Integration of metabolic fate, health effects and biokinetics predictions in an *in silico-in vitro-in vivo* approach in a tiered testing strategy

Bas J. Blaauboer
Doerenkamp-Zbinden Chair
Institute for Risk Assessment Sciences (IRAS)
Utrecht University, the Netherlands

LRI-HBM3-IRAS

The Hamner Institutes for Health Sciences

Harvey Clewell
Miyoung Yoon
Alina Efremenko
Jerry Cambell Jr.

Rijksinstituut voor Volksgezondheid en Milieu
Ministerie van Volksgezondheid, Welzijn en Sport

Emiel Rorije
Jos Bessems

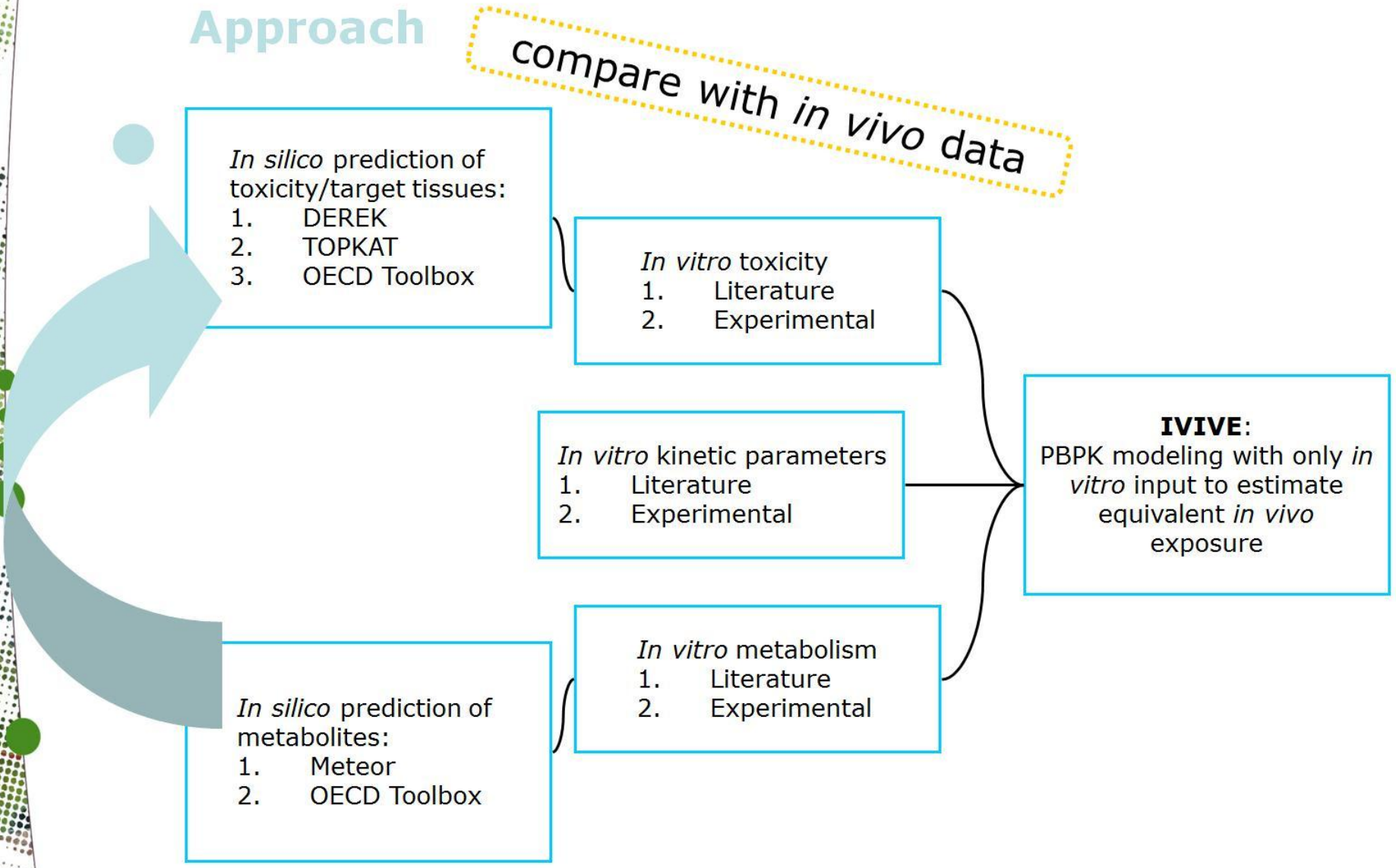
KWR Watercycle Research Institute

Merijn Schriks

