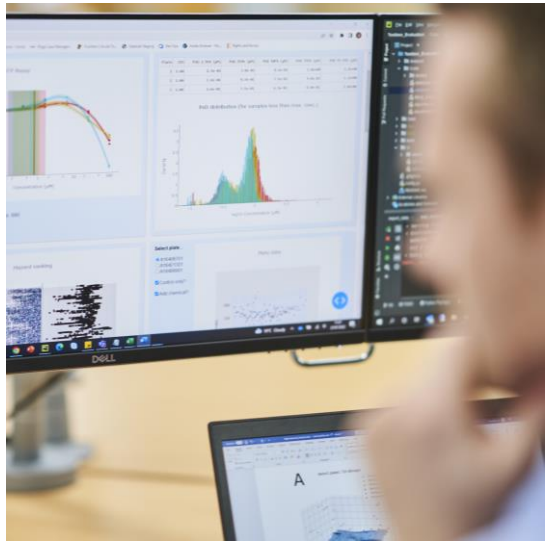
A WORKFLOW FOR TRUE DOSE CONSIDERATIONS OF IN VITRO TEST SYSTEMS WHICH ARE USED AS PART OF NEXT GENERATION RISK ASSESSMENT

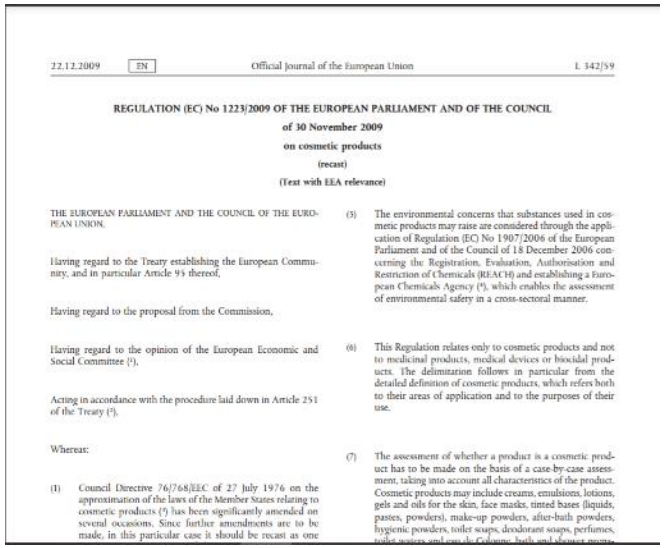
Evita Vandenbossche-Goddard



The need for non-animal safety assessments

Human Relevance

Societal Attitudes/Consumer Preference



22.12.2009 EN Official Journal of the European Union L 342/59

REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 30 November 2009
on cosmetic products
(recast)
(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 95 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Economic and Social Committee [1],

Acting in accordance with the procedure laid down in Article 251 of the Treaty [2],

Whereas:

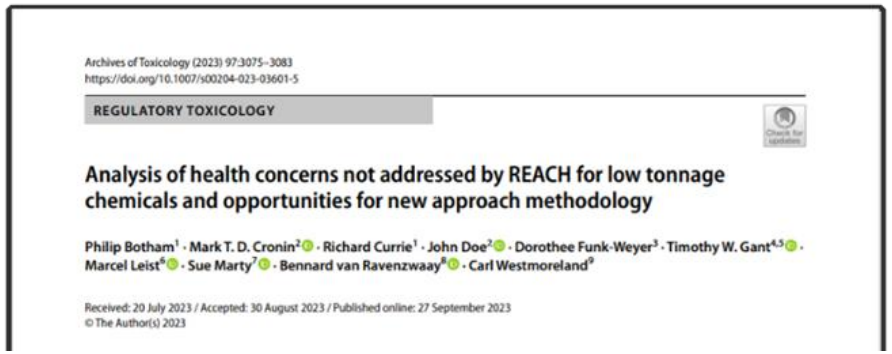
(1) Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products [3] has been significantly amended on several occasions. Since further amendments are to be made, in this particular case it should be recast as one

(3) The environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency [4], which enables the assessment of environmental safety in a cross-sectoral manner.

(6) This Regulation relates only to cosmetic products and not to medicinal products, medical devices or biocidal products. The delimitation follows in particular from the detailed definition of cosmetic products, which refers both to their areas of application and to the purposes of their use.

(7) The assessment of whether a product is a cosmetic product has to be made on the basis of a case-by-case assessment, taking into account all characteristics of the product. Cosmetic products may include creams, emulsions, lotions, gels and oils for the skin, face masks, tinted bases (liquids, pastes, powders), make-up powders, after-bath powders, hygienic powders, toilet soaps, deodorant soaps, perfumes,

Regulatory Change (e.g. EU Cosmetic regulation)



Archives of Toxicology (2023) 97:3075–3083
https://doi.org/10.1007/s00204-023-03601-5

REGULATORY TOXICOLOGY

Analysis of health concerns not addressed by REACH for low tonnage chemicals and opportunities for new approach methodology

Philip Botham[1] · Mark T. D. Cronin[2] · Richard Currie[1] · John Doe[2] · Dorothee Funk-Weyer[3] · Timothy W. Gant[4,5] · Marcel Leist[6] · Sue Marty[7] · Bennard van Ravenzwaay[8] · Carl Westmoreland[9]

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Does REACH provide sufficient information to regulate substances toxic to reproduction?

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Unilever

Unilever approach to systemic toxicity, Framework Approach: The overall goal is a human safety risk assessment

TIER 0: Problem Formulation

Characterise the chemical

Characterise the consumer exposure scenario

Collate all available information (literature mining)

Exit *if safety decision can be made*

Use of predictive tools (i.e. *in silico* QSAR models)

Use of exposure-based waiving approaches such as Threshold of Toxicological Concern (TTC)

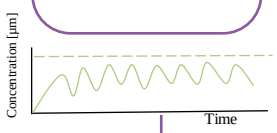
Read Across

Exit *if safety decision can be made*

Progress if safety decision can't be made

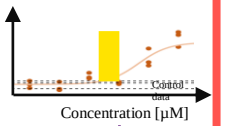
TIER 1: Data Generation

Exposure refinement including generation of relevant ADME parameter data for PBK model development



Plasma Cmax

Bioactivity data generation



Lowest platform PoD

Calculate BER

Exit *if safety decision can be made*

Progress if safety decision can't be made

TIER 2: Refine Assessment to Increase Decision Certainty

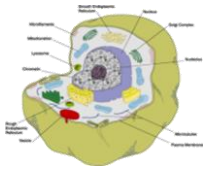
Bespoke assays to cover remaining uncertainties identified a Tier 0 or Tier 1

Further exposure refinement, e.g. consideration of transporters, metabolism ..etc...

