toxicity (Prieto, 2014)
***In vivo* study design**	Designing (further) toxicity studies (e.g. species selection based on *in vitro* metabolism species comparison) and to help their interpretation.	SCCS (2012) Notes of Guidance, REACH Guidance on TK, R.12, Commission Regulation (EU) No 283/2013
Risk assessment extrapolations	Use of chemical-specific data on ADME and/or TK instead of default Assessment Factors.	PPPR, SCCS (2012) Notes of Guidance
	TK + human urinary data to set the TWI for cadmium	EFSA (2009)
	PBTK to reduce extrapolation uncertainty and for derivation of AOELs[5]. Quantitative use of human *in vitro* ADME data.	EFSA PPR Opinion, 2006
Risk management	Persistency and bioaccumulation noted as selection criterion for the emerging chemical risk framework.	EFSA (2014), EURL ECVAM Strategy fish acute toxicity + bioaccumulation (Halder, 2014)
	Establishment of 'common assessment groups' using human metabolism (*in silico, in vitro, in vivo*) in public health issue of exposure to mixtures.	EFSA, 2014

[4] Waiving an information need (animal study): based on arguments, not deemed necessary to carry out the study.

[5] Acceptable Operator Exposure Levels as required by Regulation (EC) No 1107/2009 (PPPR).

Comparisons between human and animal ADME/TK data (*in silico*, *in vitro* or preferably *in vivo* when available) can lead to an overall reduction of animal testing by increasing the relevance of animal toxicity studies, where these are required. This is achieved by identifying and avoiding animal species with ADME/TK for the substance that is very different from human ADME/TK. This could be based on comparison of any relevant parameter or process, e.g. most relevant metabolite(s), rate of formation of a likely toxic metabolite, and allometric scalability of various kinetic parameters such as hepatic metabolic clearance, renal clearance or plasma half-life (Bessems and Geraets, 2013). In addition, risk assessment is served best when more human ADME/TK data is available. The EFSA PPR panel noted for example already in 2006 that human ADME/TK data reduce the uncertainty related to the extrapolation process from animal toxicodynamics data both in terms of species and dose, and help in assessing the relevance for humans of findings in animals (EFSA, 2006).

There are international developments as well. OECD Guidance Document 116 on chronic and carcinogenicity test guidelines (OECD, 2012) attributes significant value to information on ADME for improving the study design. The information helps to select the highest relevant dose level in order to prevent non-linear kinetics from occurring, thus enabling refinement through study design. OECD Guidance Document 151 supports the extended one generation reproductive toxicity test guideline (OECD, 2013). It states that "ADME studies should be undertaken to facilitate extrapolation from the oral to the dermal route, if this is required". Furthermore, ADME received attention at a recent OECD workshop as being an important element of IATA (OECD, 2015).

3 – Strategy to replace, reduce and refine the use of animals for human TK

Although the value of human ADME and TK data in establishing health risks of substances is widely acknowledged, concrete guidance and case studies on how to generate the *in vitro* ADME and *in silico* TK parameters and how to use this information in different decision making contexts and within IATA is largely missing. Some human *in vitro* methods to measure an ADME property exist, such as for absorption via the gastrointestinal tract or for hepatic metabolic clearance, albeit with uncertainties regarding their applicability domain. In other cases, such as renal excretion, further efforts are needed to develop suitable methods (Mostrag-Szlichtyng and Worth, 2010; Adler *et al.*, 2011; Bessems *et al.*, 2014; EFSA, 2014). In most cases however, the available methods are not standardised. This is an impediment to their use and acceptance. Therefore, in addition to the development of new methods, there is a need to characterise existing methods in a systematic manner (Adler *et al.*, 2011; Bessems *et al.*, 2014; EFSA, 2014).

With a view to replacing, reducing and refining animal testing in the assessment of toxicokinetics and systemic toxicity, EURL ECVAM has defined ***four strategic aims*** (Figure 2).

1. **ADME methods:** Development and standardisation of human *in vitro* ADME methods.
2. **Kinetic modelling:** Portals and good kinetic modelling practice.
3. **Data collection:** Analytics and databases to serve kinetic modelling.
4. **Regulatory anchoring:** Legislation and guidance on human ADME/TK data.

