Application of biokinetic modelling for *in vitro-in vivo* extrapolation (IVIVE) in chemical risk assessment

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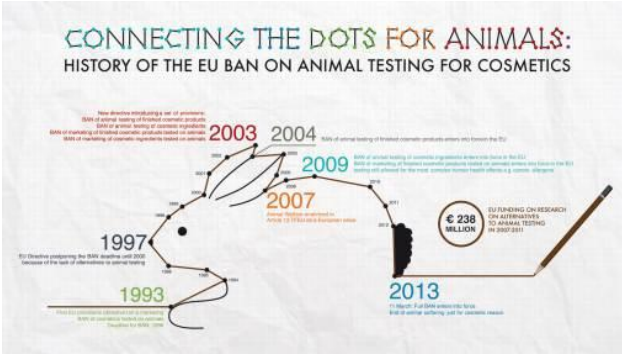
Premise

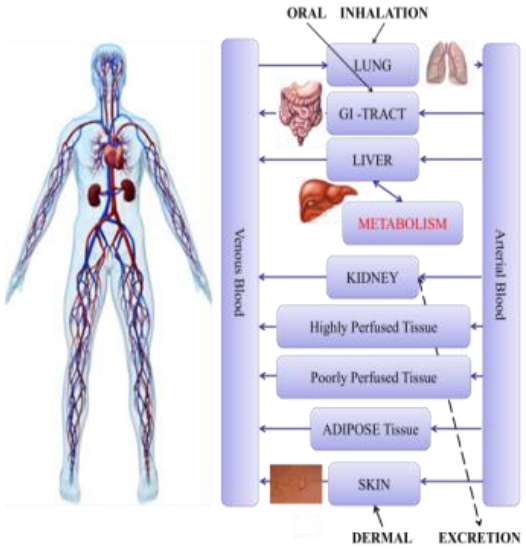
- Chemical Risk Assessment can and should be based on non-animal data
- This implies the need to use alternatives such as *in vitro* and *in silico* methods
- Especially to interpret and use *in vitro* toxicity data in combination with biokinetic data
- Biokinetic (ADME) data can be generated by *in silico* and *in vitro* models
- Mathematical modelling is the way to accurately integrate and use *in vitro* data for the design of experiments and extrapolate *in vitro* to *in vivo* for safety assessment
- Robust and reliable mathematical models are available



Pictures source: https://ec.europa.eu/growth/sectors/cosmetics/animal-testing_en
https://www.sciencemag.org/news/2019/09/us-epa-eliminate-all-mammal-testing-2035

What kinds of models are in scope?

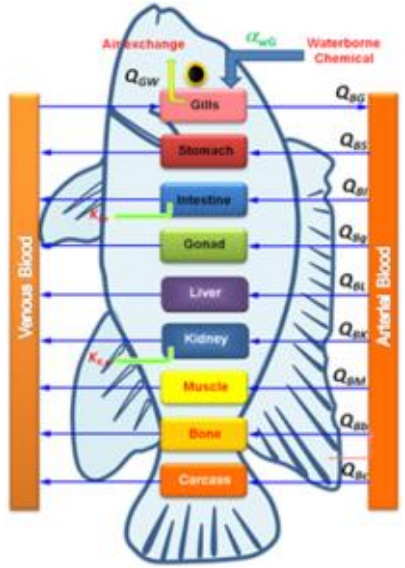
Physiologically based kinetic (PBK) model



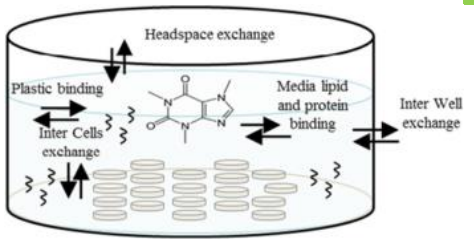
Mathematical description of the body, simulating the xenobiotic distribution into the different organs.

Throughout this presentation the more general term PBK will be used.
Noting that PBK, PBPK, PBBK and PBTK are synonyms.