Exit *Safety Decision*

Middleton et al., (2022)
Cable et al., (*in preparation*)

in vitro and in vivo dose metrics used in NGRA



In vitro

Nominally applied dose

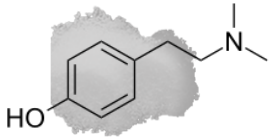
total media

free media

total cell

Free (intra) cellular

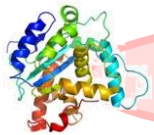
membrane / subcellular



In Vitro Biokientics

Surrogate dose metrics

PBK Modelling



Biologically effective dose

In vivo

Nominally applied dose

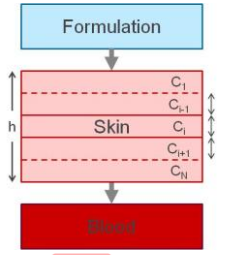
total plasma

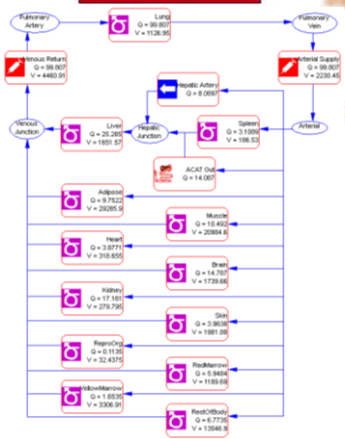
free plasma

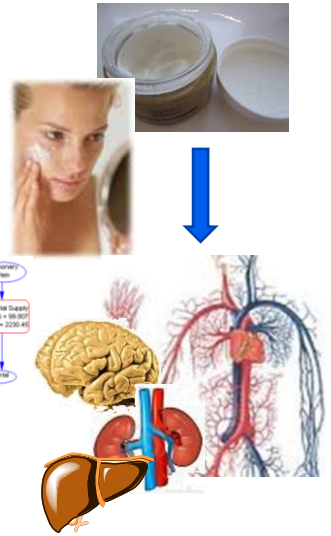
total tissue

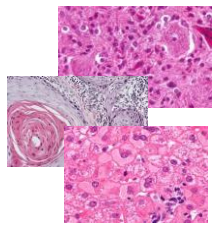
Free (intra) cellular

membrane / subcellular

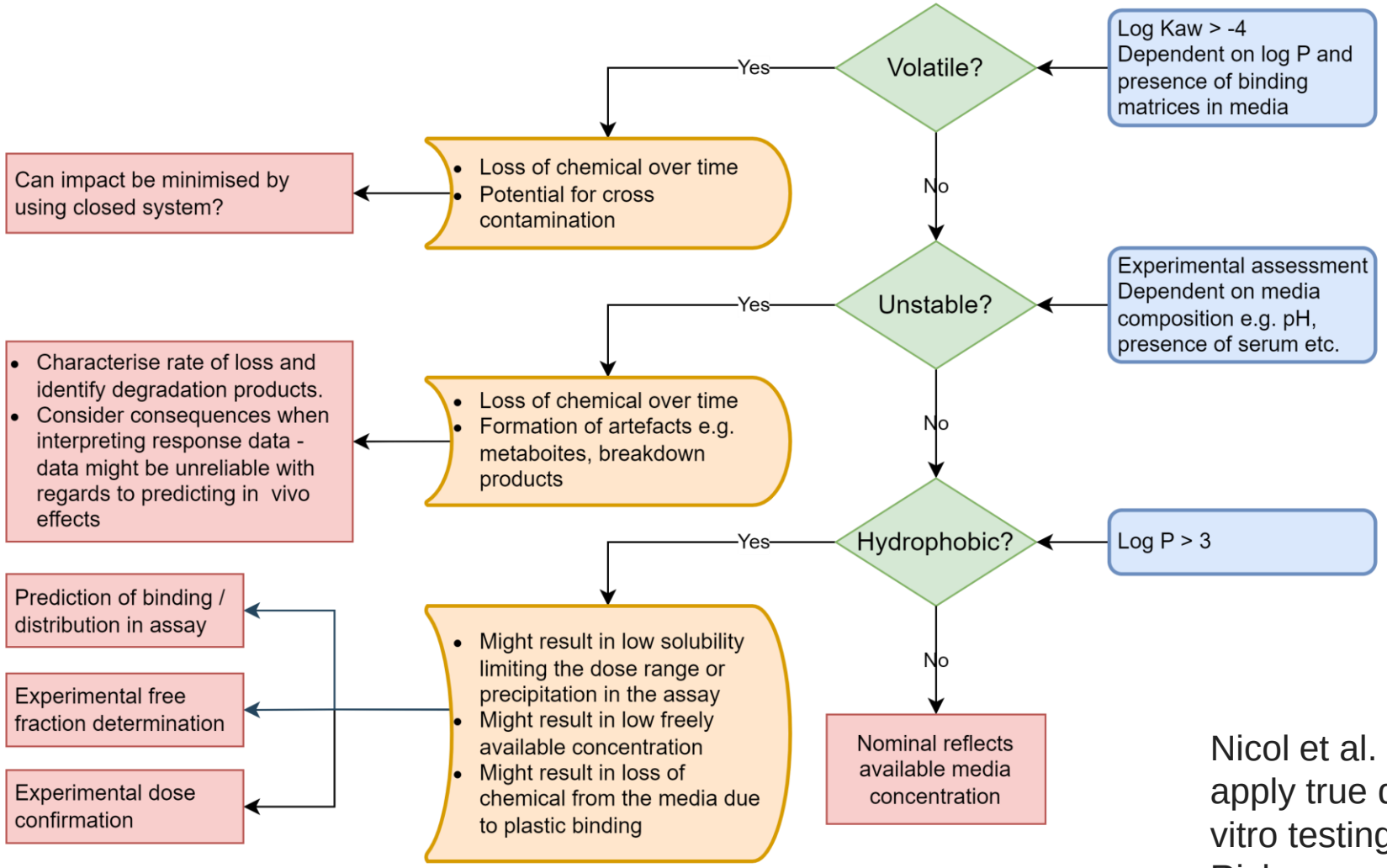








Workflow for the application of true dose considerations



Nicol et al. "A workflow to practically apply true dose considerations to in vitro testing for Next Generation Risk Assessment." *Toxicology* (2024)

