National Toxicology Program
U.S. Department of Health and Human Services

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Communities of Practice Webinar Series: Implementing computational approaches for regulatory requirements

Calculating Bioactivity Exposure Ratios (BERs) using New Approach Methods (NAMs) for Chemical Assessment

Jon Arnot[1,2], Alessandro Sangion [1], James Armitage[3]

January 29, 2024

ICCVAM Webinar

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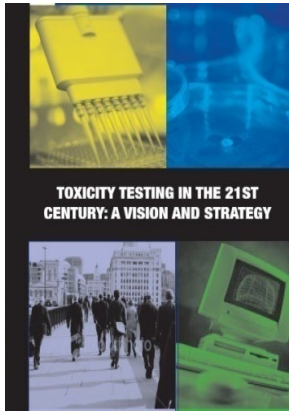
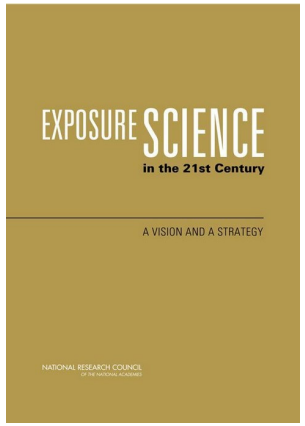
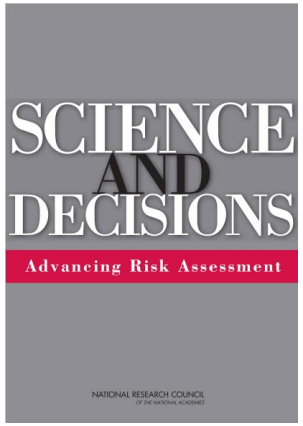
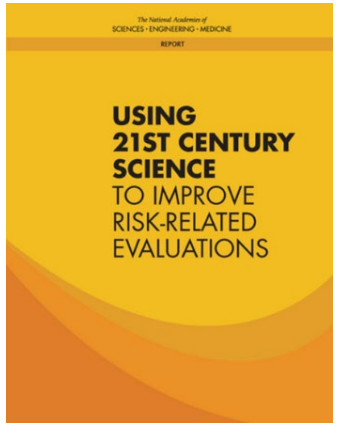
Presentation Overview

- Background: Rationale for the work & Bioactivity Exposure Ratios (BERs)
- Exposure modelling (aggregate exposure intake rates)
  - PROTEX-HT & EAS-E Suite
- In Vitro-In Vivo Extrapolation (IVIVE) to calculate Administered Equivalent Dose (AED)
  - In vitro bioassays
  - In Vitro Mass Balance Model (IV-MBM) **
  - In Vivo High Throughput Toxicokinetic (HTTK) Modelling *
- Case study calculating AEDs, aggregate exposure intake rates, and BERs







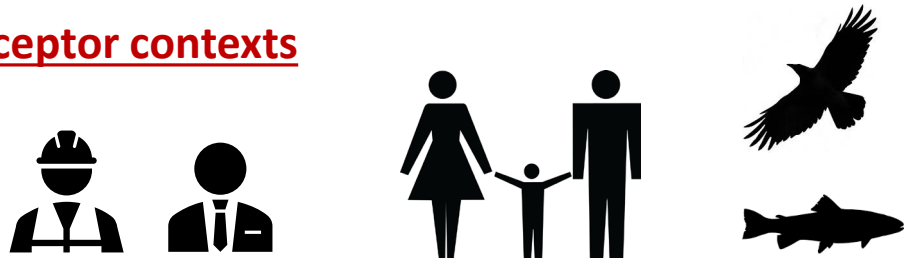


APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

Global Regulatory Situation

- Many diverse chemicals and use contexts require risk evaluation; **limited information**→ uncertainty
- Desirable to have a mechanistic understanding of toxicity **and exposure**
- Need to develop, evaluate and apply **databases** and **models** for different contexts, i.e., "**fit-for-purpose**"

Receptor contexts



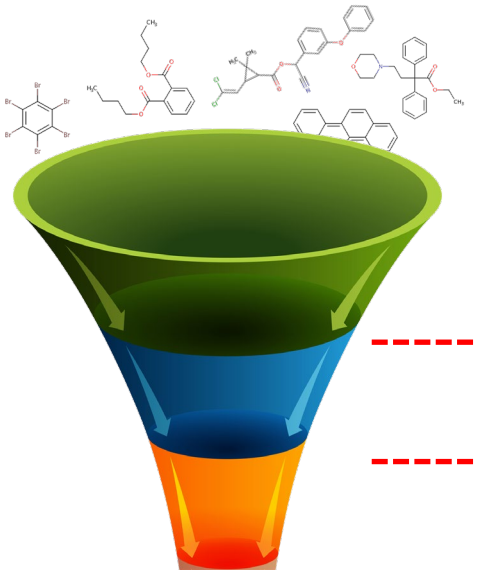
Decision contexts

Prioritization

Data Poor

Screening

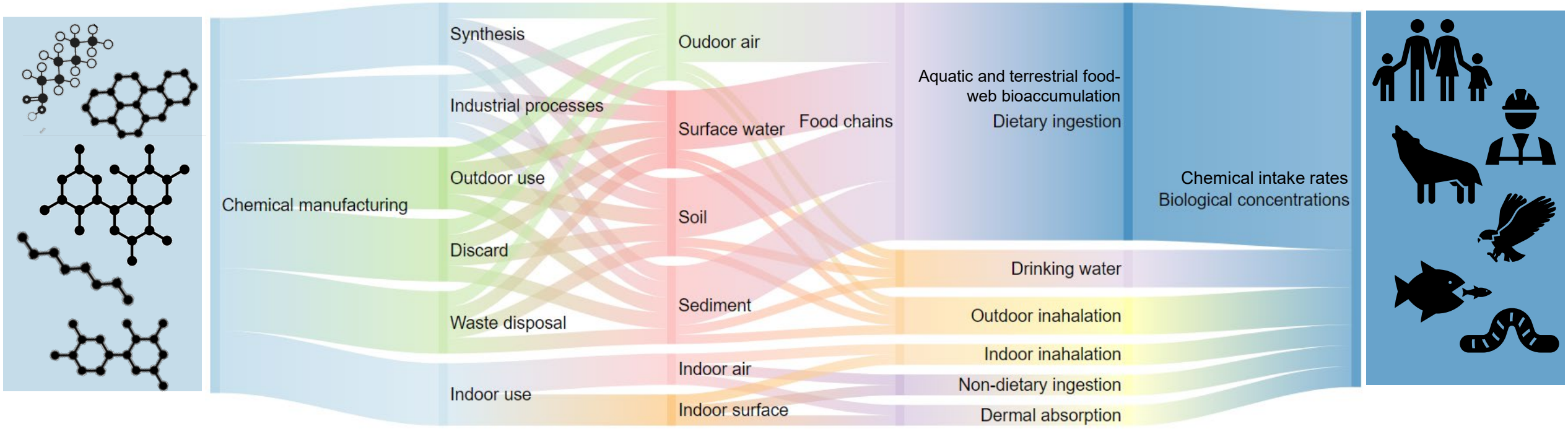
Comprehensive



MOE?

MOE?

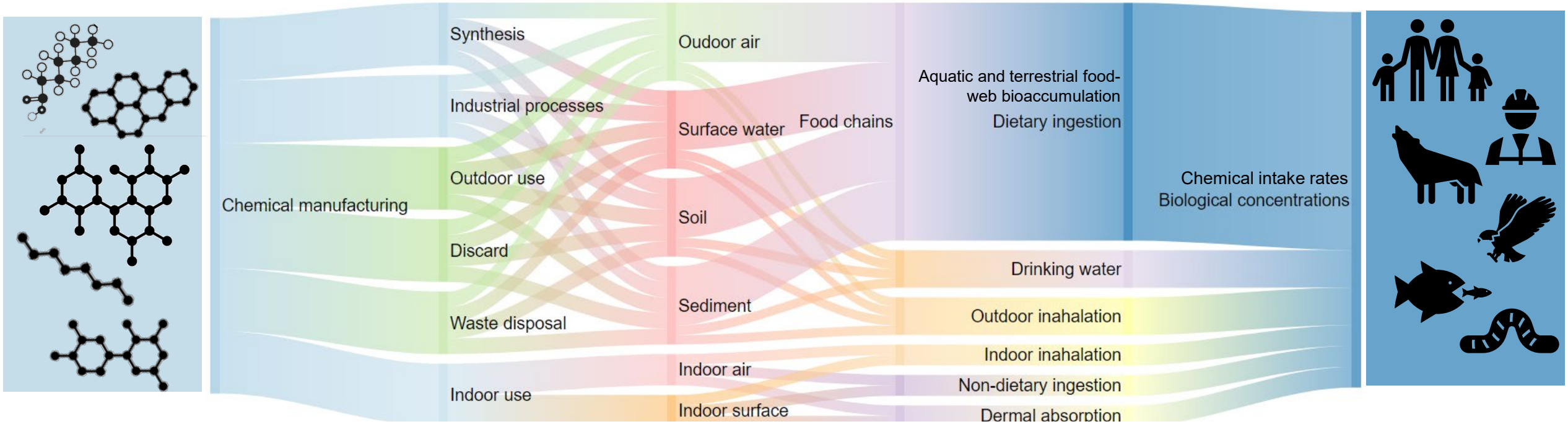
The Scope of Exposure Science: Production to Exposure



