EURL ECVAM Strategy for Toxicokinetics

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EPA, Durham, 17 Feb. 2016

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

EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing

*Article 48 of the Directive, Annex VII

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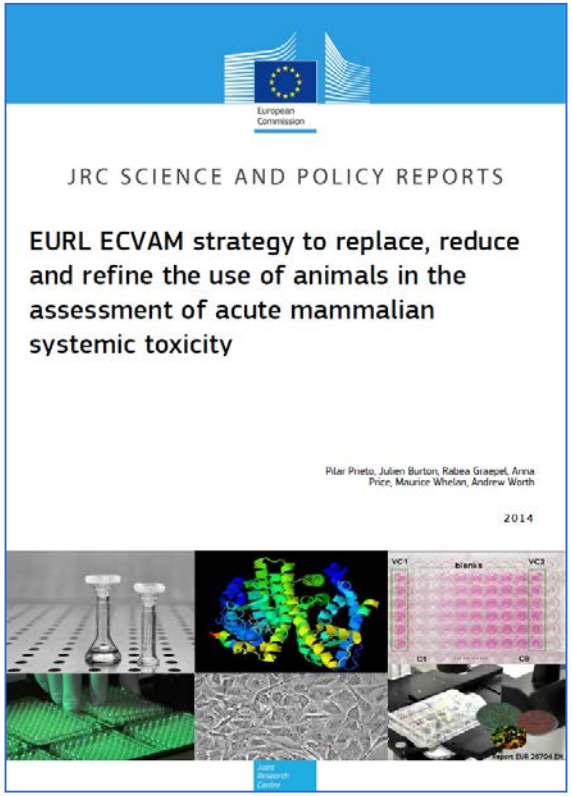
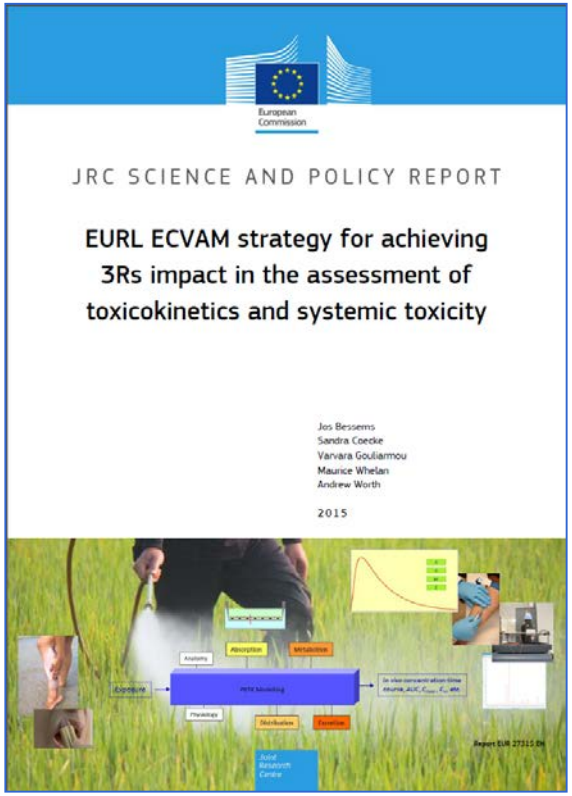
EURL ECVAM Strategy Document (July 2015)

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





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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 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)