In vitro biokinetic considerations included in OECD Guideline

Organisation for Economic Co-operation and Development

ENV/JM/MONO(2018)19

Unclassified English - Or. English

4 September 2018

ENVIRONMENT DIRECTORATE
JOIN MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

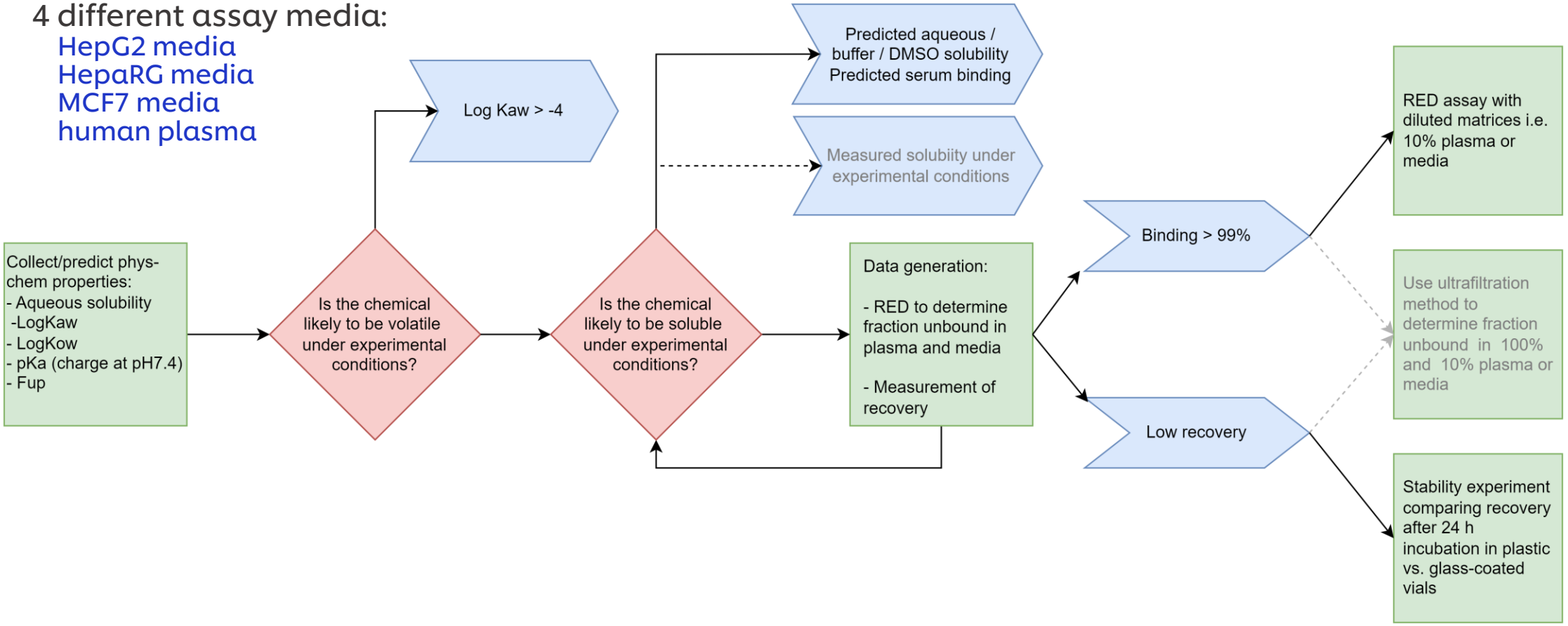
Cancels & replaces the same document of 6 August 2018

Guidance Document on Good In Vitro Method Practices (GIVIMP)