Physiologically based pharmacokinetic (PBPK) is the most widely used term for kinetic models describing the absorption, distribution, metabolism and excretion of a drug within the body. Although widely used in the pharmaceutical sector, the "PBPK" term is not strictly correct in the area of chemical risk assessment. An alternative is "PBTK" with the TK representing toxicokinetic, but this is not appropriate either (Clewell & Clewell, 2008). ***More general terms, such as physiologically based biokinetic (PBBK) or physiologically based kinetic (PBK), are thus more appropriate.***



Fate and Distribution model



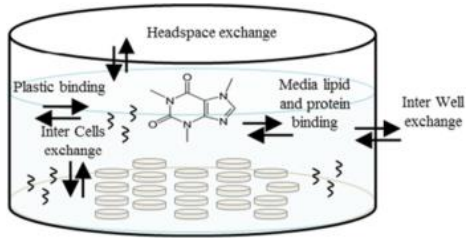
Mathematical description of the well, simulating the xenobiotic distribution into the different *in vitro* set up compartments.

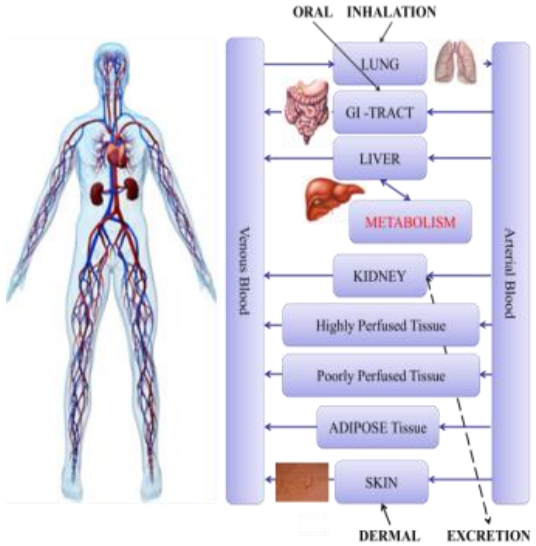
Pictures source: https://ec.europa.eu/jrc/en/publication/eurl-ecvam-workshop-new-generation-physiologically-based-kinetic-models-risk-assessment (human)
https://www.frontiersin.org/articles/10.3389/fmars.2015.00114/full (fish)

In vitro to *in vivo* extrapolation

Stream 1
Scale up of parameters

PBK model parametrisation





Stream 2
Reverse dosimetry

PBK model extrapolation

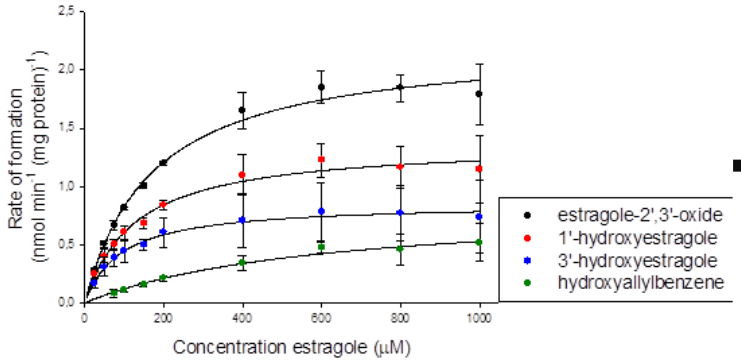
Scale-up of *in vitro* data to *in vivo* is performed by analyzing the correlation between *in vitro* and *in vivo* data or applying physiological correction factors.
in vitro data provides the parameter values for developing a model.

Translation of *in vitro* concentration effect curves into *in vivo* dose response curves.

Obtain an oral equivalent dose or a PoD.

Extrapolating adverse effects observed *in vitro* to an *in vivo* exposure.

Stream 1: Scale up of parameters



In vitro incubation rate of metabolism or clerance

nmol min-1 (mg protein)-1
nmol min-1 (mg S9 protein)-1
(measuring rate of formation)

→ V = Vmax (S) /Km + (S)

Vmax → Needs to be scaled from in vivo to in vitro
Km → assumed to be tha same as the in vivo Km (uM)

Using scaling factors (form literature); hepatocullularity values or microsomal recovery factors, non specific binding and liver weights.