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

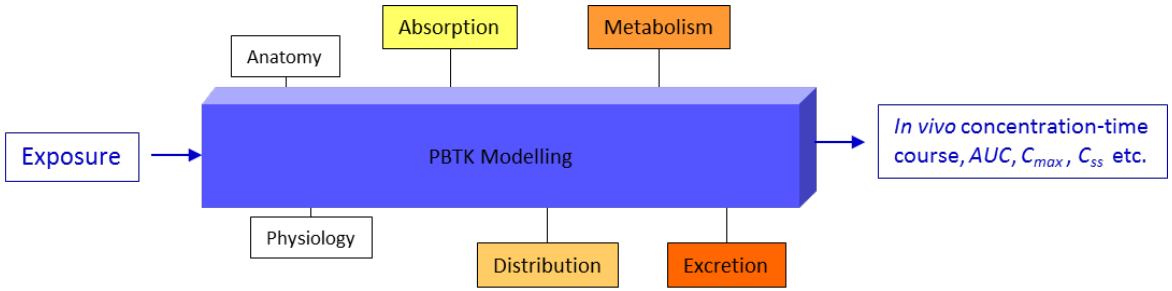
Background

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

Use cases	Examples	Source
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

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

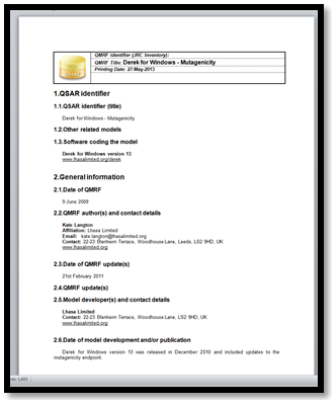
Strategic
AIM 3:
Data Collection

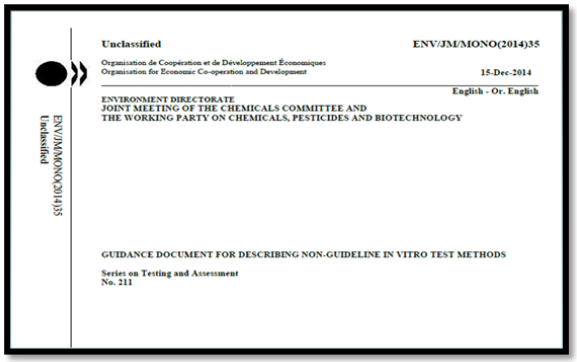
Strategic
AIM 4:
Regulatory
Anchoring

Strategic AIM 1: ADME methods

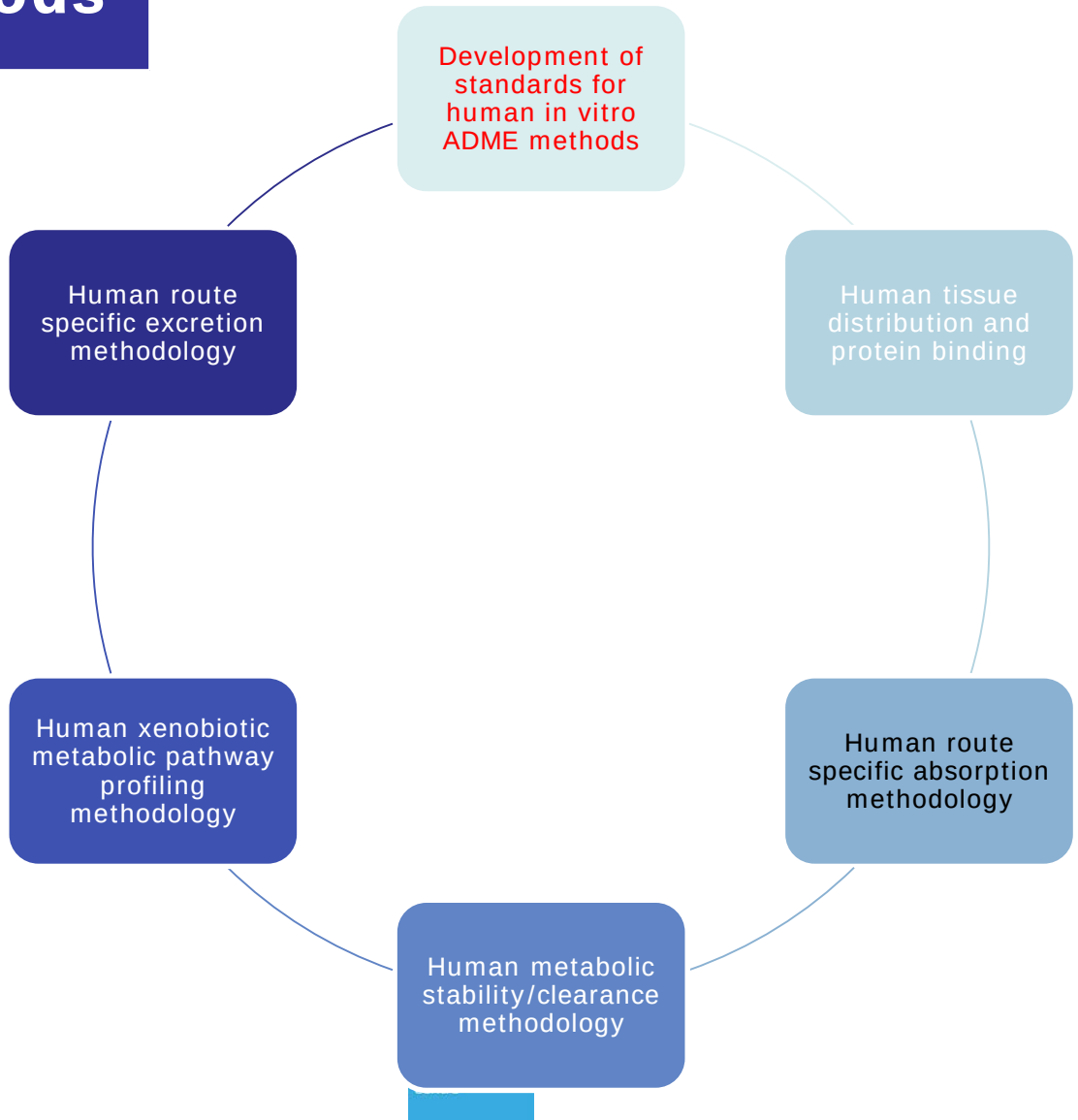
Development and standardisation of ADME/TK methods

- 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)





Strategic AIM 1: ADME methods



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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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(2009)

Institute for Health and Consumer Protection
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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.

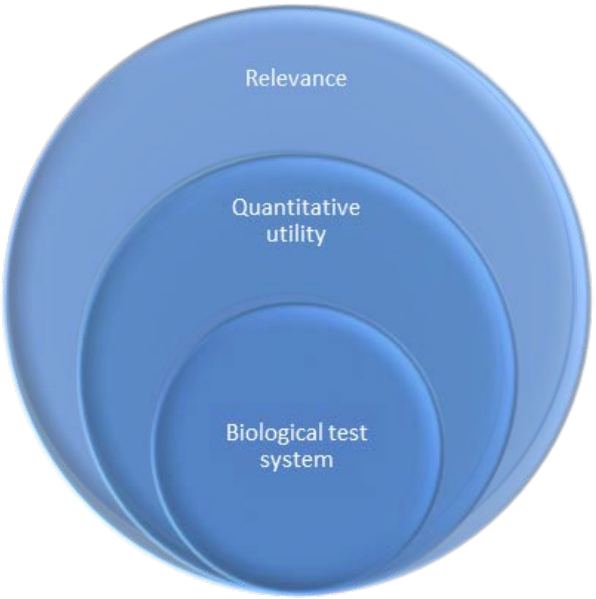
Element 2. List of Reference Chemicals:
These are used to assess the accuracy and reliability of a proposed, mechanistically and functionally similar test method. These chemicals are a representative subset of those used to demonstrate the reliability and the accuracy of the validated test method.

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.

Centre

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

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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, physiochemical, 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?

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Strategic AIM 1: ADME methods

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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

Ongoing studies to develop standards:

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

Reference chemicals with clearly defined structural, physiochemical, 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?