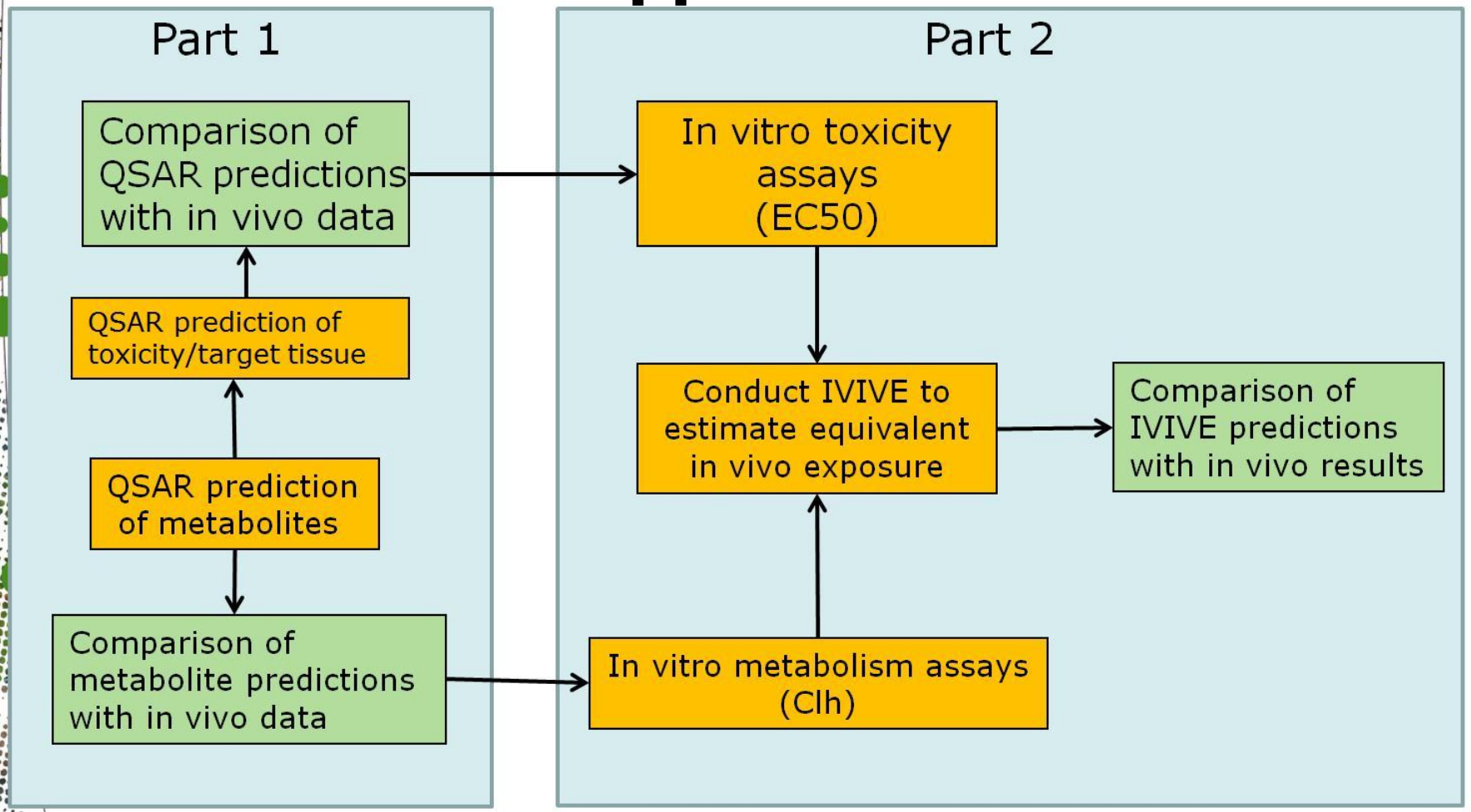
Institute for Risk Assessment Sciences

Milou ML Dingemans
Bas J. Blaauboer

Approach


compare with *in vivo* data
In silico prediction of toxicity/target tissues:
1. DEREK
2. TOPKAT
3. OECD Toolbox
In vitro toxicity
1. Literature
2. Experimental
In vitro kinetic parameters
1. Literature
2. Experimental
IVIVE:
PBPK modeling with only *in vitro* input to estimate equivalent *in vivo* exposure
In silico prediction of metabolites:
1. Meteor
2. OECD Toolbox
In vitro metabolism
1. Literature
2. Experimental

Flowchart for IRAS/Hamner Evaluation of In Vitro to In Vivo Extrapolation (IVIVE) Approach



