In Vitro to *In Vivo* Extrapolation in Predictive Toxicology

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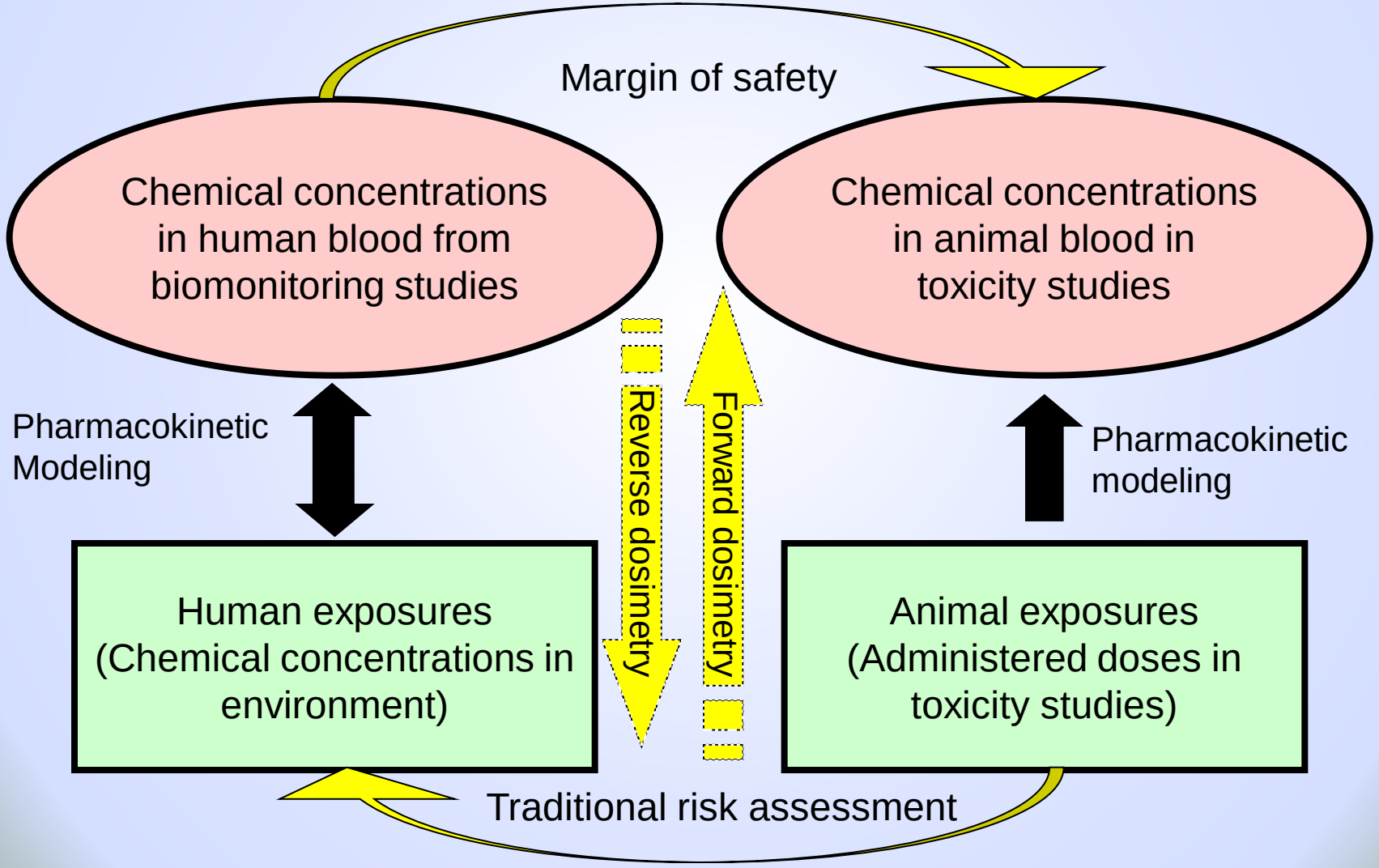
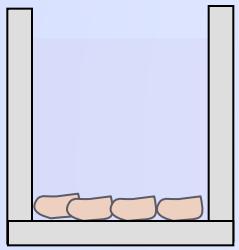
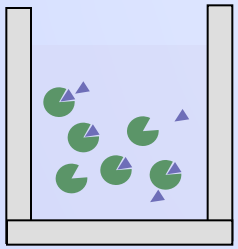
The Traditional Role of Biokinetics: Relating Animal Doses to Equivalent Human Exposures

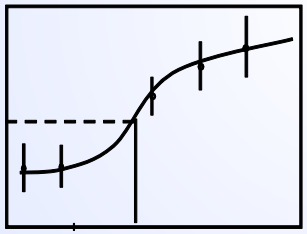


In the Future: Biokinetics Will Be **Necessary** to Relate the Nominal Concentration in an *In Vitro* Assay to the Equivalent *In Vivo* Human Exposure





***In Vitro* Toxicity Assays**



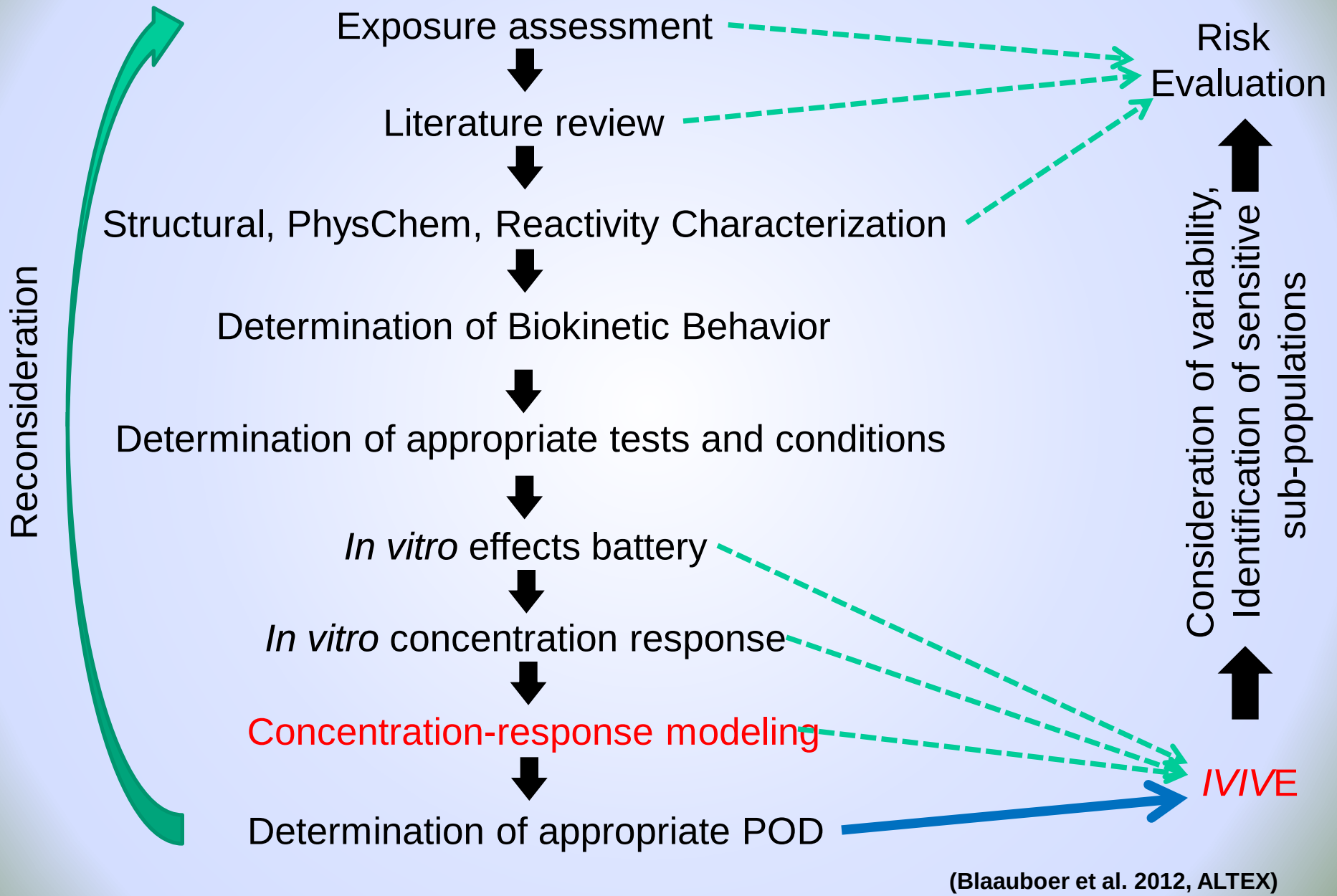
EC_{50} or Data on Concentration-Response