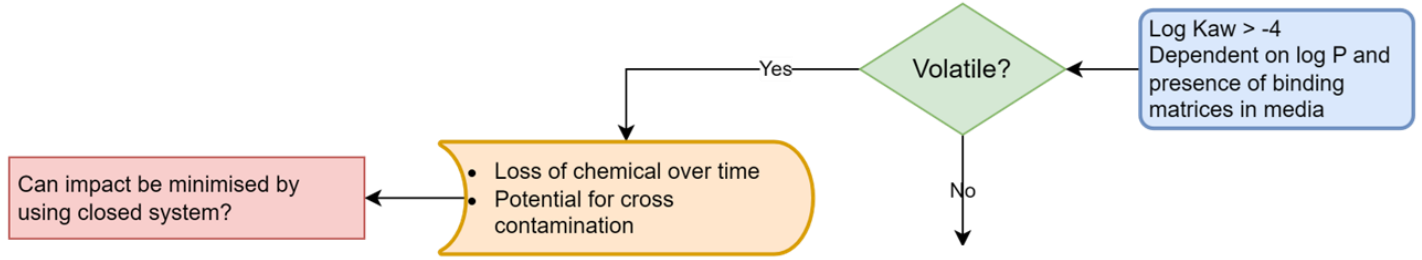
Unilever

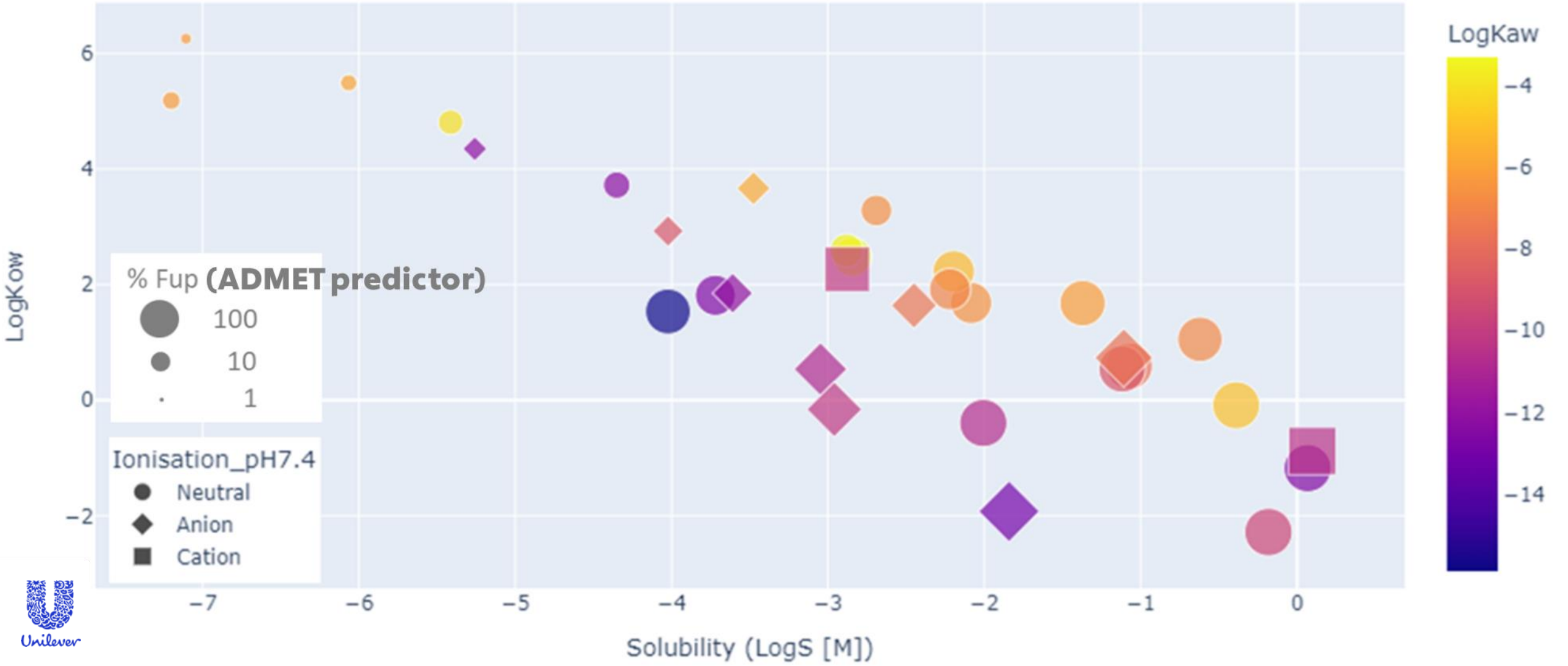
Data generation – Assessment of binding/free fraction and stability in *in vitro* assay media

- Free fraction determination using RED assay for ~ 40 chemicals
- 4 different assay media:
  - HepG2 media
  - HepaRG media
  - MCF7 media
  - human plasma



Physicochemical characteristics of chemicals in the study;





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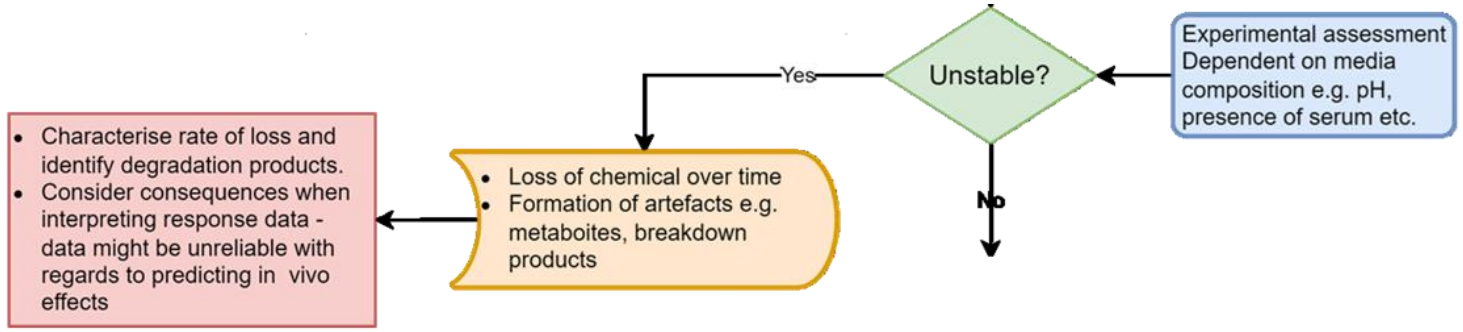
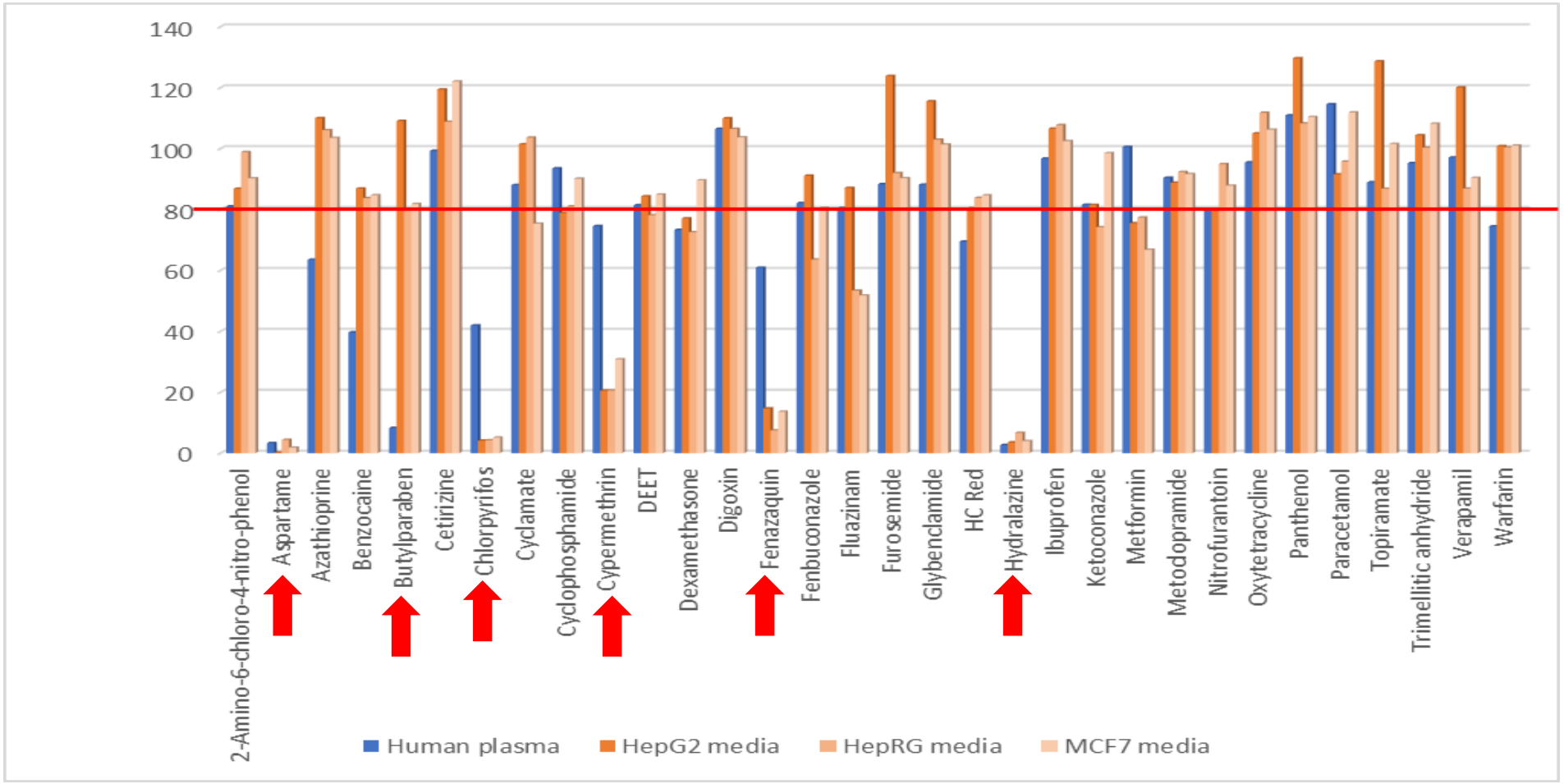
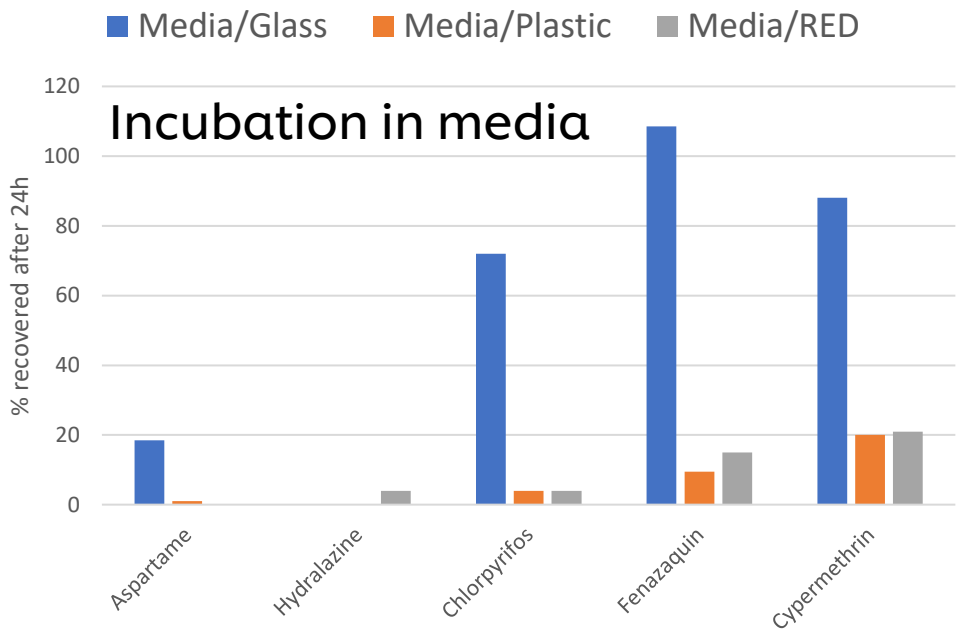
Stability results; Mass balances observed in the RED assay after 24 hours incubation