Training set

wide range of chemical classes and use categories, selected based on the availability of *in vivo* toxicity data

chemical class	Chemical	Primary use category
organic chemicals (alcohols)	2-butoxyethanol	solvent
	ethanol	solvent
	isopropanol	solvent
organic chemicals (amides)	paracetamol	(veterinary) medication
organic chemicals (carboxylic acids)	acrylamide	industrial chemical base material
	DEHP	plasticizer
	PFOA	surfactant
organic chemicals (halogenated diphenyl ethers)	decaBDE	flame retardant
organic chemicals (halogenated hydrocarbons)	chloroform	solvent
	halothane	(veterinary) medication
	trichloroethylene	solvent
organic chemicals (hydrocarbons)	retinoic acid	(veterinary) medication
organic chemicals (organophosphorus compounds)	paraoxon	pesticide
	parathion	pesticide
organic chemicals (phenols)	bisphenol A	plasticizer
heterocyclic compounds (pyrans)	coumarin	flavoring/scent
	warfarin	(veterinary) medication
heterocyclic compounds (pyridines)	nicotine	(veterinary) medication

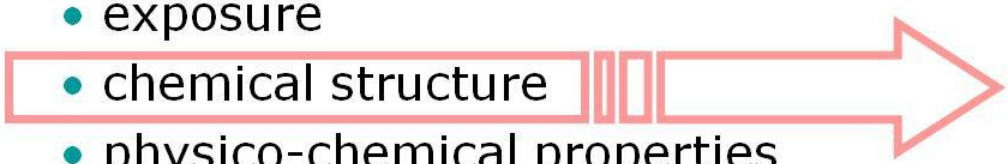
3R in chemical risk assessment – integrated testing strategies

refinement of *in vivo* testing strategies

- ethical considerations
- cost-effective considerations
- REACH

integrated testing strategies

- exposure
- chemical structure
- physico-chemical properties
- *in vitro* toxicity testing
- physiologically-based kinetic modelling



toxicity predictions

metabolism!

Methods

metabolism predictions:

METEOR (Lhasa Ltd) and the OECD QSAR Toolbox

- conjugated metabolites removed
- duplicates removed

toxicity predictions:

DEREK (Lhasa Ltd)

TOPKAT® (Accelrys)

OECD QSAR Toolbox (versions 2.0 beta and 2.1.2.865)

***in vivo* toxicity data:**

North American and EU regulatory risk assessment reports

Hazardous Substances Data Bank (Toxnet; US NIH)

Comparison of toxicity predictions with targets observed in (regulatory) experimental studies

no alerts for neurotoxicity, thyroid toxicity, reproductive system toxicity

(although these are included in DEREK)

In following slide targets are listed from risk assessment reports and HSDB

Predictions:

predicted for parent chemical	new alerts when including metabolites

ethanol *in vivo*	isopropanol *in vivo*	2-butoxyethanol *in vivo*
Risk assessment reports	Risk assessment reports	Risk assessment reports
Critical target(s): • nervous system • carcinogenicity	Critical target(s): • nervous system	Critical target(s): • nervous system • skin
Secondary target(s): • development • liver	Secondary target(s): • respiratory system • gastrointestinal system	Secondary target(s): • respiratory system • liver • kidneys
Additional targets from HSDB	Additional targets from HSDB	Additional targets from HSDB
• genotoxicity • reproductive system • cardiovascular system • hematopoietic system • immune system • liver • gastrointestinal system • adrenals • skin • eyes • bones	• cardiovascular system • hematopoietic system • immune system • liver • kidneys • skin • eyes	• carcinogenicity • hematopoietic system • immune system • eyes

predicted for parent chemical | new alerts when including metabolites

Group	Compound	CAS	developm. toxicity	carcinogenicity	hepato-toxicity	nephro-toxicity	neuro-toxicity	reproduct. system toxicity	skin toxicity
alcohols	2-butoxyethanol	111-76-2	TK-*	TK+ TB+	-	DEo	-	-	TK-* TB-
	Ethanol	64-17-5	DE+# TK+*	TK-* TB-	-	-	-	DE-	TK+* TB-
	Isopropanol	67-63-0	DE+# TK+*	TK+ TB-	-	-	-	DE-	TK- TB-
amides	Paracetamol	103-90-2	TK-	TKo* TB+	DE+#	DEo	-	-	TK- TBo
carboxylic acids	Acrylamide	79-06-1	TK+*	TK- TB+	-	-	DE+#	DE-	DE+* TK+* TB-
	DEHP	117-81-7	DE+# TK+*	TK+* TB-	DE-	DE-	-	DE+#	TKo^ TB-
	PFOA	335-67-1	TK-*	TK- TB-	-	DEo	-	-	TK+* TB-
halogenated diphenyl ethers	decaBDE	1163-19-5	TK+	DE+ TK+ TB+	-	DE-	-	-	TK+^ TB-
halogenated hydrocarbons	Chloroform	865-49-6	TK-*	DE+# TK+ TB+	DE+#	-	-	-	TK-* TB+
	Halothane	151-67-7	TK-^	DE+ TK- TB+	DE+#	DEo	-	-	TK-^ TB-
	Trichloroethylene	79-01-6	TK-	DE+ TK+* TB+	DE+	DE+#	-	-	TK+* TB-
hydrocarbons	retinoic acid	302-79-4	DE+* TK-	Deo* TK+* TB-	DE+*	-	-	-	TK+^ TB-
organophosphorus compounds	Parathion	56-38-2	TK-	DE+ TK- TB+	DE+	-	DE+	-	DE+ TK-^ TB-
Phenols	bisphenol A	80-05-7	TK-*	DE+* TK- TB-	-	DEo*	-	DE+*	DE+ TK+ TBe
Pyrans	Coumarin	91-64-5	TK-	TK+* TB+	-	-	-	-	DE+* TK-* TB-
	Warfarin	81-81-2	DEo# TK-	TK-^ TB+	DE+#	-	-	-	DE+ TK+ TBe
Pyridines	Nicotine	54-11-5	TKo	TK+* TB-	DEo	-	-	-	TK-^ TB-

