



EURL ECVAM Strategy for Toxicokinetics

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EPA, Durham, 17 Feb. 2016

The logo for EURL ECVAM, featuring the text "EURL" in large blue letters with yellow stars, and "ECVAM" in smaller blue letters below it. The background of the logo is a grid of white circles, some containing yellow stars.

EURL
ECVAM

European Union Reference Laboratory
for Alternatives to Animal Testing

The logo for the Joint Research Centre, featuring the text "Joint Research Centre" in white on a blue background.

Joint
Research
Centre

The European Union Reference Laboratory for Alternatives to Animal Testing

Established under *Directive 2010/63/EU* on the protection of animals used for scientific purposes

Key responsibilities*

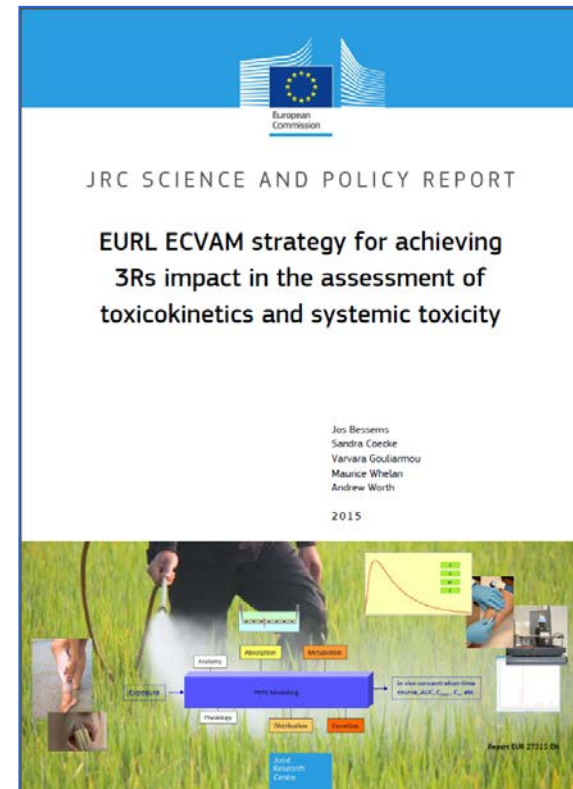
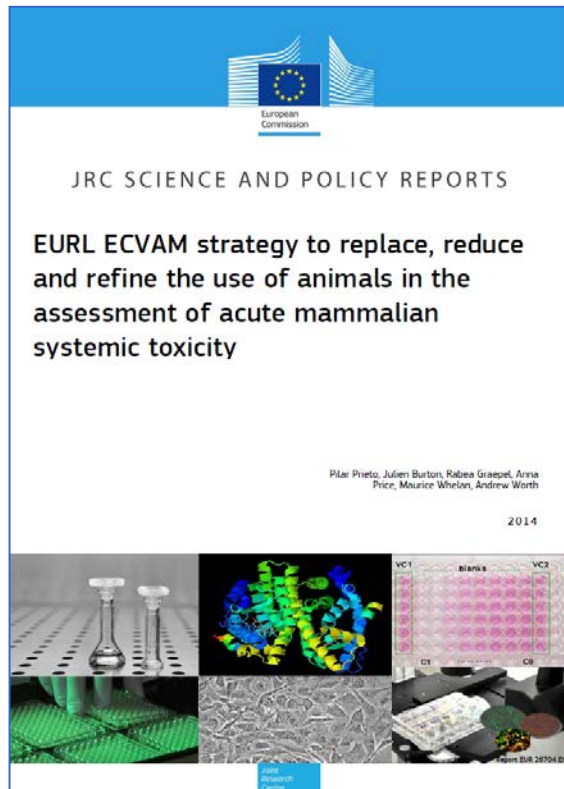
- Coordinate and promote development and use
- Coordinate validation at Union level
- Information exchange on development
- Databases and information systems
- Promote dialogue between legislators, regulators and stakeholders



*Article 48 of the Directive, Annex VII

EURL ECVAM Strategy Document (July 2015)

Opportunities for generating and making better use of toxicokinetic data in human safety assessments, ultimately avoiding the need for animal studies



Background

- Information on toxicokinetics important in human safety assessment
- Few data requirements in the EU regulatory framework

Table 1: Requirements and recommendations for ADME/TK information² in EU legal frameworks³.

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 (<i>in vitro</i> human), D, M, E and F Sometimes inhalation A <i>In vitro</i> comparative metabolism TK short-term toxicity studies	Study design (e.g. dose selection) Interspecies extrapolation Route-to-route extrapolation Residue definition (testing of metabolites)

CLP: Classification and labelling products;
 REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals;
 CPR: Cosmetic Products;
 BPR: Biocidal Products;
 PPPR: Plant protection products.

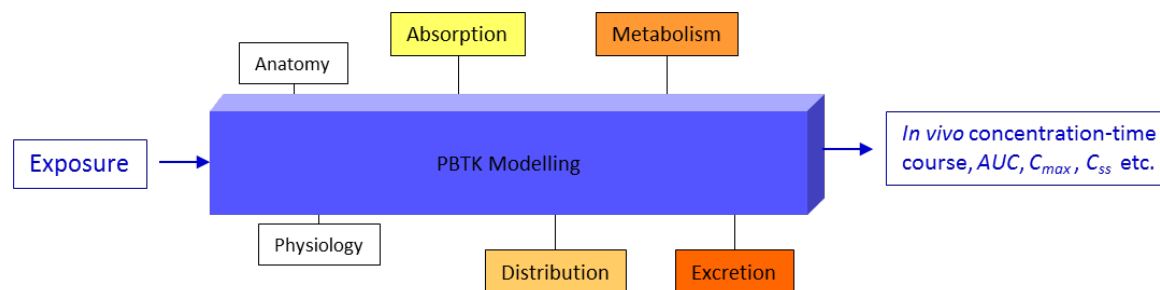
Background

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

Use cases	Examples	Source
Waiving⁴ specific <i>in vivo</i> study	Reproductive study if no systemic absorption.	BPR
	Dermal acute toxicity if no dermal absorption.	
	If somatic genotoxicant and germ cells reached, then <i>in vivo</i> 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)
	ADME and TK models are regarded to be basic elements.	ECHA report alternatives (ECHA, 2014), OECD WS Report (OECD, 2015)
IATA	Skin bioavailability critical event in adverse outcome pathway skin sensitisation.	EURL ECVAM Strategy Skin sensitisation (Casati, 2013)
	Metabolic stability/clearance + metabolite identification <i>in vitro</i> . Possibly preventing <i>in vivo</i> acute systemic tox. testing.	EURL ECVAM Strategy Acute systemic toxicity (Prieto, 2014)
<i>In vivo</i> study design	Designing (further) toxicity studies (e.g. species selection based on <i>in vitro</i> 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 <i>in vitro</i> ADME data.	EFSA PPR Opinion, 2006
Risk management	Persistence 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 (<i>in silico</i> , <i>in vitro</i> , <i>in vivo</i>) in public health issue of exposure to mixtures.	EFSA, 2014

Background

- Official (EU/OECD- 417 - 427) methods based mostly on animal procedures and only one based addressing in vitro dermal absorption (EU/OECD-428)
- Opportunities to use new (non-animal) methods and tools



AIM

The aim of the EURL ECVAM strategy is to avoid, replace, reduce and refine animal testing in the assessment of toxicokinetics and systemic toxicity of substances, showing a significant short to mid-term 3Rs impact, and at the same time laying the foundation for a risk assessment approach that is increasingly based on human ADME/TK data.