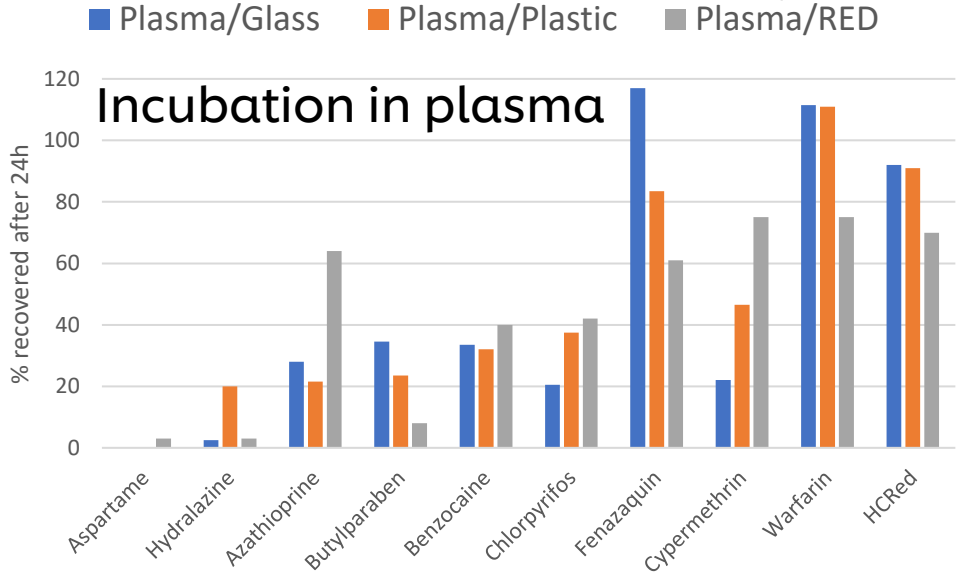




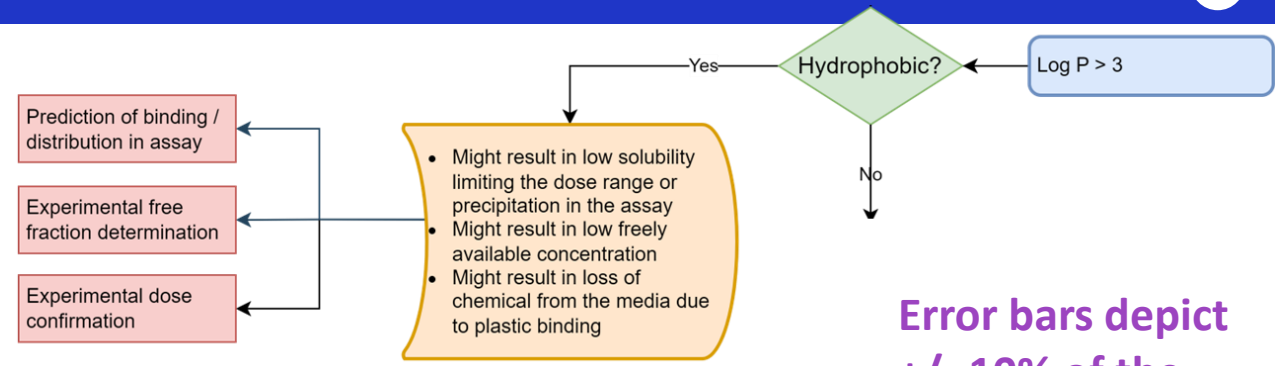
Follow up stability experiment – to identify instability or plastic binding as cause of losses

- Aspartame and Hydralazine: unstable both in plasma and media with half-lives of less than 2 h indicating rapid chemical degradation.
- Fenazaquin: incubation plasma in glass or plastic and in media in glass vessels full recovery after 24 h; however, only 10% of chemical were recovered from media incubations in plastic demonstrating that plastic binding rather than instability are responsible for the observed losses.
- Chlorpyriphos and Cypermethrin: low recoveries under all conditions, both instability and plastic binding are likely to affect the dose available in an in vitro assay experiment.