Risk = Exposure / Effect

Properties of chemicals | **Use characteristics** | **Environmental characteristics** | **Receptor characteristics**

The Scope of Exposure Science: Production to Exposure



Chemical properties, production volumes & use information

Physiologically-based BioKinetic (PBK) models for various receptors

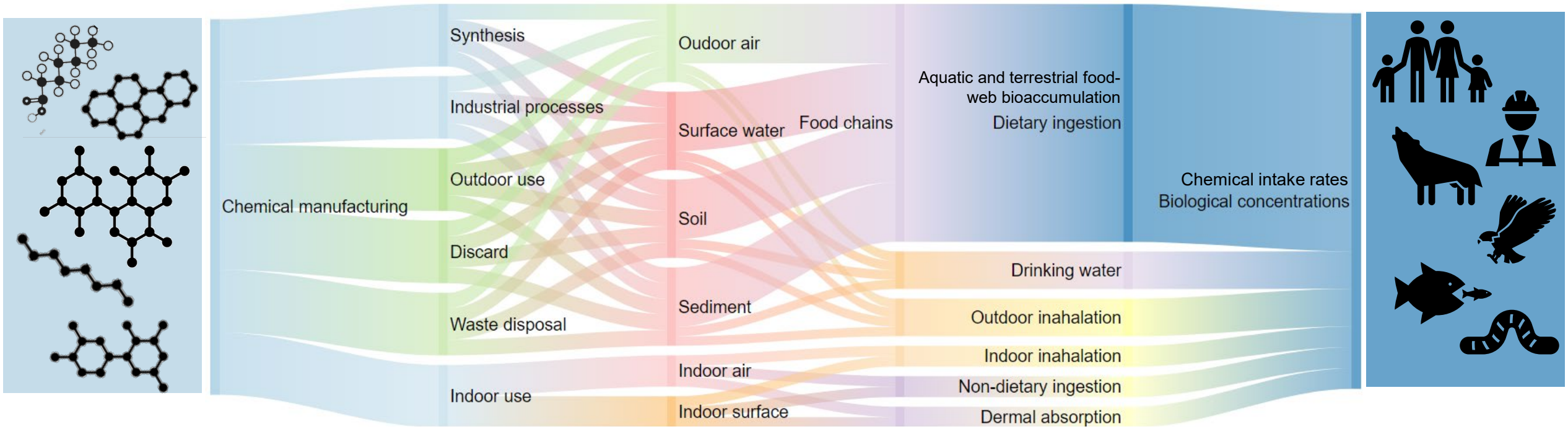
Life cycle chemical emission rates

Food web bioaccumulation models

Multi-media environmental fate & exposure models (outdoor **and indoor systems**)

Exposure pathways, single route and **aggregate exposure** estimates

The Scope of Exposure Science: Production to Exposure



PROduction-To-EXposure High-Throughput (PROTEX-HT) model

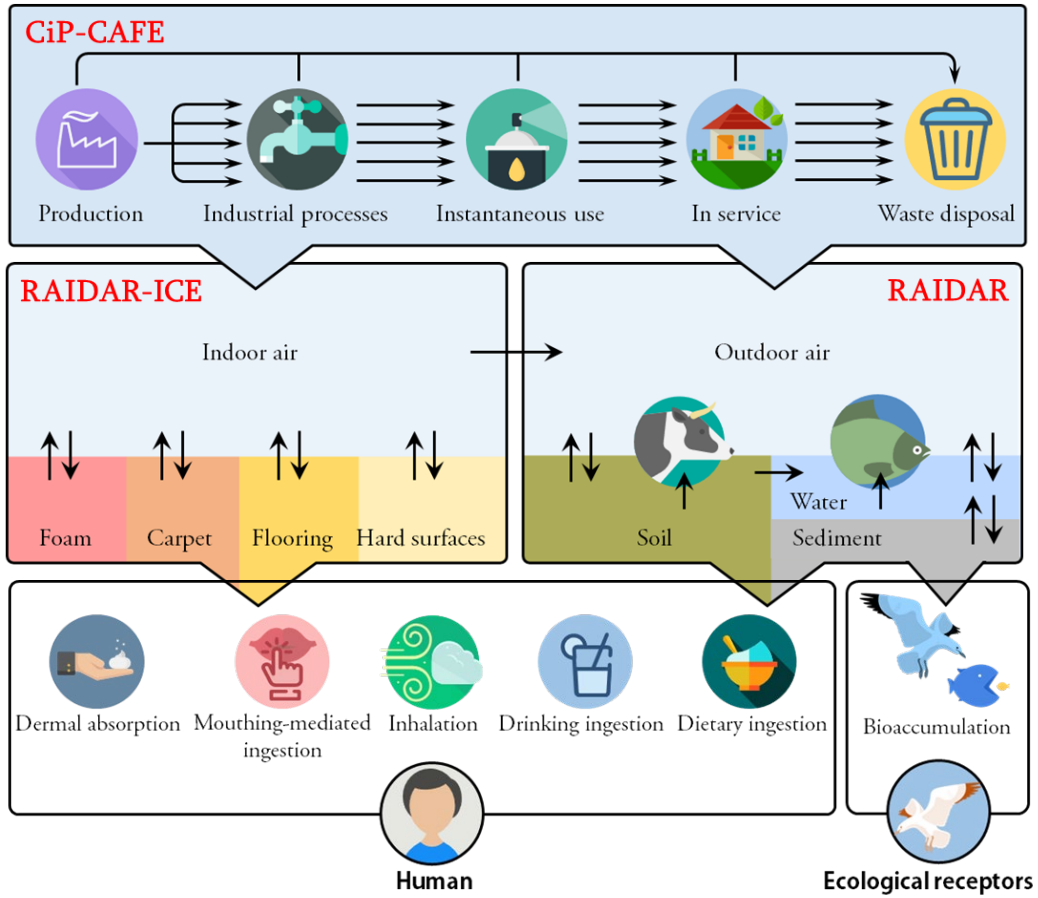
Consolidation of mechanistic (process-driven) mass-balance models

Predictions for multimedia concentrations and external and internal exposure under representative conditions

Requires only chemical structure, production volume (and functional use)

PROTEX-HT

- Simulating aggregate human exposure and ecological exposure: “One Health” approach
- Input parameters: Production Volume, Chemical SMILES (structure), Functional Use Category



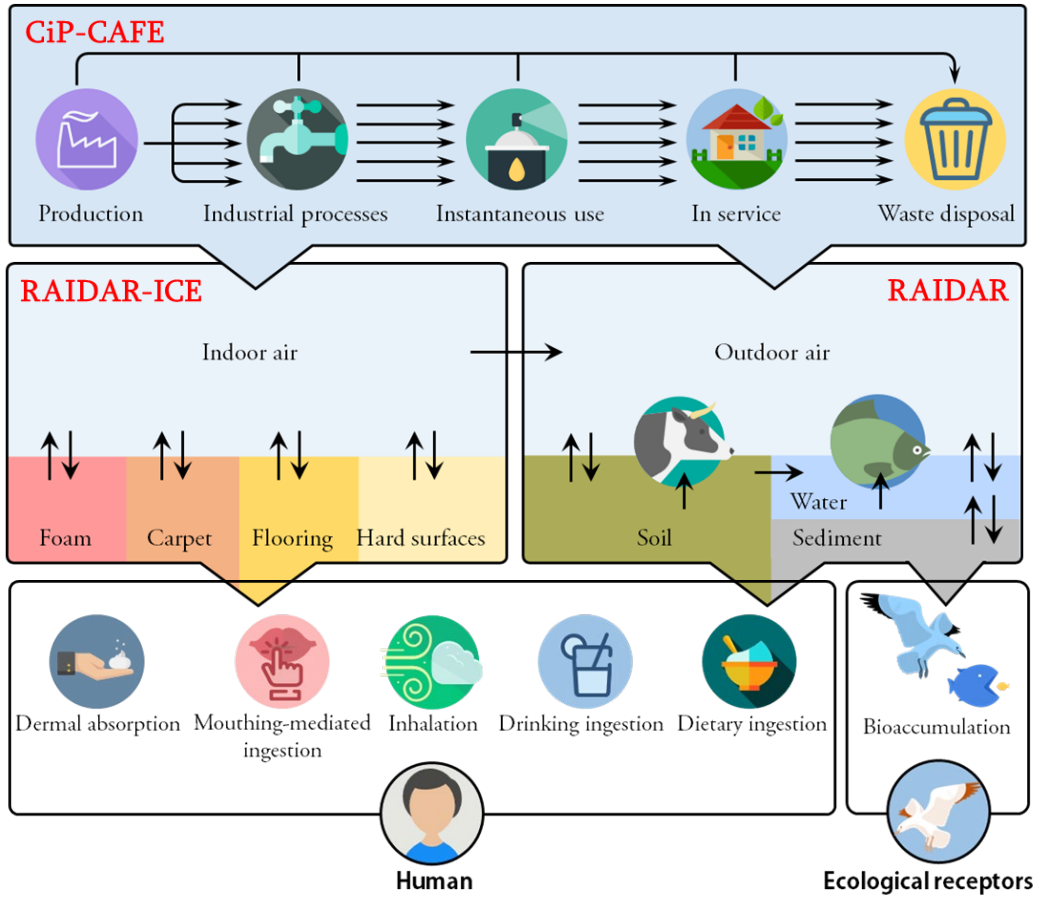
Output:

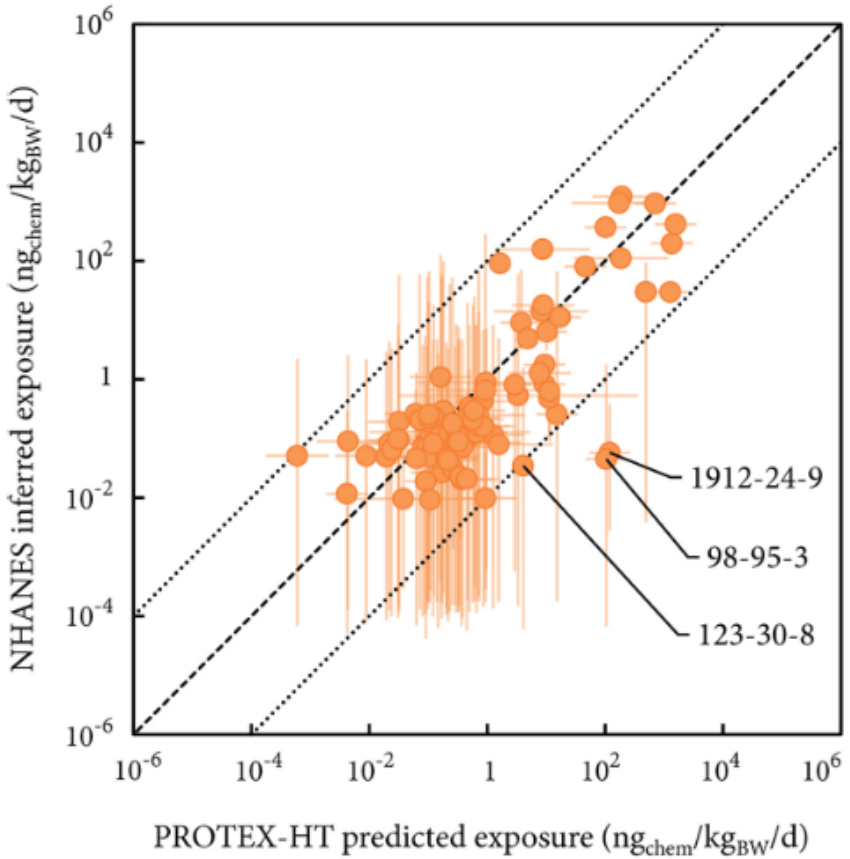
- Chemical emission rates
- Chemical concentrations in multi-media (air, water, soil, sediment, various ecological and agricultural organisms, humans)
- External exposure rates: route-specific intake rates & aggregate intake rates
- Internal exposure concentrations: body, blood, urine
- Risk / safety estimates when hazard data included

Li et al., EHP 2021

PROTEX-HT

- Simulating aggregate human exposure and ecological exposure: “One Health” approach
- Input parameters: Production Volume, Chemical SMILES (structure), Functional Use Category





Li et al., EHP 2021

Exposure And Safety Estimation (EAS-E) Suite

- Free, user-friendly online platform of new and existing data and tools **www.eas-e-suite.com**
- Integrates curated databases, OECD validated QSARs, and environmental fate (P/LRTP), B/TK and exposure models to aid **chemical assessments for ecological and human health** & **chemical safety and sustainability**
- Facilitates model parameterization and data queries based on **CAS**, **SMILES** or **name** entry using built-in databases (~70K chemicals); *options for user-preferred information to replace system "defaults"*
- For chemicals not in the built-in database: *model parameterization for chemicals with only SMILES notation*

Chemical properties & $t_{1/2}$s for >70K organic chemicals

IFSQSAR and ppLFER models for chemical properties and $t_{1/2}$s

EPA OPERA QSAR models for chemical properties and $t_{1/2}$s

QSARINS for biotransformation and total elim. $t_{1/2}$s (fish & humans)

CiP-CAFE: mass flow model to predict emission rate & release throughout life-cycle

RAIDAR: mass balance for environmental fate, exposure

RAIDAR-ICE: mass balance for indoor fate human exposure

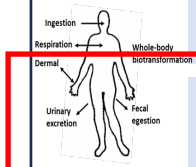
POINT SOURCE: mass balance, dilution models for eco & human exposure

F-PEST: environmental fate & distribution, persistence, long-range transport, mobility

BET: bioaccumulation estimation tool: lab & field, aquatic & air-breathing

PROTEX-HT: aggregate human exposure & risk

Dermal exposure models ("IH-SkinPerm", EPA CEM, ECETOC TRA)



EAS-E Suite HTTK models (incl. rTK & IVIVE) for fish, humans, rat; EPA httk

IV-MBM: mass balance model for fate & disposition in in vitro assays

In vitro and in vivo TK data: critically evaluated values for fish, rodents, humans

IVIVE: Administered Equivalent Dose (AED)

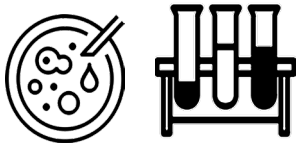
Effect Concentration (EC): Initial nominal medium concentration in the in vitro (µM) system

AED: the "real world" exposure dose expected to give a plasma (or blood) concentration corresponding to an in vitro bioactivity, e.g., in vitro Point of Departure (POD)

$$AED = \frac{EC_X}{C_{SS}} \times \frac{1\,mg}{kg_{BW} \times day}$$

C_{SS}: The steady-state blood or plasma concentration corresponding to an oral exposure of 1 mg/kg-BW/d assuming 100% absorption

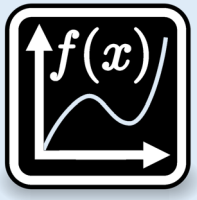
IVIVE: Administered Equivalent Dose (AED)



In vitro Effect Concentration
EC (µM)

In Vitro Dosimetry

IV-MBM
(In Vitro Mass Balance Model)

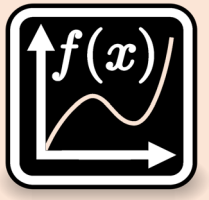


$$AED = \frac{EC_X}{C_{SS}} \times \frac{1\,mg}{kg_{BW} \times day}$$

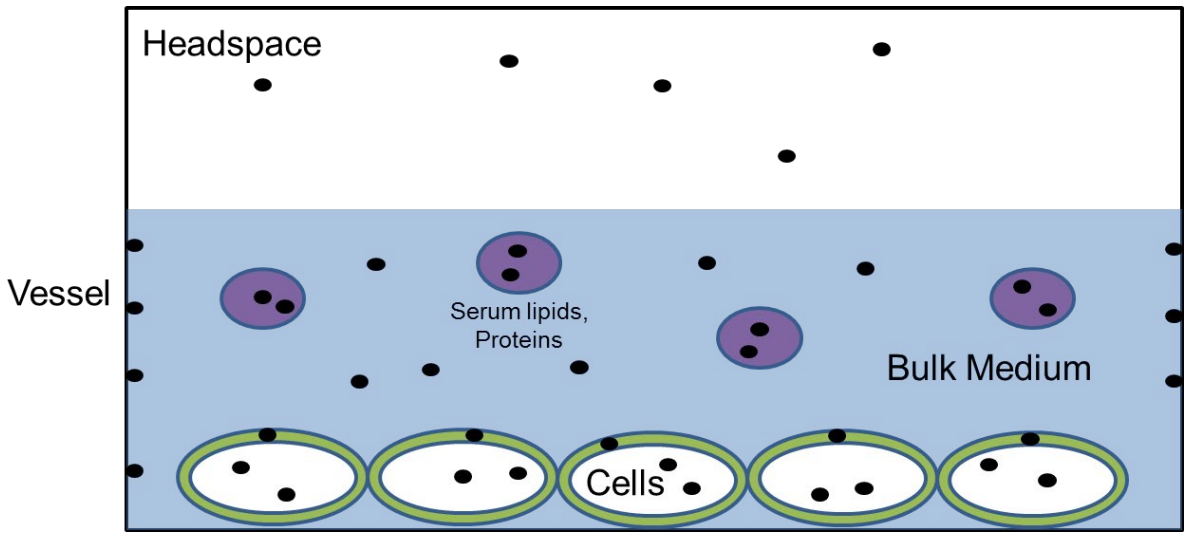
Blood concentration at
steady-state C_{SS} (µM)

High-Throughput Toxicokinetics

1Co-PBK
(1 compartment physiologically based kinetic model)



