New Approach Methodologies to Prioritize and Identify Key Components of UVCBs

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Background

Over half of chemicals in commerce are classified as chemical substances of **u**nknown or **v**ariable composition, **c**omplex reaction products and/or **b**iological materials (UVCBs). Some UVCBs originate from natural products (e.g., essential oils and petroleum products), while others are developed to meet performance criteria (e.g., surfactant mixtures). Examples of UVCBs include:

Petroleum & petroleum products

Natural extracts (e.g. essential oils)

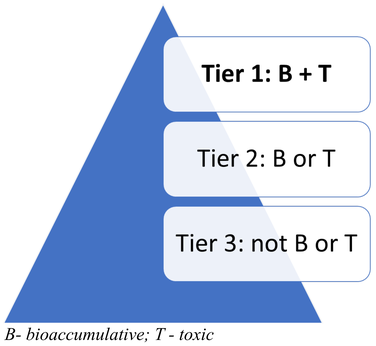
Fats and oils (e.g. vegetable oil)

Commercial surfactant mixtures

The Challenge

Individual UVCBs are poorly defined at the chemical structure and weight fraction levels, making traditional exposure and risk assessment methodologies poorly suited for evaluating UVCB safety. As such, there is a need for new methods to further define UVCB compositions and categorize exposure and hazard potential.

Approach



B- bioaccumulative; T - toxic

This research proposes a tiered approach for prioritizing UVCB components for in-depth chemical compositional analysis via high resolution mass spectrometry (HRMS) based on parallel in vitro bioactivity and metabolism assays.

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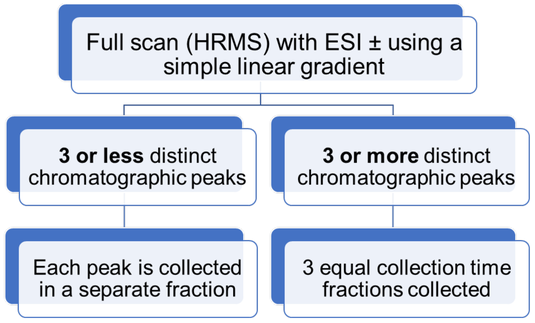
References: Baron, M. G., et al. (2017). Pharmaceutical metabolism in fish: using a 3-D hepatic in vitro model to assess clearance. PLoS One, 12(1), e0168837.
Houston, J. B., et al. (2007). Evaluation of cryopreserved human hepatocytes as an alternative in vitro system to microsomes for the prediction of metabolic clearance. Drug metabolism and disposition, 35(2), 293-301.

UVCB Fractionation

UVCBs will be initially characterized by HRMS using full-scan (m/z 150-2,000) MS1 data collected in both positive and negative electrospray ionization modes. Following initial characterization, subfractions will be collected using a liquid chromatography system equipped with an automated fraction collector.

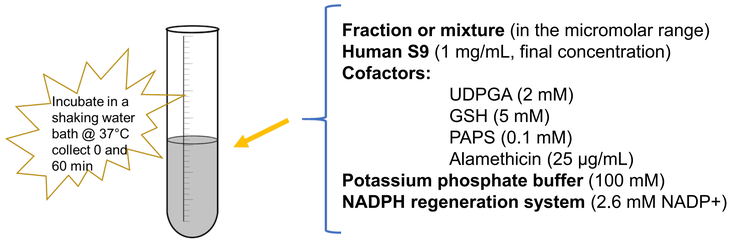


UVCBs and their associated fractions will then be assessed in parallel bioaccumulation and bioactivity assays.

In Vitro Assays

Metabolism

The metabolism and potential bioaccumulation of individual UVCB features will be estimated via a substrate depletion approach using an incubation system consisting of human liver subcellular fractions (S9) and cofactors that support both Phase I and II biotransformation.



Reference pharmaceuticals with varying levels of intrinsic hepatic clearance ($CL_{INT,HEPATIC}$) will be used as positive controls (Houston 2007; Baron 2107).

