Critical review of in vitro dosing methods for hydrocarbon UVCB substances: An invitation to contribute to new and ongoing research

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INTRODUCTION

New approach methodologies (NAMs), which include *in vitro* testing, are used to support regulatory chemical assessments. *In vitro* data can be used in multiple contexts, from prioritization and screening to supporting chemical grouping and read-across. It is likely that eventually, *in vitro* tests will replace whole organism *in vivo* testing. A new challenge will be to determine fit for purpose methods for dosing hydrocarbon UVCBs, including petroleum substances (PS), in *in vitro* test systems. However, the applicability domain of many *in vitro* assays may not cover the variety of chemical properties found in PS. Furthermore, each dosing method presents different advantages and disadvantages for PS (Table 1).

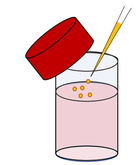
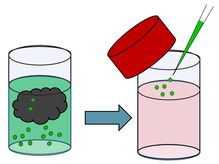
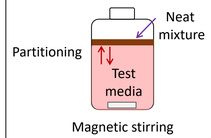
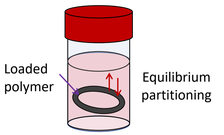
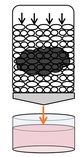
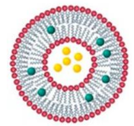
The ability to deliver and maintain stable PS exposure in *in vitro* test systems is challenged by:

1. High surface area to volume ratio of most multi-well plates, which increases the likelihood of sorption to plate walls.
2. Inability to seal some test vessels; volatile constituents can escape open test vessels and may contaminate neighbouring plate wells.
3. Poor solubility of hydrophobic constituents in biological media
4. Compatibility with small testing volumes
5. Presence of lipids and proteins in biological media may differentially bind individual constituents.

OBJECTIVE

Concawe is commissioning a critical systematic review to further our understanding of *in vitro* methods, their challenges, and their applicability in (eco)toxicological assessments of PS.

Table 1. Examples of different in vitro dosing methods and their advantages and disadvantages for petroleum UVCBs.

	Direct Addition	Solvent Carrier	Solvent extraction	Media Accommodated Fractions	Passive dosing	Generator system	Dosing with particle vectors/carriers
Description	 Direct addition with/out agitation to enhance bioavailability	 Dissolve substance in miscible solvent	 Extract substance with solvent (e.g., DMSO) and spike extract	 Stir substance with test medium, allow to separate and use medium for testing	 Dose substance via a polymer or sorbent into biological medium	 Dosing via substance-coated wells or saturator columns	 Targeted dosing of substances with lipophilic particulate (biological) vectors
Advantage	Easy	High dosing concentrations	Mimics lipid-water partitioning depending on solvent used	Realistic dosing scenario	Continuous delivery and concentration	Continuous delivery and concentration	Overcomes solubility challenges for delivery to cells and tissues
Disadvantage	Solubility limitations, possible toxicity of particulate or bulk material	Risk of dosing past solubility, possibility of co-solvent effects	Selective solubility of certain constituents in the solvent, possibility of co-solvent effects	Time consuming, fractions may contain particulates, difficult to implement at micro-scales	Polymer must be small enough to fit in the vessel. Resulting concentrations may be too low for detection	Bulky set up, not suitable for liquids, limited use	Highly specialized technology unexplored for UVCB delivery

CRITICAL REVIEW: Assess the feasibility of *in vitro* testing for hydrocarbon UVCBs and petroleum substances

Application of in vitro test methods in chemical assessments
- Hazard characterization
- Chemical grouping & read across
- Risk assessment

Overview of difficult to test substances, including PS UVCBs, and their properties
What properties make *in vitro* testing of substances difficult?

Overview of common in vitro (eco)toxicity test set ups
Are standard assay set ups suitable? What adaptations can be made?

Overview of dosing options and their applicability to in vitro tests
What are their advantages and limitations? Are they easy to implement in regulatory testing?

Evaluation of in vitro test conditions and their influence on chemical exposure
What conditions influence chemical distribution in test systems? What tools are available to assess this?

Feasibility of in vitro dosing methods and recommendations for future work
What is best suited for PS UVCBs? What adaptions should be made? What is the likelihood of EU regulatory acceptance?

Review outputs will be used to identify relevant in vitro tests and dosing methods and adaptations best suited for PS in future research and regulatory testing efforts

CONTACT US

To view our Request for Proposal, please contact us or visit our LinkedIn page via the QR Code. If you are interested in hearing about how this work progresses, or would like to share ideas or discuss this topic for PS or other UVCB substances, we would be happy to hear from you. Please contact leslie.saunders@concawe.eu or delina.lyon@concawe.eu to stay in touch!