LINK: <https://eurl-ecvam.jrc.ec.europa.eu/eurl-ecvam-publishes-its-strategy-in-the-area-of-toxicokinetics>

Strategy for Toxicokinetics

Strategic
AIM 1:
ADME methods

Strategic
AIM 2:
Kinetic modelling

Strategic
AIM 3:
Data Collection

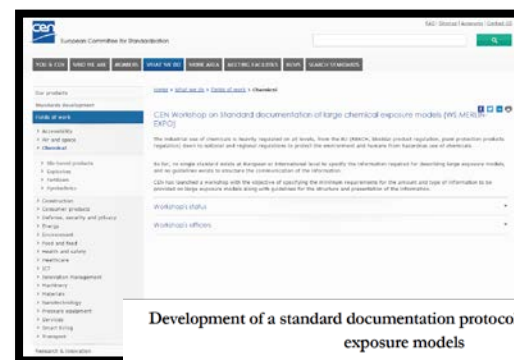
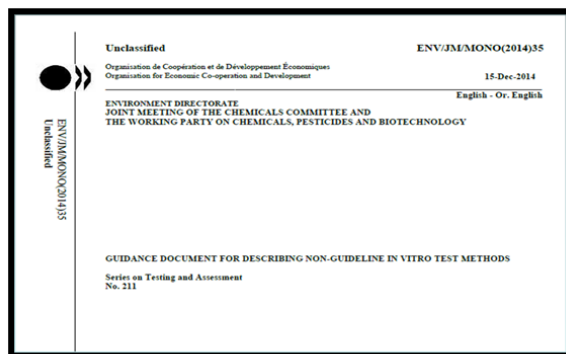
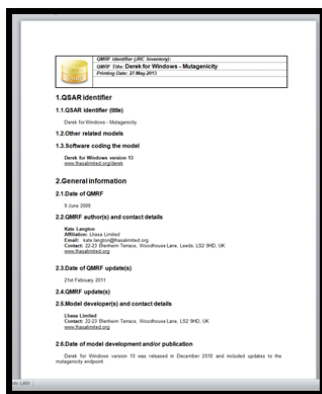
Strategic
AIM 4:
Regulatory
Anchoring

Strategic AIM 1: ADME methods



Development and standardisation of ADME/TK methods

- Need **quality assurance framework** that covers *in vitro*, *in silico* and human data
- Reporting standards already adopted for QSAR & non-guideline *in vitro* methods
- Need standards for PBK models (→ CEN WA Merlin Expo)

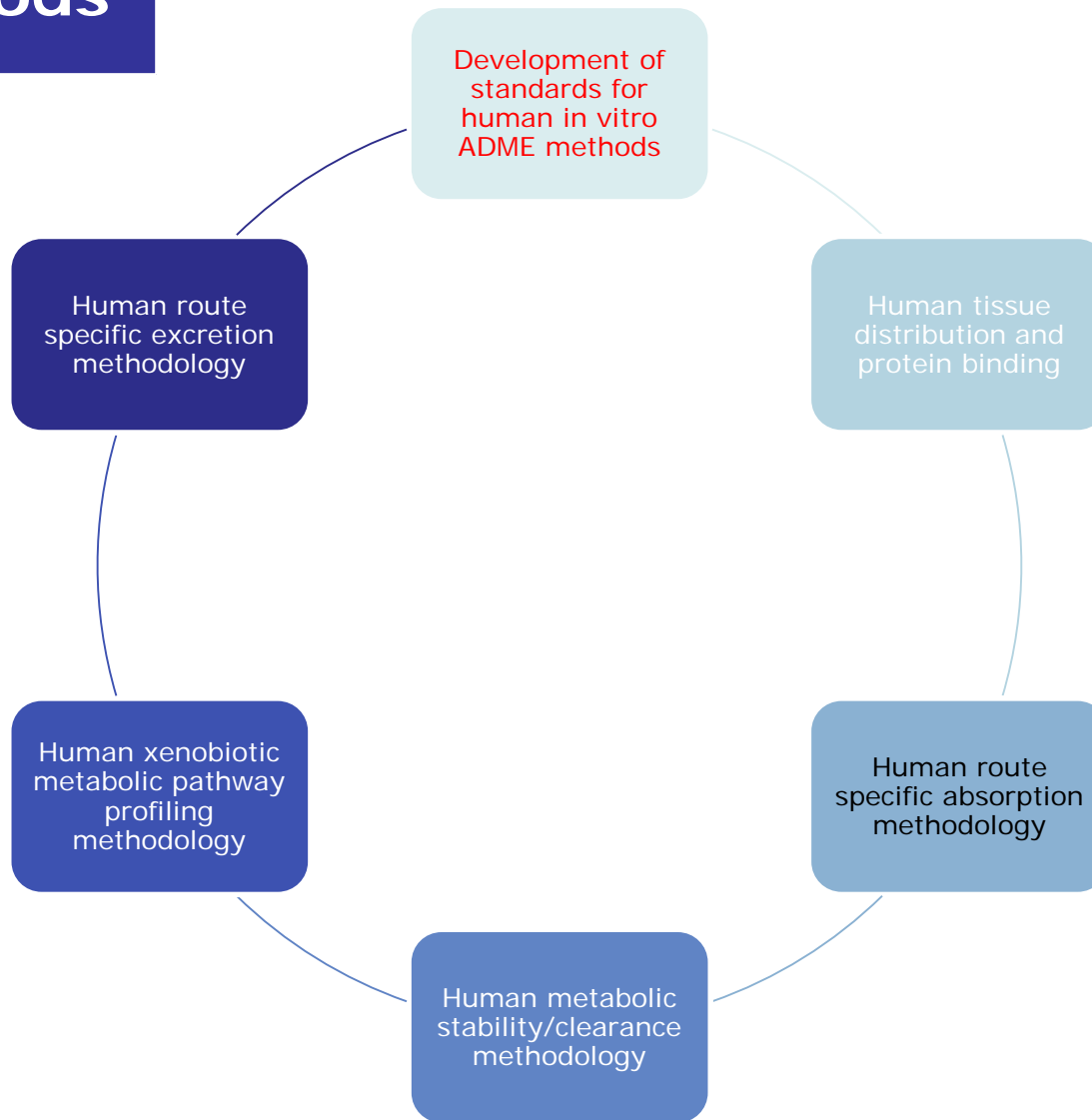


Development of a standard documentation protocol for communicating exposure models

Ciffroy P.¹, Altenpohl A.², Fair G.³, Fransman W.⁴, Paini A.⁵, Radovanovic A.⁶, Simon-Coxu M.⁷, Suci N.⁸, Verdonck F.⁹



Strategic AIM 1: ADME methods





EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

(2009)

Institute for Health and Consumer Protection
In-Vitro Methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

Performance Standards
for
***In-Vitro* Skin Irritation Test Methods**
based on Reconstructed Human Epidermis (RhE)

Purpose of Performance Standards

- 1) PS-based equivalence validation studies concerning (a) similar and (b) modified test methods.
- 2) for the assessment of the performance of test methods without intending on formal validation



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JOINT RESEARCH CENTRE

(2009)

Institute for Health and Consumer Protection
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European Centre for the Validation of Alternative Methods (ECVAM)

Performance Standards

Element 1. Essential Test Method Components:

These consist of essential structural, functional, and procedural elements of a validated test method that should be included in the protocol of a proposed, mechanistically and functionally similar test method. These components include unique characteristics of the test method, critical procedural details, and quality control measures.