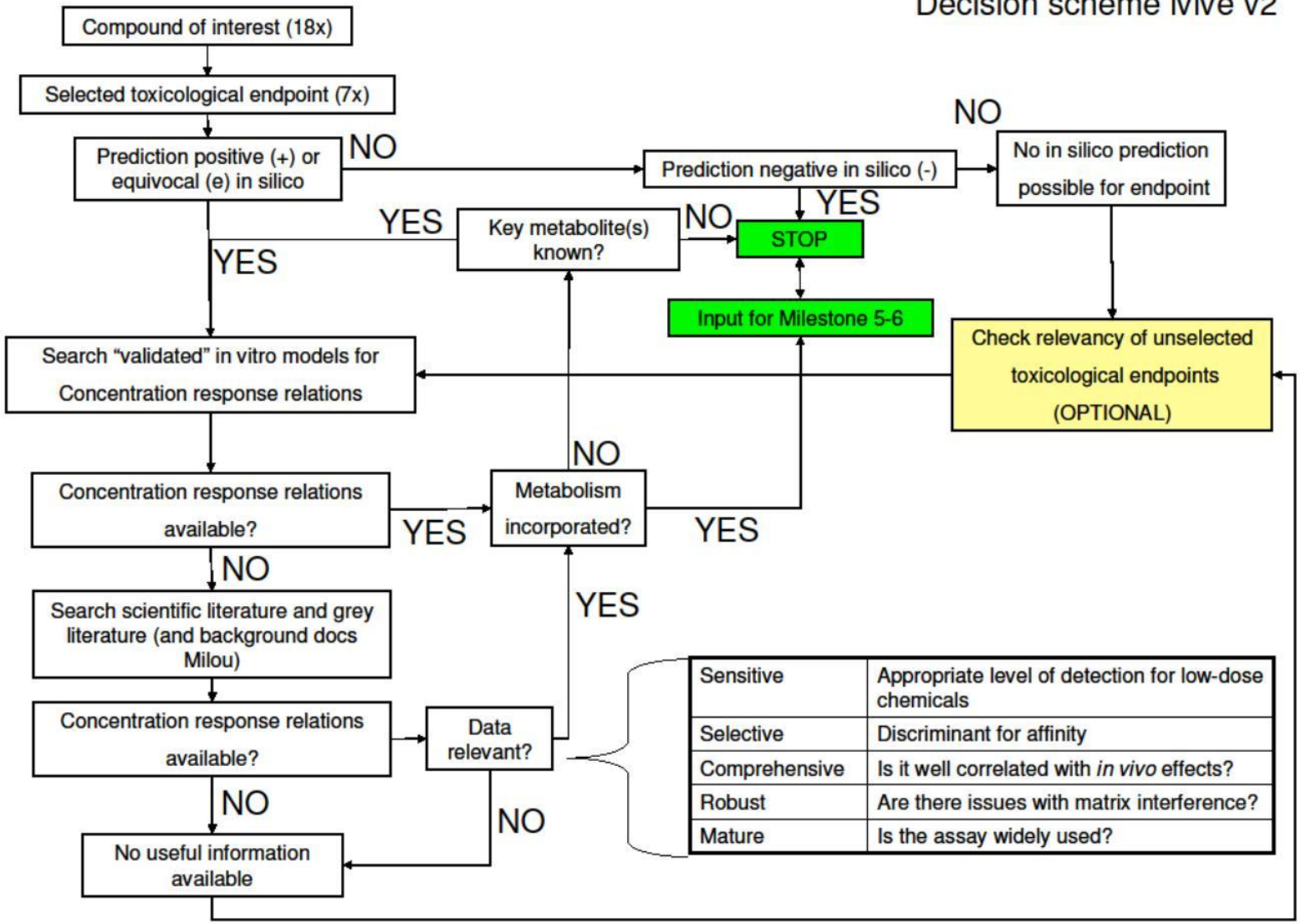
Part 1a: Prediction of Metabolism

- The OECD Toolbox was able to correctly predict the primary metabolite responsible for the toxicity of 9 of the12 chemicals investigated in this study where toxicity is due to a metabolite
- However, a number of other metabolites were also predicted, including many that have not been detected in vivo
- The prediction of nontoxic or low-yield metabolites makes the process of investigating possible metabolite toxicity more difficult and time-consuming