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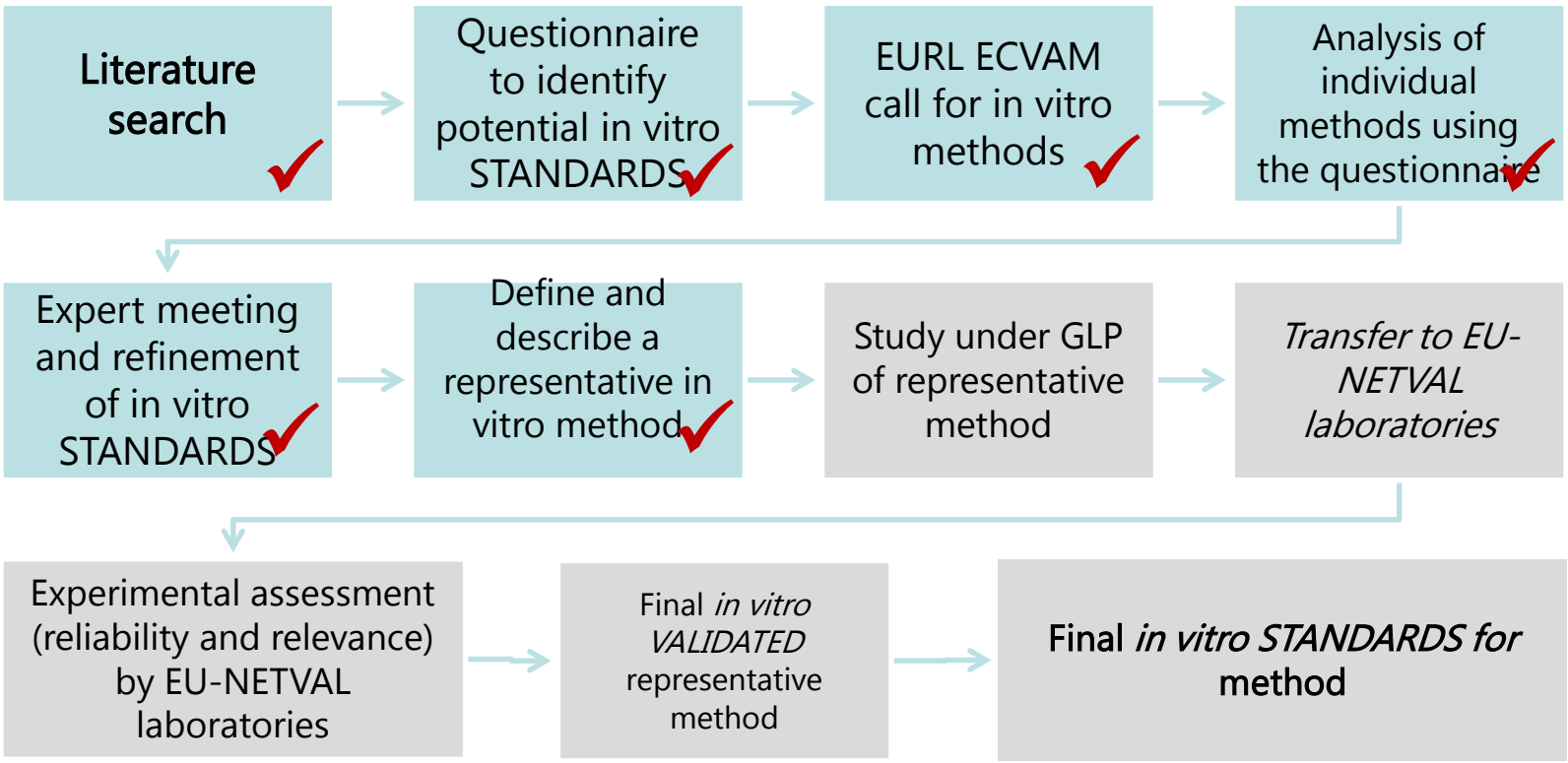
Strategic AIM 1: ADME methods