Element 2. List of Reference Chemicals:

These are used to assess the accuracy and reliability of a proposed, mechanistically and functionally similar test method. These chemicals are a representative subset of those used to demonstrate the reliability and the accuracy of the validated test method.

Element 3. Target Values for Reliability and Predictive Capacity (Accuracy):

These are the performance requisites that should be achieved by the proposed test method when evaluated using the minimum list of RC, i.e. reliability and predictive capacity that should be achieved by the proposed test method when testing the RC.

Strategic AIM 1: ADME methods



Validation framework for *in vitro* methods based on standards

Primary level: characterisation of the basic properties and functionality of the biological test system

Intermediate level: validation of the method's utility to measure the endpoint in qualitative and quantitative terms

Application level: validation of the method in terms of its potential to serve specific domains of application

A complete set of 'nested' standards serves all three levels of characterisation and validation of an *in vitro* method



Strategic AIM 1: ADME methods



Validation Standards

What kind of information should be reported to describe the method and its performance?

Reporting

Standardised templates for describing the characteristics of a method and how it used to generate results

Chemical

Reference chemicals with clearly defined structural, physicochemical, mechanistic, toxicological and toxicokinetic properties

Which reference chemicals should be used to fill the required information?

Procedural

(Experimental) Protocols

How these reference chemical should be applied to fill in the required information?

Data Processing -Evaluation-

Reproducibility & goodness of fit!!!

How should we Validate the method?

Strategic AIM 1: ADME methods



Validation Standards

What kind of information should be reported to describe the method and its performance?

Reporting

Standardised templates for describing the characteristics

of a method and how it is used to generate results

- Standards for CYP induction method
- Standards for AR Transactivation Assay methods
- Standards for Clearance methods

*Reference chemicals
with clearly defined structural,
physicochemical, mechanistic,
toxicological and
toxicokinetic properties*

(Experimental) Protocols

*Reproducibility
&
goodness of fit!!!*

Which reference chemicals should be used to fill the required information?

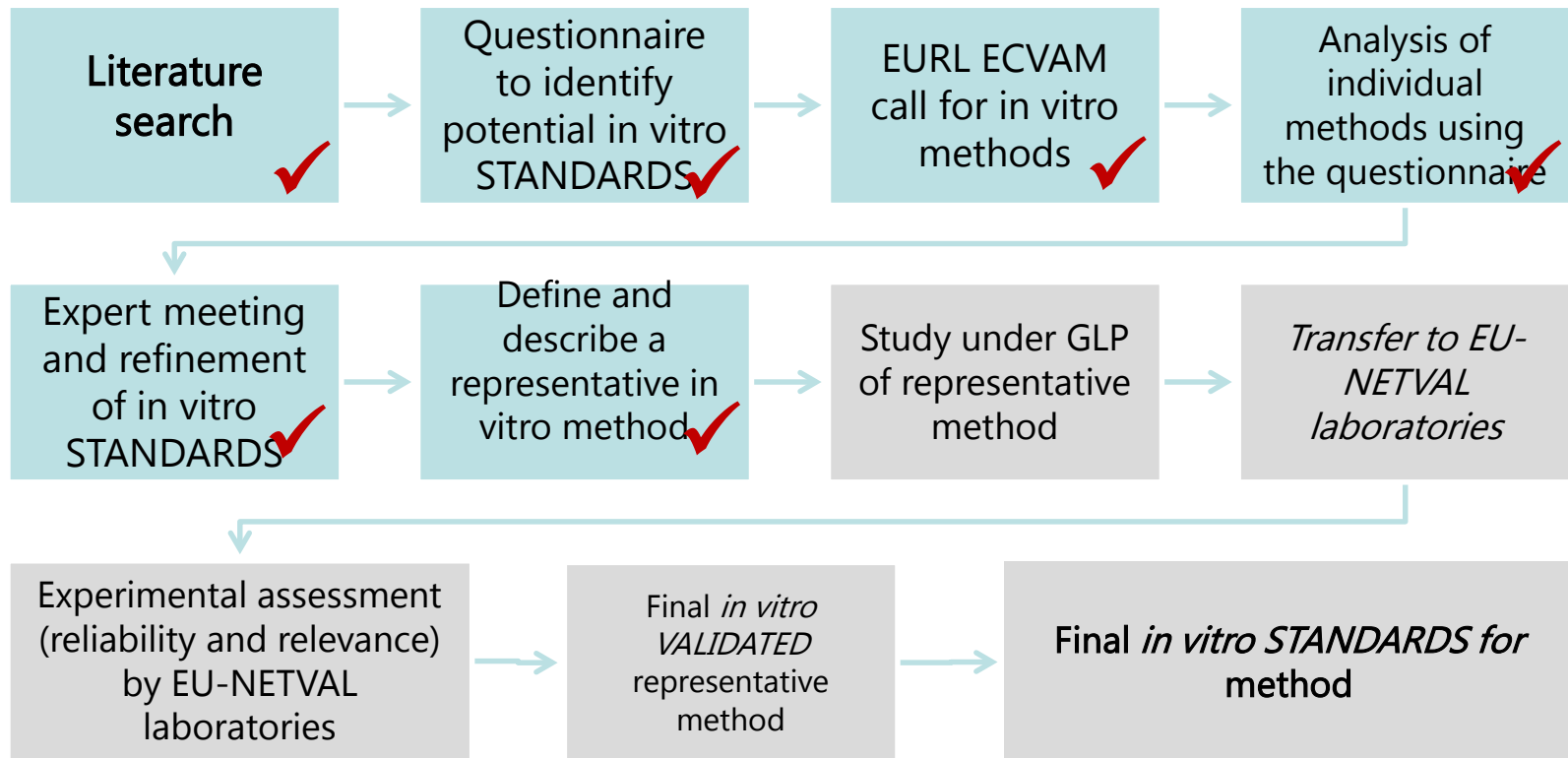
How these reference chemical should be applied to fill in the required information?

How should we Validate the method?