- Cyps abundance
- S9 abundance
- protein abundance (HLM)

→ Vmax, *in vivo* umol hr^{-1}
Clint,H, *in vivo* uL/min/g Liver

In vitro to *in vivo* extrapolation of parameters

→ Incorporate of the scaled *in vivo* parameters in the PBK model
Liver model: Well stirred, parallel tube, dispersion

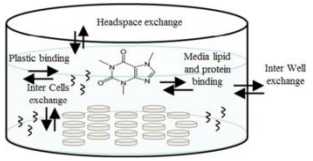
Yoon et al., 2012 June 2012
Critical Reviews in Toxicology 42(8):633-52
10.3109/10408444.2012.692115
Punt A. (2009) WUR PBK model course

Stream 2: Reverse dosimetry

Difference in exposure

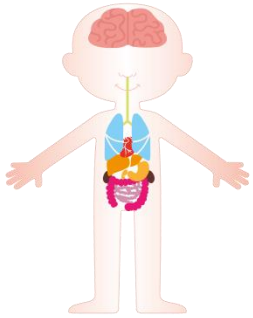
Effect measured in vitro

- 5 or 10 % serum
- Single cells
- High concentration
- Non bioaccumulation
- Plastics/Evaporation
- Short exposure
- Batch and experimental set up variability



Effect measured in vivo

- 100 % serum
- Connected complex cell system
- Low concentration
- Bioaccumulation
- No plastic/No evaporation
- Long exposure
- Inter-individual variability

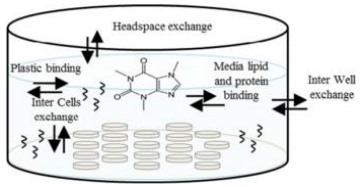


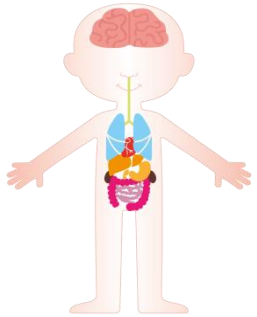
Difference in dose metrics

Maybe best dose metric: internal concentration

Adapted from presentation by Rendal et al., 2017,
NC3R event London 15-16 February 2017

Stream 2: Reverse dosimetry - Steps





Nominal Concentrations

Experimental dilutions

Exposure dose

mg/kg BW

- Armitage model (2014)
- Kramer model (2010)
- Zaldivar model (2016)
- Proenca et al (2021)

Distribution math models

PBK models

- Httk
- PKSim
- SymCip
- Simulation plus
- IndusChemFate
- MeGEN
- Berkley Madonna/Matlab/R
- Among others