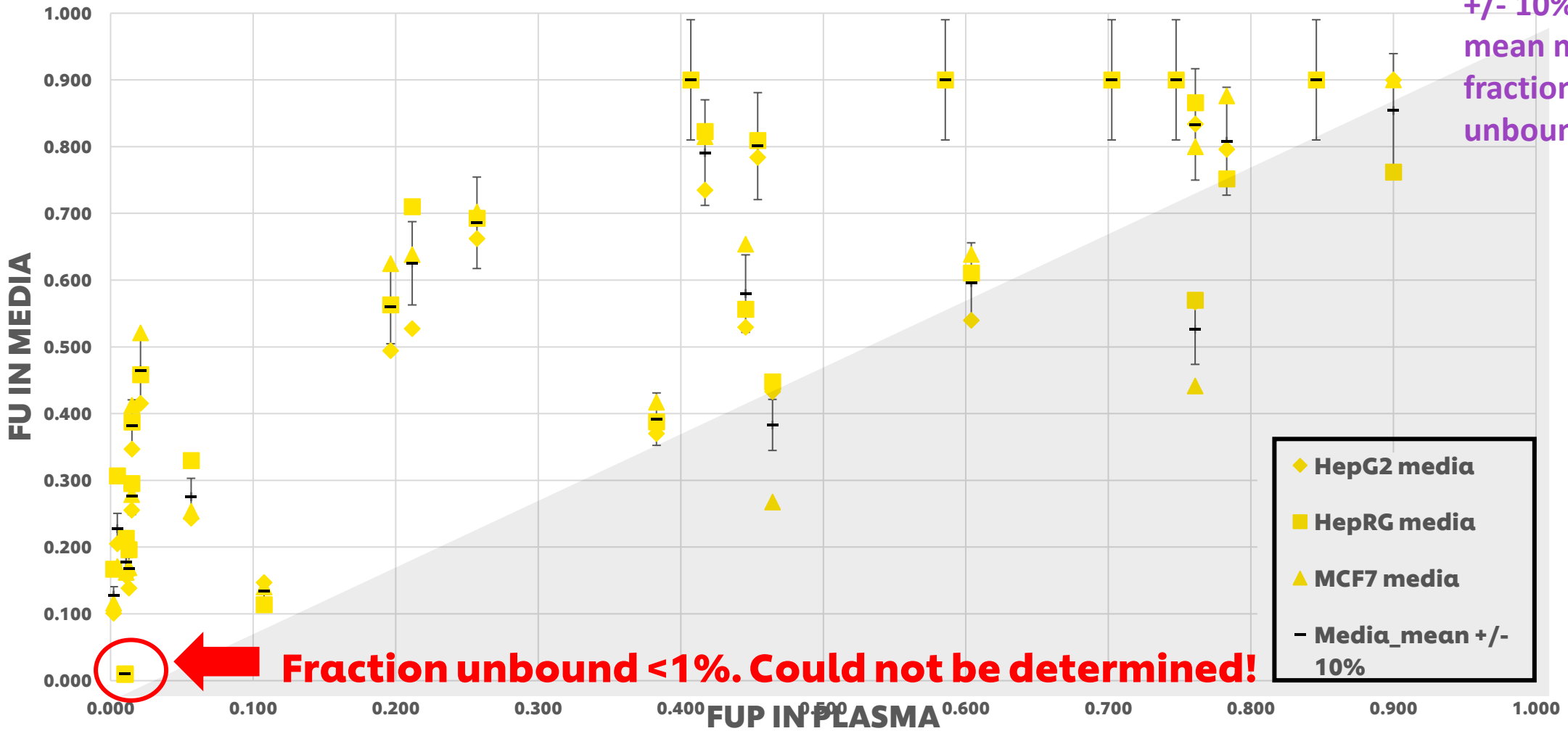




Comparison of fraction unbound in plasma and fraction unbound in three different in vitro assay media