In Vitro Bioactivity Assays

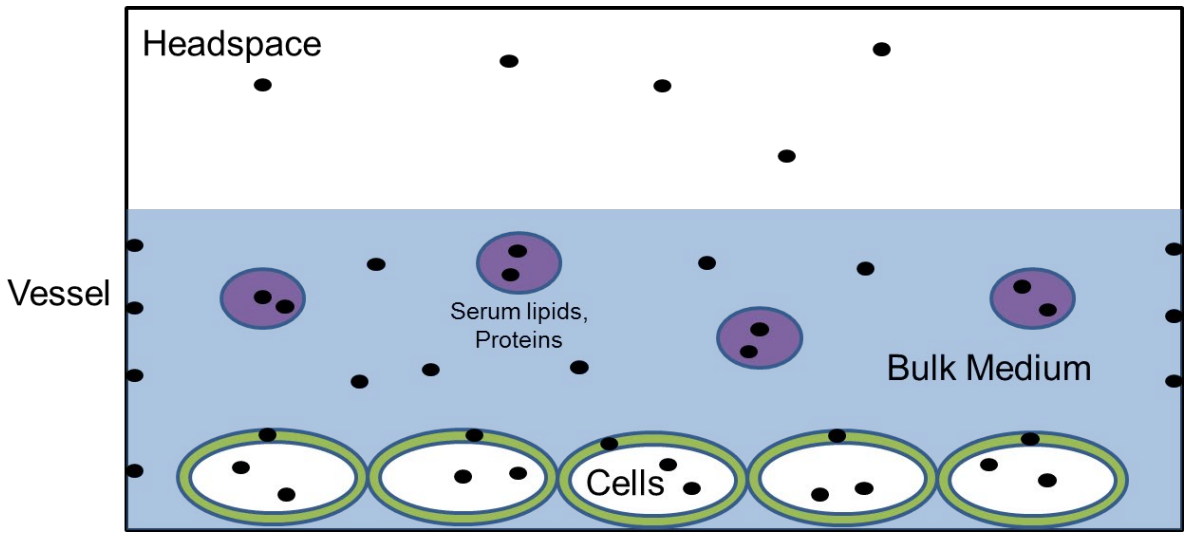


Main Issues

- **Dose-Response is typically reported based on nominal medium concentrations** (i.e., mass of chemical added / volume of medium)
- **BUT: in vitro disposition (i.e., distribution) is chemical- and assay-specific**
- In vitro conditions may not be reflective of the in vivo conditions (i.e., medium composition ≠ blood composition)
- There is a need to put in vitro effects data into the proper context to inform the hazard and risk assessment of chemicals

Armitage et al., ES&T 2014

In Vitro Mass Balance Model (IV-MBM)



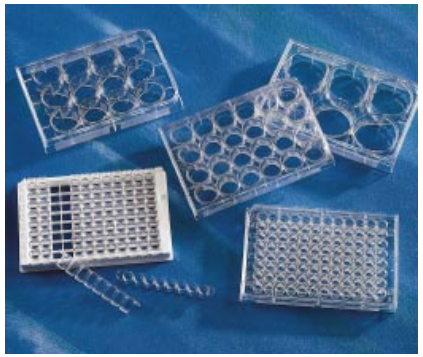
Solution

- **Calculate the distribution (disposition) of organic chemicals in in vitro test systems based on <u>chemical-specific partitioning properties and assay-specific properties</u>**

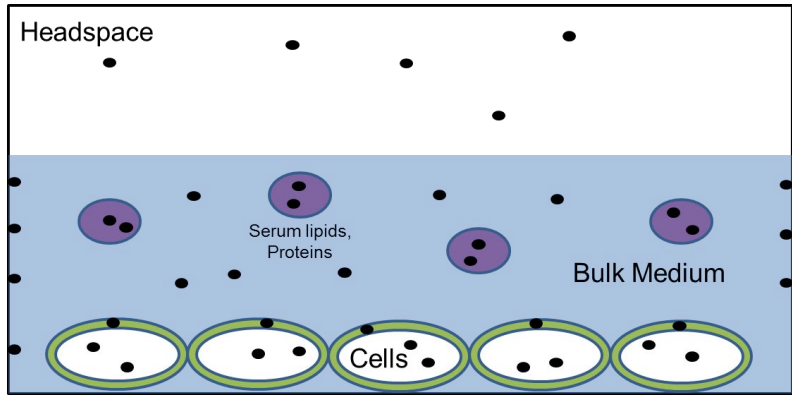
- Applicable to neutral organics and many IOCs
- Includes more explicit guidance on parameterization of the numerous in vitro test systems (e.g., well plate characteristics, cell seeding)

Armitage et al., ES&T 2014

IV-MBM: Concepts



Reality



Model

1. Chemical is added to bulk medium

2. Final in vitro disposition (i.e., distribution) is a function of:
   i. test system (assay) properties
   ii. partitioning properties (chemical)

Mass balance equations

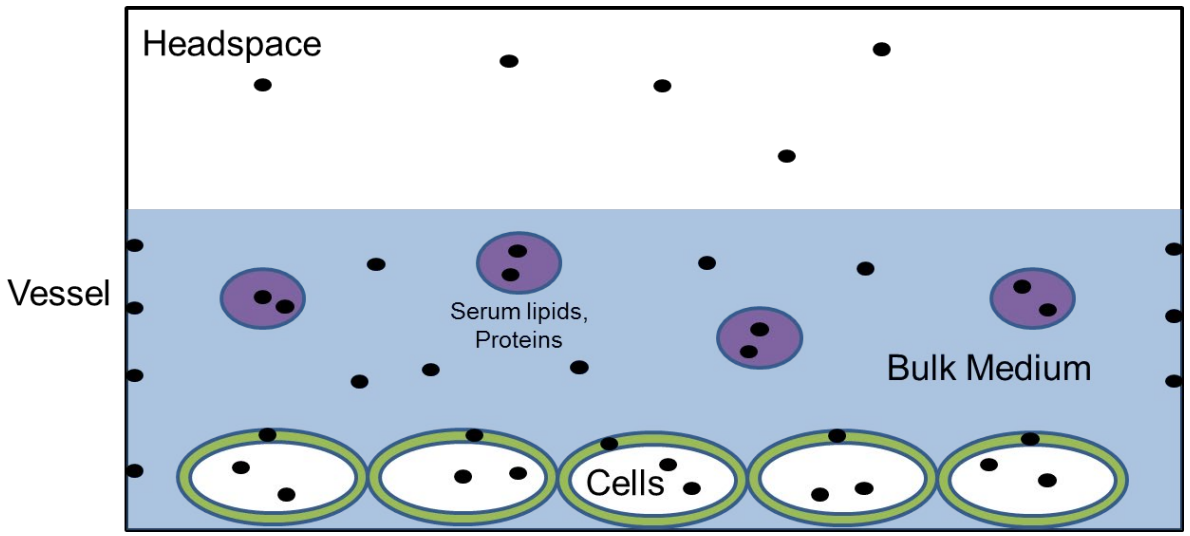
$$C_{Free} = \frac{Total\ Mass\ Added}{V_W + K_{AW} * V_A + K_{SaW} * V_{Sa} + K_{SlW} * V_{Sl} + K_{DsW} * V_{Ds} + K_{CW} * V_C + K_{PlW} * A_{Pl}}$$

Partition ratios:

- Air-Water
- Serum albumin-Water
- Storage lipid-Water
- Dissolved organic matter-Water
- Cell-Water
- Plastic-Water

Armitage et al., Toxics 2021

IV-MBM: Assumptions

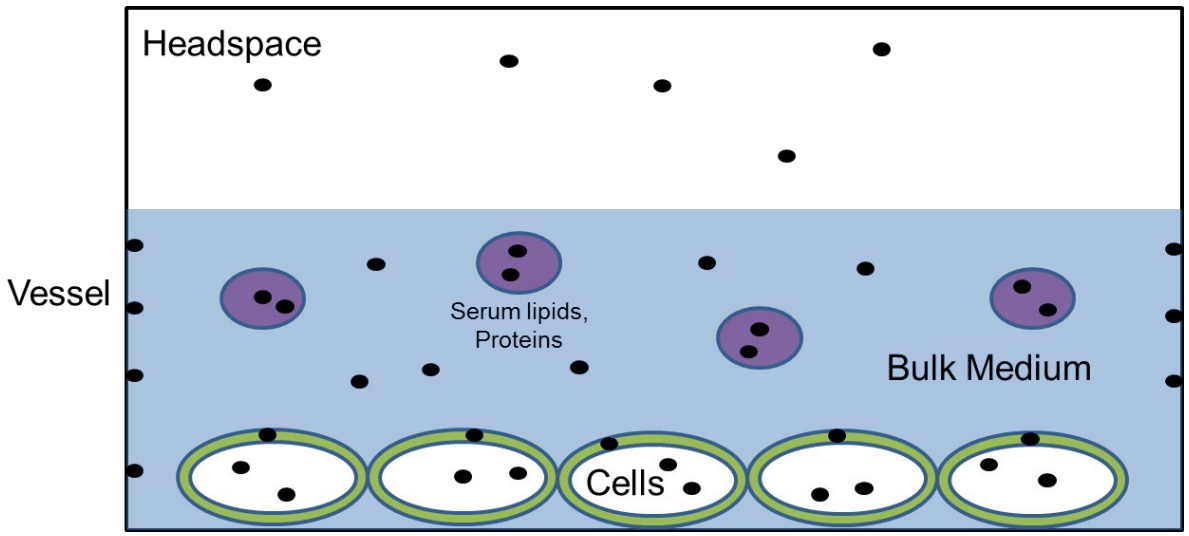


Equilibrium Partitioning (EQP)

- "Static" i.e., uptake kinetics not considered
- Assay environment considered well mixed
- Single dose only
- No biotransformation/degradation
- No changes to exposure scenario