Strategic AIM 1: ADME methods



Process followed to generate standards



Strategic AIM 1: ADME methods

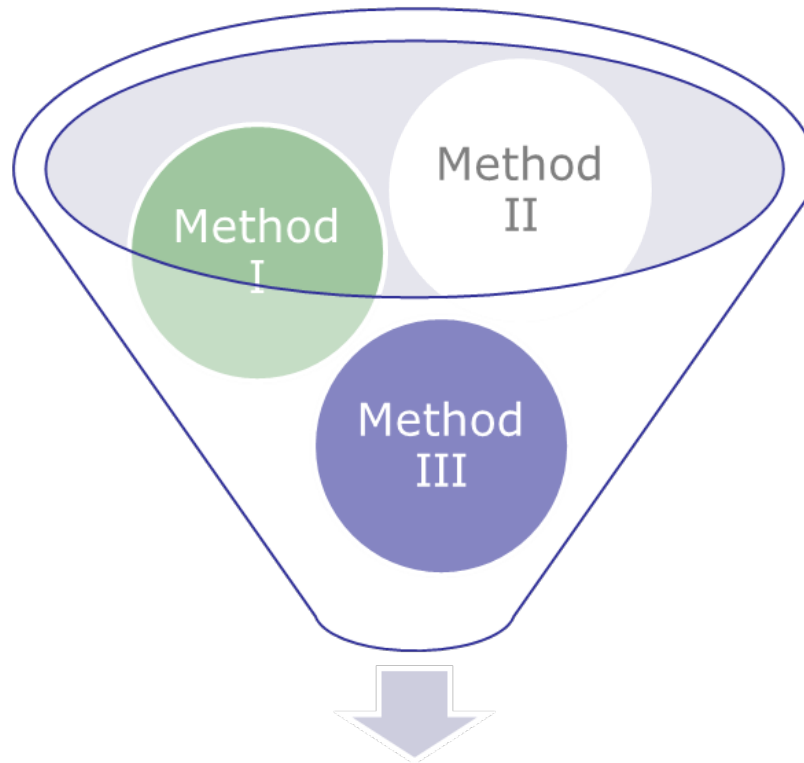


Process followed to generate standards

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Standard



Strategic AIM 1: ADME methods



Development of standards for human in vitro ADME methods

Methods to measure the permeability of external and internal membranes

Route of exposure	OECD TG
Dermal	TG428
Oral/inhalation	None
Others	none

Methods to measure lipid (storage/bioaccumulation) and protein affinity/binding and distribution.

Human route specific absorption methodology

Human tissue distribution and protein binding

Human metabolic stability/clearance methodology

Clearance Case study
Dr. V. Gouliarmou

Human route specific excretion methodology

Human xenobiotic metabolic pathway profiling methodology

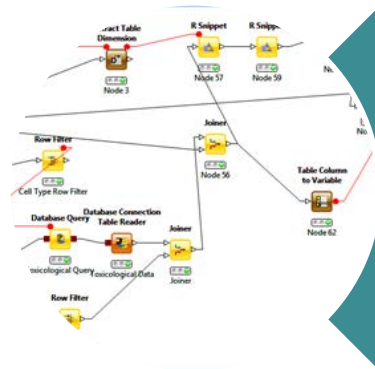
urinary and biliary
Saliva, sweat, hair, tears, nails, exhalation

Biomonitoring DATA

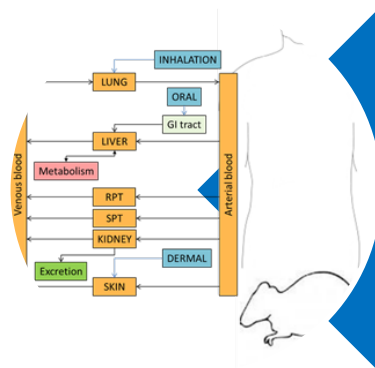
Metabolomics
Endogenous and exogenous
Low MW molecule