QIVIVE*

Estimate of Human Equivalent Dose

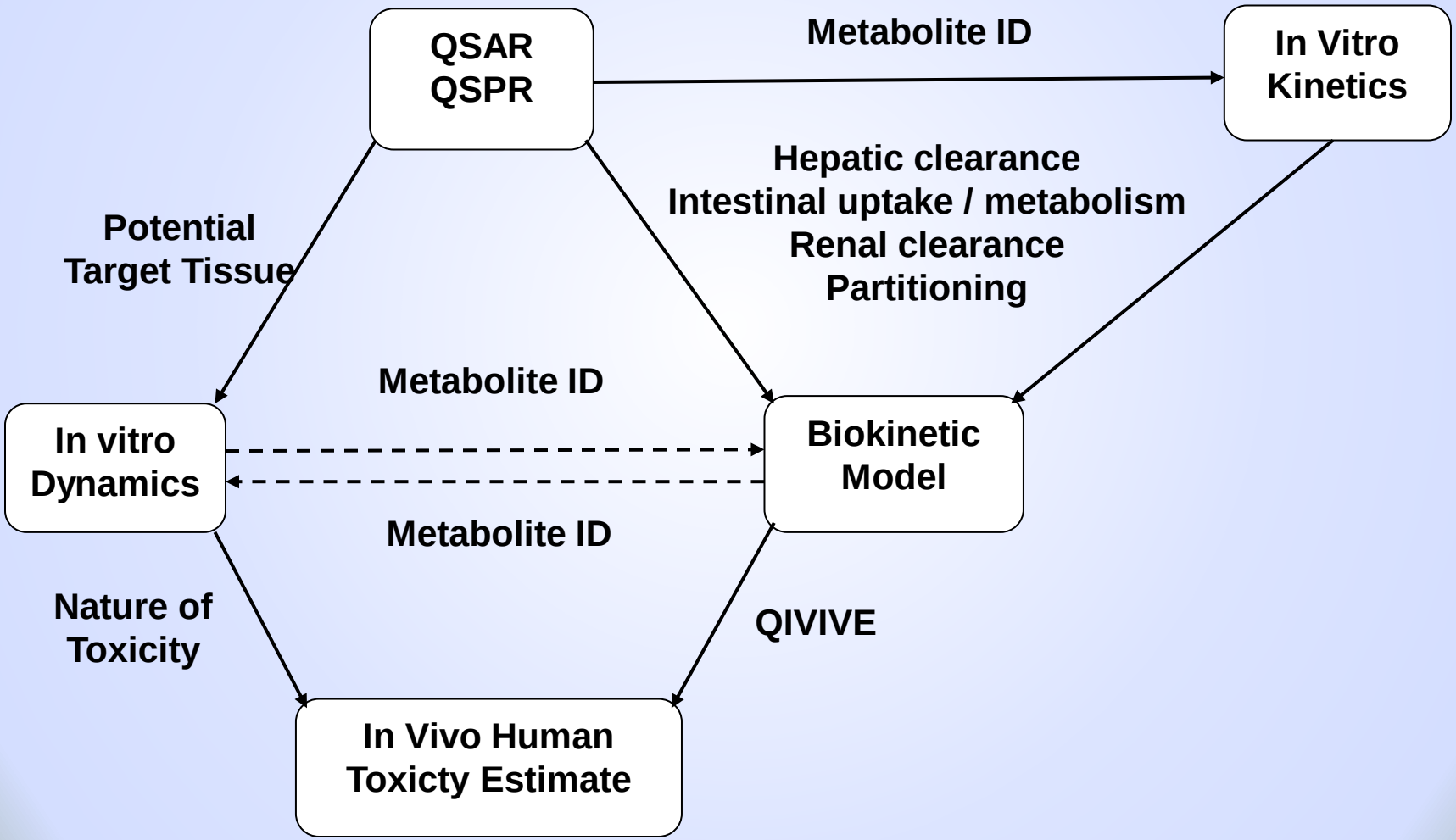
*** Quantitative *In Vitro* to *In Vivo* Extrapolation**

Step-wise *In Vitro* Based Risk Evaluation Approach



(Blaauboer et al. 2012, ALTEX)