Armitage et al., 2014, 2021

IV-MBM: Key Outputs



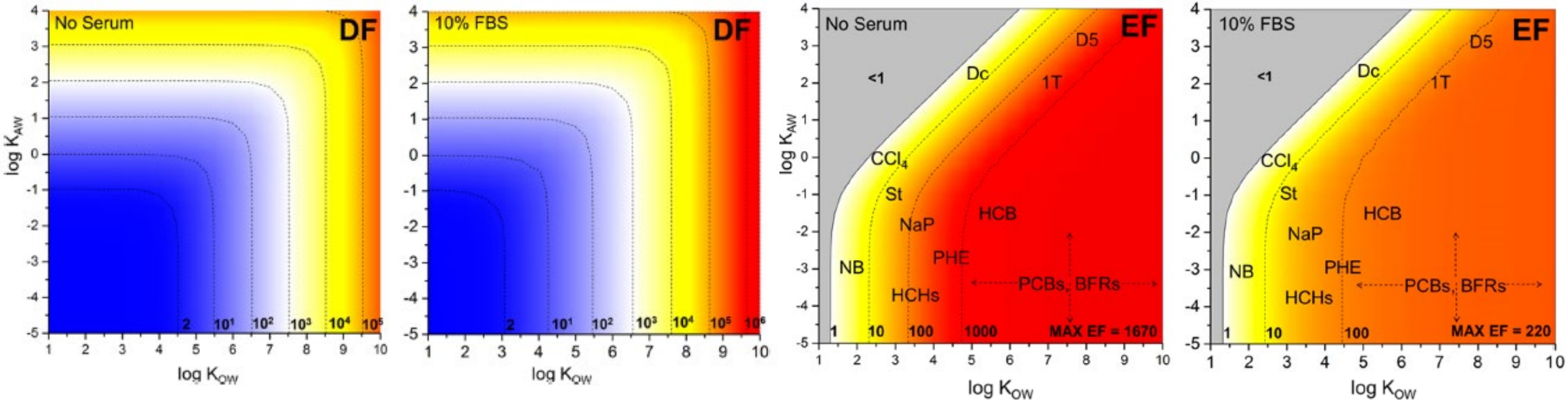
- Predicted masses and mass fractions
- Predicted concentrations in bulk medium, **C_{Free}**, cells, membranes, head space…
- IVIVE: In vitro POD extrapolated to an "in vivo blood concentration"
  - C_{Free} x Blood-water partitioning → i.e., directly comparable to total blood concentration
- Warning for possible issues:
  "Volatility issue"
  "Solubility issue"
  "Plastic sorption"
  "Cytotoxic burst" (Baseline toxicity)

Armitage et al., 2014, 2021

Implications: Expected EQP Chemical Distribution

$$Depletion\ Factor\ (DF) = \frac{C_{Nominal}}{C_{Free}}$$

$$Enrichment\ Factor\ (EF) = \frac{C_{Cell}}{C_{Nominal}}$$



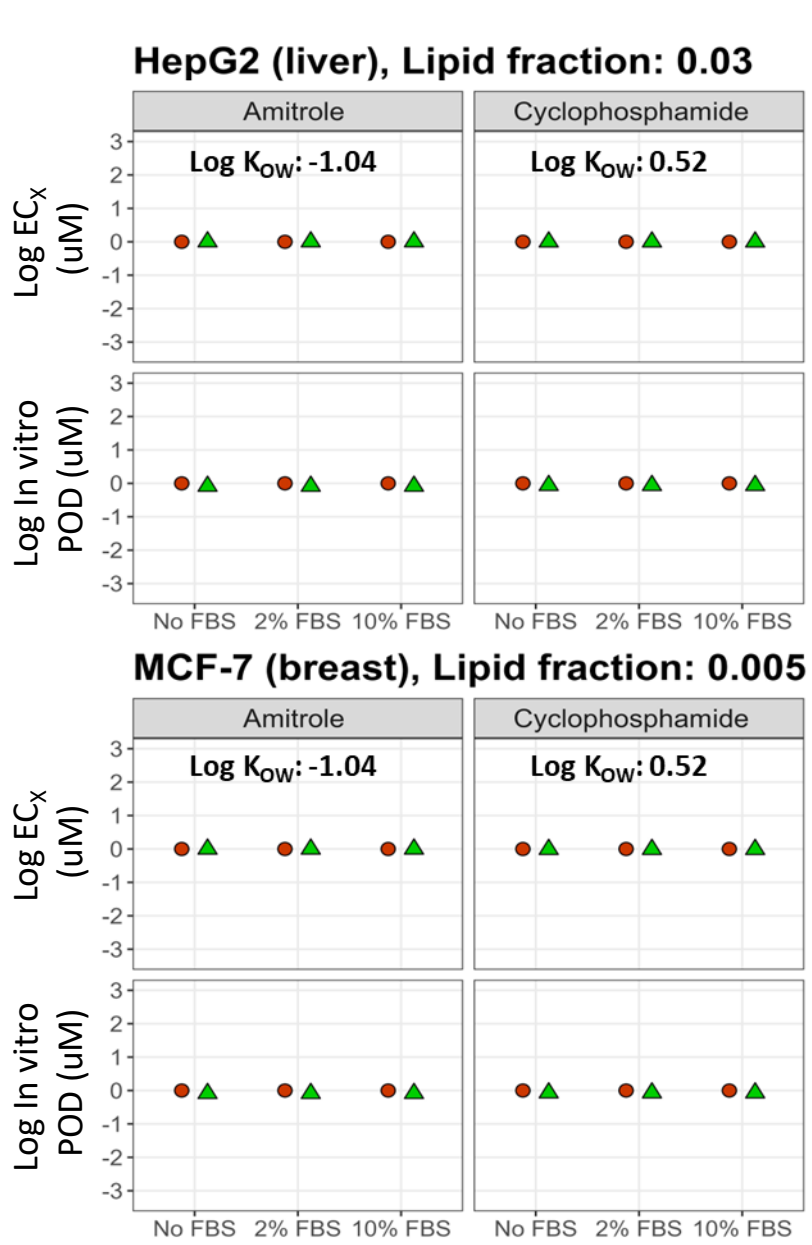
$C_{NOMINAL}$ = e.g., AC50, EC50, AC10, etc.

K_{OW} = octanol-water partitioning

K_{AW} = air-water partitioning

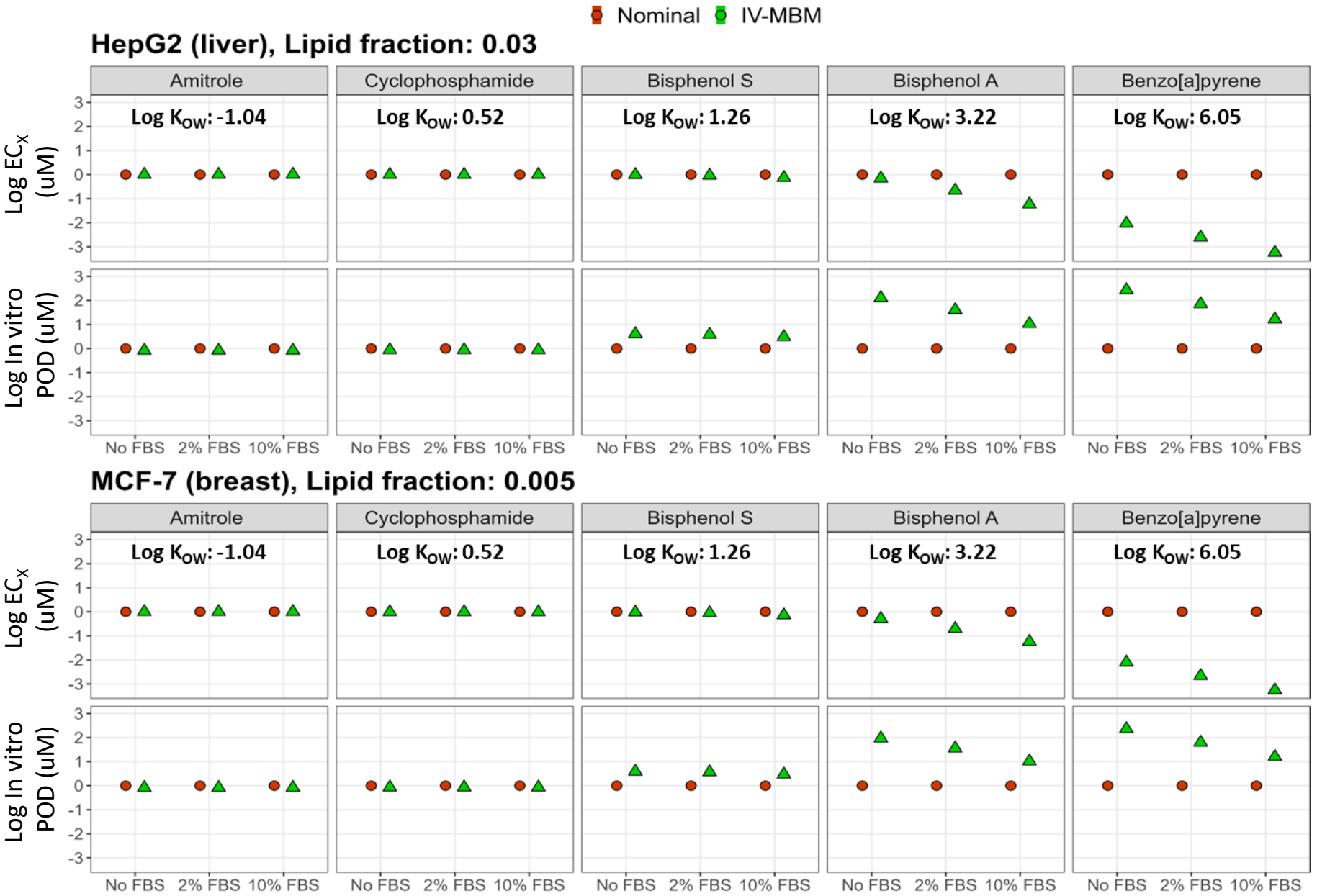
Armitage et al., ES&T 2014

Implications: Illustrative Example



- Different cell lines have different compositions (e.g., different relative lipid contents, proteins)
- A specific assay can be parameterized differently (e.g., different level of added FBS) changing its relative composition
- These differences in relative composition affect chemicals differently according to their physical chemical properties