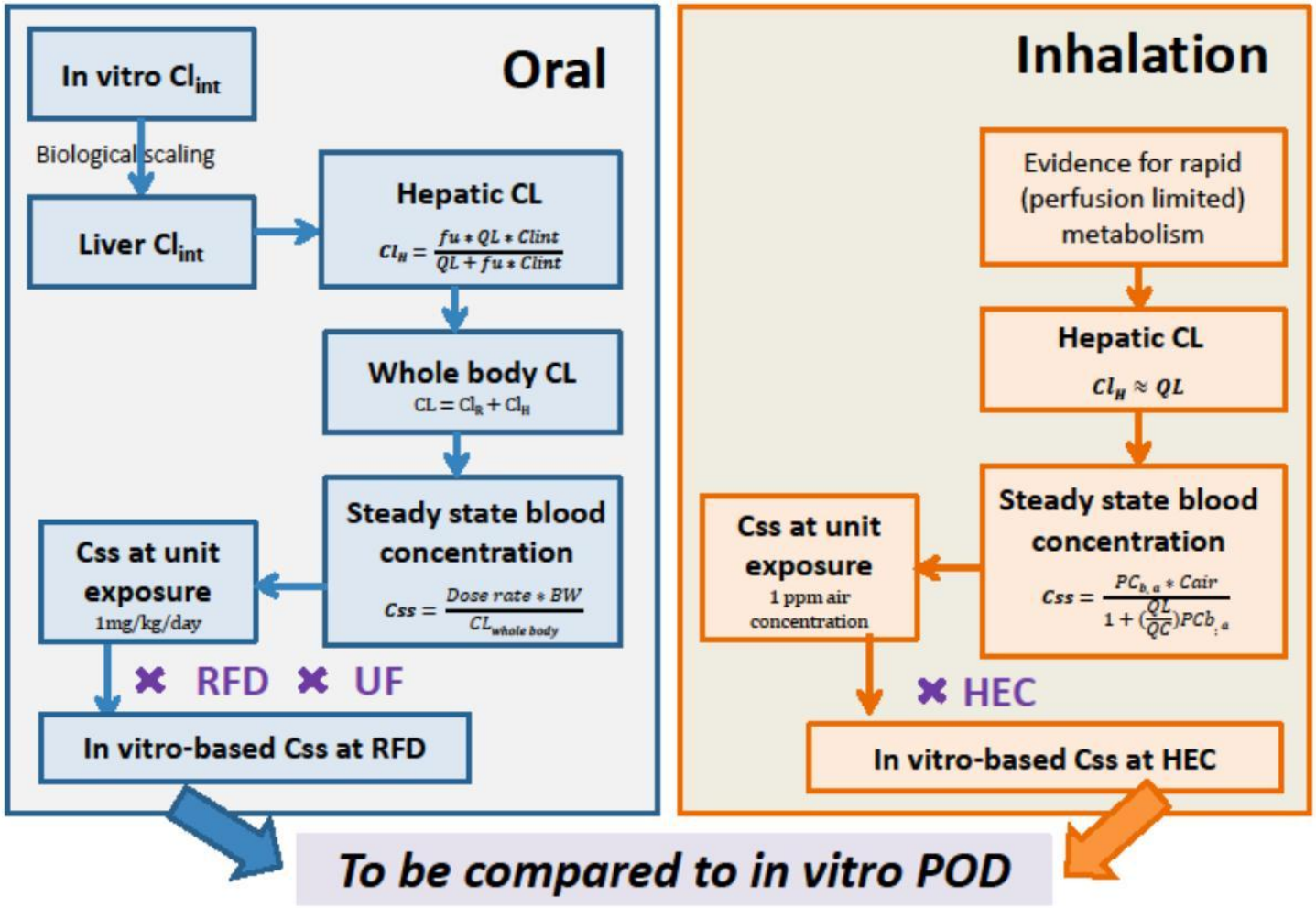
Part 1b: Prediction of Toxicity

- The critical toxic endpoint was successfully predicted for 11 of the 17 compounds evaluated
- By including QSAR-predicted metabolites in QSAR prediction of toxicity, the average sensitivity across the endpoints tested increased from 0.35 to 0.55, compared to predictions based on parent chemical alone