Free Concentrations

Free conc

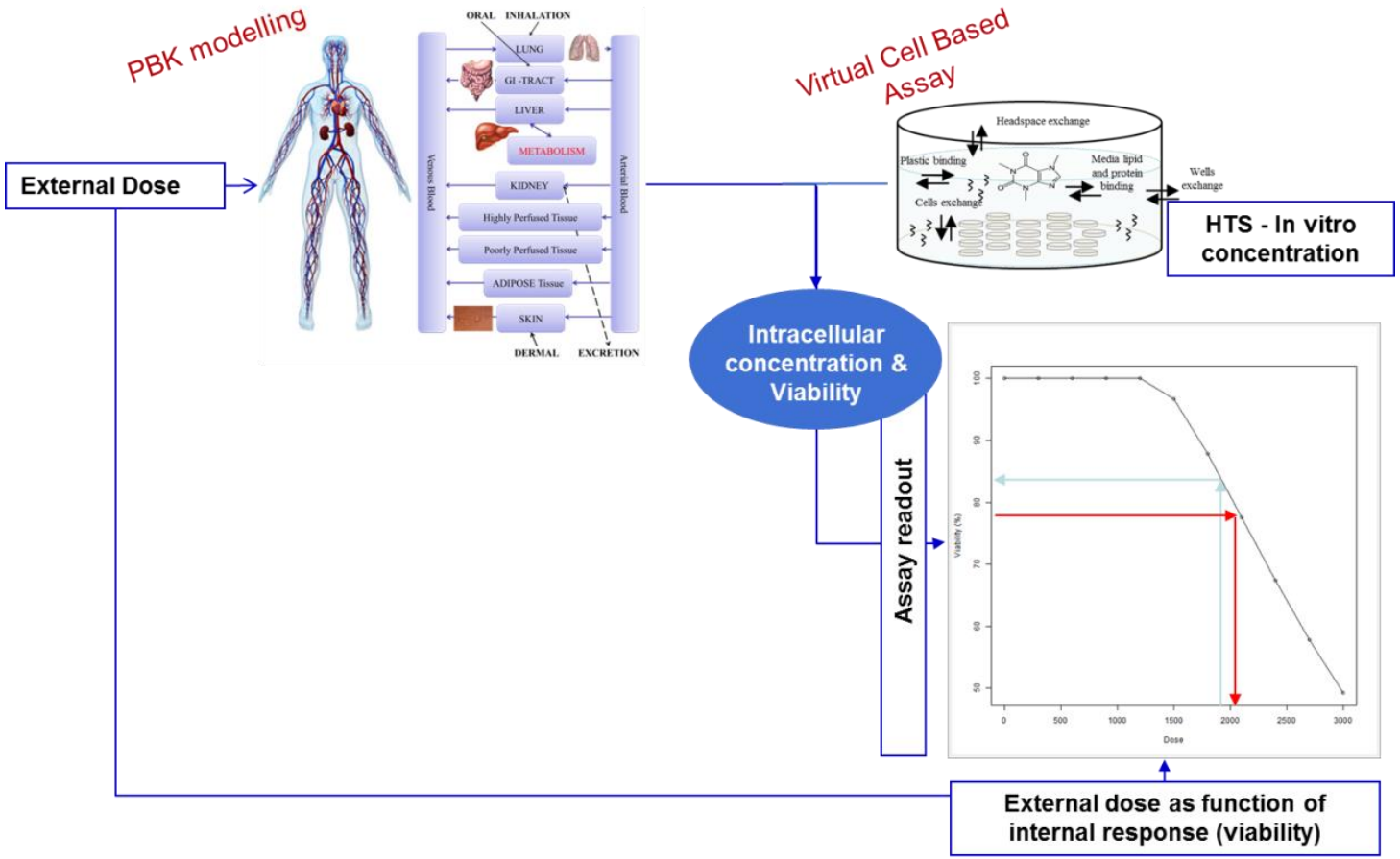
Organ concentration

Cmax & AUC

*Extrapolation Translation

*Assumption that the free concentration in the assay and the organ concertation can be considered =
Paini et al., 2017, Tox in vitro – OECD IATA 2020 – under review Systemic Toxicity of Phenoxyethanol – Pistollato et al., 2021 Rep.Tox

Stream 2: Reverse dosimetry – endpoint



This strategy has been applied to a number of **toxicological endpoints** including **developmental toxicity, genotoxicity, acute toxicity and hepatotoxicity, nephrotoxicity, neurotoxicity, and, more recently, endocrine disruption**.

Source for the different endpoint: Gubbelsvan Hal et al. 2005, Verwej et al. 2006, Forsby and Blaauboer 2007, Paini et al. 2010**, Louisse et al. 2010**, 2015; **Wetmore et al., 2012**, Strikwold et al. 2013, 2017; Li et al. 2017; Abdullah et al. 2016, Zhang et al. 2018, Fabien et al., 2019.