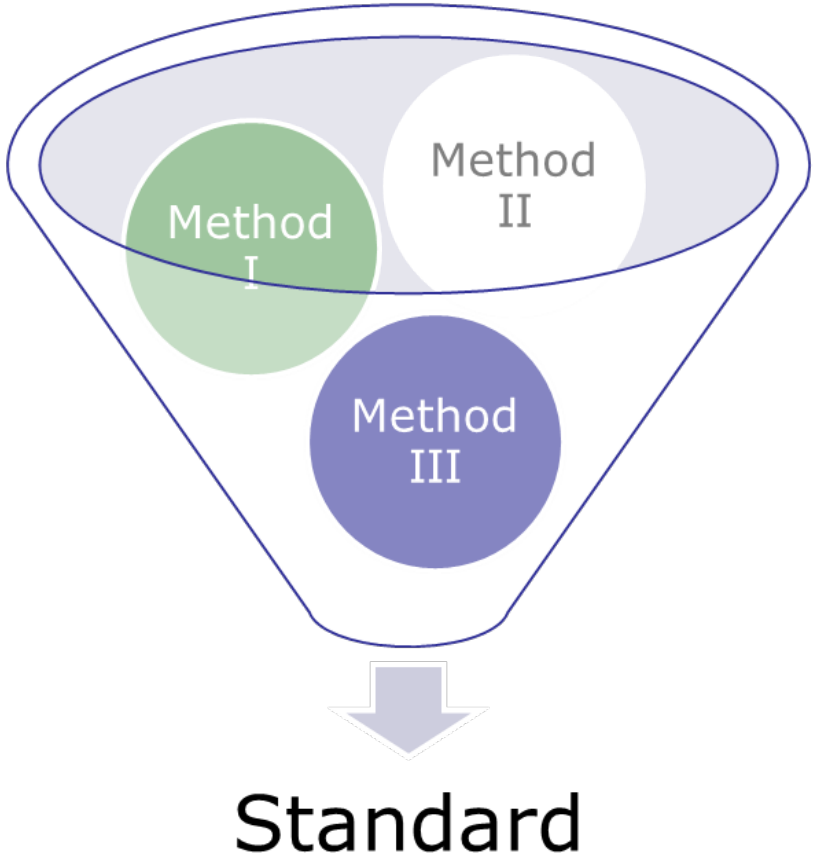
Process followed to generate standards



hhm clearance

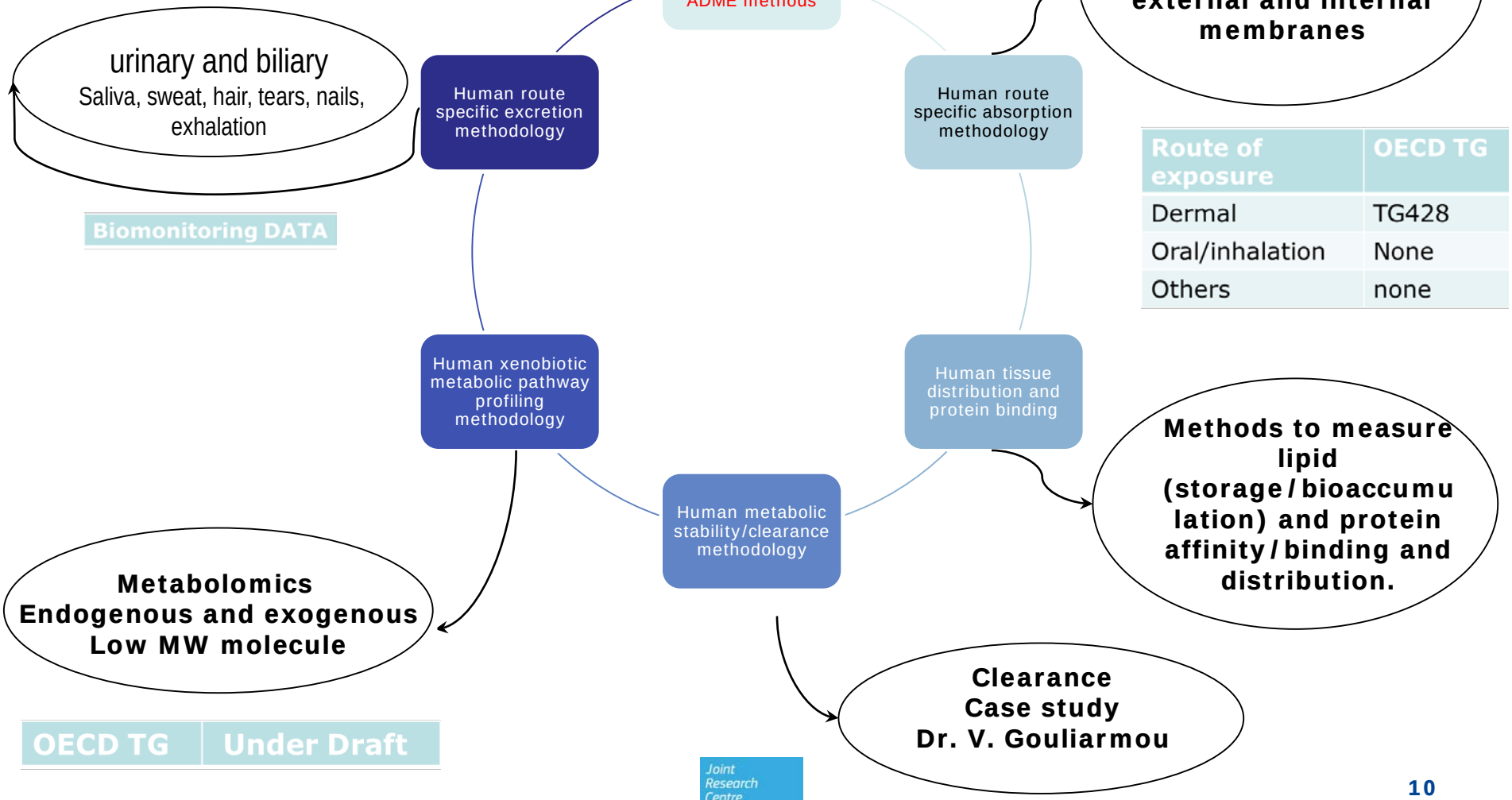
Strategic AIM 1: ADME methods

Process followed to generate standards



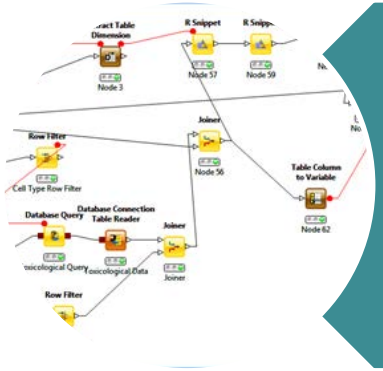
hhm clearance

Strategic AIM 1: ADME methods

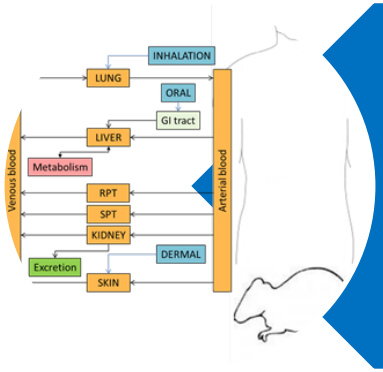


Route of exposure	OECD TG
Dermal	TG428
Oral/inhalation	None
Others	none

Strategic AIM 2: Kinetic modelling



Comprehensive web-based kinetic modelling portals



Good kinetic modelling practice

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Strategic AIM 2: Kinetic modelling

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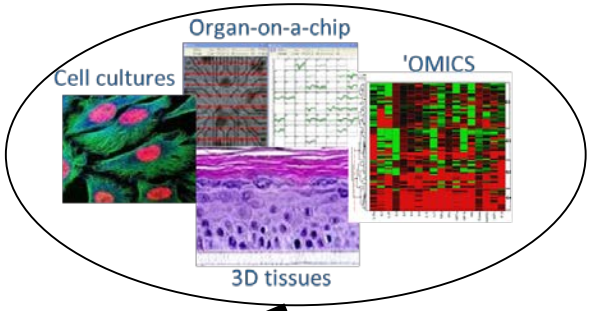
Comprehensive web-based kinetic modelling portals

