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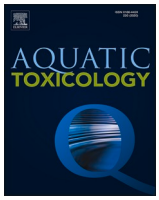

Recommendations for the advancement of oil-in-water media and source oil characterization in aquatic toxicity test studies

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A B S T R A C T

During toxicity testing, chemical analyses of oil and exposure media samples are needed to allow comparison of results between different tests as well as to assist with identification of the drivers and mechanisms for the toxic effects observed. However, to maximize the ability to compare results between different laboratories and biota, it has long been recognized that guidelines for standard protocols were needed. In 2005, the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) protocol was developed with existing common analytical methods that described a standard method for reproducible preparation of exposure media as well as recommended specific analytical methods and analyte lists for comparative toxicity testing. At the time, the primary purpose for the data collected was to inform oil spill response and contingency planning. Since then, with improvements in both analytical equipment and methods, the use of toxicity data has expanded to include their integration into fate and effect models that aim to extend the applicability of lab-based study results to make predictions for field system-level impacts. This paper focuses on providing a summary of current chemical analyses for characterization of oil and exposure media used during aquatic toxicity testing and makes recommendations for the minimum analyses needed to allow for interpretation and modeling purposes.

1. Introduction

In 2005, the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) protocol described a standard method for reproducible water-accommodated fraction (WAF) and chemicallyenhanced WAF (CEWAF) preparation, and recommended specific analytical methods and analyte lists for comparative toxicity testing to inform oil spill response and contingency planning (Singer et al., 2000; Aurand and Coelho, 2005). The original CROSERF protocol recommended limited numbers of samples to be collected and analyzed; and included guidance for chemical characterization of exposure media that was based on the best available technology for that time, balancing analytical resolution with budgetary expectations. These and other CROSERF recommendations were intended to encourage test results with direct applicability to questions related to dispersant use (i.e., WAF vs CEWAF toxicity), as well as to facilitate comparisons of the relative toxicity of oils and the relative sensitivity of several test species. Compositional analyses of oil were based on three subgroups of chemical compounds including total petroleum hydrocarbons (TPHs), volatiles, and compound-specific semi-volatiles. TPH compounds greater than ten carbons in size (x >C10) were analyzed by gas chromatography with flame ionization detector (GC-FID) using baseline integration encompassing hydrocarbons from C10 – C36 (Aurand and Coelho, 2005). The analysis of volatiles (x <C10) could be performed by GC-FID or by gas chromatography with mass spectrometry detection (GC–MS), provided the minimum target analytes could be identified. Analysis of compound-specific semi-volatile analytes was not required under the original CROSERF protocol due to the cost and time requirements for the method at the time. In general, toxicity was found to correlate with the contents of aromatic compounds in oils where benzene, toluene, ethylbenzene and xylenes (BTEX) in the volatiles oil fraction were thought to be at least partially responsible for acute toxicity and that the toxicity of

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other compounds were assumed to be proportional to the BTEX. However, as the scope of oil toxicity studies expanded beyond the original intention of the CROSERF protocol, analytical procedures were updated to reflect the importance of semi-volatiles (e.g., polycyclic aromatic compounds, PACs) in causing both acute and chronic effects (French-McCay 2002; Hodson et al., 2007, 2017, 2019; Adams et al., 2014; Bornstein et al., 2014; Meador and Nahrgang 2019) (Table 1A).

To meet emerging needs and toxicity research interests, "modified"-CROSERF protocols became widely used. At the same time, technological advancements resulted in greater resolution of compounds and compound types (Prince and Walters, 2022; Wise et al., 2022). This has both expanded the list of recommended oil compounds (Table 1B) and reduced the associated costs for basic GC analyses. However, measured analytes reported are inconsistent across studies and many even omit analytes of particular importance for the interpretation of toxic effects and toxicity modeling. Despite the availability of analytical guidelines and updated analyte lists, a troubling number of studies report only nominal concentrations (or loading rate) of oil and dispersant (if used) (e.g., Bejarano et al., 2014; Coelho et al., 2013; NASEM, 2020).

This paper represents 1 of 9 papers in the special series for "Modernizing CROSERF". The topics include a review of CROSERF critiques and modifications (Loughery et al., 2023), the use of biological effects models (French-McCay et al., 2023), experimental conduct (Stubblefield et al., 2023), considerations for phototoxicity (Alloy et al., 2023), media preparation (Parkerton et al., 2023a), reporting and communication (Bejarano et al., 2023) and interpretation (Parkerton et al., 2023b). Recommendations from the series are summarized in Lee

Table 1A
Minimum recommended analytes previously proposed: Original CROSERF protocol compounds in *italics* (Aurand and Coelho, 2005) where semi-volatiles and paraffin, isoparaffin, olefin, naphthene, aromatic (PIONA) analytes depended upon oil used; Revised list where semi-volatiles became recommended as well as analytes marked by * (NOAA, 2014).