How to accurately integrate *in vitro* data

Stream 1
Scale up

Stream 2
Reverse dosimetry

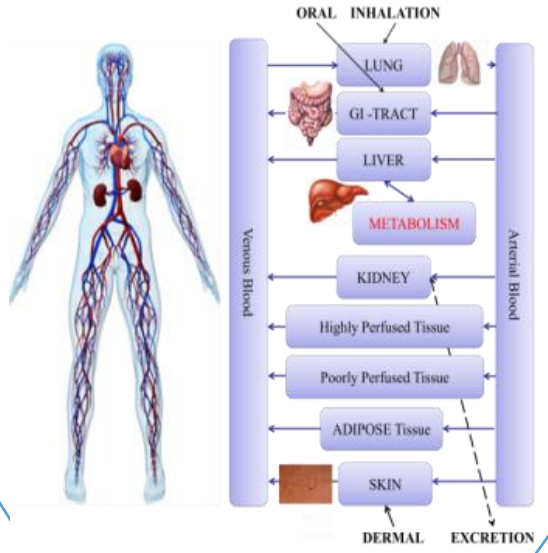
Several PBK models
available in the literature

PBK model parametrisation

IVIVE

PBK model extrapolation

QIVIVE



How to gain confidence???

Connected Streams
PB(P)K modelling

Connected Streams

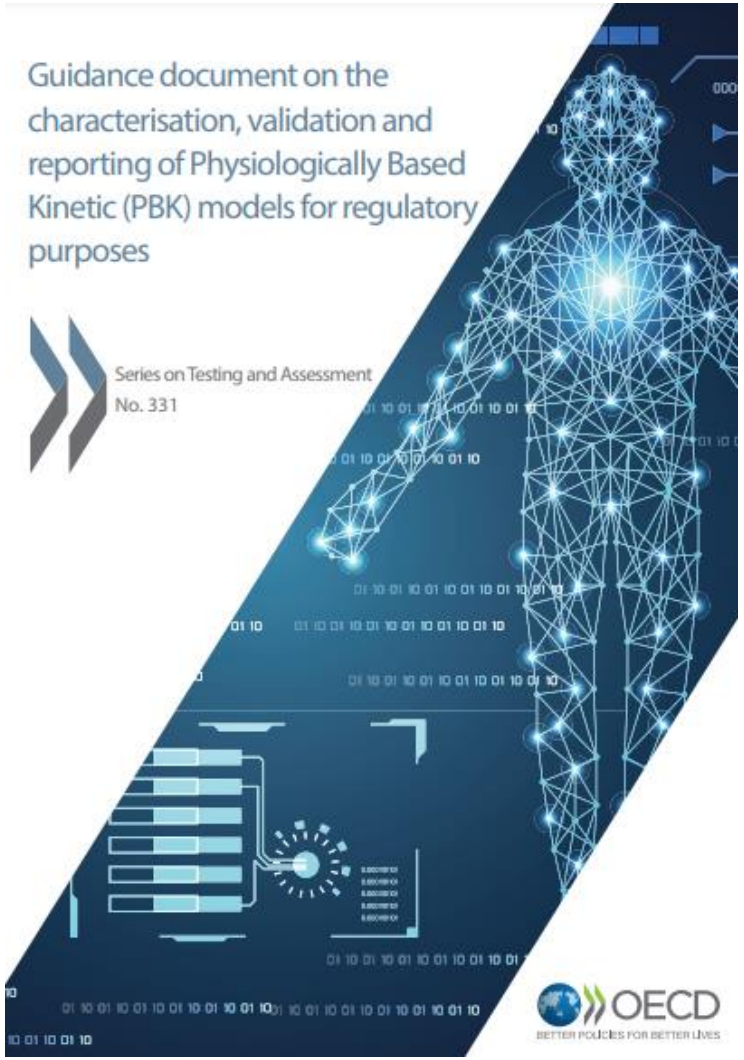
In vitro **input parameters → OECD TG & GD (OHTs) or GIVIMP**