The first three are intended to enhance the availability and usefulness of the necessary tools while the fourth is intended to foster a regulatory evolution towards stronger requirements for ADME and TK information based on non-animal and human-relevant approaches. In the following paragraphs, the four strategic aims are further explained and translated into concrete objectives.

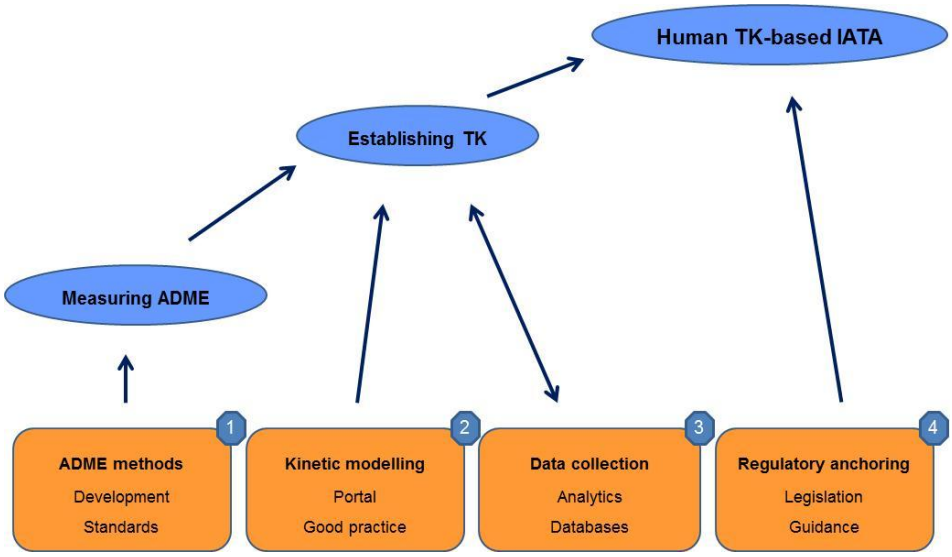

Figure 2: Four strategic aims to facilitate generation and use of human ADME and TK data.

3.1 Strategic aim 1: Development and standardisation of human ADME methods

In order to promote the acceptance of non-animal ADME/TK data for regulatory purposes, an international quality assurance framework needs to be established (SCHER, 2013; Coecke *et al*. 2014). This framework should be applicable to ADME data generated by *in vitro* test methods and QSARs, TK data generated by integrated PBTK models, as well as human *in vivo* data obtained in monitoring programs or volunteer studies (SCHER, 2013).

Some elements of this framework are already established or under development. For example, the QSAR Model Reporting Format (QMRF) and QSAR Prediction Reporting Format (QPRF) are internationally recognised standards for reporting the characteristics of QSAR models, and the quality of QSAR predictions, respectively[6]. More recently, the OECD has published a guidance document on how to characterise and describe non-guideline *in vitro* test methods (OECD, 2014). Guidance on the characterisation, documentation and application of PBTK models has been published by the World Health Organisation (WHO/IPCS, 2010).

Other elements of this framework need to be developed. In particular, standards for *in vitro* ADME methods will provide a means of characterising and comparing *in vitro* methods which typically provide the same kind of information (ADME property) but which may differ considerably in terms of the underlying test systems and experimental protocols used. Different domains (varying physicochemical properties, magnitude of output parameter) may require different standards.

Lastly and as mentioned earlier, for several ADME endpoints, test methods need to be developed, improved or their applicability domain widened.

Objective 1.1 – Development of standards for human in vitro ADME methods

In order to make better use of human *in vitro* methods for ADME properties, there is a need to develop a framework aiming to (a) describe a method including the characteristics of the test system and the results of the test method in an objective and standardised way, (b) to assess the performance of the method (reliability and relevance) and (c) to define its applicability in terms of the ranges of physicochemical properties of substances, its measurement outputs and the time-scales for which the test system is valid.