APP

Prediction of ADME Parameters

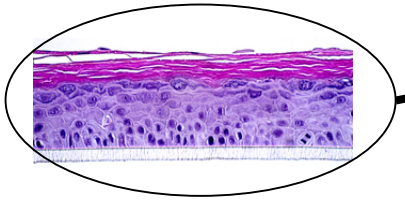
In vitro Methods



Database for model building

TK

In silico tools



DB existing models

PC

Your One-Stop-Shop for All Your BioKinetics Needs

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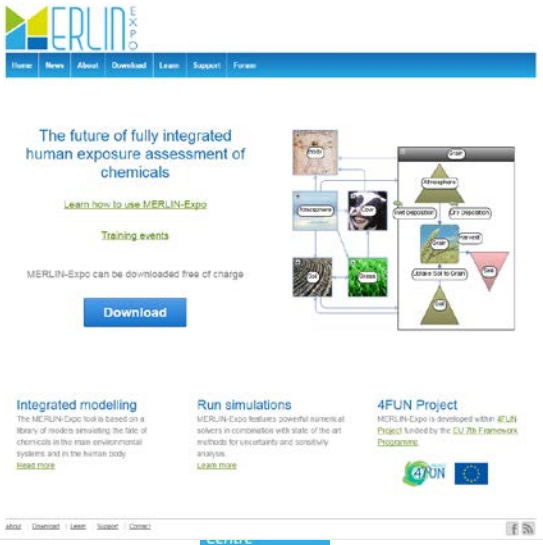
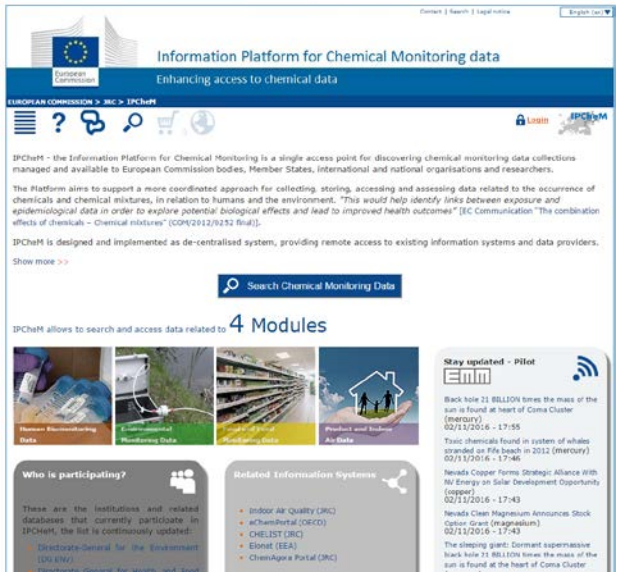
Strategic AIM 2: Kinetic modelling

European Commission

Comprehensive web-based kinetic modelling portals





Strategic AIM 2: Kinetic modelling

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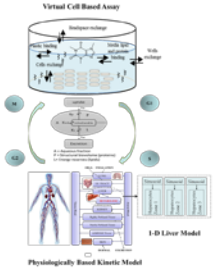
Good kinetic modelling practice

0. Hypothesis

1. Definition of conceptual model



2. Translation to math. equation

Example equation liver:

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

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, rgenoud, ReacTran, PFME, and AICcmodavg

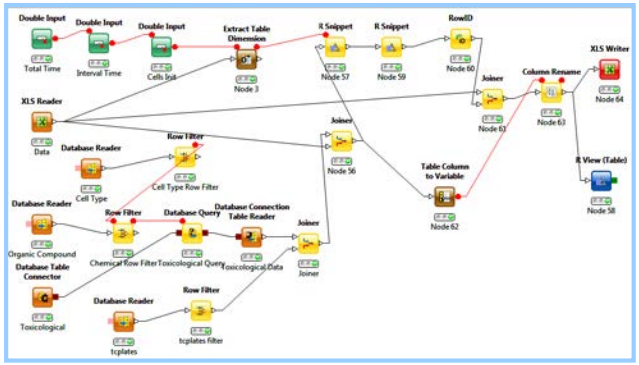
5. Evaluation of model performance

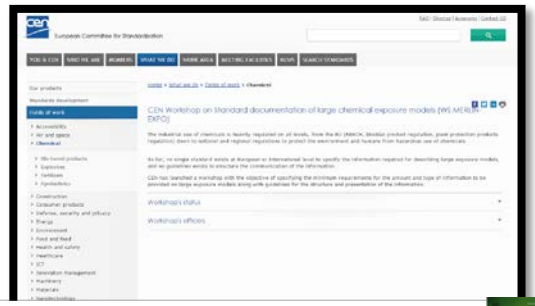
In vitro data
Human in vivo data available in literature.
Then Sensitivity analysis

6. Model Predictions

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

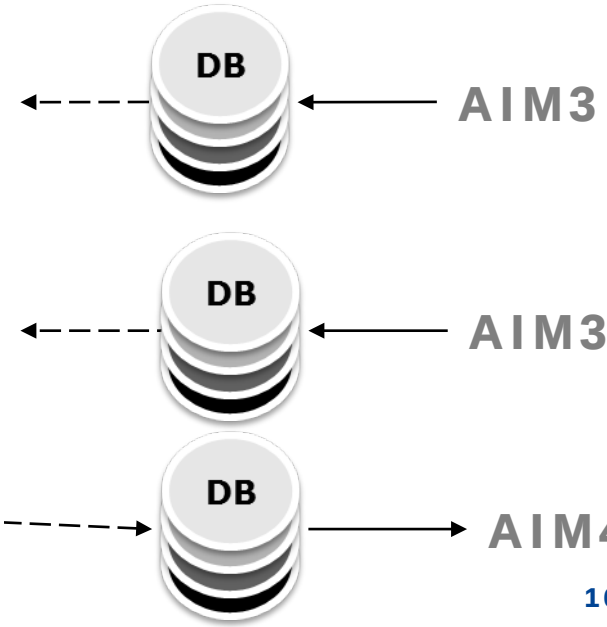
7. Model Reporting and Dissemination





Development of a standard documentation protocol for communicating exposure models

