Critical review of *in vitro* dosing methods for petroleum UVCBs

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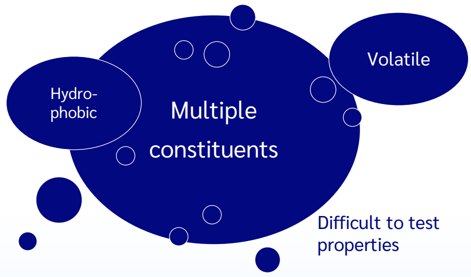
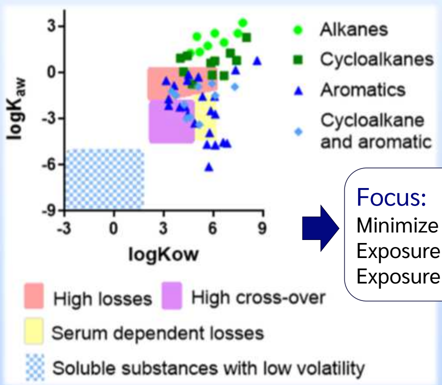
INTRODUCTION

- *In vitro* tests will be used in place of whole organism *in vivo* testing for regulatory chemical assessments.
- Petroleum UVCB substances (Unknown, Variable composition, Complex reaction products, or Biological origin) typically contain a large number and variety of hydrophobic and (semi)volatile hydrocarbon constituents.
- Establish, maintain and confirm defined test substance concentrations throughout the test is challenging for *in vitro* tests for these substances.
- A systematic review on the state of science of *in vitro* dosing methods, their challenges, and their applicability in (eco)toxicological assessments of petroleum UVCBs.



OBJECTIVE: ADDRESS LOSSES AND OTHER CHALLENGES

- Plastic well plates have high surface area to volume ratios that increase the potential for sorption to plate walls;
- Volatile constituents can potentially cross-contaminate adjacent wells;
- Poor solubility of hydrophobic constituents in biological media containing lipids and proteins may lead to differential binding.



Focus:
Minimize losses
Exposure confirmation
Exposure control

Figure 1: Chemical space with observed chemical behavior in a plastic 96-well microplate after incubation for 24 hours at 37°C (Birch *et al.*, 2019). The data points represent different subclasses of petroleum hydrocarbons covering a carbon number range from C8 to C20 (Birch *et al.*, 2018).

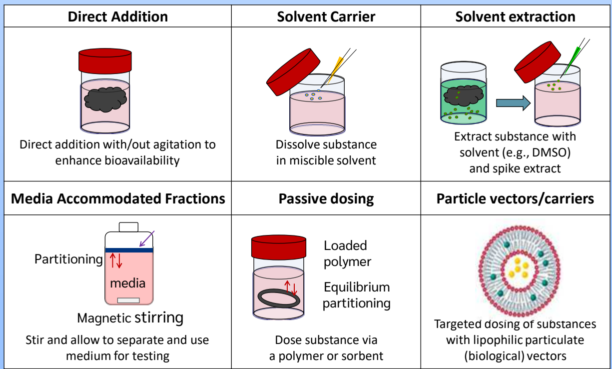


Figure 2: Examples of possible *in vitro* dosing methods for petroleum UVCBs

METHOD: CRITICAL LITERATURE REVIEW SEARCH STRATEGY

Search term strategy for in vitro test overview

- Testing search terms using AbstractSifter v7
- PubMed 3846 papers; Web of Science 452 papers
- Positive seeds 53 papers → SWIFT ActiveScreener For relevant papers selection

Citation search for difficult to test properties

- Identified 6 key papers on topics
- Sorption and loss challenges; Exposure control
- Identified 155 relevant papers

Overview of *in vitro* tests for petroleum UVCBs and applicable dosing methods

Figure 2: Flow of literature search strategy: A wide literature search was initiated in PubMed and Web of Science, and 4285 papers are being screened using the tool SWIFT ActiveScreener (Howard et al., 2020). In parallel, a citation search was performed based on 6 papers, each relevant to the topics of Sorption and loss challenges and Exposure control.

Search terms	*In vitro* *	Ames **	FET ***
Direct addition + UVCB	294	67	3
Solvent carrier + UVCB	64	11	0
Passive dosing + UVCB	10	2	0
MAF****+ UVCB	3	0	0
WAF****+ UVCB	12	13	14
Passive dosing + hydrophobic	19	3	5
UVCB	8	4	1
Petroleum + volatile	2450	407	15
Petroleum + hydrophobic	355	34	3
Petroleum + complex	200	51	3
Multi-constituent	24	3	0

***In vitro:** *In vitro* Techniques OR cell culture OR "*in vitro*" OR cell-based

Ames test: mutagenicity tests OR mutagens OR mutagenicity

OR mutagenic or "gene mutation" OR "ames" " OR "comet assay"

*****FET test:**(zebrafish AND embryo) OR FET test

******MAF/WAF:** Media/Water-accommodated fraction

Table 1: Heat map of the number of PubMed articles retrieved using AbstracSifter V7 (Adkins et al., 2022) for 3 categories of assays combined with different descriptors for test compounds + dosing method.

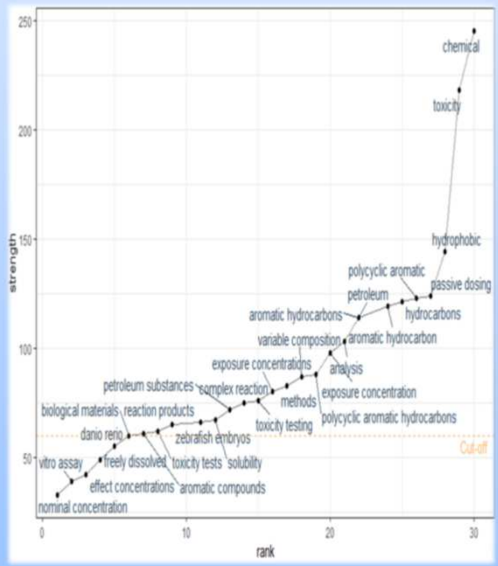


Figure 4: Key words relevance analysis using litsearchr R tool (Grames *et al.*, 2019) from 53 papers identified by expert judgement, and later imported in the SWIFT ActiveScreener tool as positive seeds.

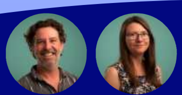
FUTURE IMPACT

- Will help address the critical challenge of petroleum UVCB dosing and improve the reliability of testing.
- Will ultimately help improve the risk assessment of petroleum UVCBs.
- Will aid hazard and risk assessments of other non-petroleum UVCBS.

NEXT STEPS

1. Evaluation of the relevance of selected literature
2. Compilation of a list of *in vitro* methods used for UVCB testing
3. Evaluation of the applicability of different dosing methods
4. Development of recommendations

References: Adkins *et al.*, (2022). U.S. Environmental Protection Agency.
Birch *et al.*, (2019). Chem. Res. in Toxicol. 32, 1780-1790.
Birch *et al.*, (2018). Env. Sci. & Technol., 52(4), 2143–2151.
Grames *et al.*, (2019). Methods Ecol Evol. 10: 1645–1654.
Howard *et al.*, (2020).Environment international, 138, 105623.



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