Group	Analytes	
Volatiles	*Methylpentanes*	*Benzene*
	Hexanes (C0-C2)	*Toluene*
	Cyclopentane	*Ethylbenzene*
	Dimethylpentanes	*p-xylene*
	Cyclohexane	*m-xylene*
	Heptanes (C0-C2)	*o-xylene*
	Cycloheptane	*n-propylbenzene*
	Octane	*C3-benzenes*
	Nonane	
Semi-volatiles	*Naphthalene (C0-C4)* (each reported)	*Benzo(a)anthracene*
	Biphenyl	Chrysene + Triphenylene *
	Dibenzofuran *	Chrysenes (C1-C4) *
	Acenaphthylene *	*Benzo(b)fluoranthene*
	Acenaphthene *	Benzo(j + k) fluoranthene *
	Fluorenes (C0-C3) (each reported)	Benzo(a)fluoranthene *
	Anthracene *	*Benzo(e)pyrene*
	Phenanthrene *	*Benzo(a)pyrene*
	Phenanthrenes (C1-C4)/Anthracenes (C1-C4) *+	*Perylene*
	Benzo(b)fluorene *	*Indeno(1,2,3-c,d) pyrene*
	Fluoranthene	*Dibenzo(a,h)anthracene*
	Pyrene *	*Benzo(g,h,i)perylene*
	Pyrenes (C1-C4)/Fluoranthenes (C1-C4) *	
	Dibenzothiophenes (C0-C4)	Decalins (C0-C4)

Optional Analytes		
Semi-Volatiles	Benzo(b)thiophene	Phenols (C0-C5)
	Naphthobenzothiophenes (C0-C4) *	
PIONA	n-Paraffins	Naphthenes
	Isoparaffins	Aromatics
	Olefins	

+ Need to differentiate if study includes photo-oxidation.

Table 1B
Recommended analytes to be measured in source oil and exposure media during toxicity testing: Minimum target analytes proposed in CROSERF (*italics*); Phenols (in process water); Volatiles (**bold**).

Group	Compound	Abb
Decalins	Decalin	DE
	C1-decalins	DE1
	C2-decalins	DE2
	C3-decalins	DE3
	C4-decalins	DE4
Naphthalenes	*Naphthalene*	*N*
	C1-naphthalenes	*N1*
	C2-naphthalenes	*N2*
	C3-naphthalenes	*N3*
	C4-naphthalenes	*N4*
2–3 ring PAHs and identified dibenzothiophenes	Benzo(b)thiophene	BT
	Biphenyl	*B*
	Acenaphthylene	ANY
	Acenaphthene	ANA
	Dibenzofuran	DBF
	Fluorene	*F*
	C1-fluorenes	*F1*
	C2-fluorenes	*F2*
	C3-fluorenes	*F3*
	Phenanthrene	P
	Anthracene	A
	C1-phenanthrenes/ anthracenes	P1
	C2-phenanthrenes/ anthracenes	P2
	C3-phenanthrenes/ anthracenes	P3
	C4-phenanthrenes/ anthracenes	P4
	Dibenzothiophene	*D*
	C1-dibenzothiophenes	*D1*
	C2-dibenzothiophenes	*D2*
	C3-dibenzothiophenes	*D3*
	C4-dibenzothiophenes	*D4*
4–6 ring PAHs	*Fluoranthene*	*FL*
	Pyrene	PY
	C1-fluoranthrenes/pyrenes	FL1
	C2-fluoranthenes/pyrenes	FL2
	C3-fluoranthenes/pyrenes	FL3
	Benz[a]anthracene	*BA*
	Chrysene	C
	C1-chrysenes	C1
	C2-chrysenes	C2
	C3-chrysenes	C3
	C4-chrysenes	C4
	Benzo[b]fluoranthene	*BBF*
	Benzo[k]fluoranthene	BKF
	Benzo[e]pyrene	*BEP*
	Benzo[a]pyrene	*BAP*
	Perylene	*PE*
	Indeno[1,2,3-c,d]pyrene	*IN*
	Dibenz[a,h]anthracene	*DBA*
	Benzo(g,h,i)perylene	*BPE*
C0-C5 phenols	Phenol	PH
	C1-phenols	PH1
	C2-phenols	PH2
	C3-phenols	PH3
	C4-phenols	PH4
	C5-phenols	PH5
BTEX	***Benzene***	
	Toluene	
	Ethylbenzene	
	m-xylene	
	p-xylene	
	o-xylene	
C3-benzenes	**Propylbenzene**	
	1-Methyl-3-ethylbenzene	
	1-Methyl-4-ethylbenzene	
	1,3,5-Trimethylbenzene	
	1-Methyl-2-ethylbenzene	
	1,2,4-Trimethylbenzene	
	1,2,3-Trimethylbenzene	