Decision scheme ivive v2

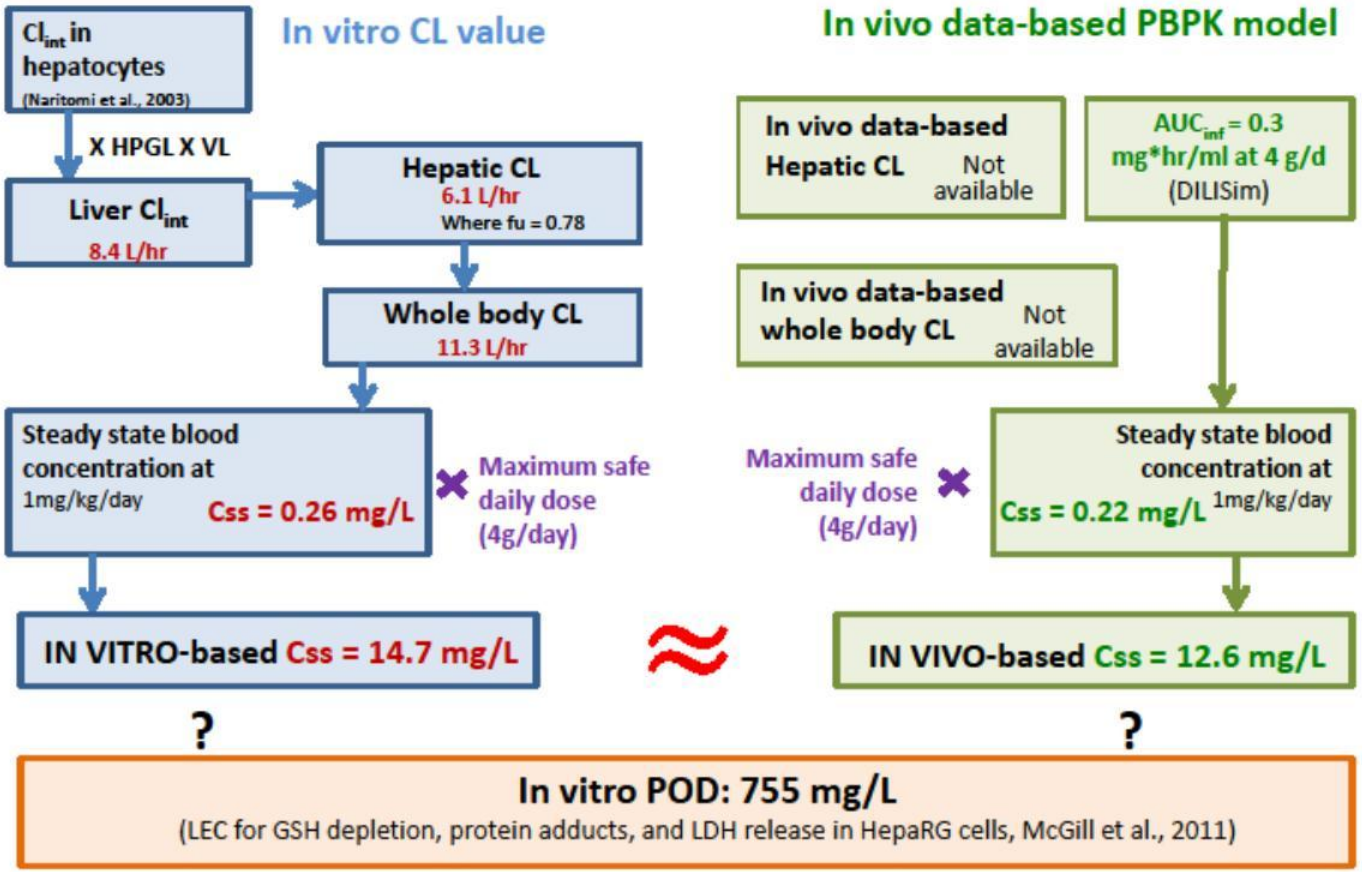


Part 2: In Vitro to In Vivo Extrapolation



Evaluation of IVIVE approach for Paracetamol

In vitro-based Css vs. PBPK model-based/in vivo data-based Css vs. in vitro POD

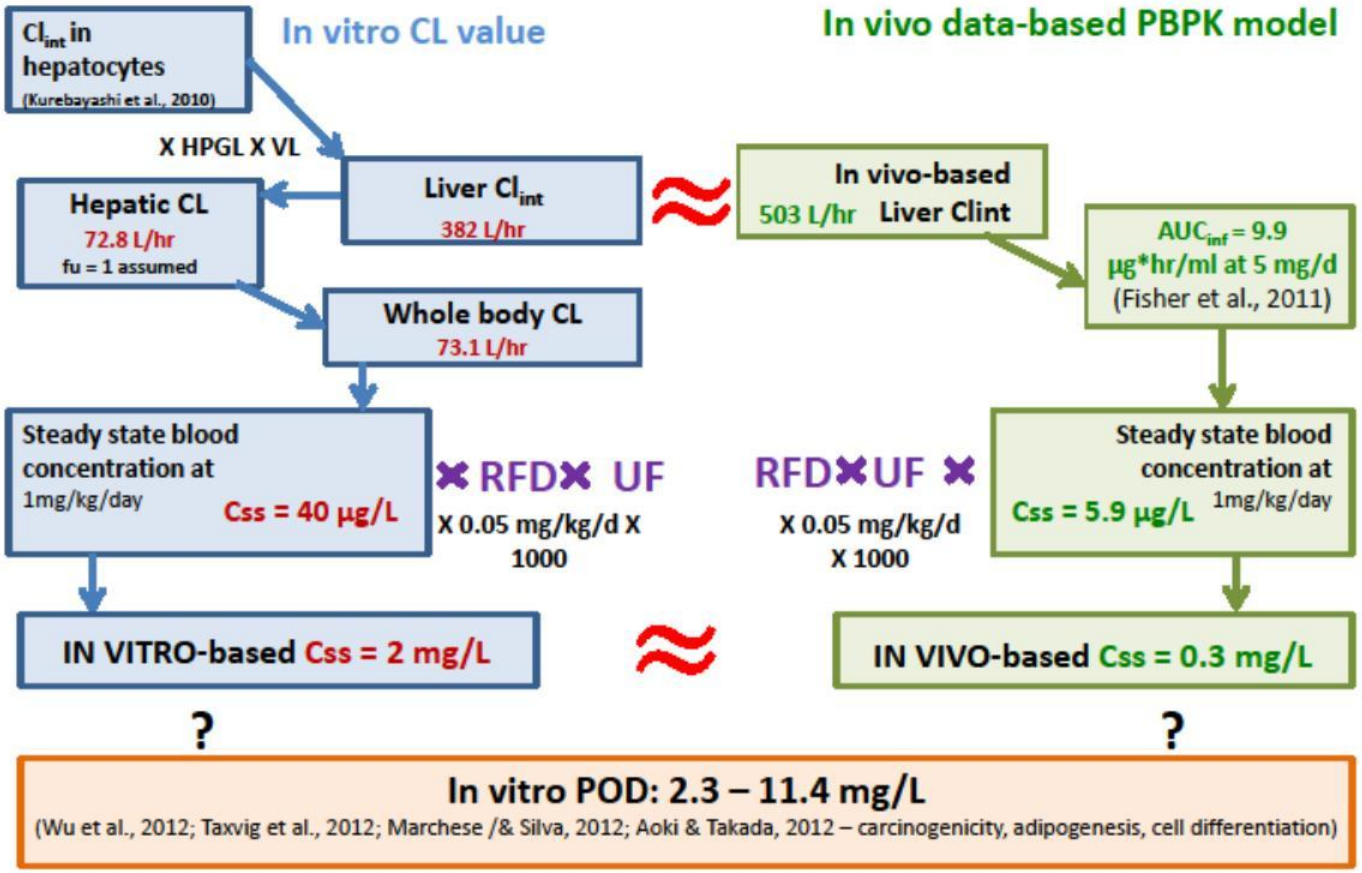


Summary: Paracetamol

- Critical toxic endpoint:
  - Predicted: hepatotoxicity, among others
  - In vivo: hepatotoxicity
- Toxic metabolite prediction:
  - Prediction: no oxidative metabolism
  - In vivo: toxicity due to oxidation to reactive metabolite
- IVIVE:
  - In vitro predicted Css for 1mg/kg/d: 0.26mg/L
  - In vivo Css for 1 mg/kg/d: 0.22 mg/L
- Toxicity estimate:
  - In vitro predicted: 14.7mg/L
  - In vivo: 755 mg/L
- Problem:
  - Toxicity due to production of reactive metabolite
  - Lack of metabolic competence in *in vitro* assays