- High – propranolol; $CL_{INT,HEPATIC}$ = 50 mL/min/kg
- Mid – quinidine; $CL_{INT,HEPATIC}$ = 17 mL/min/kg
- Low – atenolol; $CL_{INT,HEPATIC}$ = 5.1 mL/min/kg

The abundance of a feature observed at sixty minutes will be divided by that observed at zero minutes, and this value will be converted to a percentage and reported as "% remaining at 60 min".

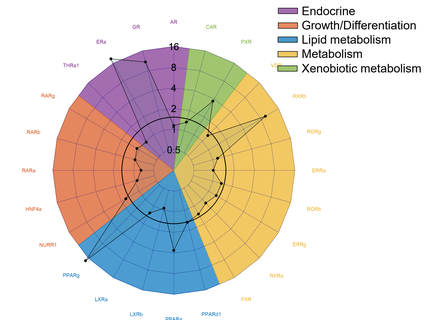
In Vitro Assays Cont'd.

Bioactivity

Bioactivity of UVCB fractions will be evaluated via Attagene's trans-FACTORIAL assay to assess interaction of test samples with 24 human nuclear receptors in the liver HepG2 cell line, a method previously used for testing contaminated surface waters.

#	Name:	Nomenclature:	Ligands:
1	AR	NR3C4	Testosterone, 6-Fluorotestosterone
2	CAR	NR1I3	Xenobiotics, CITCO
3	ERα	NR3A1	Estradiol-17, 4-OH tamoxifen
4	ERRα	NR3B1	Orphan
5	ERRγ	NR3B3	DES, 4-OH tamoxifen
6	FXR	NR1H4	Bile acids, CDCA
7	GR	NR3C1	Cortisol, dexamethasone
8	HNF4α	NR2A1	Orphan
9	LXRα	NR1H3	Oxysterols, T0901317
10	LXRβ	NR1H2	Oxysterols, T0901317
11	NURR1	NR4A2	Orphan
12	PPARα	NR1C1	Fatty acids, leukotriene B_4, fibrates
13	PPARδ	NR1C2	Fatty acids
14	PPARγ	NR1C3	Fatty acids, thiazolidinediones
15	PXR	NR1I2	Xenobiotics, Rifampicin
16	RARα	NR1B1	Retinoic acid
17	RARβ	NR1B2	Retinoic acid
18	RARγ	NR1B3	Retinoic acid
19	RORβ	NR1F2	Orphan
20	RORγ	NR1F3	Orphan
21	RXRα	NR2B1	9-cis-Retinoic acid
22	RXRβ	NR2B2	9-cis-Retinoic acid
23	TRα	NR1A1	Thyroid hormones
24	VDR	NR1I1	Vitamin D, 1,25-dihydroxyvitamin D_3
25	GAL4	yeast	negative control

End points measured using the trans-FACTORIAL assay. From: Attagene.com



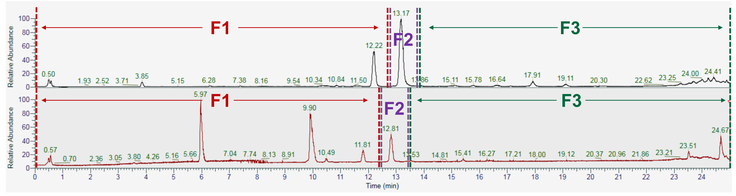
An example radar plot of trans-FACTORIAL end points. Adapted from: B. R. Blackwell.

Analytical Characterization

Features and fractions scoring the highest in both assays will be prioritized for in depth structural characterization using non-targeted, HRMS techniques, and potentially further fractionation and bioassay tests. When possible, tentative identifications will be confirmed with authentic standards and concentrations will be estimated.

Preliminary Data

The efficacy of this approach will be tested in case studies with two commercial UVCBs: a surfactant and a nonylphenol mixture. Initial HRMS characterization and fractionation is underway.



*Example total ion chromatogram (TIC) for a surfactant mixture. Top panel – ESI positive mode; bottom panel – ESI negative mode. **F1** – ionic surfactants; **F2** – fluorinated surfactants; **F3** – non-ionic surfactants.*

Implications

Generated data will aid modelers in assessing UVCB exposure and hazard potential in support of risk assessment for complex chemical mixtures.