(*continued on next page*)

Table 1B (*continued*)

Group	Compound	Abb
Other VOCs	**Isopentane** **n-C5 (Pentane)** ***Cyclopentane*** ***2-Methylpentane*** **3-Methylpentane** ***n-C6 (Hexane)*** **Methylcyclopentane** **Cyclohexane** **2,3-Dimethylpentane** **3-Methylhexane** **n-C7 (Heptane)** **Methylcyclohexane** **2,4-Dimethylhexane** **2-Methylheptane** ***n-C8 (Octane)*** ***n-C9 (Nonane)*** **n-C10 (Decane)** **1,2,4,5-Tetramethylbenzene** **n-Pentylbenzene**	

et al. (2023).

The current paper gives a comprehensive overview of oil and exposure media analytical methods that are currently relevant for toxicity test characterization, from simple to complex. The discussion section starts with describing the minimum recommended analyses proposed. Then, the rationale for addition of more types of analyses is discussed. Studies aimed at improving knowledge of the mechanistic drivers of toxicity, as well as being able to enhance biological effects models, would benefit from more detailed analytical information beyond the minimum level. Finally, the conclusion section summarizes the recommendations proposed by this paper. Technical terms are used, and methods selected from several fields of chemistry including analytical, environmental, and petroleum. Consequently, supplemental information (SI) includes definitions of the technical terms, brief descriptions of the analytical methods, and sample storage and handling procedures.

2. Discussion

A minimum set of analyses are recommended so that toxicity results can fulfill minimum data comparability and model needs. From there, toxicologists can choose the level of details, sophistication, cost, and complexity that align with the research objectives of their studies. Analytical methods could be relatively simple such as using fluorescence to monitor experimental progress, to being more complex to collect data needed for developing specific mechanistic knowledge for toxic effects, through to using state of the art analytical techniques to identify new types of toxic compounds. For each purpose, there are different categories of oil and water analyses that are recommended, as well as specific lists of analytes to be included. An overview of the suggested methods is given below.

In addition, the use of analytical data requires toxicologists to be aware of the proper validation methods required for the chosen analyses. Method validation includes but is not limited to calibration range and linearity, method detection limits (MDL), method quantitation limit (MQL), precision and bias, accuracy, uncertainty, and robustness studies such as those recommended by the International Organization for Standardization [ISO] 17025, 2017; US EPA, 2003. Brief summaries of experimental protocols are given in the Analytical Methods section under "Method Validation Protocols" (Shoari and Dubé, 2018; US EPA, 1991).

Finally, the potential usefulness of toxicity results has grown with the development of integrated oil fate and biological effects models that combine the results of toxicity studies to predict the potential effects of oil spilled in aquatic environments. Oil fate models predict the concentrations of various groups of compounds in space and time. Tables 2 and 3 show the lists of pseudo-components used for two examples oil fate models including SIMAP (French-McCay et al., 2021) and OSCAR (Reed et al., 2001; Reed and Aamo, 1995). Biological effects models use the Toxic Unit (TU) approach to estimate the toxicity of a mixture of narcotic chemicals (McCarty and Mackay, 1993; Swartz et al., 1995; Di Toro et al., 2000) and oil-derived mixtures (French-McCay, 2002; McGrath and Di Toro, 2009; McGrath et al., 2005, 2018; Redman et al., 2017). Examples of higher and lower resolution hydrocarbon blocks used in PETROTOX models are shown in Tables 4 and 5, respectively. Coupled fate and effects models use the predicted exposure concentrations to evaluate toxic effects of dynamic mixtures of compounds with varying toxicity (French-McCay et al., 2023). Based on considerations that have been highlighted in numerous reviews (Redman and Parkerton, 2015; Adams et al., 2017; Bejarano, 2018; Hodson et al., 2019; Loughery et al., 2023; Lee et al., 2023), this project seeks to modernize the analytical approach of the CROSERF protocol. The revised analytical recommendations for toxicity studies not only provide at least the minimum data needed for interpretation of toxicity results but will also be sufficient for secondary purposes such as data inputs for fate and effects models.

Table 2
Pseudo-component definitions used in SIMAP applications: Quantifies groups of petroleum compounds in droplet and dissolved phases using high temperature simulated distillation curves and speciated hydrocarbon GC analysis methods (in wt%); Contents of Aliphatic (AL) Groups are determined by subtracting the contents of Aromatic (AR) Groups from the oil contents in each boiling range (French-McCay et al., 2021). [Note: AR groups are operationally defined as including compounds that are soluble or semi-soluble.].