Furthermore, the OECD reporting standard for non-guideline *in vitro* methods (OECD, 2014) needs to be evaluated for its applicability to ADME methods. Additional standards may need to be established to characterise and compare different methods within a given class of methods (that generate the same ADME parameter). A few other important issues need to be taken into consideration here. One issue is the fact that not all ADME methods are necessarily designed to be directly predictive of an *in vivo* parameter as such. This means that the classical validation based on a direct comparison of a human *in vitro* ADME method data against human *in vivo* data is not meaningful[7]. Prediction methods as complex as PBTK models would be needed to interpret the human *in vitro* ADME data. Nevertheless, simple ADME parameters obtained using standardised

[6] JRC QSAR Model Database and QSAR Model Reporting Formats. https://eurl-ecvam.jrc.ec.europa.eu/databases/jrc-qsar-model-database-and-qsar-model-reporting-formats.

[7] In this context, this could mean total *in vivo* absorption (relative to the applied dose) measured over 7 days. Whereas an *in vitro* absorption method may deliver a flux, being an absorption rate per unit area (e.g. µmol min^{-1} cm^2).

human *in vitro* methods could be used directly such as for priority setting and ranking (rates of absorption) as well as in the context of IATA.

Objective 1.2 – Human route-specific absorption methodology

For *in vitro* dermal absorption, an OECD TG does exist (TG 428). However, this TG needs critical review and/or revision as the current method is focussed mainly on determining the relative amount of substance systemically absorbed and much less so on establishing dermal flux values (through the skin) and the underlying determinants such as dermal diffusion coefficients that may be needed for PBTK modelling (Bessems *et al.*, 2014). EFSA has published opinions on the adaptation and improvement of this TG, especially in relation to the interpretation and standardised reporting of results of OECD TG 428 (EFSA, 2011; EFSA, 2012). For other routes, OECD TGs and validated test methods are lacking.

It is important to improve and/or standardise a set of representative *in vitro* methods that can be used to derive harmonised standards for this class of test methods (which measure the permeability of external membranes). This is relevant to methods for assessing exposure via skin as well as by inhalation or ingestion. Although this formally applies to tissue distribution, a further step will be to improve and develop equivalent test methods and standards for *in vitro* methods that measure the passive permeability of internal membranes, such as the blood-brain barrier and the placental barrier. Obviously, in the end the same applies to active transport across barriers.

Objective 1.3 – Human tissue distribution and protein binding methodology

Several *in vitro* test methods that measure parameters that drive the distribution (partitioning coefficients, protein binding), including an indication of their level of development, are described elsewhere (Bessems *et al.*, 2014). Distribution is a key driver of phenomena like persistency. For example, high fat solubility increases the risk of persistency and bioaccumulation.

A set of standards to improve the quality and traceability of PBTK input parameters such as partitioning coefficients and protein binding is crucial. Improvements and widening of applicability domains may be needed as well.

Objective 1.4 – Human metabolic stability/clearance methodology

Human metabolic clearance levels (or their absence, defined as metabolic stability) in liver, skin and lungs being the most relevant portals of entry, are important determinants of bioavailability as well as the (pre-systemic) elimination of substances from the body. These are essential pieces of information for PBTK modelling. Metabolic clearance might even be the most influential parameter that determines terminal elimination half-life (and thus persistency and risk for human bioaccumulation), systemic toxicity upon dermal exposure, as well as inter-individual variability in plasma levels. Harmonised standards are needed since the methods submitted to EURL ECVAM and available in the scientific literature vary considerably. For example, there are differences in the *in vitro* test system (e.g. subcellular fraction, primary cells, cell lines, liver slices) and the ability to deal with fast or slowly cleared compounds (Brandon *et al.*, 2003; Di and Obach, 2015).

Objective 1.5 – Human xenobiotic metabolic pathway profiling methodology

The identification of the main and most relevant human metabolites serves multiple applications. For example, the identification of common metabolites for two substances in a mixture might trigger the assessment to be based on the properties of the common active metabolite. As a multitude of methods is used under various performance requirements, a set of standards needs to be developed for this class of methods. Induction and inhibition of biotransformation enzymes are important process as well. Induction is currently the subject of a draft OECD TG.