Implications: Illustrative Example



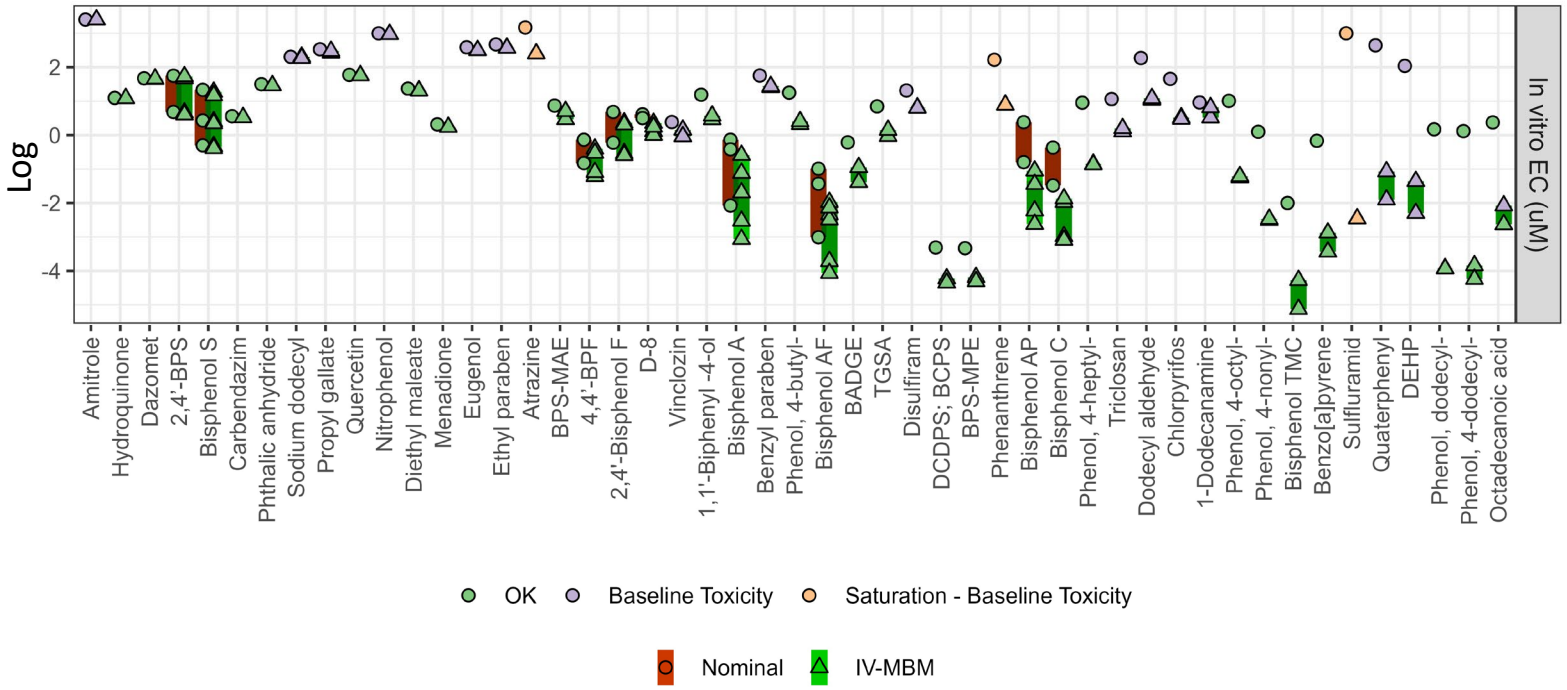
$C_{Free} \leq C_{NOMINAL}$

$C_{Blood} > C_{NOMINAL}$

$C_{Free} \leq C_{NOMINAL}$

$C_{Blood} > C_{NOMINAL}$

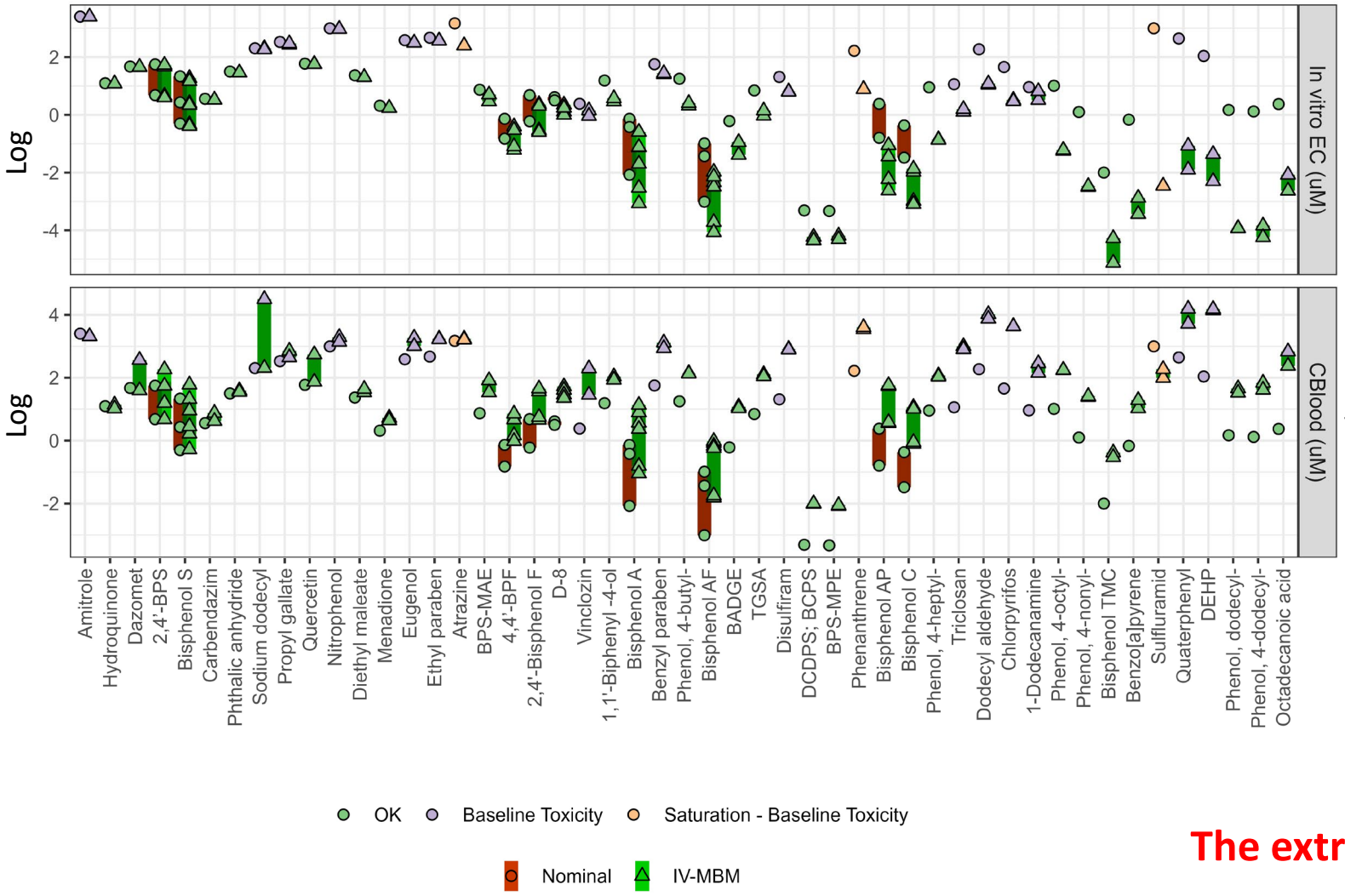
Case Study: IVIVE of Bioactivity Data



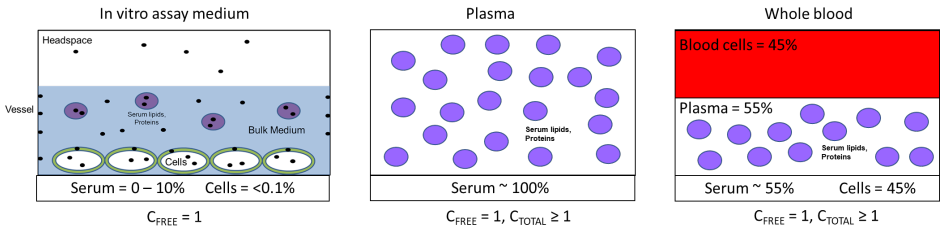
Bioactivity data based on nominal concentrations are problematic because **in vitro disposition** is not considered and can vary by chemical and assay