OECD TG Under Draft

Strategic AIM 2: Kinetic modelling



Comprehensive
web-based
kinetic
modelling
portals

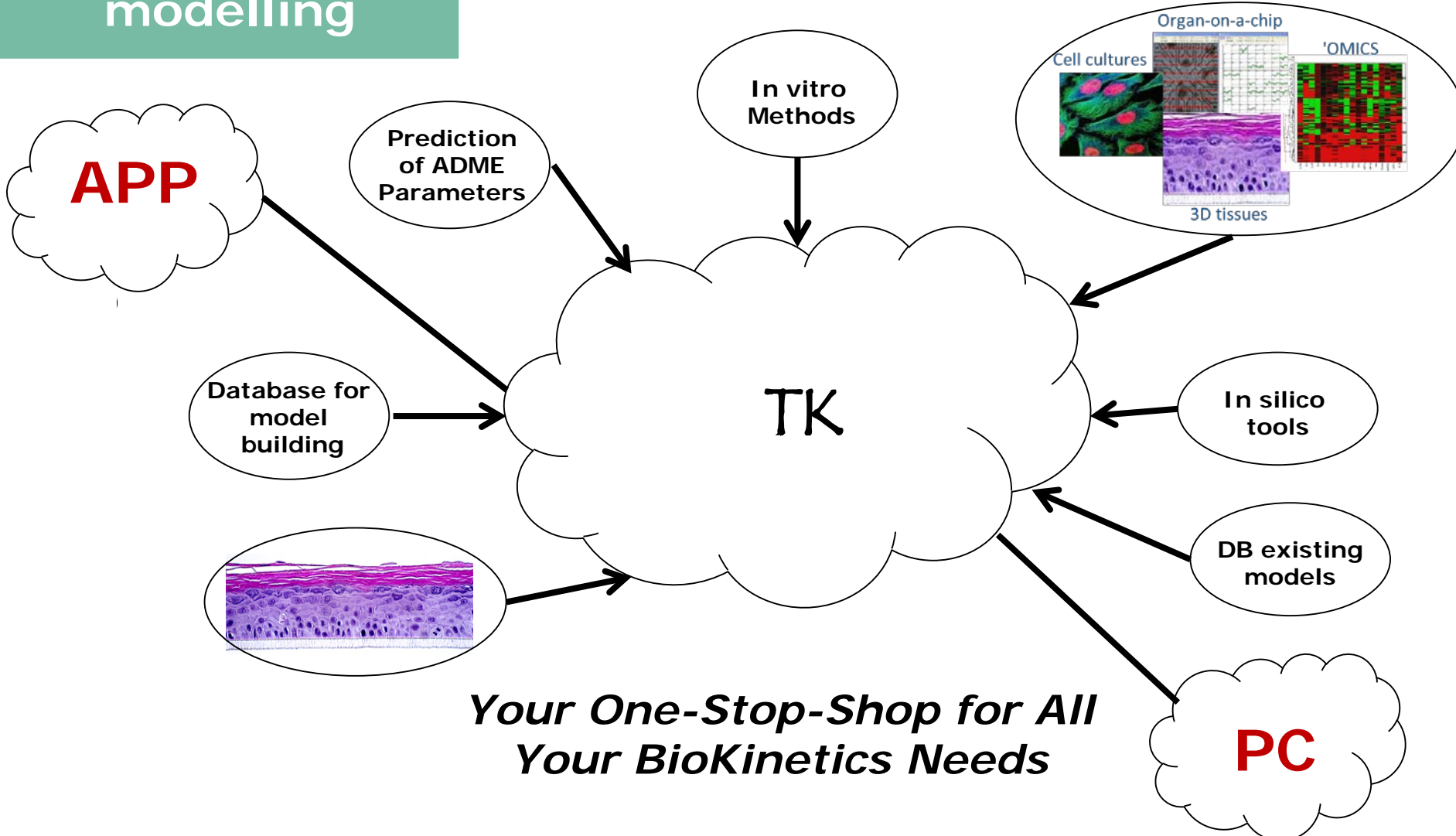


Good kinetic
modelling
practice

Strategic AIM 2: Kinetic modelling



Comprehensive
web-based
kinetic
modelling
portals



*Your One-Stop-Shop for All
Your BioKinetics Needs*

Strategic AIM 2: Kinetic modelling



Comprehensive web-based kinetic modelling portals

COSMOSpace

ABOUT CONTACT LOGIN

Welcome to COSMOS Space

COSMOS Space provides a free storage and interactive functionalities for researchers (in Predictive Toxicology and Cheminformatics) supported by the COSMOS project funded by EC FP7 and Cosmetics Europe.

Open for Innovation KNIME WebPortal

Version 3.9.3

Username:

Password:

Login

MIDAS PORTAL

Modelling Inventory Database & Access Services

Welcome to the MIDAS Portal

MIDAS stands for "Modelling Inventory Database & Access Services". It is a database of models that are in use in JRC. Accessible from within the Commission Network, MIDAS allows model users and policy makers from JRC and other DGs to find models and their descriptions, to assess the use of these models for impact assessment and policy support, and to access related datasets, model descriptions and documents.

Skin Permeability Estimation

Abstract: The skin permeability coefficient (P_{sk}) is estimated based on calculated physico-chemical descriptors.

Description: Dermal absorption is of great importance for risk assessment in many fields. Therefore the purpose of this workflow is to estimate the skin permeability coefficient (P_{sk}) from an arbitrary organic compound represented as SMILES string. The estimator for information can then be used for risk assessment and toxicology.

Workflow diagram showing nodes: Double Input, CSV Reader, Joiner, R Snippet, XLS Writer, etc.

ERLIN-Expo

The future of fully integrated human exposure assessment of chemicals

Learn how to use MERLIN-Expo

Training events

MERLIN-Expo can be downloaded free of charge

Download

Search

Inventory

Browse

Library

Wiki

Statistics

Whiteboard - Beta

Information Platform for Chemical Monitoring data

Enhancing access to chemical data

IPChEM - the Information Platform for Chemical Monitoring is a single access point for discovering chemical monitoring data collections managed and available to European Commission bodies, Member States, international and national organisations and researchers.

Search Chemical Monitoring Data

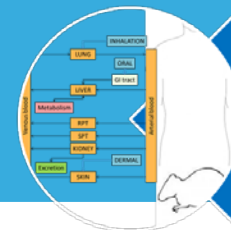
4 Modules

Stay updated - Pilot

Who is participating?

Related Information Systems

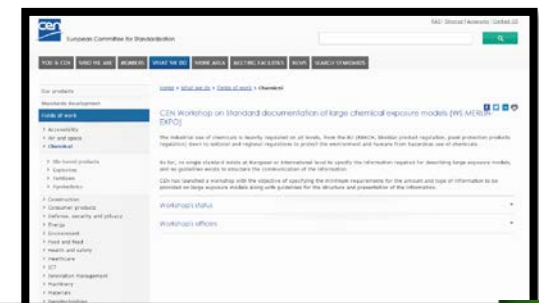
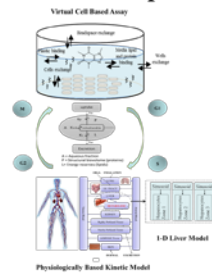
Strategic AIM 2: Kinetic modelling



Good kinetic modelling practice

0. Hypothesis

1. Definition of conceptual model



Development of a standard documentation protocol for communicating exposure models

Ciffroy P.¹, Altenpohl A.², Fait G.³, Fraunman W.⁴, Patis A.⁵, Radovanovic A.⁶, Simon-Cornu M.⁶, Susic N.⁷, Verdoock F.⁸

2. Translation to math. equation

Example equation liver:

$$dA/dt = + k_A * A_{GI} - QL * (CA - CL/PL) - V_{max} * C_{L_chemical} / (K_m + C_{L_chemical})$$

Annotations: Uptake from GI tract, Transport from arterial to venous blood, Metabolism

Rietjens et al., 2011



3. Define parameters

- Physiological and anatomical: tissue volumes, blood flow rates
- Physicochemical: partition coefficients
- Biochemical: uptake constant, metabolic parameters.
- [Literature, in vitro, in silico predictions QSARs]

4. Solving the equation

R packages: deSolve, PK, rgeonmod, RscnTran, PFME, and AICComodary

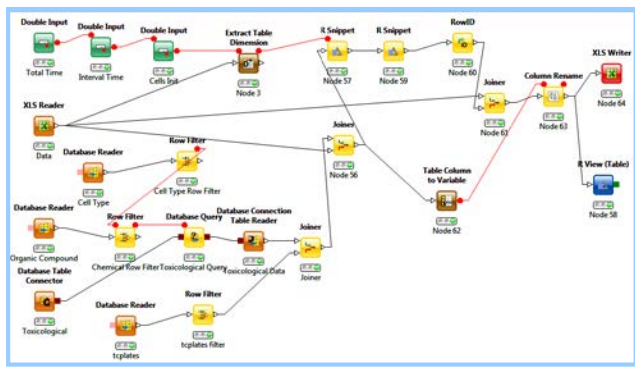
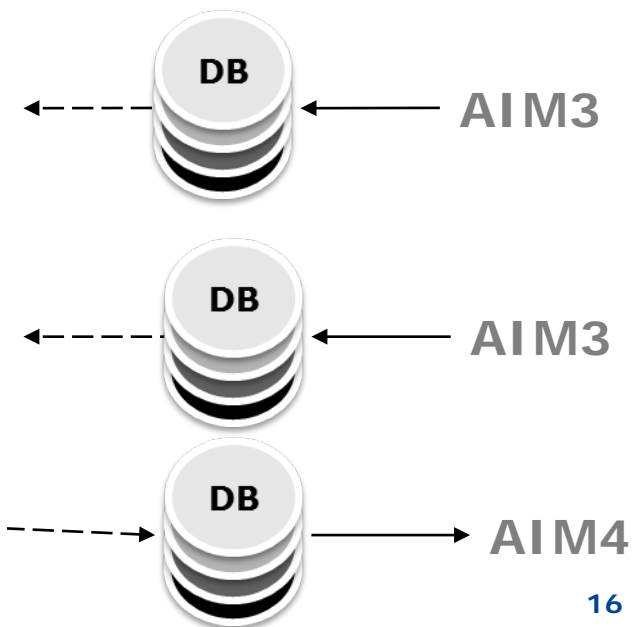
5. Evaluation of model performance