Objective 1.6 – Human route-specific excretion methodology

Urinary and biliary excretion pathways are the two most relevant excretion pathways. To our knowledge, there are no *in vitro* methods available at the moment for urinary excretion because of the complex renal mechanism of formation of primary urine, passive and active reuptake and the dependence of these mechanisms on differences in pressure and osmolarity between blood and primary urine. For biliary clearance, some *in vitro* methods have been reported (De Bruyn et al., 2013), but further development and standardisation work is necessary to identify representative methods based on which harmonised standards can be established. Although passive excretion has been suggested to suffice for 'Tier 1 PBTK modelling', it is clear that in the future, excretion based on active transporters needs to be taken into account as well.

3.2 Strategic aim 2: Kinetic modelling

In order to facilitate the use of PBTK modelling in the risk assessment process, there is a need to make *in silico* ADME prediction tools as well as PBTK modelling tools readily accessible and easy to apply, and to establish good practice in kinetic modelling (WHO/IPCS, 2010).

Objective 2.1 – Comprehensive web-based kinetic modelling portals

Kinetic modelling includes classical kinetic modelling approaches, PBTK modelling, and also *in silico* prediction of ADME parameters. It is generally felt to be quite a complex process that requires experts in TK, mathematical modellers and regulatory risk assessors to work together (WHO/IPCS, 2010; Bessems *et al*., 2014). The establishment of comprehensive 'one-stop' web-based kinetic modelling portals is needed to provide a collaborative environment to facilitate the development and use of kinetic models. Such portals should contain or link to freely available kinetic modelling tools and databases (objective 3.2).

Objective 2.2 – Good kinetic modelling practice

To facilitate the regulatory acceptance of PBTK models, good practice needs to be established for the development and documentation of models based on already available guidance (WHO/IPCS, 2010). It is necessary to develop standard reporting formats, equivalent in purpose to the QSAR Model Reporting Format (QMRF) and QSAR Prediction Reporting Format (QPRF), for presenting sufficient details of model construct and application. These reporting formats should include assumptions made concerning the mode of action, the most relevant qualitative dose metric

(parent or metabolite), the most relevant quantitative dose metric (*AUC*, C_{max}, rate of formation) and address uncertainty issues (see also 3.4). This will help kinetic model developers to take into account all the necessary considerations and facilitate uptake of kinetic modelling approaches in decision making.

The formation of a PBTK modelling expert group, ideally by an international organisation, would be an appropriate means of developing good PBTK modelling practice.

3.3 Strategic aim 3: Data collection - Generation and storage of ADME and TK data

To support objectives 1 and 2, a concerted action to stimulate generation and collection of data is needed. These data should ideally be quality-assured and stored in readily accessible (and preferably free-to-use) databases in order to make the process of establishing human ADME and TK parameters as transparent and efficient as possible (Bessems *et al.*, 2014). Sharing of proprietary data could enable the generation of a large, high quality database of 'paired' *in vitro* and *in vivo* human data on the same substance. This could be used to investigate the predictive value of *in vitro* data (Leist *et al.*, 2014). Databases are a necessary building block for ready to use PBTK modelling and should become an important part of comprehensive web-based PBTK modelling platforms (see objective 2.1).

Objective 3.1 – Collection of human in vitro ADME and in vivo TK information

There is considerable need for standardised *in vitro* generation of ADME parameters. Ideally, these ADME parameters should be stored in open-access curated databases. This would speed up the development of *in silico* (QSAR) ADME prediction tools as well as the parameterisation of PBTK models.

Simultaneous measurements of external exposure and systemic exposure in order to establish real-life human TK are scarce. A concerted effort is necessary to collect and store *in vivo* human ADME and TK information by building on human exposure monitoring programmes (e.g. dietary intake studies, occupational monitoring) as well as on human biomonitoring programmes (e.g. measurements in biofluids). If doubts remain and more specific human *in vivo* benchmarking is required, controlled human microdosing in volunteer studies could be performed with the use of specific isotope labelling (Madeen *et al.*, 2015).

Objective 3.2 – Databases

In order to facilitate human PBTK modelling, centralised publicly available (web-based) databases facilities containing the following are crucial:

- A collection of human ADME data obtained by *in vitro* measurements.
- Anatomical and physiological data including their variation in the human population. An example is the RIVM Interspecies database (http://www.interspeciesinfo.com);
- *In vivo* human TK information. An example is the EURL ECVAM KinParDB (https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/toxicokinetics).