In silico **input parameters → OECD QSAR GD - QMRF**

Evaluation/qualification/validation PBK model → OECD PBK model GD

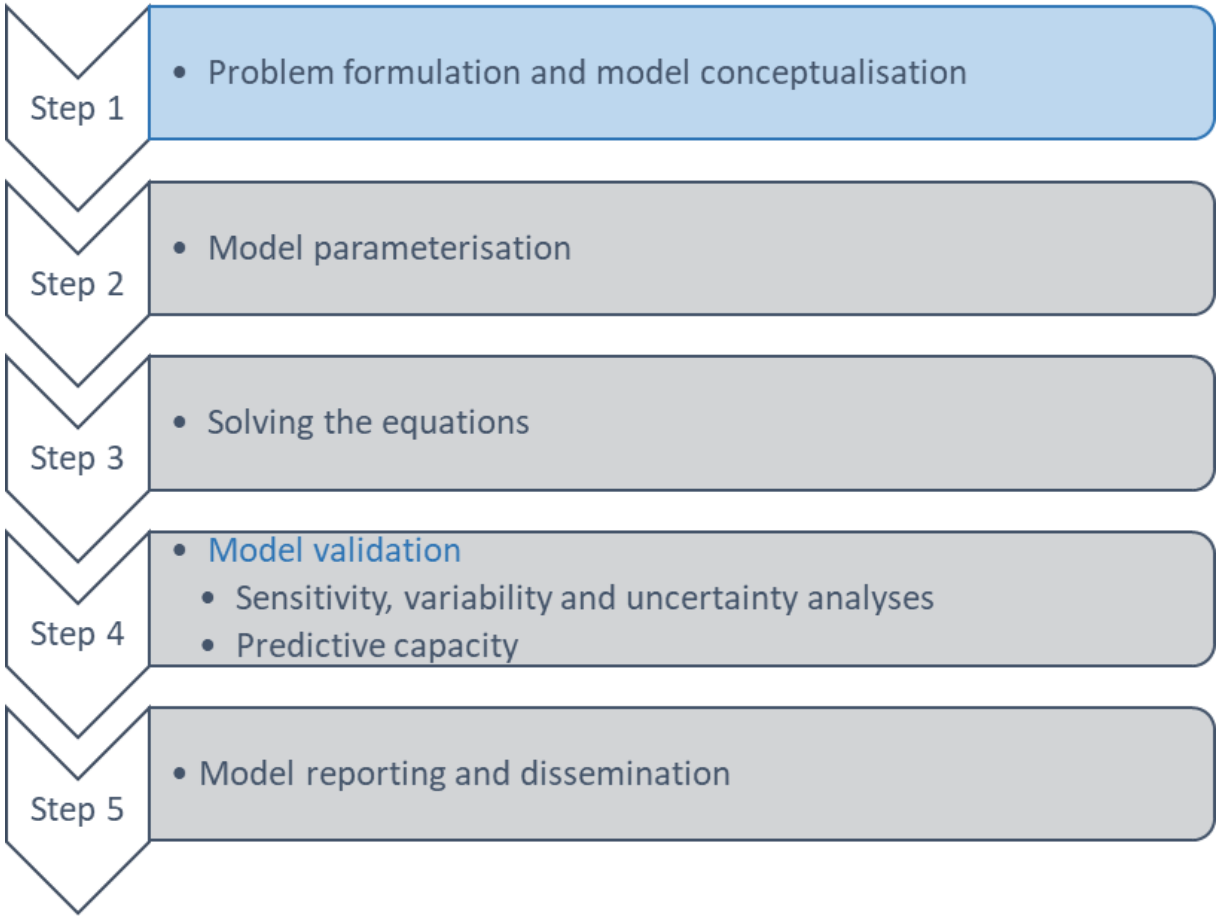
OECD PBK model GD
Purpose and scope

- Provide guidance on characterising, reporting, and evaluating PBK models used in regulatory assessment of chemicals
- Address challenges associated with developing and evaluating PBK models for chemicals without *in vivo* kinetic data
- Promote the use of PBK models in regulatory risk assessment and facilitate dialogue between model developers and users