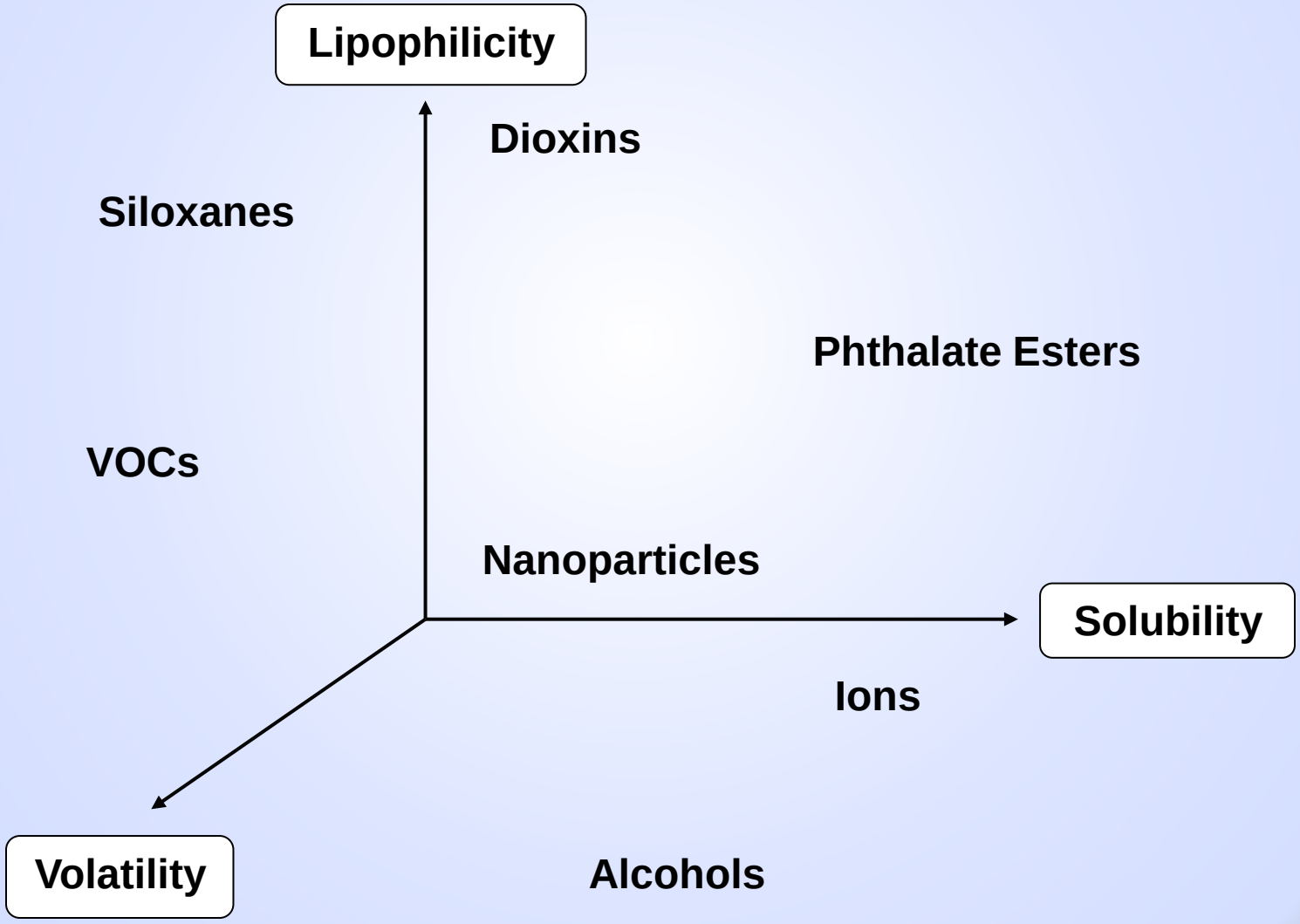
QIVIVE Approach



QSAR/QSPR for QIVIVE

- Multiple methods available
  - Correlations
    - Physical Chemical Properties
      - Kow, Sw, Hb/g, pKa, permeability
      - partition coefficients
  - Fragment- or rule-based systems
    - metabolism (qualitative)
      - Derek/Meteor, OECD Toolbox
    - 3-D docking (some cyps)
- Limitation
  - Availability and quality of training data for the wide range of chemical property classes (particularly for “non-druglike” compounds)

Physico-Chemical Classification



Considerations for QIVIVE

Pharmacokinetic factors that affect ***in vivo*** toxicity but are not appropriately reflected in ***in vitro*** toxicity tests:

- Bioavailability
- Transport
- Protein binding
- Clearance
  - Metabolic
  - Renal
  - Biliary
  - Exhalation

In Vitro Metabolism

- Kinetics
  - Primary hepatocytes
  - Tissue slices
  - Microsomes, cytosol
  - Recombinant cyps
  - Organotypic cultures
- Identification of primary metabolites
  - QSAR (Meteor, Multicase)
  - GC/MS, LC/MS

Complications Due to Metabolism

- Metabolite could be responsible for toxicity
  - Toxicity would not be observed in test cells with low metabolic competence
- Circulating metabolite could be toxic in tissue other than liver
  - brain (trichlorethanol from chloral hydrate)
  - kidney (reactive thiol from glutathione conjugate)

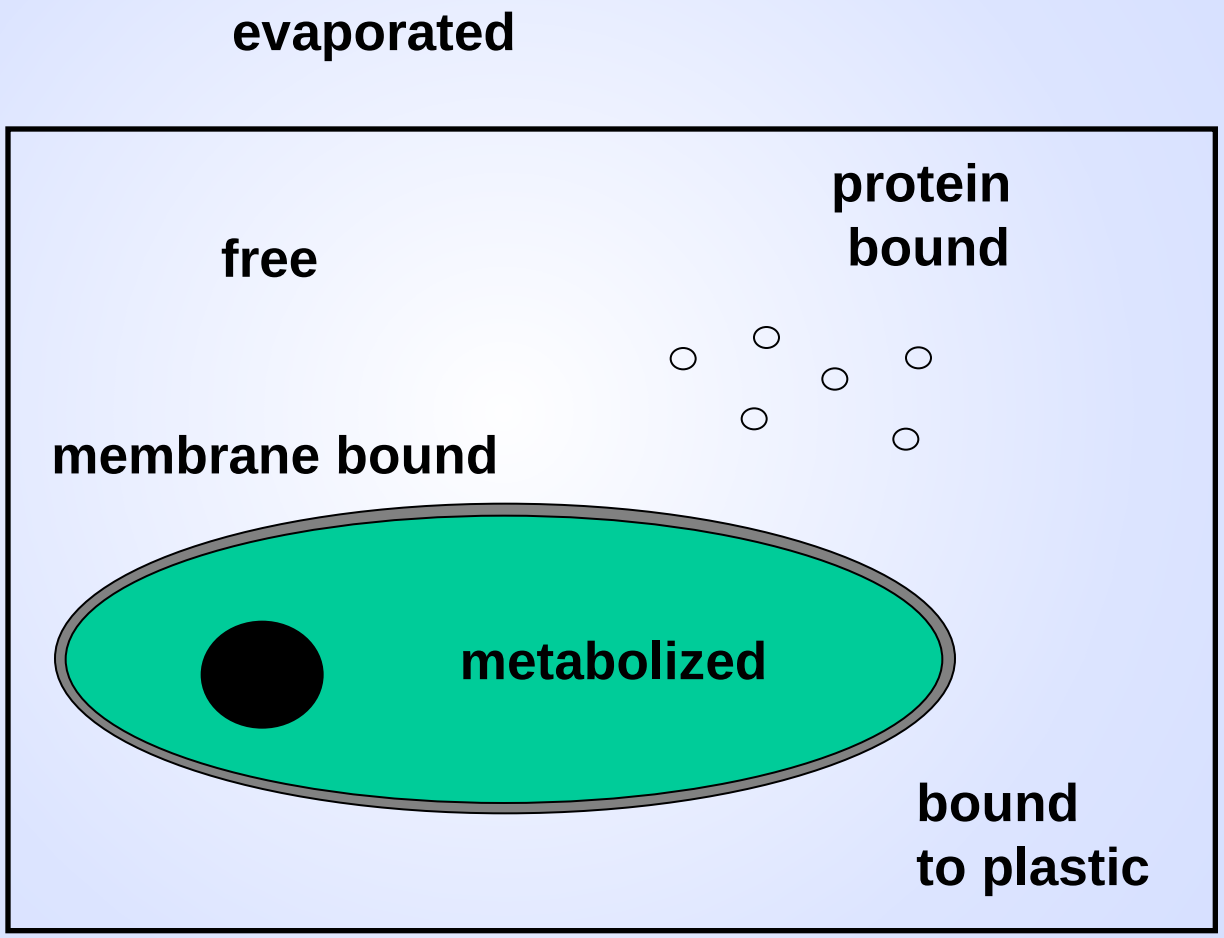
Other Factors

- Urinary clearance
  - water soluble chemicals
  - based on glomerular filtration rate
  - Ignores active transport
- Ventilatory clearance
  - volatile chemicals
  - based on alveolar ventilation rate
- Poor absorption
  - Estimate bioavailability (e.g., Caco2)