Sparse but relevant kinetic data may already be available in various databases from the JRC and others that were not specifically designed for storage of ADME data, such as DB-ALM, the DataBaseservice on ALternative Methods (http://ecvam-dbalm.jrc.ec.europa.eu), the QSAR Model Database (http://ihcp.jrc.ec.europa.eu/our_databases/jrc-qsar-inventory) and databases that participate in the OECD eChemPortal (http://www.echemportal.org/echemportal/propertysearch/page.action?pageID=0).

Objective 3.3 – Sampling strategies, methods, preparations and analytical determination

Sampling strategies, sampling methods, sample pre-treatment and final analytical determination and quantification are all necessary for the transition to a human-based IATA. This pertains to *in vitro* ADME testing (Broeders *et al.*, 2012) and to the collection of human *in vivo* data on TK (biomonitoring and human volunteer studies). This also concerns *in vitro* toxicity testing where the *in vitro* kinetics should be measured, i.e. the concentration-time profile of the chemical and/or metabolites intracellular as well as in the incubation medium (Blaauboer, 2010; Wilmes *et al.*, 2013). And it pertains as well to animal toxicity testing as long as it is still performed in order to improve study design and value for risk assessment (Creton *et al.*, 2012). The issue was identified previously as a high priority (Bessems *et al.*, 2014), i.e. 'high-throughput and low cost analytical facilities to measure chemicals in physiological media'.

3.4 Strategic aim 4: Regulatory anchoring of human ADME and TK

The inherent value of human ADME and TK information in risk assessment is obvious, be it based on *in silico, in vitro* and/or *in vivo* approaches. However, it is not always obvious which particular ADME parameter (e.g. relative absorption or absorption rate), or which TK parameter (e.g. AUC or C_{max}) is needed for a particular decision. Clarity is often lacking too regarding if and how human variability should be taken into consideration and what level of uncertainty is tolerable for various ADME/TK information elements.

Objective 4.1 – Guidance on the use of human ADME and TK data in IATA

Reliable information on human ADME parameter values is helpful in the development and application of IATA. For example, extremely low absorption rates could justify the waiving of certain animal bioassays (analytical precision becomes very important here, see objective 3.3), local (skin) bioavailability is a key consideration for assessing skin sensitisation, and low whole body clearance, indicated by low urinary excretion and/or high metabolic stability, indicates human persistency of a substance. Further guidance is therefore needed on the use of ADME/TK data in IATA for specific endpoints, including how to characterise the uncertainty in conclusions drawn from these data. Uncertainty may result, for example, from the reliability and relevance of the underlying ADME/TK methods.

Objective 4.2 – Evolution of legislative anchoring of human ADME and TK information

There is growing awareness that human-relevant ADME and TK data are instrumental for a much stronger role of non-animal approaches in regulatory risk assessment. This implies a transition from the current animal-based testing paradigm to one that is based on 21st century science with greater reliance on human-relevant *in vitro* testing and human *in vivo* data (US NAS, 2007; SCHER, 2013; US EPA, 2014). In order to achieve this, there is a need for a stronger regulatory anchoring of human *in silico* and *in vitro* ADME data, of human PBTK modelling tools to integrate human ADME data as well as of collecting human *in vivo* data where possible to support this.

3.5 Timelines

In Figure 3, an overview is presented of the strategic aims and related objectives with indicative timelines for meeting the objectives. This assumes optimal conditions in terms of the availability of necessary expertise and resources.