- IV-MBM applied to express concentration in term of C_{Free} (i.e., concentration that is bioavailable)
- Differences between nominal and freely-dissolved phase concentrations increase with increasing hydrophobicity
- Potential issues are highlighted by the IV-MBM

Case Study: IVIVE of Bioactivity Data



In vitro assay medium is NOT THE SAME as plasma or blood; differences in composition (e.g., volume fraction of serum lipids, proteins, cells) lead to differences in BIOAVAILABILITY



Multiply C_{Free} by the blood-water partition coefficient to get an equivalent C_{blood}

The extrapolated C_{Blood} is the in vitro POD for the AED calculations

C_{SS} Calculation

The steady-state blood or plasma concentration corresponding to an oral exposure of 1 mg/kg-BW/d (assuming 100% absorption):

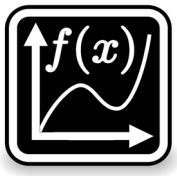
$$C_{SS} = \frac{Dose\ x\ AE}{VD_{SS}\ x\ k_T}$$

- Dose: 1 ($mg/kg_{BW}/d$)
- AE – Absorption Efficiency: 1 (100% absorption)
- VD_{SS} - Volume of distribution at steady state: (L_{Blood}/kg_{BW})
- **k_T – total elimination rate constant: (1/d)**

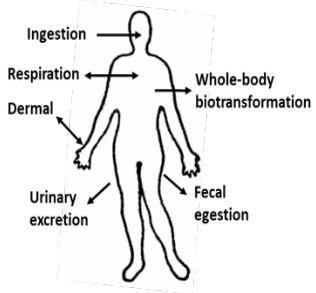
$$HL_T = \frac{\ln(2)}{k_T}$$

Empirical k_T Data

Direct k_T Prediction:
QSAR



Indirect k_T prediction:
HTTK models (need biotransformation)



k_T - Direct Prediction: QSAR

QSARs developed from **in vivo** data for human total elimination HL (HL_T) **and biotransformation HL (HL_B)**

Environmental Science & Technology

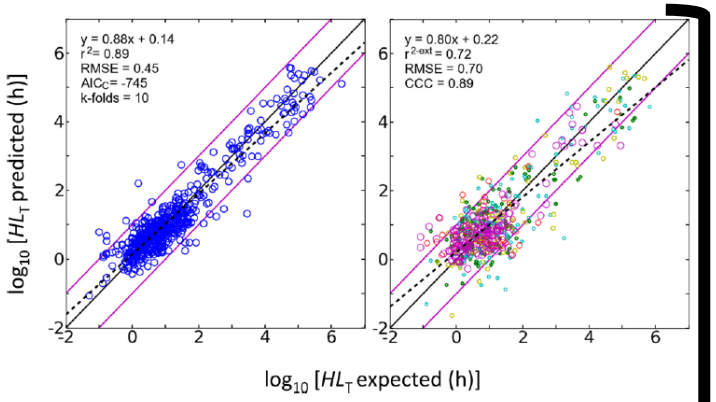
Article

pubs.acs.org/est

Estimating Screening-Level Organic Chemical Half-Lives in Humans

Jon A. Arnot,*[†,‡] Trevor N. Brown,[†,§] and Frank Wania[‡]

Iterative Fragment Selection (IFS)

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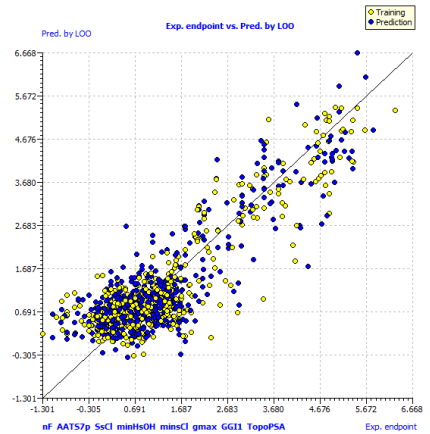
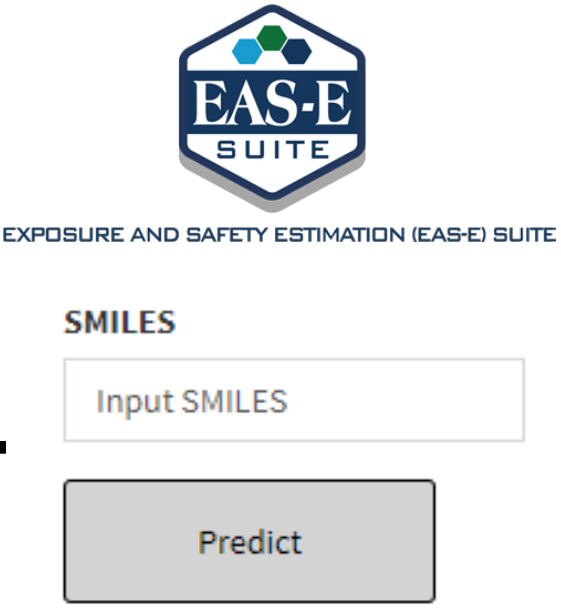
Development of human biotransformation QSARs and application for PBT assessment refinement

Ester Papa [a,*], Alessandro Sangion [a], Jon A. Arnot [b,c,d], Paola Gramatica [a]

Molecular descriptor

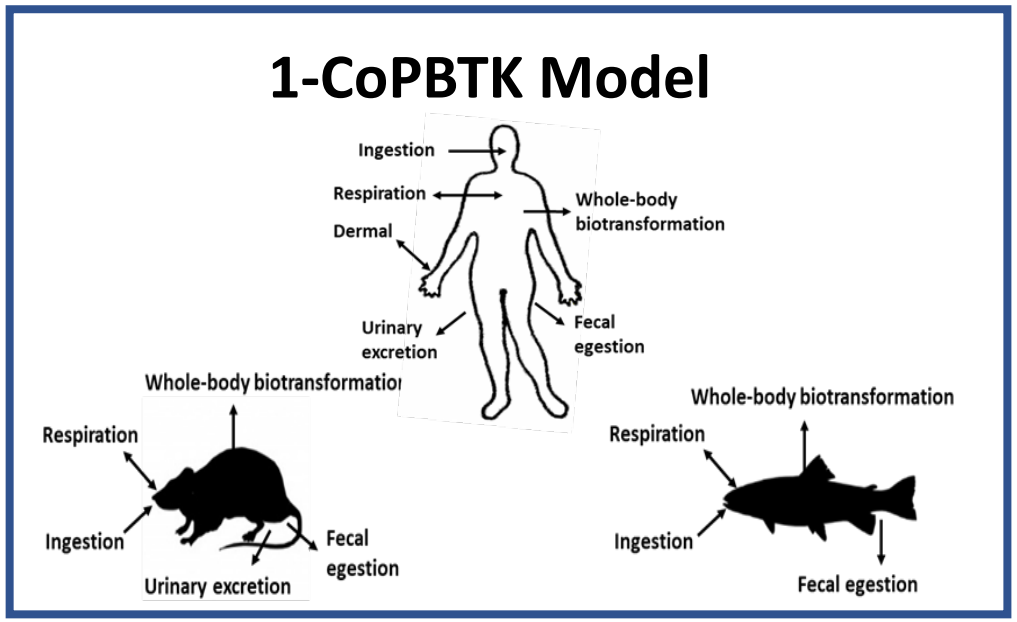




Consensus approach to improve predictions of individual models and enlarge Applicability Domain (AD).

k_T - Indirect Prediction: HTTK

General one-compartment physiologically based toxicokinetic (1Co-PBTK) model that can be parameterized to different mammals implemented in the EAS-E Suite platform