Ciffroy P.[1], Altenpohl A.[2], Fait G.[3], Fransman W.[4], Paini A.[5], Radovnikovic A.[6], Simon-Cornu M.[8], Suciu N.[7], Verdonck F.[8]



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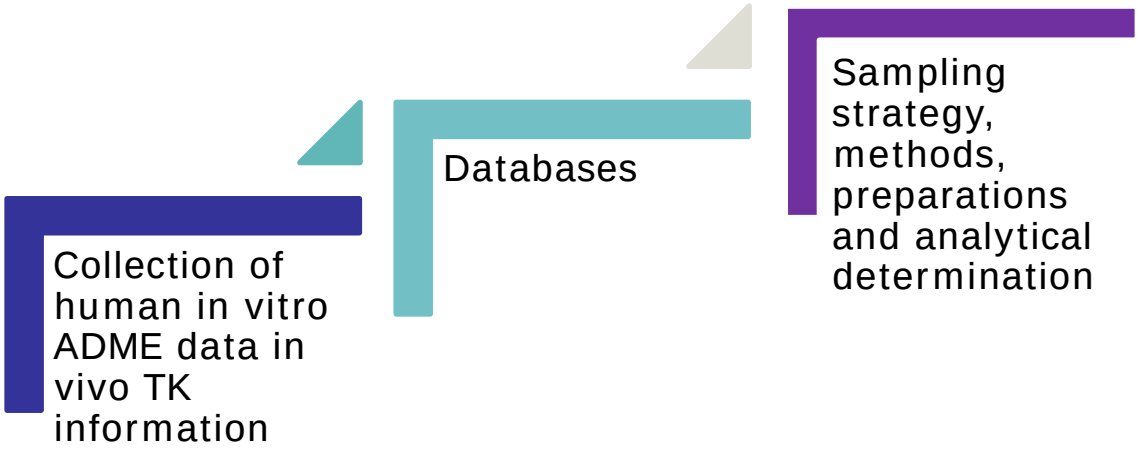
Strategic AIM 3: Data Collection



Strategic AIM 3: Data Collection

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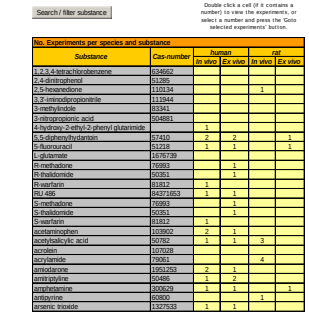
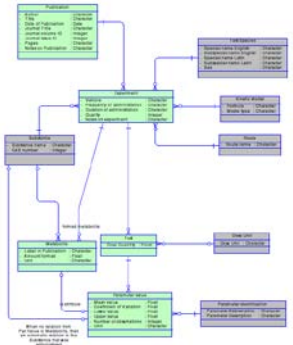
Collection of human in vitro ADME data in vivo TK information

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://qsardb.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