Contents of OECD Guidance Document

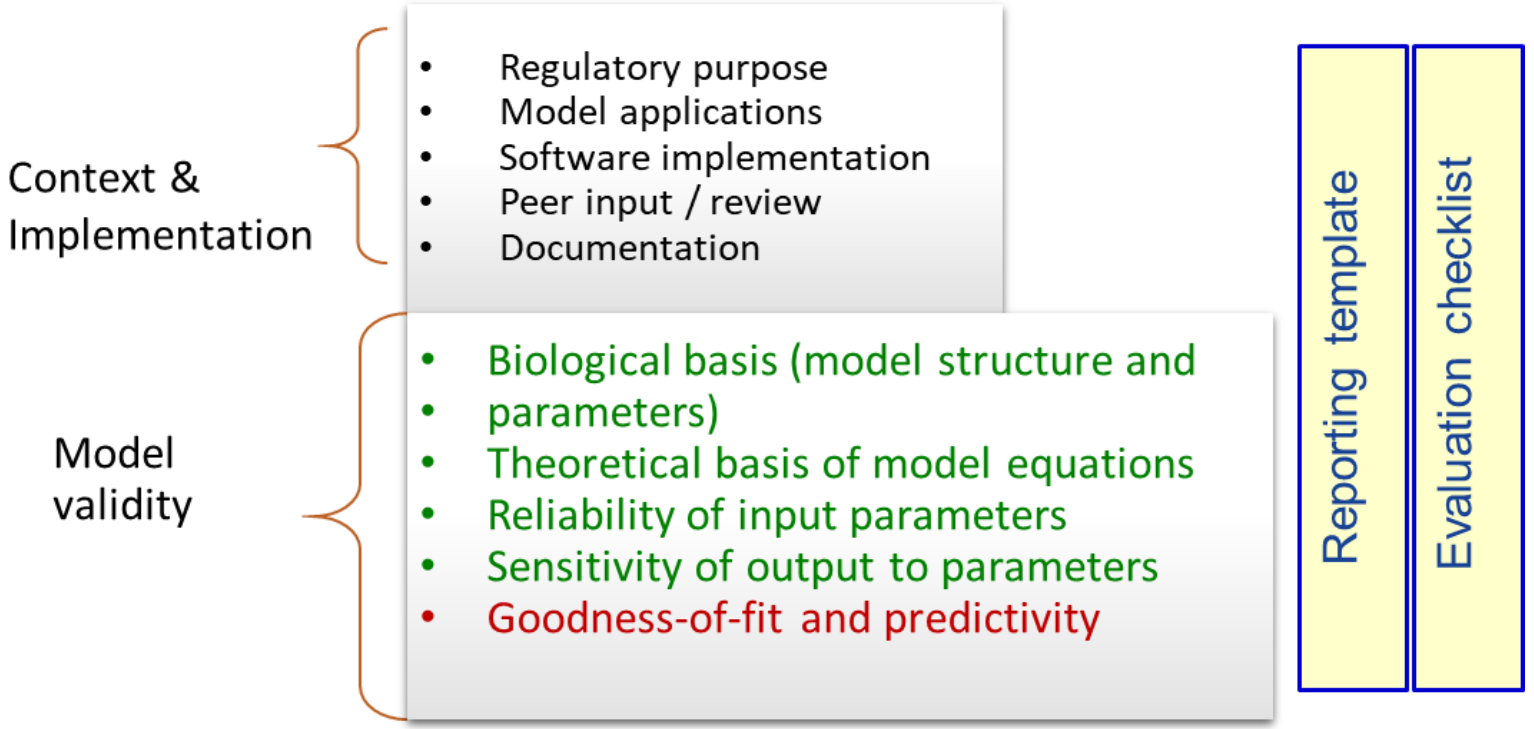
1. PBK Model workflow



Scientific workflow for characterising and validating PBK models, with emphasis on the use of *in vitro* and *in silico* data for absorption, distribution, metabolism and excretion (ADME) parameters, and in scenarios where *in vivo* kinetic data are limited or unavailable to parameterise model parameters

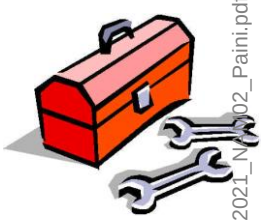
Contents of OECD Guidance Document

2. Regulatory assessment framework of PBK models



Develop an assessment framework for evaluating PBK models, with emphasis on the major uncertainties underlying the model predictions.