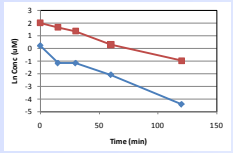
Key Problem for QIVIVE

- Problem: Typically No Measurement of Free Chemical Concentration during *In Vitro* Assay
  - Nominal concentration (Dose/Volume) unacceptable for QIVIVE
- Possible Approach:
  - Measure media concentration
  - Determine binding in media vs. plasma
  - Estimate media:cell partition
  - Check mass balance with media and cell concentrations
- The only thing worse than no data is bad data

Processes Affecting Free Concentration

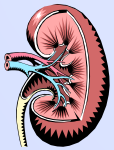


QIVIVE and Reverse Dosimetry for Interpreting *In Vitro* Assay Results



Hepatocellular Clearance

Plasma Protein Binding



Estimated Renal Clearance

Prediction of in vivo clearance

Reverse Dosimetry

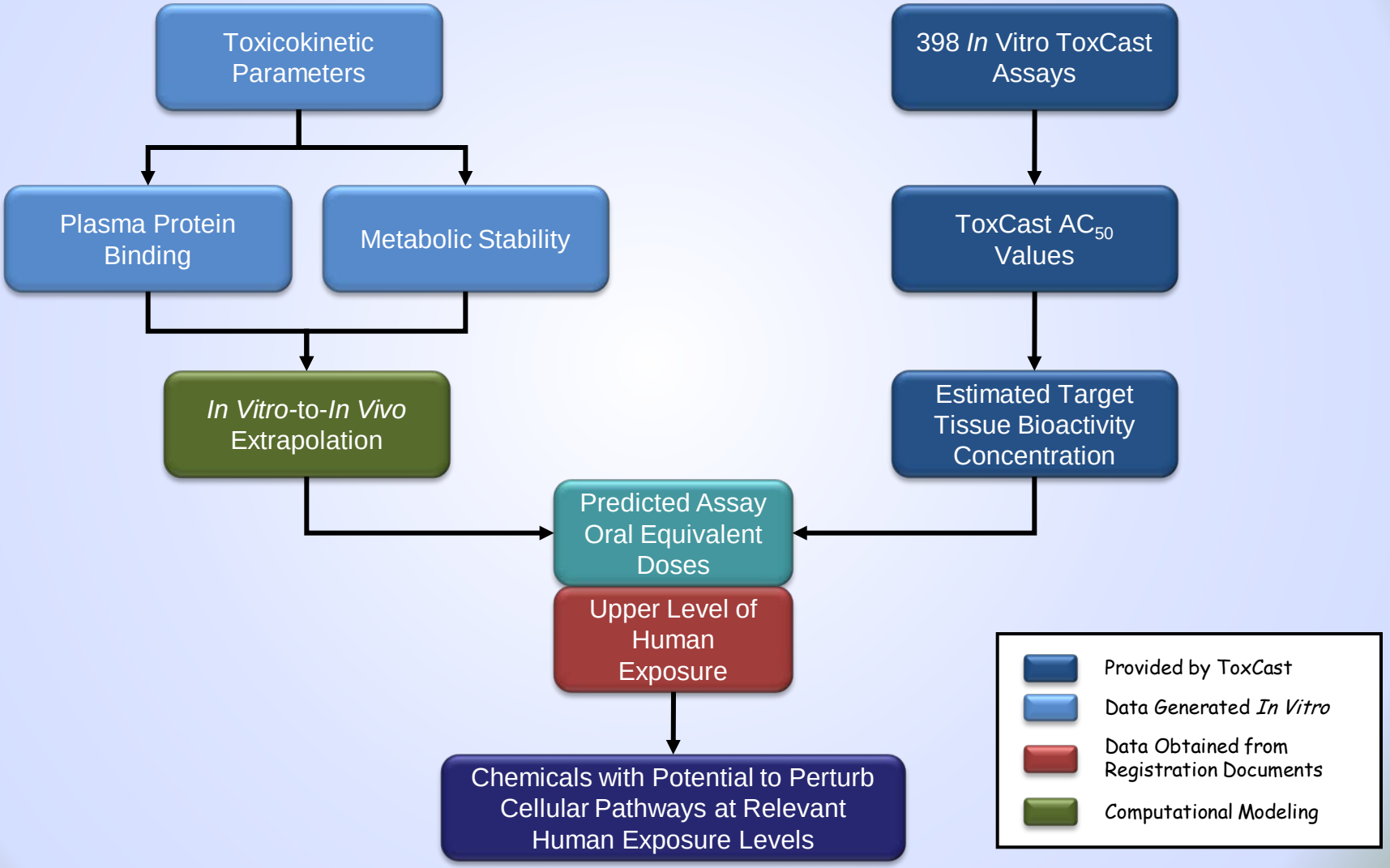
Equivalent Exposure

Concentration from in vitro assay

Comparison of *in vitro*-to-*in vivo* extrapolation results with estimates based on *in vivo* PK (Wetmore *et al*. 2012)

Chemical	In Vivo Derived Css (µM)	IVIVE Restrictive (fub) Css (uM)	IVIVE, Non-restrictive Css (uM)
2,4-dichlorophenoxyacetic acid	**9.05-90.05**	39.25	39.25
Bisphenol-A	**< 0.13**[d]	0.35	0.06
Cacodylic acid	**1.80**	3.06	3.06
Carbaryl	**0.03**	0.04	0.03
Fenitrothion	**0.03**	17.91	0.10
Lindane	**0.46**	13.21	0.07
Oxytetracycline dihydrate	**0.36**	2.00	2.00
Parathion	**0.17**	24.63	0.14
Perfluorooctanoic acid	**20,120**[g]	55.34[g]	0.4[g]
Picloram	**0.27**	57.63	0.37
Thiabendazole	**0.45**	13.76	13.76
Triclosan	**2-10**	1.56	0.01

Application: Defining Dosimetry in High Throughput Toxicity Screens



Results From Reverse Dosimetry Analysis

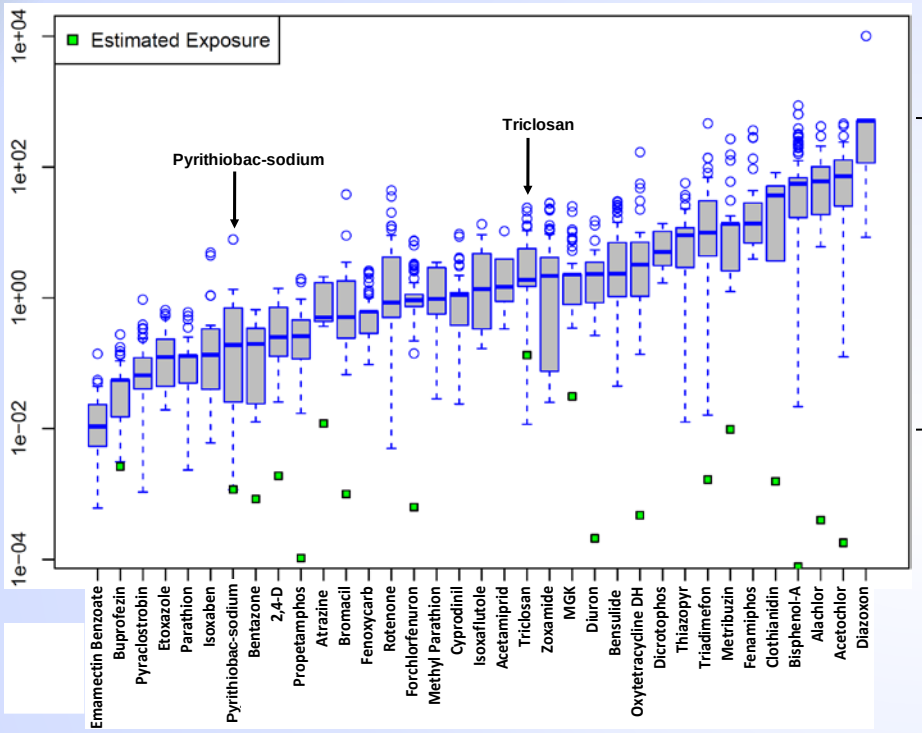
The Same EC50 Does Not Imply the Same Exposure!