DB ALM

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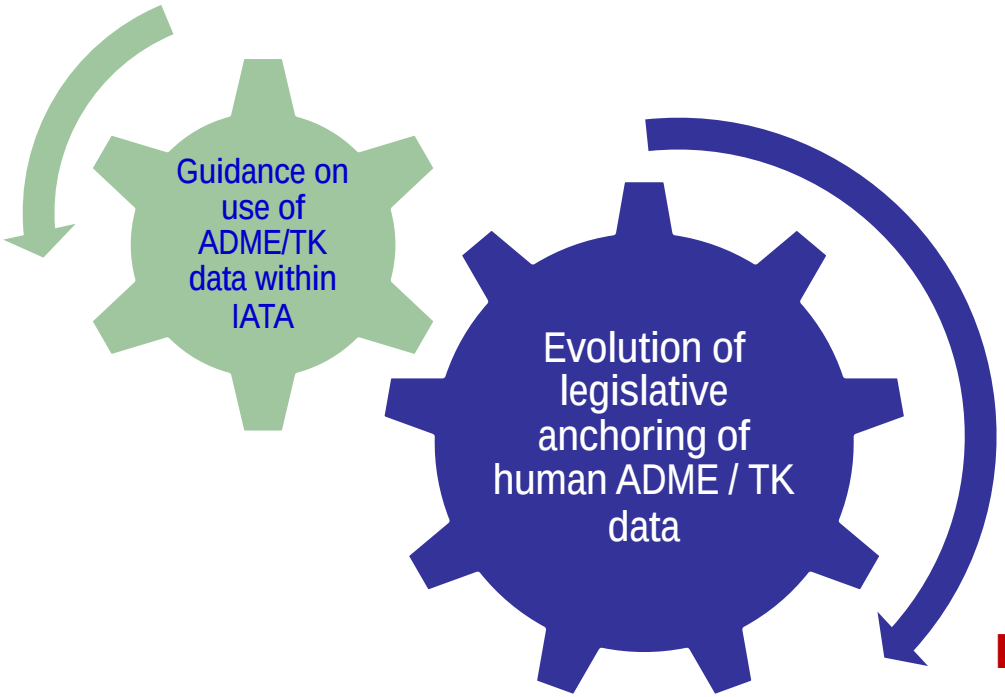
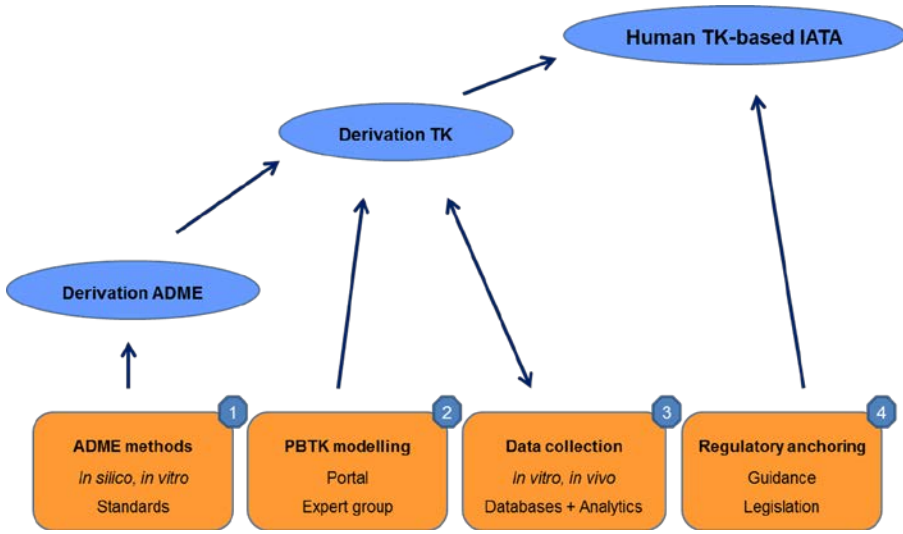
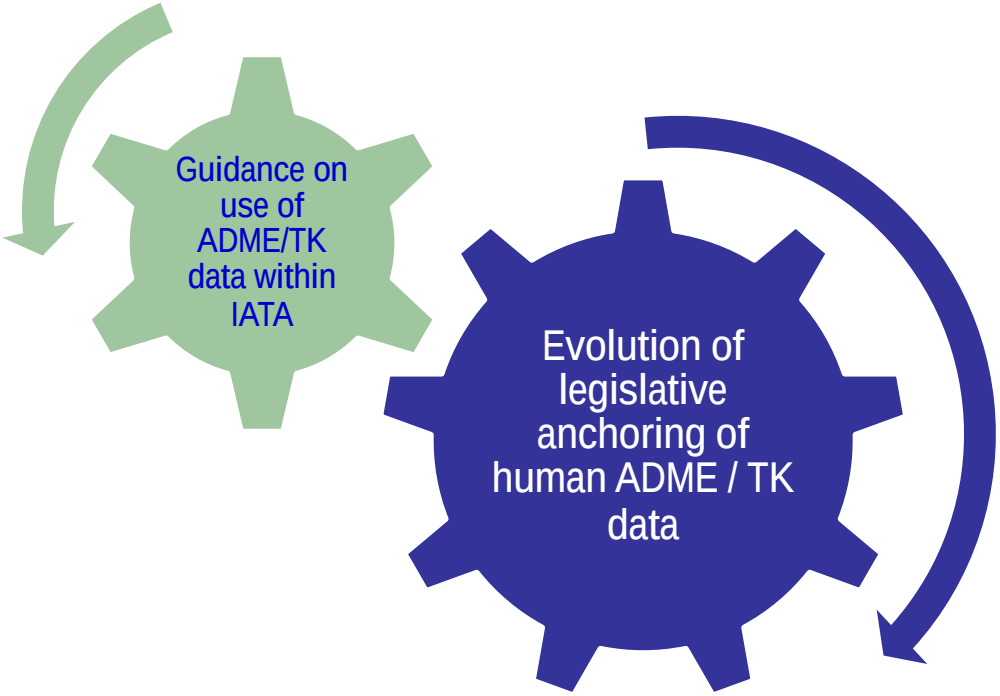
Strategic AIM 4: Regulatory anchoring





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

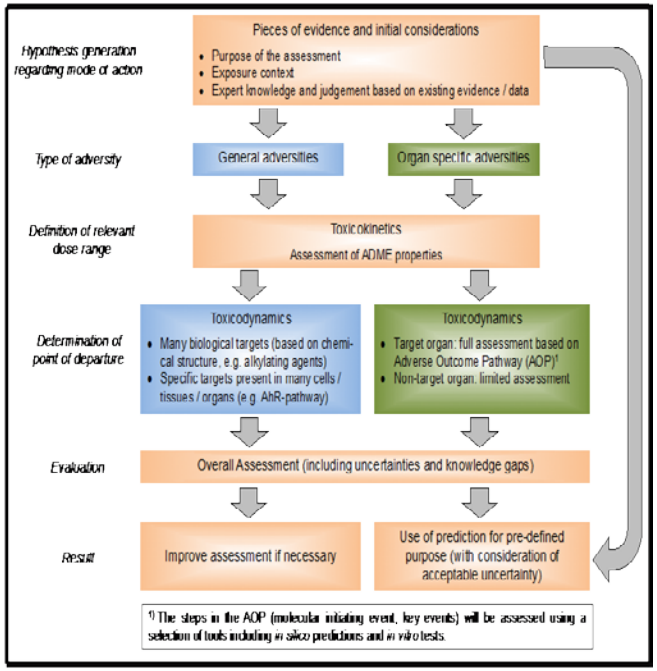
Strategic AIM 4: Regulatory anchoring



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 cmax, 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.