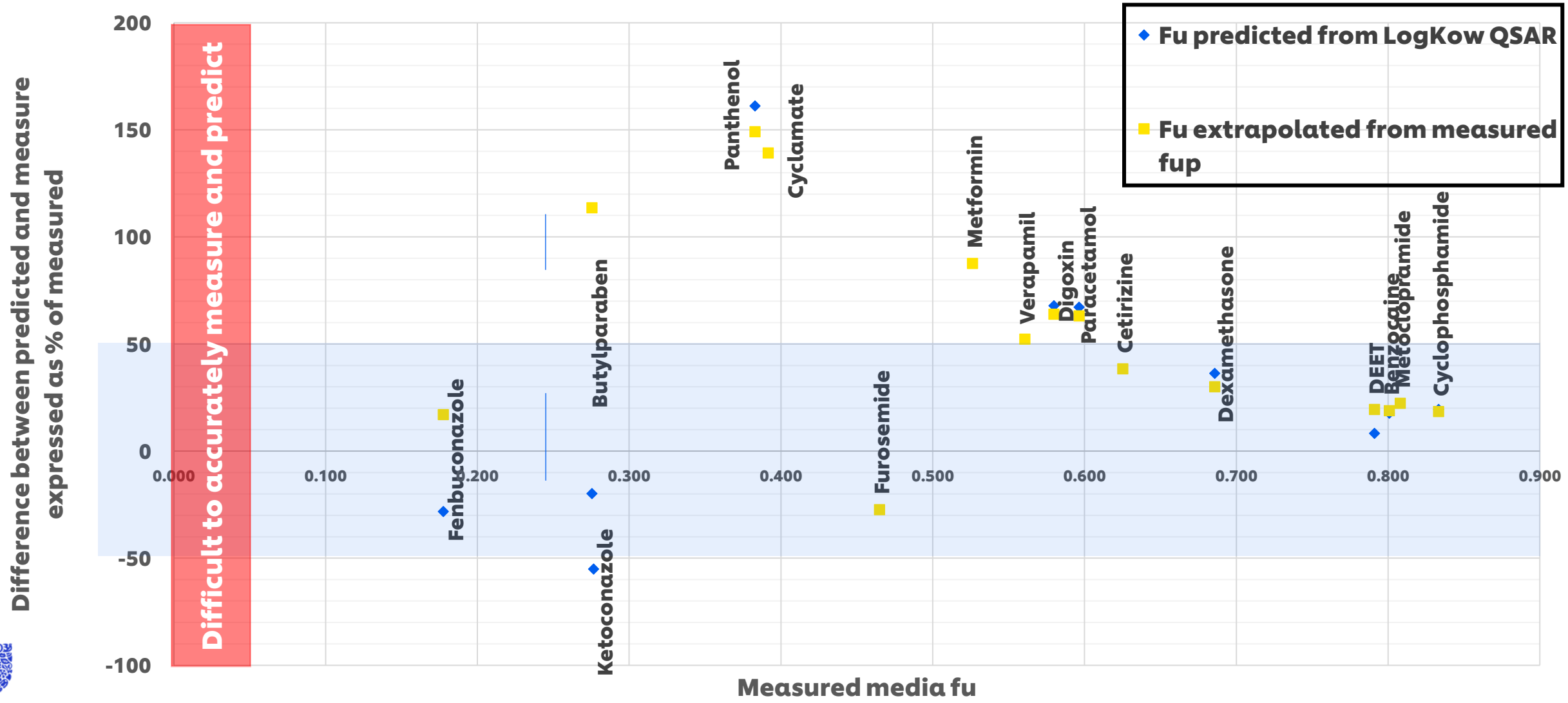


Error bars depict +/- 10% of the mean media fraction unbound.

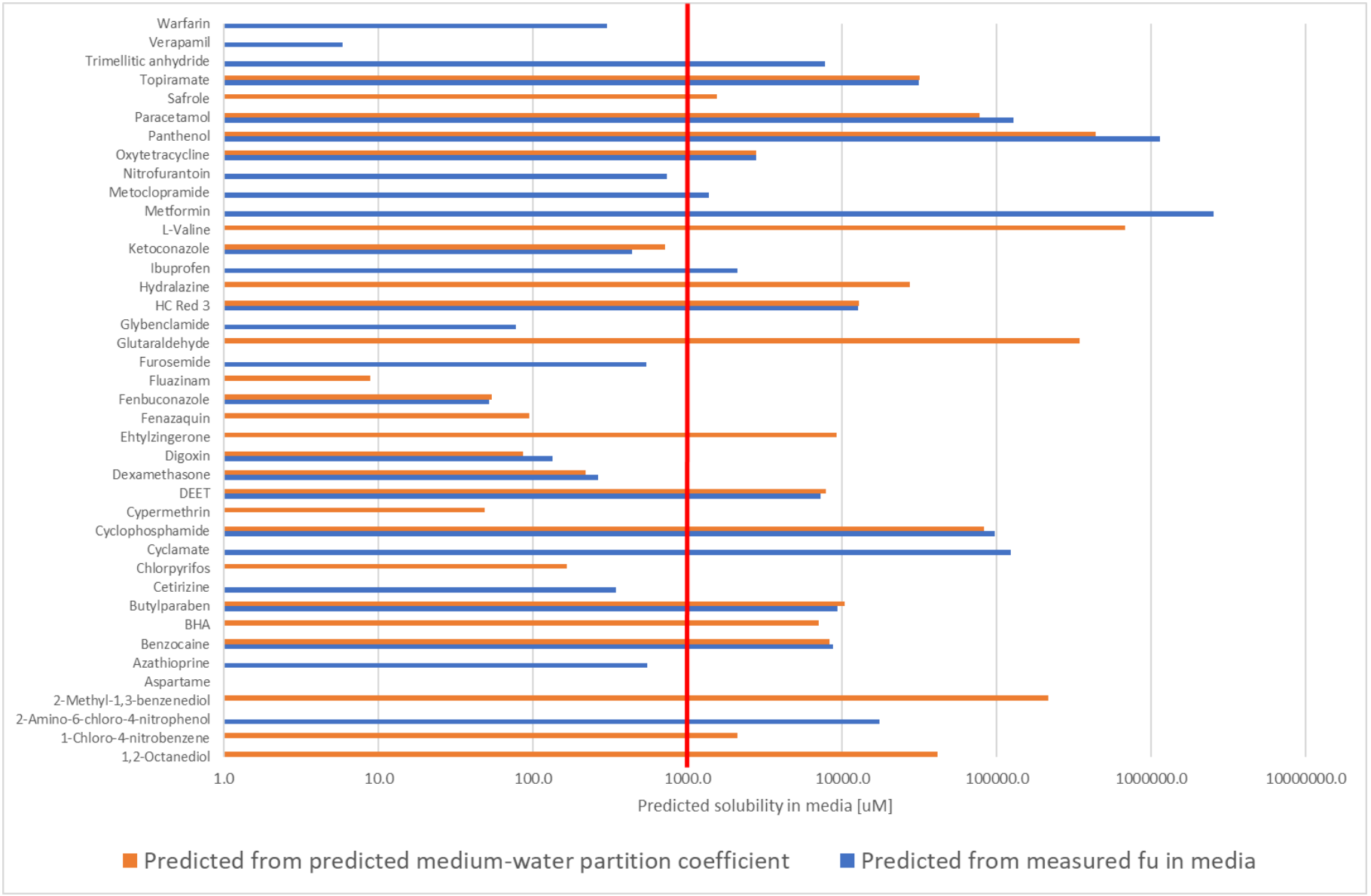


Fraction unbound <1%. Could not be determined!

Difference between predicted fraction unbound in plasma and measured values for the three media types



Solubility results; Predicted media solubility



Can models provide us with all the answers?