Chemical	ToxCast Endpoint	Minimum EC50 or LEL (uM)	Est Oral Equivalent (mg/kg/day)
Acetamiprid	**BSK_BE3C_uPAR**	**1.481**	**0.384**
Atrazine	**BSK_KF3CT_IP10**	**1.481**	**1.215**
Bromacil	**BSK_BE3C_IP10**	**1.481**	**0.888**
Forchlorfenuron	**BSK_BE3C_uPAR**	**1.481**	**1.277**
Metribuzin	**BSK_hDFCGF_MMP1**	**1.481**	**6.577**
Isoxaflutole	**BSK_hDFCGF_EGFR**	**1.481**	**1.209**
Dicrotophos	BSK_hDFCGF_PAI1	1.481	2.632
Clothianidin	BSK_hDFCGF_EGFR	1.481	7.580
Diazoxon	BSK_KF3CT_IP10	1.481	0.266
Oxytetracycline	**BSK_BE3C_IL1a**	**1.481**	**0.567**
2,4-D	**BSK_BE3C_IL1a**	**1.481**	**1.389**

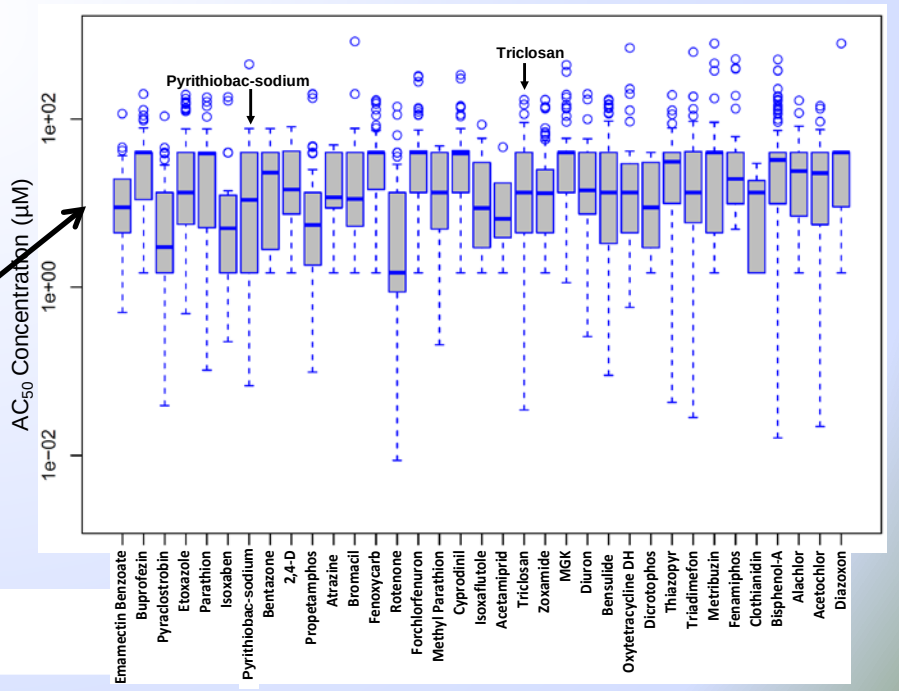
Similar LEL Values

Different Oral Equivalents

This Simple Implementation of IVIVE for HTS Has Demonstrated that Kinetics is Crucial (Rotroff *et al*. 2010)

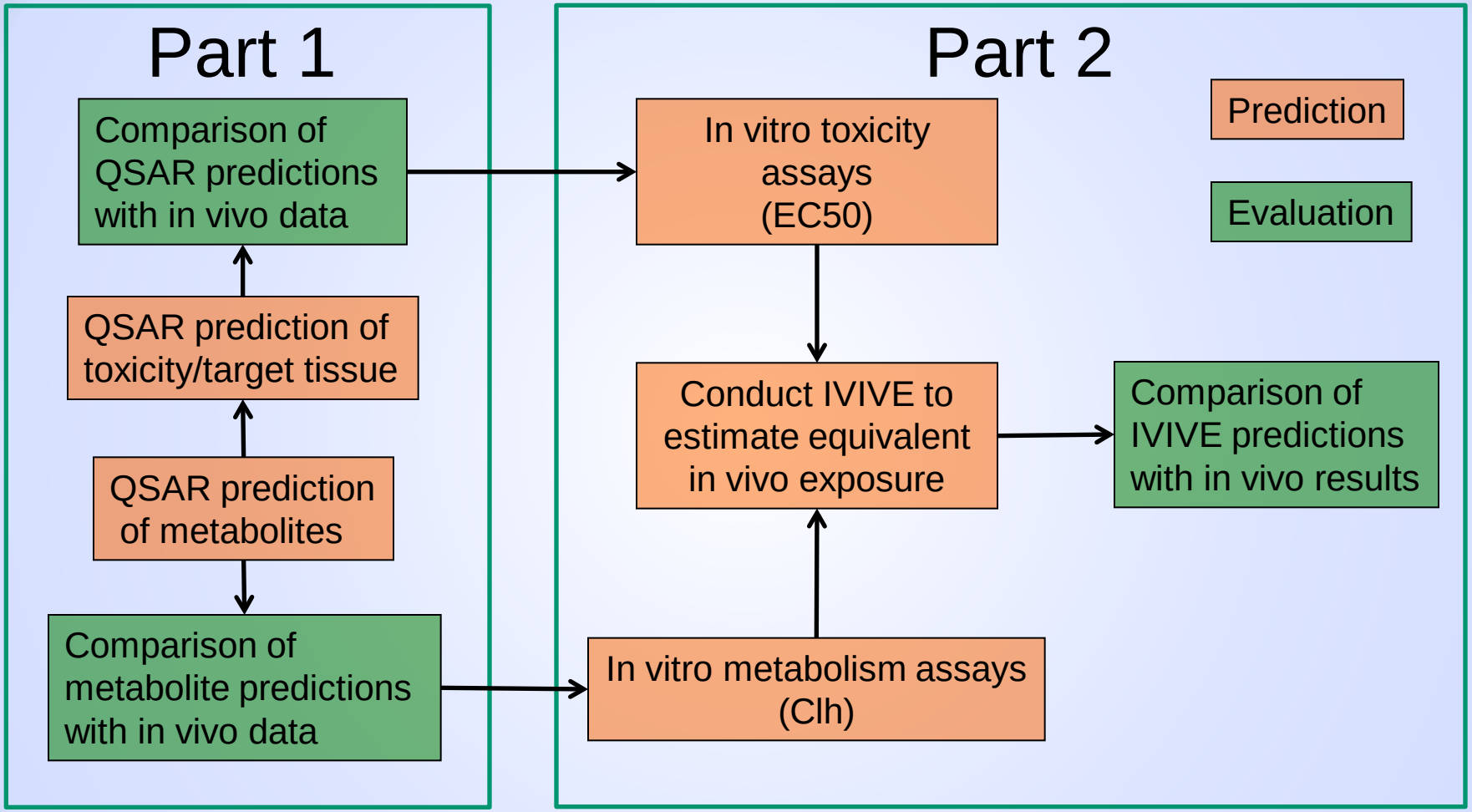


Greater than 4 orders of magnitude difference in potencies *in vivo*



By themselves, in vitro assay effect concentrations are quantitatively meaningless for risk assessment