AB INITIO

Joint Research Centre

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

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AB INITIO

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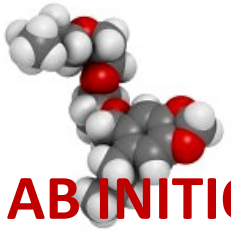
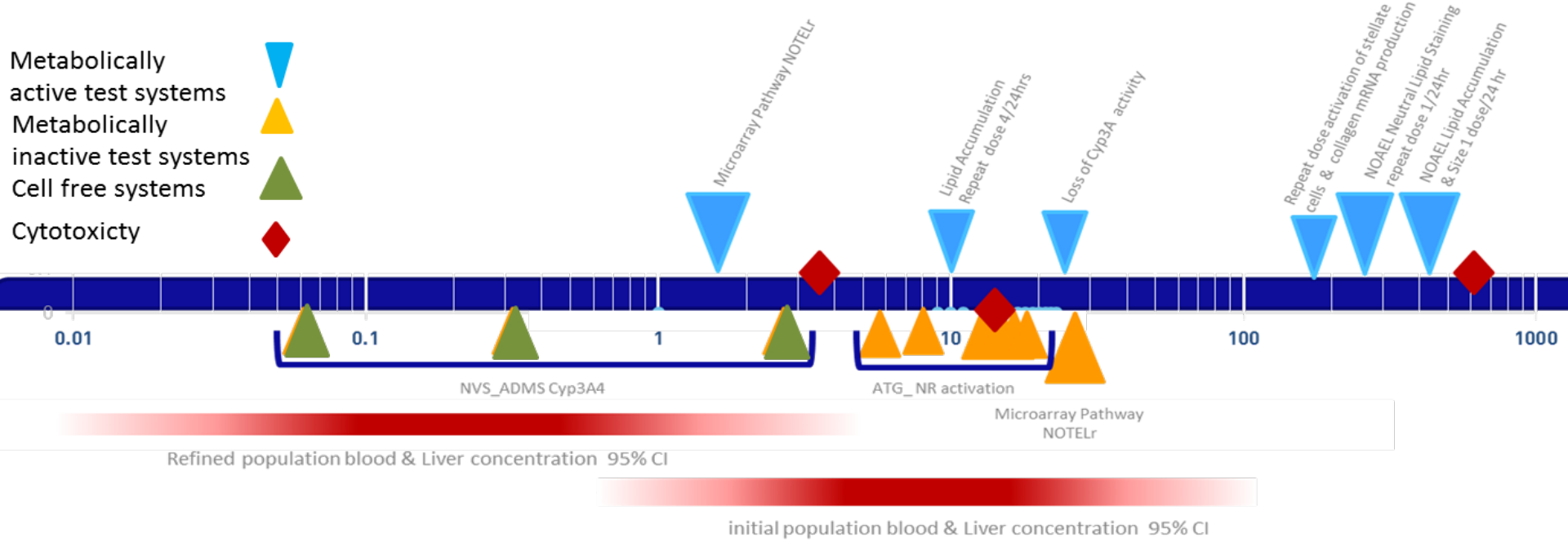


AB INITIO

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.



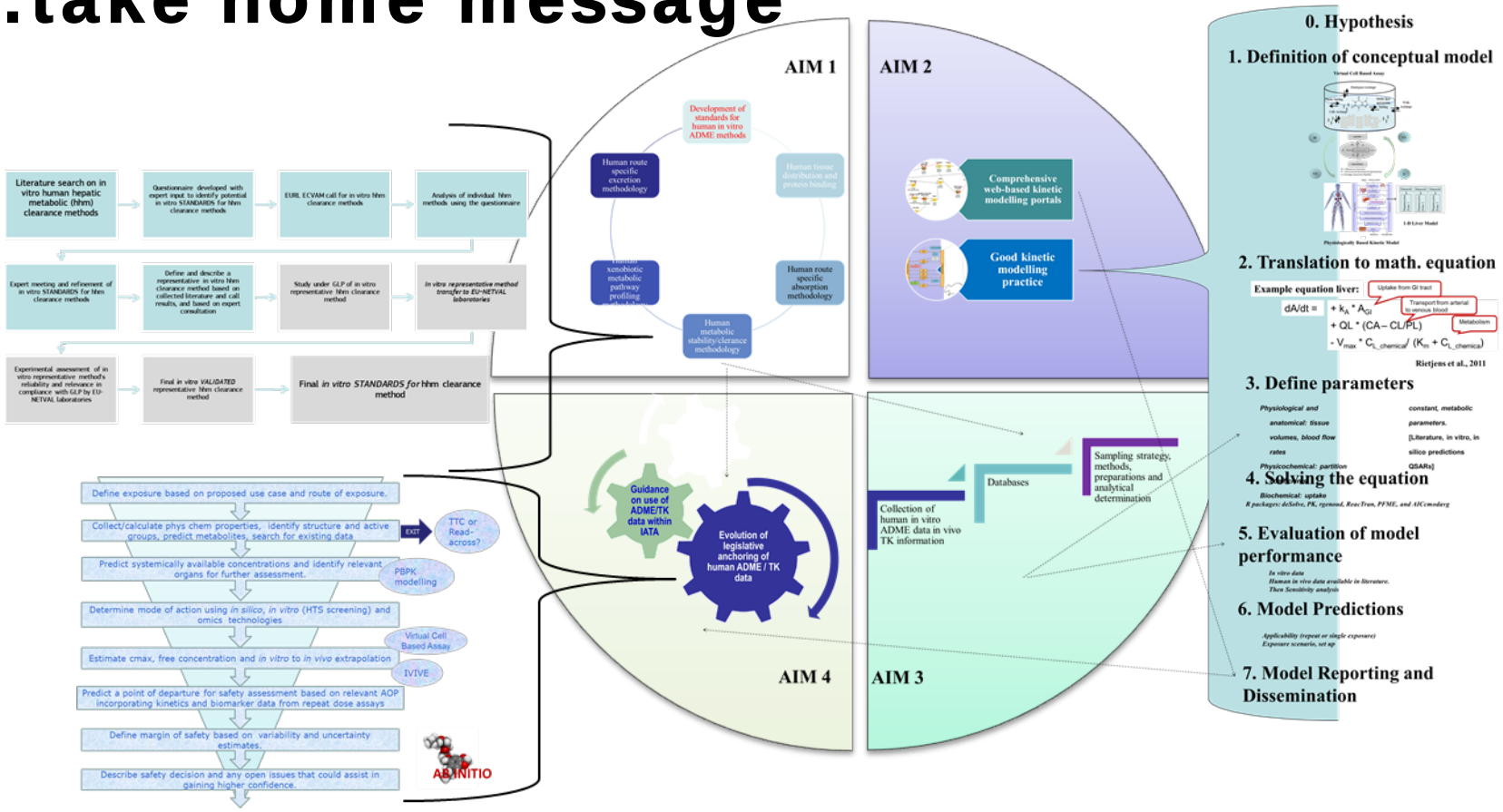
The figure illustrates predicted liver and blood concentrations of PBO alongside in vitro assay results overlap.

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

European Commission

…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.

Joint Research Centre

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