In vitro data
Human in vivo data available in literature.
Then Sensitivity analysis

6. Model Predictions

Applicability (repeat or single exposure)
Exposure scenario, set up

7. Model Reporting and Dissemination



Strategic AIM 3: Data Collection



Strategic AIM 3: Data Collection



Collection of
human *in vitro*
ADME data in
vivo TK
information

Databases

Sampling
strategy,
methods,
preparations
and analytical
determination

- Human *in vitro* ADME methods & data collection
- JRC DB ALM → <http://ecvam-dbalm.jrc.ec.europa.eu/beta/>
- QSAR databases → <http://qsar.db.jrc.it/qmrf/>
- Human *in vivo* TK data
- ECVAM KinPar database → <https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/validation-regulatory-acceptance/systemic-toxicity/toxicokinetics#available-for-downloading-are>

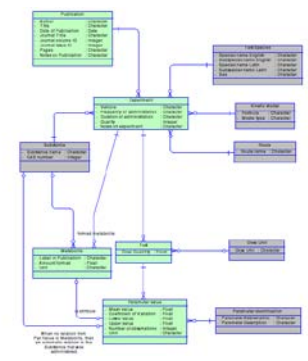
• Online RIVM document: Data Collection on kinetic parameters of substances, Noorlander et al., 2008

- Anatomical and physiological data
- RIVM Interspecies database → <https://www.interspeciesinfo.com/>
- Integration of databases with modelling platform

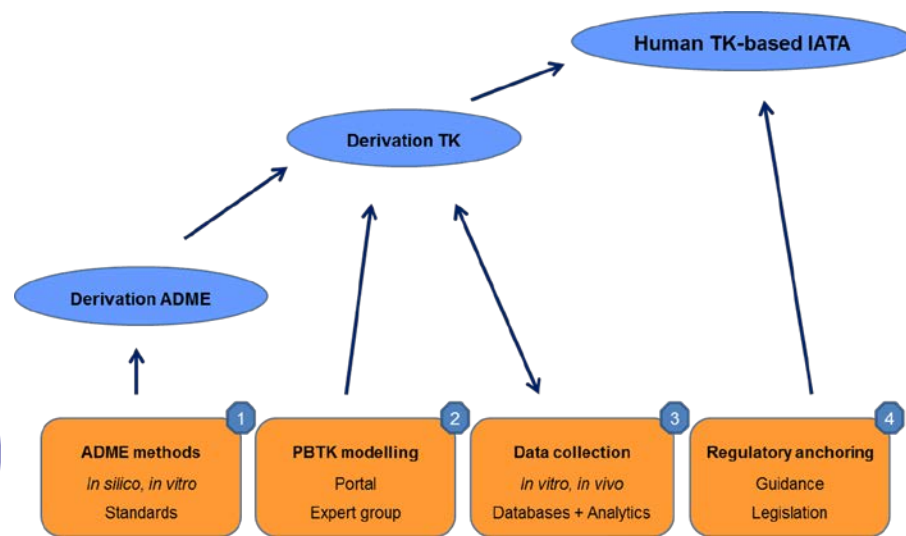
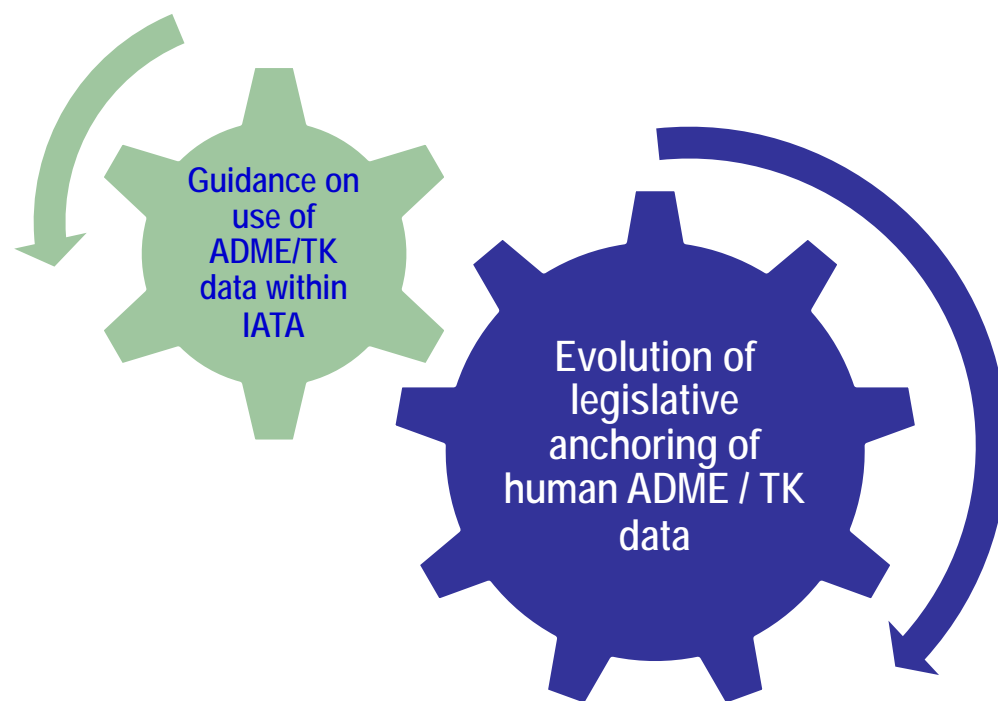
Search/filter substance

Double click a cell of a certain number to see the experiments, or select a number and press the Open selected experiments button.

Substance	Exp. number	Human		rat	
		In vitro	Ex vivo	In vitro	Ex vivo
1,3-Cyclohexanedione	03032				
2,4-Diaminobenzonitrile	01285				
2-Chloroethanol	110134				1
3,3'-Dimethylsuccinonitrile	111344				
3-Methylindole	03341				
4-Aminobenzonitrile	02081				
4-Ethoxy-2-ethyl-2-phenylglutamide	01410	1	1		
5-Cyano-2-hydroxytoluene	01410	1	2		1
6-Chloroindole	01716	1	1		
Caproic acid	1078739			1	
Cinchonidine	72003		1		
D-Naloxone	02381		1		
Diazepam	01812			1	
Diethyl ether	0401023		1		
Dimethyl sulfoxide	70003		1		
Diphenhydramine	02001			1	
Droperidol	01812			1	
Ethylmethylphenol	1078739		1		
Glutamic acid	02302		1		3
Ibuprofen	107209				
Indomethacin	72003				4
Insulin	1001200		1		
Insulin lispro	02440		1		2
Insulin zinc	02020		1		1
Insulin human	02020		1		1
Insulin porcine	107209		1		1