IRAS/RIVM/Hamner Evaluation of In Vitro to In Vivo Extrapolation (IVIVE) Approaches



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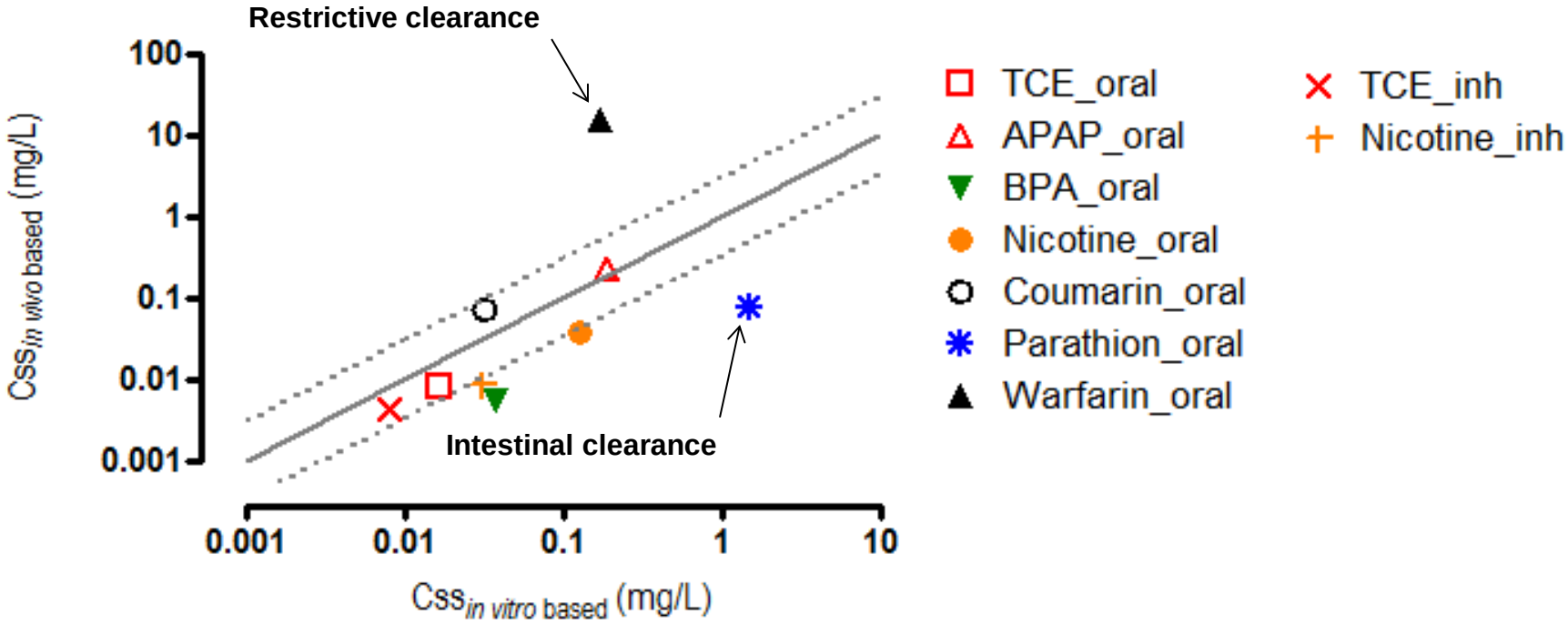
IRAS/RIVM/Hamner Evaluation of In Vitro to In Vivo Extrapolation (IVIVE) Approaches

QSAR Prediction of Metabolites

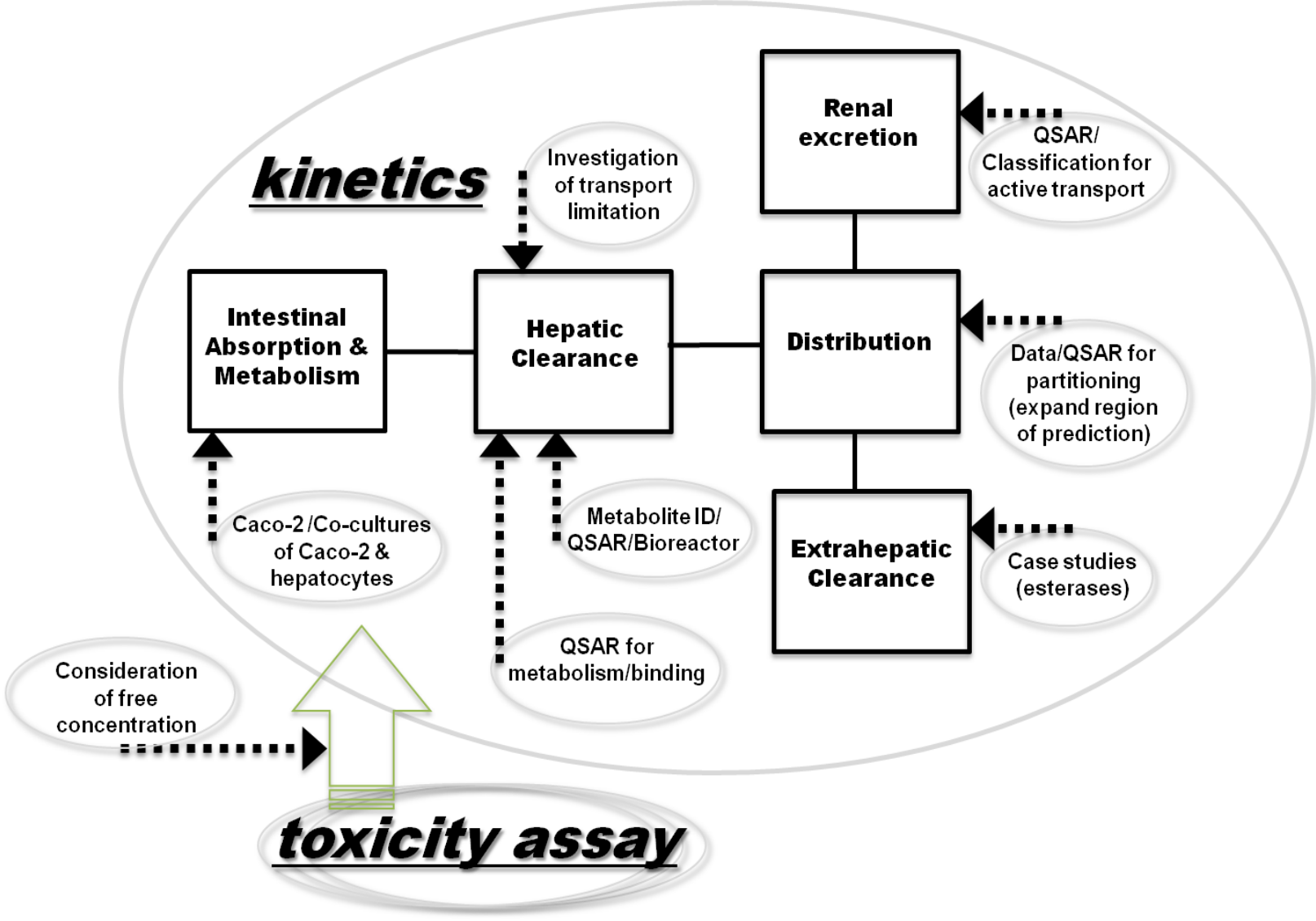
- The OECD Toolbox was able to correctly predict the primary metabolite responsible for the toxicity of 9 of the 12 chemicals investigated in this study where toxicity is due to a metabolite
- However, a number of other metabolites were also predicted, including many that have not been detected in vivo
- The prediction of nontoxic or low-yield metabolites for makes the process of investigating possible metabolite toxicity more difficult and time-consuming