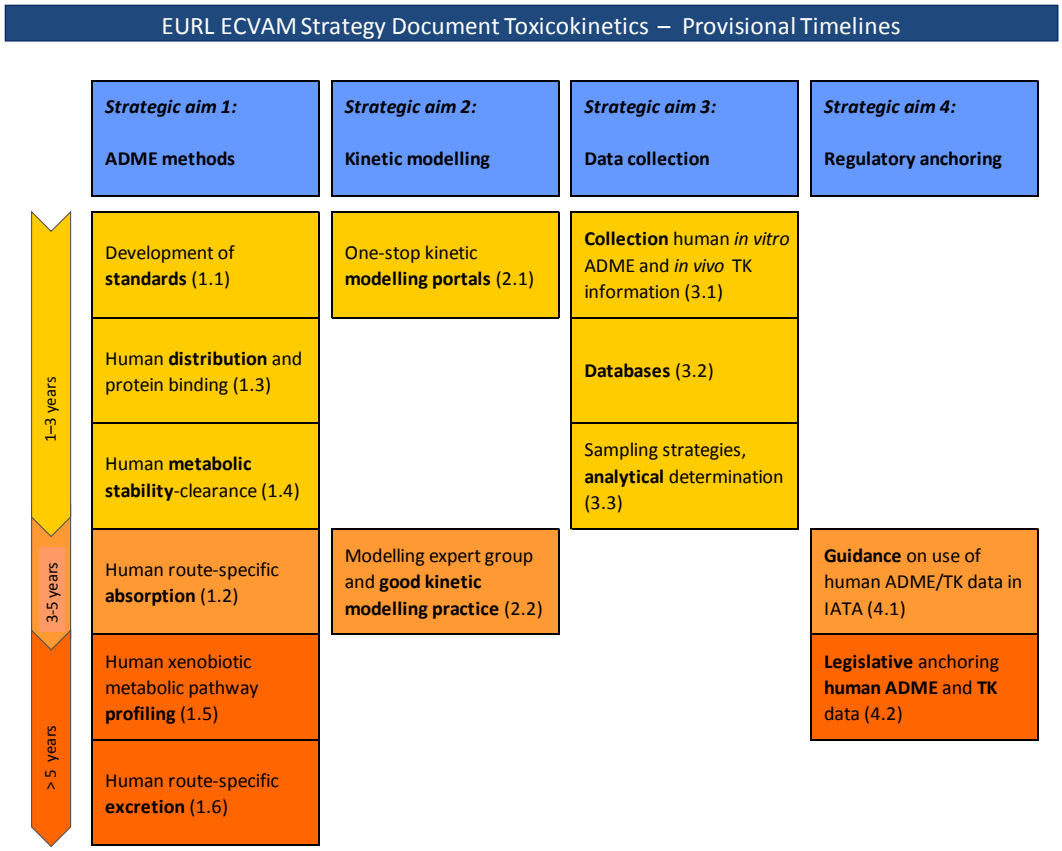

Figure 3: Indicative timelines for critical developments in the human ADME and TK areas.

4 – Conclusions

This document presents EURL ECVAM's strategic view on how to achieve a significant 3Rs impact in the assessment of toxicokinetics and systemic toxicity. Grouped under four strategic aims, a number of objectives and related activities have been identified to foster the generation and more intelligent use of human toxicokinetic data. In particular, the development of standards and a quality assurance framework to support the acceptance of human ADME/TK methods, and the targeted generation of relevant *in silico* and *in vitro* toxicokinetic data and their integration via PBTK modelling is expected to have a significant impact on the 3Rs. These activities will also lay the foundation for a more human-relevant approach to the safety assessment of chemicals. This strategy document is also intended to provide a framework for the prioritisation of alternative test methods for validation by EURL ECVAM towards regulatory acceptance and use.

EURL ECVAM has already taken some important steps consistent with these strategic aims. For example, a pilot project delivered a kinetic parameters database (KinParDB) as well as a user-friendly kinetic modelling tool (KinCalTool). More recently, EURL ECVAM has started to explore the development of standards for human *in vitro* hepatic metabolic clearance methods. Furthermore, with a view to developing a risk assessment approach based entirely on non-animal methods, EURL ECVAM has been working closely with other partners within the SEURAT-1 initiative to explore the use of *in silico* models in performing route-to-route extrapolations and *in vitro*-to-*in vivo* comparisons.

The strategy outlined here is intended to be inclusive. EURL ECVAM has an important role to play in its implementation, but achievement of the objectives will depend on the proactive and coordinated engagement of multiple stakeholders. EURL ECVAM will continue to review its work programme in the light of developments in this field and with a view to providing added value at the EU and international levels.

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