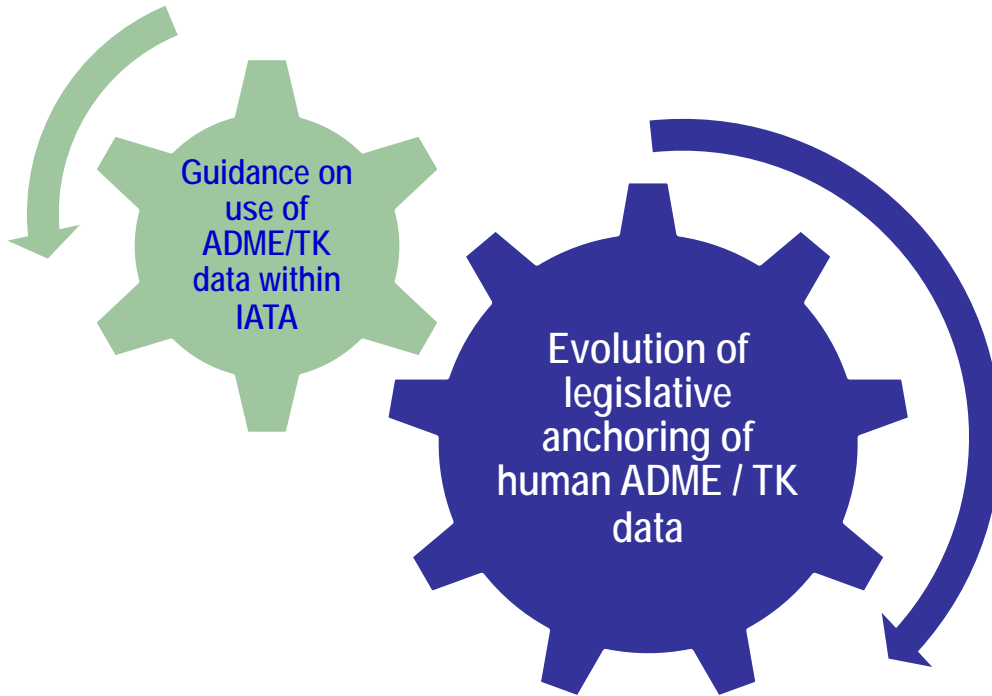


Strategic AIM 4: Regulatory anchoring



Four strategic aims to facilitate generation and use of human ADME and TK data in a IATA

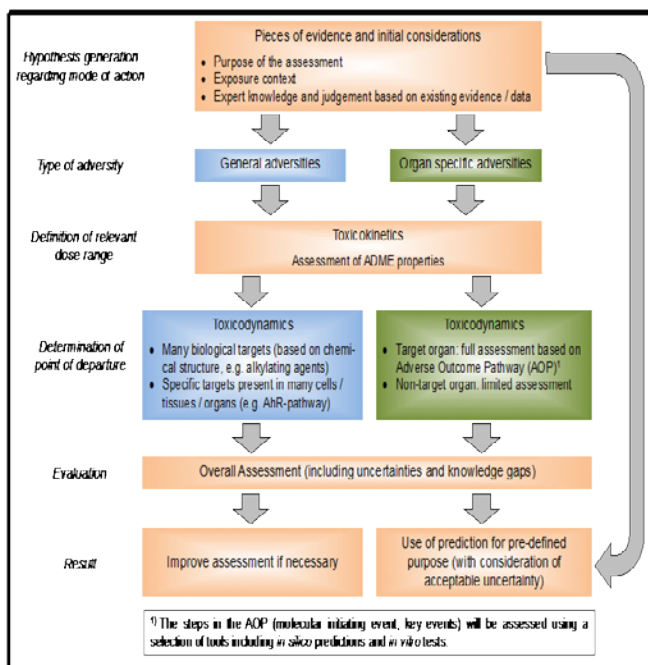
Strategic AIM 4: Regulatory anchoring



USE CASES SEURAT-1 Project

1. TTC
2. READ-ACROSS
3. AB INITIO

The SEURAT-1 ab initio case study



- ❖ Building a logic decision workflow combining *in silico* knowledge & predictions and *in vitro* data
- ❖ Aiming on an integrated risk assessment relying only on alternative methods
- ❖ Identifying remaining weaknesses and knowledge gaps to further advance alternative assessment approaches

Seurat 1 Ab initio team:
Berggren E, Bois FY, Mahony C, Ouedraogo G,
Paini A, Richarz AN, White A.

Define exposure based on proposed use case and route of exposure.

Collect/calculate phys chem properties, identify structure and active groups, predict metabolites, search for existing data

EXIT

TTC or Read-across?

Predict systemically available concentrations and identify relevant organs for further assessment.

PBPK modelling

Determine mode of action using *in silico*, *in vitro* (HTS screening) and omics technologies

Virtual Cell Based Assay

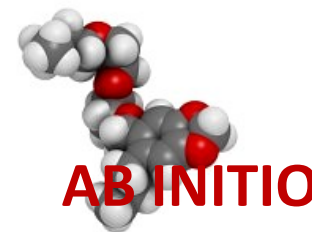
Estimate c_{max} , free concentration and *in vitro* to *in vivo* extrapolation

IVIVE

Predict a point of departure for safety assessment based on relevant AOP incorporating kinetics and biomarker data from repeat dose assays

Define margin of safety based on variability and uncertainty estimates.

Describe safety decision and any open issues that could assist in gaining higher confidence.



Define exposure based on proposed use case and route of exposure.

Collect/calculate phys chem properties, identify structure and active groups, predict metabolites, search for existing data

EXIT

TTC or Read-across?

Predict systemically available concentrations and identify relevant organs for further assessment.

PBPK modelling

Determine mode of action using (omics, modelling) and

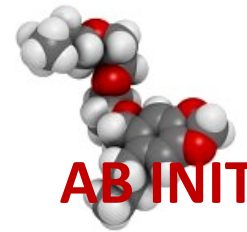
Virtual Cell Based Assay

Estimate c_{max} , free concentration, extrapolation

IVIVE

Predict a point of departure for relevant AOP incorporating kinetics and biomarker data from repeat dose assays

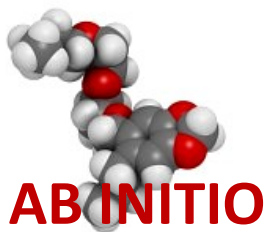
Define margin of safety based on variability and uncertainty estimates.



AB INITIO

Describe safety decision and any open issues that could assist in gaining higher confidence.