IRAS/RIVM/Hamner Evaluation of In Vitro to In Vivo Extrapolation (IVIVE) Approaches

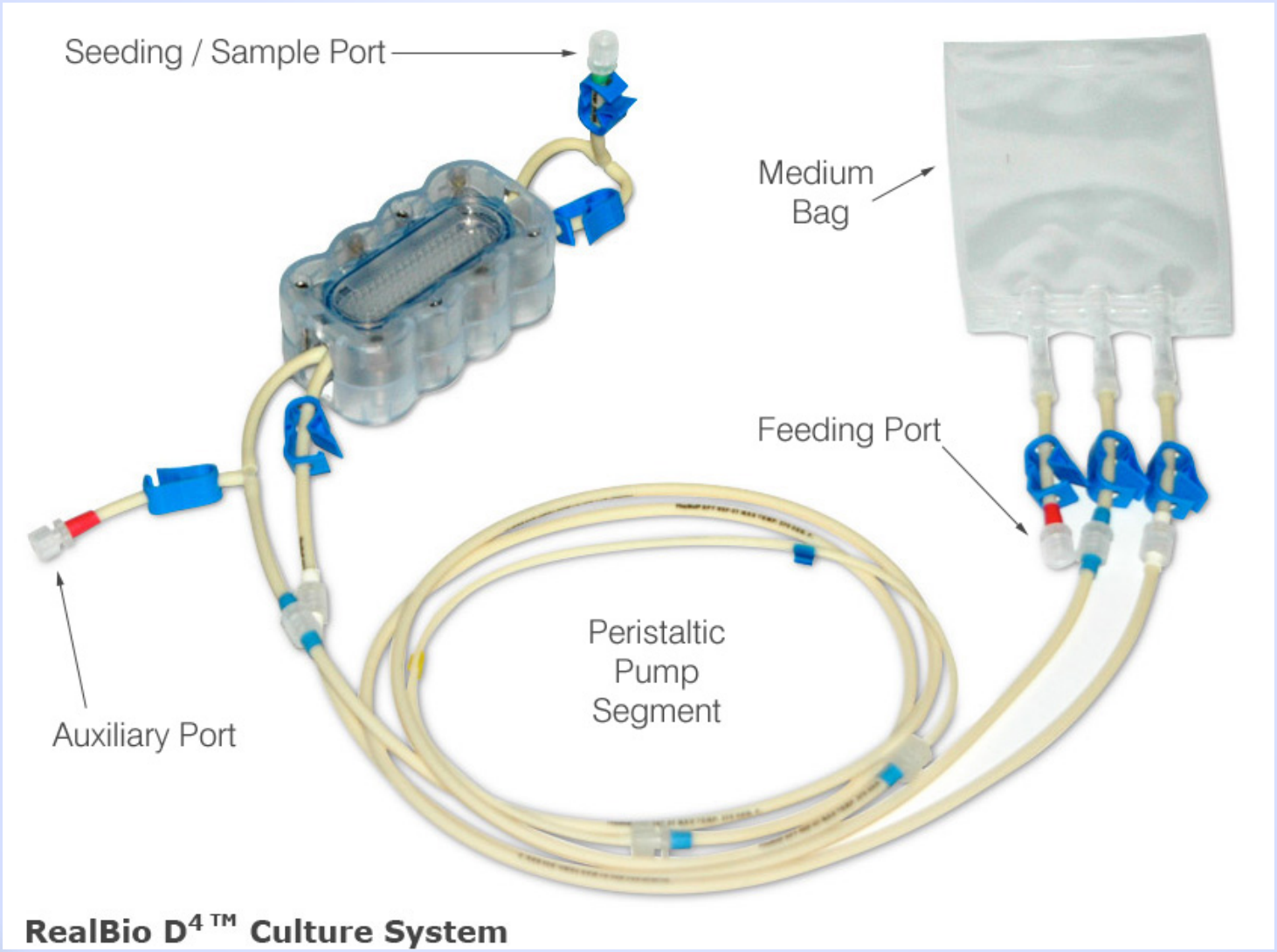
Comparison of Css estimates based on in vitro- and in vivo-based Clints