Evaluation of IVIVE approach for Bisphenol-A

In vitro-based Css vs. PBPK model-based/in vivo data-based Css vs. in vitro POD

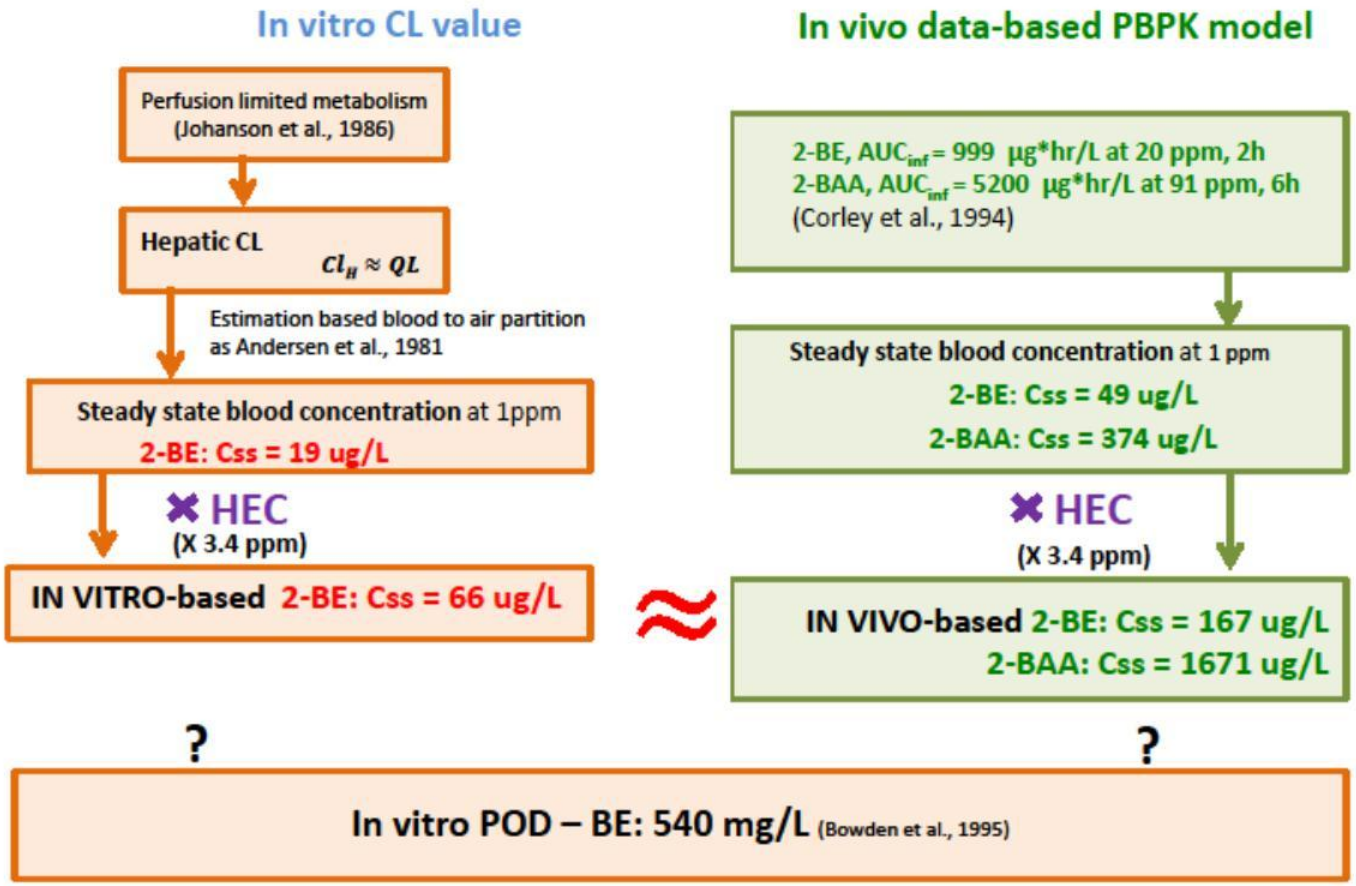


Summary: Bisphenol A

- Critical toxic endpoint prediction:
  - Predicted: reproductive system toxicity
  - In vivo: decreased body weight gain
- Toxic metabolite prediction: N/A
- IVIVE:
  - In vitro predicted Css for 1mg/kg/d: 40 ug/L
  - In vivo Css for 1 mg/kg/d: 5.9 ug/L
- Toxicity estimate:
  - In vitro predicted: 2 mg/L
  - In vivo: 2.3-11.4 mg/L
- Problem: uncertain mode of action for *in vivo* effect

Evaluation of IVIVE approach for 2-Butoxyethanol

In vitro-based Css vs. PBPK model-based/in vivo data-based Css vs. in vitro POD



Summary: 2-Butoxyethanol

- Critical toxic endpoint prediction:
  - Predicted: hepatotoxicity (metabolite)
  - In vivo: heptotoxicity (metabolite)
- Toxic metabolite prediction:
  - Predicted: 2-butoxyacetic acid, among others
  - In vivo: toxicity due to 2-butoxyacetic acid
- IVIVE:
  - In vitro predicted Css for 1 ppm 2-BE: 19 ug/L
  - In vivo Css for 1 ppm 2-BE: 49 ug/L
- Toxicity estimate:
  - In vitro predicted: 66 ug/L
  - In vivo: 540 mg/L
- Problem:
  - Toxicity due to metabolite
  - Lack of metabolic competence in *in vitro* assays

Conclusion

- Structure >> toxicity:
  - qualitatively reasonably good predictions of toxic endpoints, especially if metabolism is included
- In vitro toxicity >> prediction of in vivo toxicity:
  - quantification: problem: choice of POD and UF
  - good estimates if in vitro data also take into account metabolism

Perspective