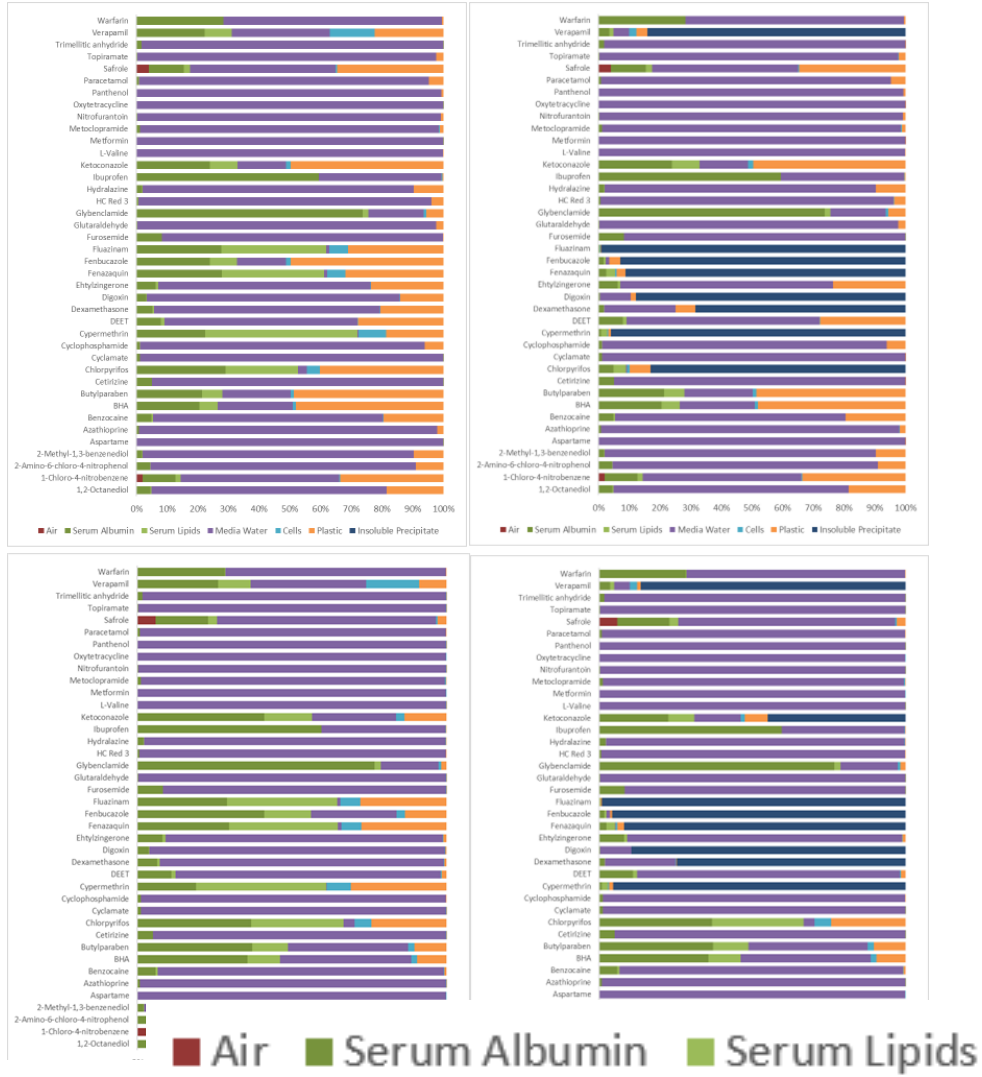
Application of mass balance distribution modelling to 40 case study chemicals –Prediction of steady state mass distribution:

Armitage vs2 model – considering binding, volatility and solubility simultaneously

- Can not consider stability
- Only predicts situation at equilibrium, but some kinetic processes are very slow (evaporation, precipitation)
- Volatility difficult to predict due to difficulty to define headspace (plates are not a closed system)
- Based on simple logP based QSARs with little validation and therefore high degree of uncertainty
- Not easily applicable to ionisable chemicals – requires adjustment factors which introduce further uncertainty

Armitage et al., (2021)

Left: for 1 µM test concentration
Right: for 1000 µM test concentration
Top: Plastic binding prediction based on QSAR option 1.
Bottom: Plastic binding prediction based on QSAR option 2.



Dose confidence matrix: overview of identified potential True Dose challenges for test chemicals

	Phys-chem parameter / in vitro factor					
Chemical name	Volatility	Stability	Plastic binding	Ratio fu media / fup	Aqueous or DMSO solubility	Media solubility
1,2-Octanediol						
1-Chloro-4-nitrobenzene						
2-Amino-6-chloro-4-NP						
2-Methyl-1,3-benzenediol						
Aspartame						N/A
Azathioprine						
Benzocaine						
BHA						
Butylparaben						
Cetirizine						
Chlorpyrifos						
Cyclamate						
Cyclophosphamide						
Cypermethrin						
DEET						
Dexamethasone						
Digoxin						
Ehtylzingerone						
Fenazaquin						

	Phys-chem parameter / in vitro factor					
Chemical name	Volatility	Stability	Plastic binding	Ratio fu media / fup	Aqueous or DMSO solubility	Media solubility
Fluazinam						
Furosemide						
Glutaraldehyde						
Glybenclamide						
HC Red 3						
Hydralazine						
Ibuprofen						
Ketoconazole						
L-Valine						
Metformin						
Metoclopramide						
Nitrofurantoin						
Oxytetracycline						
Panthenol						
Paracetamol						
Safrole						
Topiramate						
Trimellitic anhydride						
Verapamil						
Warfarin						

Unilever

What is the impact on risk assessment?

In vitro distribution

- **Plastic binding:** $C_{max} < C_{nominal}$
- **Distribution between cells and media-water:**
- **Difference in serum binding:** for the same total concentration. C_{free} in vivo < C_{free} in vitro
- **Active transport:** Relationship between free intracellular and free extracellular concentration assumed to be the same for in vitro and in vivo. ?

In vitro loss processes

- **Volatility:** Loss of chemical over time Experimental artefacts from cross-contamination. Loss of chemical over time. $AUC_{actual} < AUC_{nominal}$
- **Stability:** Loss of chemical over time. Composition of the dose changes over time. $AUC_{actual} < AUC_{nominal}$
- **Solubility:** Experimental artefacts from chemical precipitation. $C_{max} < C_{nominal}$

BER risk assessment

Unilever

Acknowledgements

- Beate Nicol
- Charlotte Thorpe

- Richard Newman
- Dawn Yates
- Hiral Patel

- And the wider Unilever team!

Nicol, Beate, et al. "A workflow to practically apply true dose considerations to in vitro testing for Next Generation Risk Assessment." *Toxicology* (2024): 153826. https://doi.org/10.1016/j.tox.2024.153826

Thank You

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