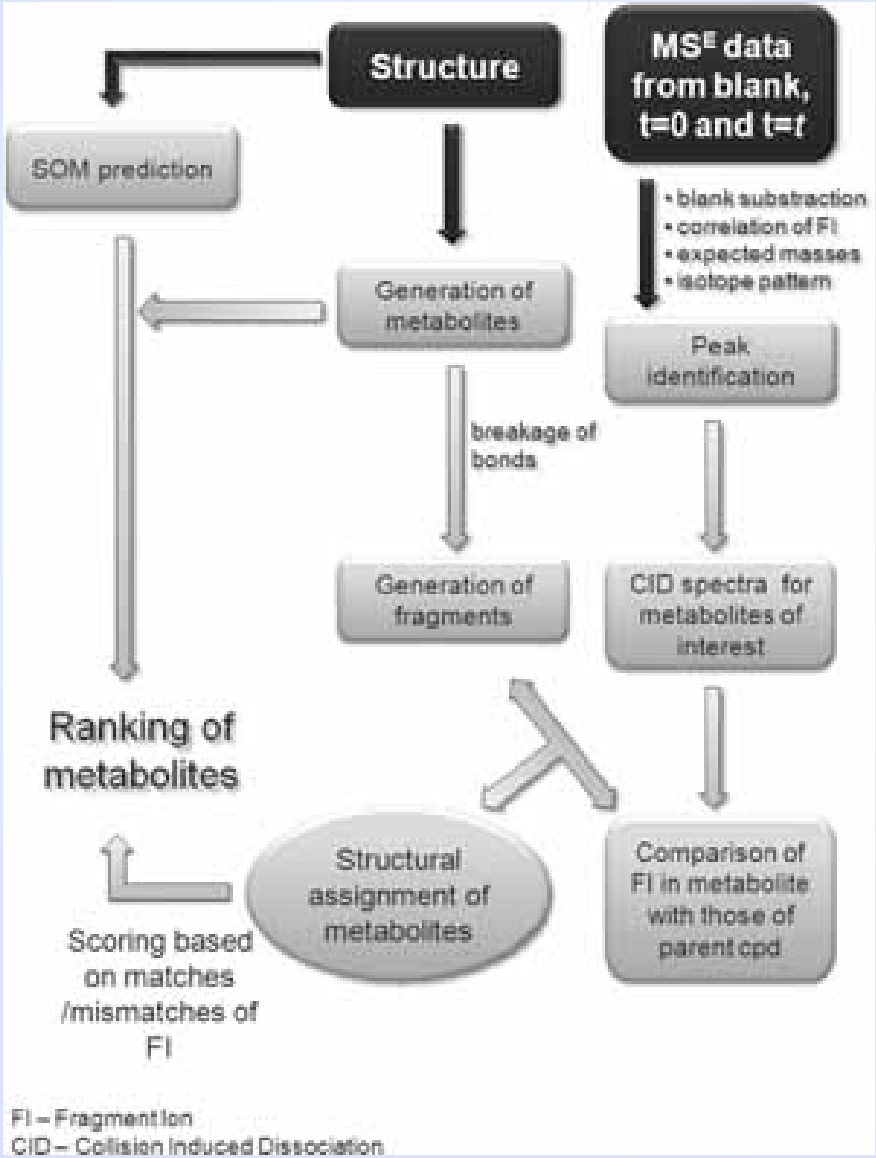
Experimental research to improve QIVIVE



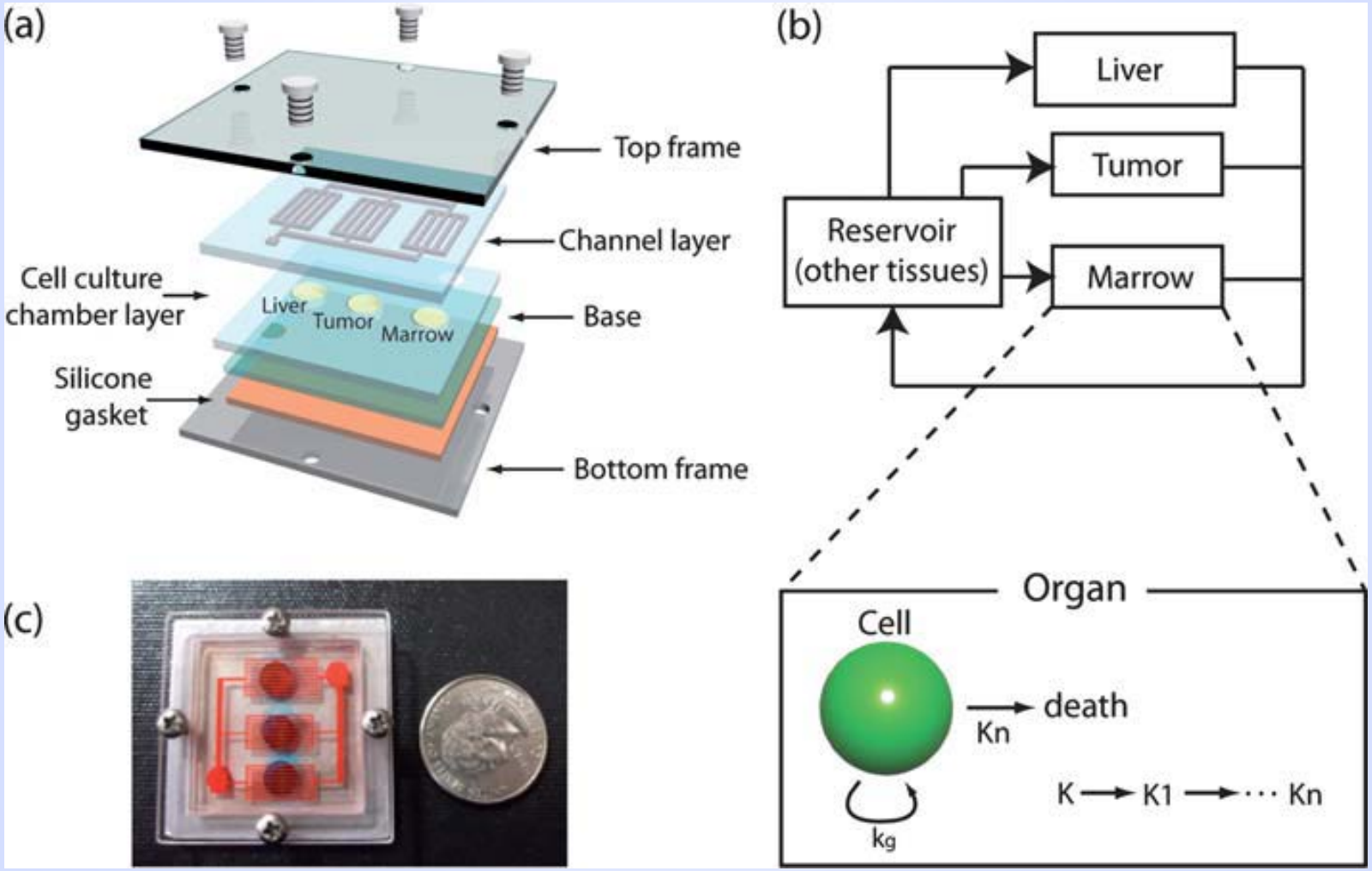
In Vitro Liver Bioreactor for Metabolite Identification



Mass-Metasite: Enhanced metabolite identification with semi-automated software for structural elucidation



Microfluidic Human on a Chip



(Sung et al. 2010)

Requirements for *in vitro* based risk assessment

Characterization of free concentration in cell-based assays
- binding
- metabolism
- active transport

***In vitro* models**
- concurrent intestinal absorption/metabolism
- dermal absorption
- blood/brain barrier
- hepatocyte clearance
- pathway/metabolite ID/kinetics (organotypic)
- renal clearance

Data collection to support QSPR modeling
- metabolite identification
- protein binding in cell-based assays
- tissue partitions (some classes of compounds)
- restricted vs unrestricted hepatic clearance
- metabolism rates
- gut absorption/metabolism (non-druglike cmpds)
- transporter substrates/renal clearance

QIVIVE case studies
- classes of physicochemical properties
- different metabolism pathways
- parent vs stable metabolite vs reactive metabolite
- portal of entry vs liver vs remote toxicity

Development of generic PBPK modeling platforms
- user friendly, open access
- database for physiological parameters
- inhalation, dermal, and oral exposure
- multiple parallel metabolic pathways

transatlantic think tank for toxicology

"The difficulty lies, not in the new ideas, but in escaping from the old ones."
John Maynard Keynes (1883-1946)

"This report, by its very length, defends itself against the risk of being read."
Winston Churchill (1874-1965)

t4 Report*

A Roadmap for the Development of Alternative (Non-Animal) Methods for Systemic Toxicity Testing

David A. Basketter [1,§], Harvey Clewell [2,§], Ian Kimber [3,§], Annamaria Rossi [4,§], Bas Blaauboer [5], Robert Burrier [6], Mardas Daneshian [7], Chantra Eskes [8], Alan Goldberg [9], Nina Hasiwa [10], Sebastian Hoffmann [11], Joanna Jaworska [12], Thomas B. Knudsen [13], Robert Landsiedel [14], Marcel Leist [15], Paul Locke [9], Gavin Maxwell [16], James McKim [17], Emily A. McVey [18], Gladys Ouédraogo [19], Grace Patlewicz [20], Olavi Pelkonen [21], Erwin Roggen [22], Costanza Rovida [23], Irmela Ruhdel [24], Michael Schwarz [25], Andreas Schepky [26], Greet Schoeters [27], Nigel Skinner [28], Kerstin Trentz [29], Marian Turner [30], Philippe Vanparys [31], James Yager [32], Joanne Zurlo [9], and Thomas Hartung [33,§]