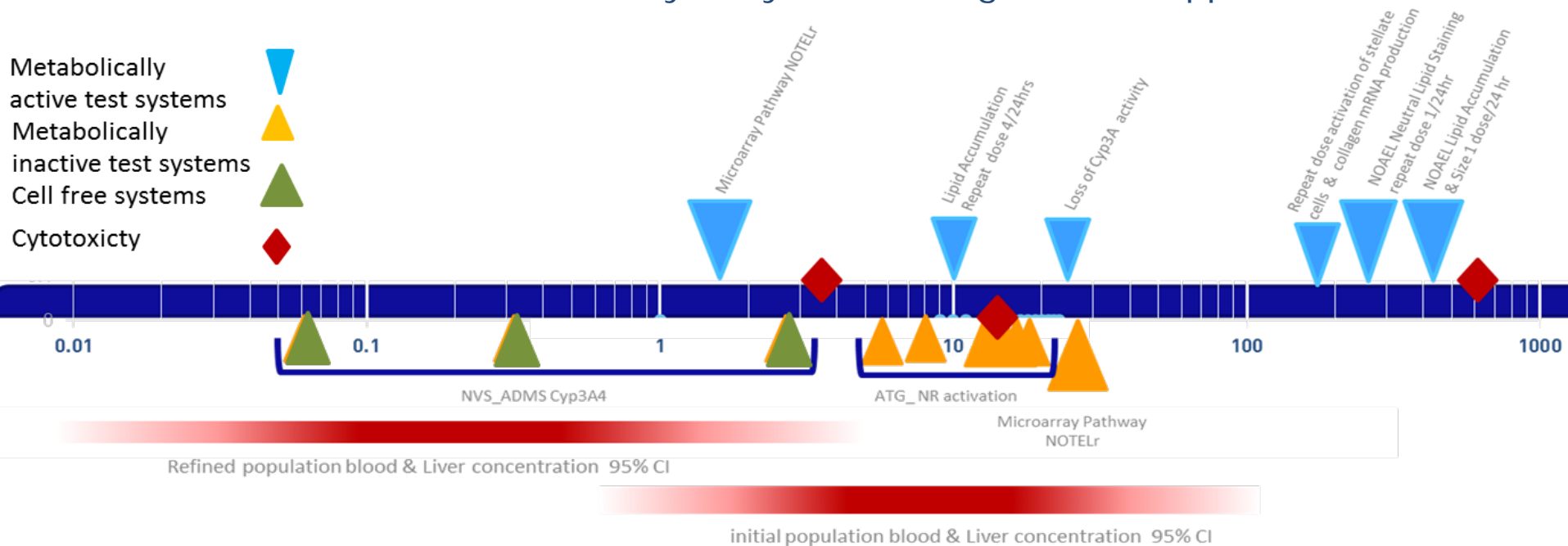




Our case study:

Can we safely use 12.5% Piperonyl butoxide (PBO) in a body lotion applied twice a day (corresponding to 144.797mg/kg/day)?

Even with the remaining variability and uncertainty it appears there is not an adequate margin of safety for a use scenario of 12.5% PBO in a daily body lotion using the new approach data.

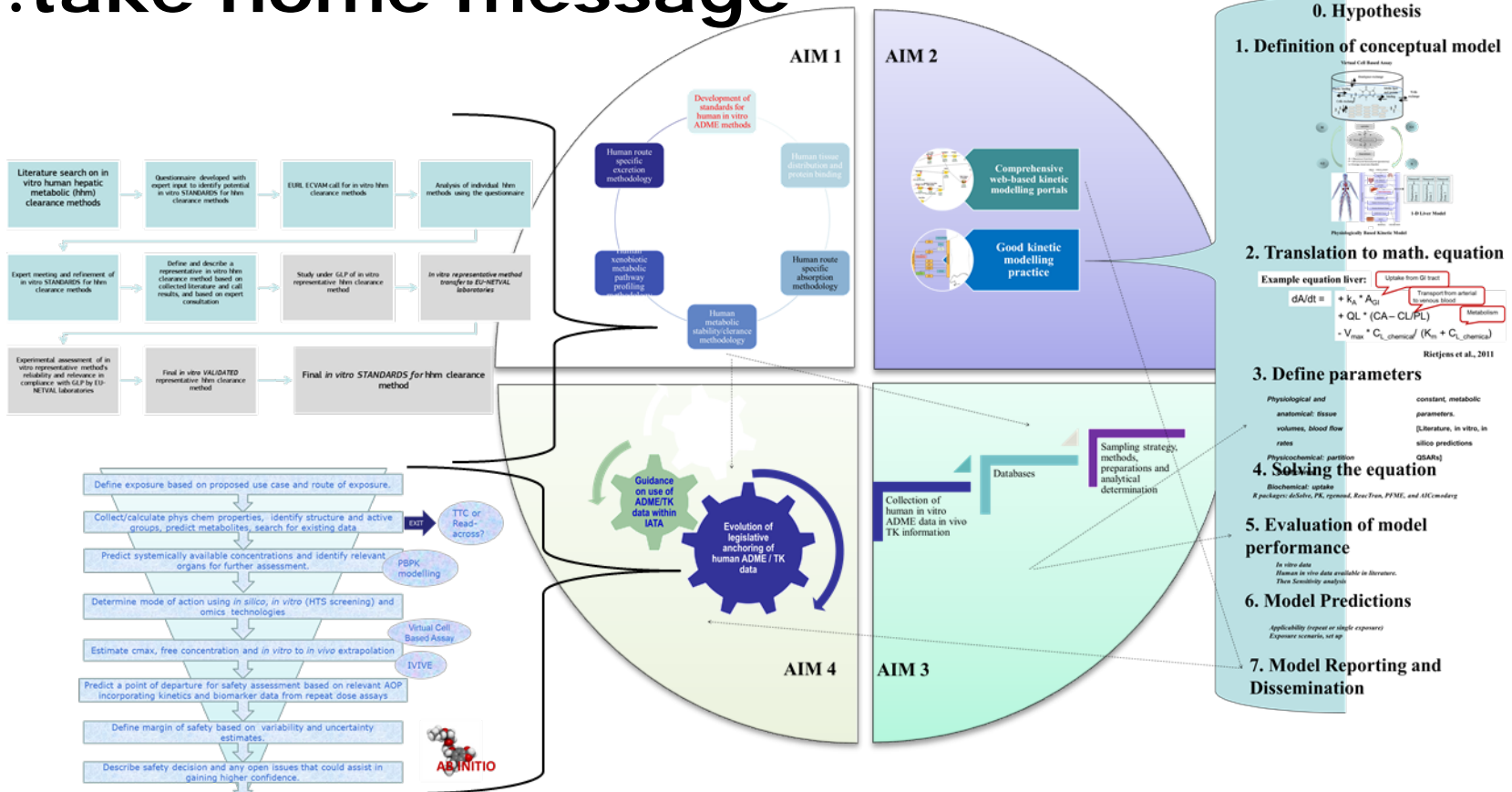


The figure illustrates predicted liver and blood concentrations of PBO alongside in vitro assay results overlap.

In conclusions

- Better design of *in vitro* toxicity studies & biokinetic models
- Better documentation of biokinetic models and *in vitro* toxicity methods
- Develop a Risk Assessment based approach on only *in silico*, *in vitro* and *in vivo* human data, without use of animals methods or new animals data.
- Integrated Approaches to Testing and Assessment (IATA)
- Laying common grounds for TK in several areas of toxicology (ENV, NANO, ACUTE, MIX) with an organized knowhow
- Support the regulatory decision making process

...take home message



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 and international collaboration.

Acknowledgements

Jos Bessems, Sandra Coecke, Varvara Gouliarmou, Andrea Richarz, Elisabet Berggren, Andrew Worth, Maurice Whelan.