$$k_T = (k_{RO} + k_E + k_B + k_U + k_G)$$

k_{RO}; k_E; **k_B**; k_U; k_G; Respiratory loss, Fecal egestion,

Biotransformation; Renal, Growth

EAS-E SUITE

EXPOSURE AND SAFETY ESTIMATION (EAS-E) SUITE

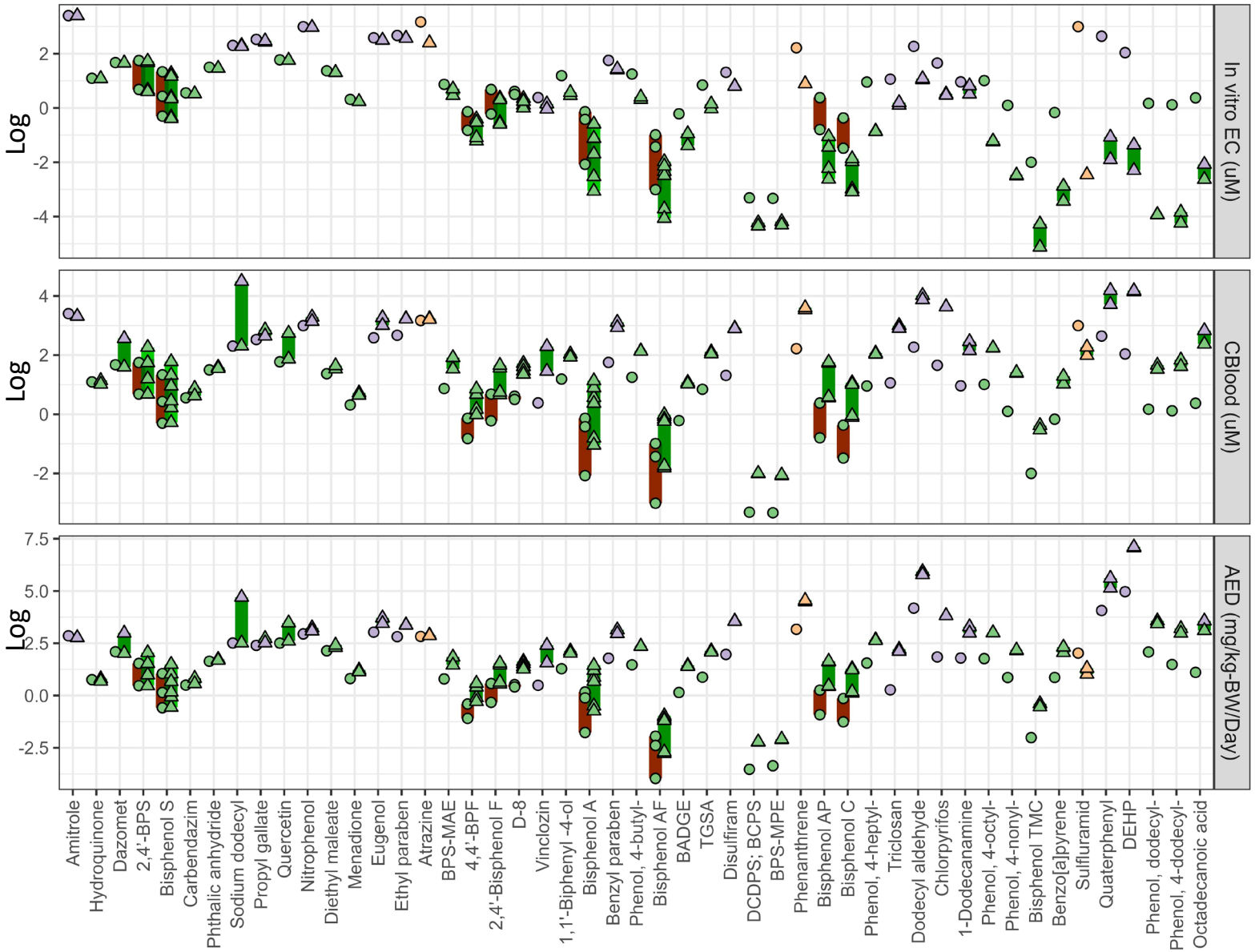
Diet absorption efficiency (ED)

Biopartitioning data to replace octanol assumption

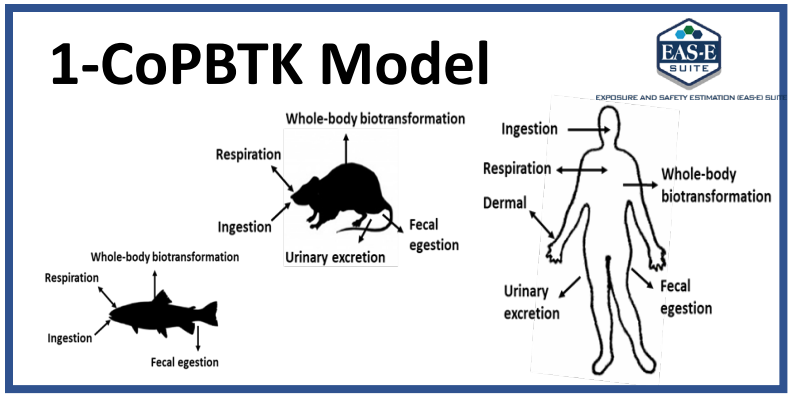
H^+ Ionogenic Organic Chemicals (IOCs)

k_B from empirical in vivo data, or QSAR (Arnot et al., 2014; Papa et al., 2018) or from in vitro (hepatocytes + IVIVE models) or combining these data sources to address a key source of uncertainty in extrapolating in vitro bioactivity data

Case Study: AED of Bioactivity Data



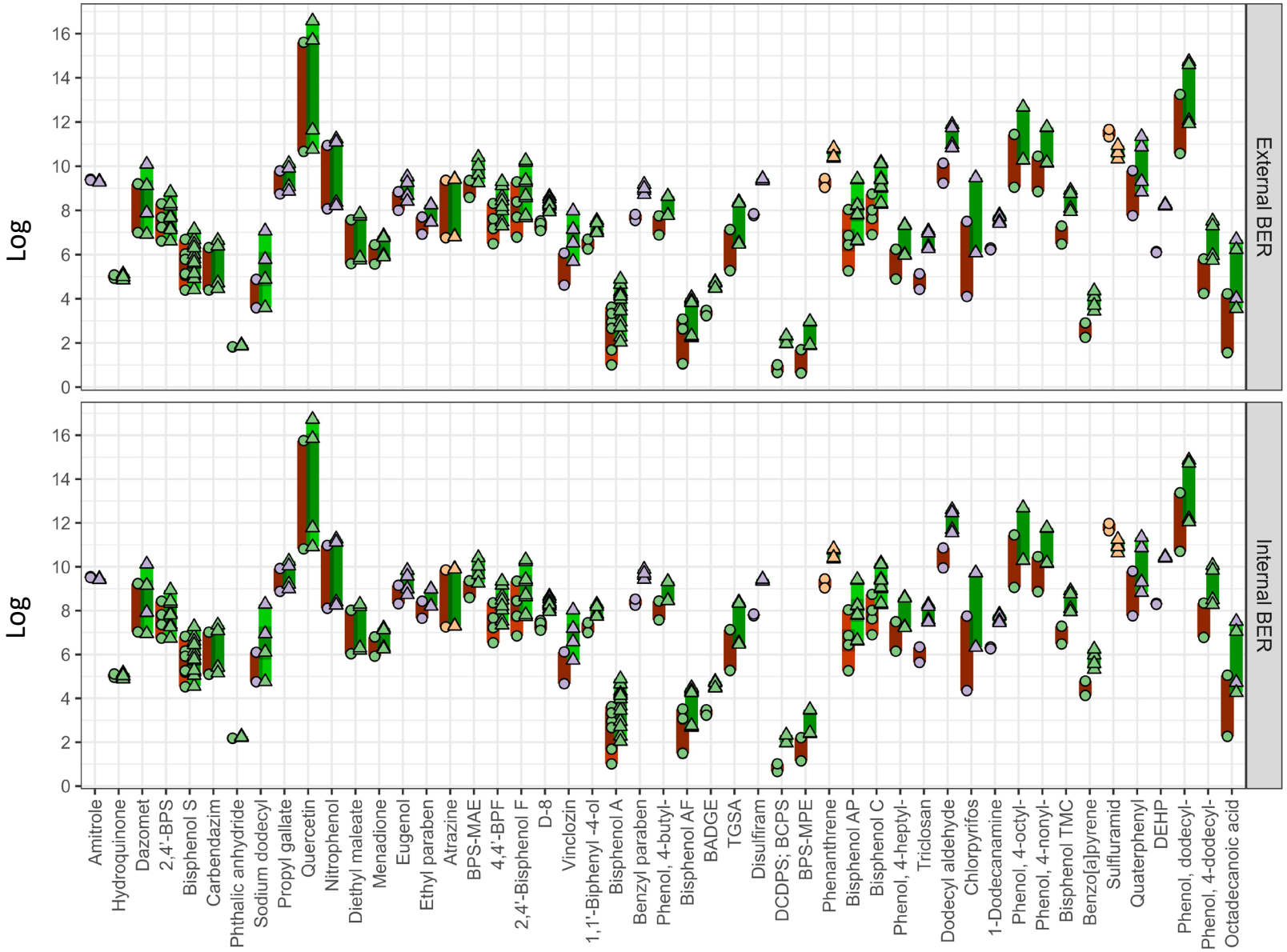
- General PBK model to estimate steady state blood concentration (C_{SS}) corresponding to 1 mg/kg BW/day dose
- AED necessary to generate a C_{Blood} equivalent to an in vitro EC, assuming a 100% absorption efficiency



OK Baseline Toxicity Saturation - Baseline Toxicity

Nominal IV-MBM

Case Study: BER



- BER synthetized in vitro bioactivity information and exposure estimate
- Results change if nominal concentration is used or if in vitro EC is extrapolated to in vivo condition with the IV-MBM model
- PBK model can be applied to investigate the differences between external (intake) and internal (uptake) exposures

Summary

- Various models and assumptions can be developed and evaluated for applying in vitro data
- Models are hypothesis generating machines for experimental testing **-> scientific method!**
- Need to test IVIVE assumptions (e.g., $C_{nominal} \neq C_{blood}$ or $C_{nominal} \approx C_{blood}$) and models (e.g., IV-MBM) and exposure models
- **Know your chemical** (key properties!); **know your (assay) system** (key parameters!)
- Measured, high quality biopartitioning data are needed for IOCs to address uncertainty in AED and BER
- **Be quantitative & explicit** –> align exposure doses across systems for hazard data comparisons & for risk

- **EAS-E Suite** addresses many challenges for **safe and sustainable** chemical production and use:
  - **Facilitates the application of scientific advancements** for decision-making
  - **Provides public access to many computational tools** for chemical evaluations
  - **Provides opportunities** for coordinated and systematic efforts and guidance to address uncertainty in chemical exposure and risk assessment
  - **Improves communication** (outreach, training, education) among stakeholders

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