Contents of OECD Guidance Document

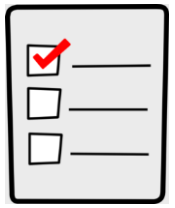


3. PBK model Evaluation tool box

1. Model Reporting Template

		Uncertainty in variability of the input parameter estimates		
		High	Medium	Low
SENSITIVITY	High		Parameter 1	Parameter 3 Parameter 4 Parameter 7
	Medium		Parameter 2	Parameter 10
	Low			Parameter 12 Parameter 13

2. Evaluation Checklist



3. Overall Evaluation Matrix (adapted from WHO 2010)

	LEVEL OF CONFIDENCE: NONE		HIGH
Biological basis	The model parameters, structure or assumptions are consistent with neither the biology nor the current state of knowledge regarding the kinetics of the chemical.	The biological basis of some model parameters, structural elements or assumptions is questionable.	The model parameters and structure have reasonable biological basis and are consistent with available kinetic data in several experiments using a single set of input parameters .
Model simulations of data	Model is unable to reproduce the shape (i.e. bumps, valleys) of the kinetic time course curves, neither for the chemical of interest nor for a suitable	Model reproduces the shape of part but not all of the kinetic time course curves, either for the chemical of interest or suitable analogue.	Model reproduces consistently all kinetic data, including the shape of time course profiles for chemical of interest.
Uncertainty in input parameters and model output; Sensitivity of model output to	No uncertainty and sensitivity analyses were performed	Local Sensitivity Analysis supports the robustness of the model.	Global Sensitivity Analysis supports the robustness of the model.

PUBLICATIONS ON TESTING AND ASSESSMENT

No. 331 Guidance Document on the Characterisation, Validation and Reporting of PBK Models for Regulatory Purposes (Glossy - Mono - Annex IV)

Thirteen case studies (listed in Annex 4)

Case Study I: Generic PBK model for farm animal species: Cattle (*Bos taurus*), Swine (*Sus scrofa*), Sheep (*Ovis aries*) and Chicken (*Gallus gallus domesticus*)

Lautz et al. (2019 a,b; 2020 a,b)

Case Study II: Generic PBK models for four fish species

Grech et al. (2017, 2018 a,b; 2019)

Case Study XIII: Generic Human one compartment and QIVIVE PB-K models

Wiecek et al. (2019 a,b)

Case Study VIII

PBK model application in species and route to route extrapolation

Bessems et al., 2017

Case study IX

Caffeine PBBK model to predict MoIE for risk assessment

IATA caffeine CS

Case study X

IVIVE-PBPK model for phenyl-1,4-dihydropyridine calcium channel antagonists

Gardner et al.

Case XI

Using high-throughput pharmacokinetic simulation and in silico property predictions to predict herbicide absorption and bioavailability

Clark Robert D

Case Study XII

Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products

Moxon et al. 2020

https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Case Study III

In vitro-to In vivo extrapolation (IVIVE) by PBTK modelling

Fabian et al. 2019

Case Study IV

PBK model predictions using data from analogues

Paini et al., 2021

Case Study V

Physiologically based pharmacokinetic (PBK) model for acrylonitrile in humans

Takano et al 2010

Case Study VI

PBK model predictions for monoisononyl phthalate

Miura et al.,2019

Case Study VII

Quantitative Proteomics-based Bottom-up PBK Modeling to Predict Chemical Exposure in Humans

Chan et al. 2019

Sources

OECD PBK model GD webinar

https://www.oecd.org/chemicalsafety/testing/webinars-on-testing-and-assessment-methodologies.htm

OECD PBK model GD (n 331)

Case Studies to illustrate (ANNEX IV)

https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Take Home

- Characterising *in vitro* and *in vivo* biokinetics is going to be critical for determining the relevance and context of your results → IVIVE!

- Connected Streams → Integration!

- As the risk assessment community increase its dependence on *in vitro* systems and NAMs, more PBK models are being developed without the use of *in vivo* data → Confidence!

Affiliation

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- As from 1st of October Senior Scientist, esqLABS GmbH, alicia.paini@esqlabs.com