Aromatic Group #	Includes	Aliphatic Group #	Boiling Point Range (°C)	Includes
AR1	BTEX	AL1	<150 °C	Unresolved aliphatics
AR2	C3-benzenes	AL2	150–180 °C	Unresolved aliphatics
AR3	C4-benzenes	AL3	180–200 °C	C9-C10 Alkanes
AR4	Decalins	AL4	200–230 °C	C11-C12 Alkanes
AR5	C0-C2 Naphthalenes, C0-C2 Benzothiophenes, biphenyl, acenaphthene, acenaphthylene	AL5	230–280 °C	C13-C16 Alkanes
AR6	C3-C4 Naphthalenes, C3-C4 Benzothiophenes, dibenzofuran	AL6	280–300 °C	C17-C18 Alkanes
AR7	C0-C3 Fluorenes, C0-C1 dibenzothiophenes, C0-C1 phenanthrenes	AL7	300–350 °C	C19-C20 Alkanes
AR8	C0-C2 pyrenes & fluoranthenes, C2-C3 dibenzothiophenes, C2-C3 phenanthrenes, chrysene	AL8	350–380 °C	C21-C23 Alkanes
AR9	Soluble alkanes, Isoalkanes, Cycloalkanes (C5-C8)	AL9	(N.A.)	Dispersant
Residual	Insoluble PACs and other aromatics	Residual	>380 °C	Residual includes both aromatics and aliphatics

2.1. Analytical considerations for toxicity testing

During design of toxicity tests, analyses need to be considered for the source oil (fresh or weathered) used to create the WAF, as well as for the

Table 3
Pseudo-component definitions used in SINTEF OSCAR model (Reed et al., 2001) using GC analyses and boiling point distribution data.

No.	Group description
1	C1-C4 gasses (dissolved in oil)
2	C5-saturates (*n*-/iso/cyclo)
3	C6-saturates (*n*-/iso/cyclo)
4	Benzene
5	C7-saturates (*n*-/iso/cyclo)
6	C1-Benzene (Toluene)
7	C8-saturates (*n*-/iso/cyclo)
8	C2-Benzene (xylenes)
9	C9-saturates (*n*-/iso/cyclo)
10	C3-Benzenes
11	C10-saturates (*n*-/iso/cyclo)
12	C4- and C5- Benzenes
13	C11-C12 (total sat + aro)[1]
14	Phenols (C0-C4 alkylated)
15	Naphthalenes 1 (C0-C1-alkylated)
16	C13-C14 (total sat + aro)
17	Unresolved Complex Mixture (UCM: C10 to C36)
37	metabolite 1
38	metabolite 2
18	Naphthalenes 2 (C2-C3-alkylated)
19	C15-C16 (total sat + aro)
20	PAH 1 (Medium soluble PAHs (3 rings-non-alkylated <4 rings)
21	C17-C18 (total sat + aro)
22	C19-C20 (total sat + aro)
23	C21-C25 (total sat + aro)
24	PAH 2 (Low soluble PAHs (3 rings-alkylated, 4–5+ rings)
25	C25+ (total)

[1] Saturates (sat) and aromatics (aro).

Table 4
An example of PETROTOX hydrocarbon blocks for kerosene generated from high-resolution GCxGC analyses (Redman et al., 2015).

Hydrocarbon Block	Starting Boiling Point (°C)	Ending Boiling Point (°C)	Aliphatic (weight%)	Aromatic (weight%)
1	34	127	0.80	0.17
2	127	178	11.15	4.87
3	178	222	31.37	7.71
4	222	259	24.54	4.74
5	259	292	10.95	1.09
6	292	321	1.71	0.10
7	321	349	0.24	0.01
8	349	373	0.04	0.00
9	373	395	0.02	0.00
10	395	412	0.02	0.00
11	412	449	0.03	0.00
12	449	454	0.01	0.00
Residual	>454[1]		0.44	–

[1] Petroleum compounds that boil at temperatures greater than 454 °C, when present, are assumed to not contribute to toxicity and so are not used in the PetroTox model.

Table 5
Suggested minimum resolution hydrocarbon blocks for kerosene [generated from high-resolution GCxGC analyses (Redman et al., 2015) (French-McCay et al., 2023)]. These data could also be generated using the total petroleum hydrocarbon – fractionated (TPHF) method recommended.

Hydrocarbon Block	Starting Boiling Point (°C)	Ending Boiling Point (°C)	Aliphatic (weight%)	Aromatic (weight%)
1		<68	0.02	0.00
2	68	127	0.78	0.17
3	127	178	11.15	4.87
4	178	222	31.37	7.71
5	222	292	35.49	5.83
6	292	349	1.95	0.11
7	>349		0.56	–

water samples collected before and after the addition of oil, and during and at the end of the test. If the oil is being subsampled for ongoing toxicity tests, considerations for sample handling, storage, and analyses for oil quality are needed to ensure that the oil composition remains constant over the full series of tests being performed. Specific recommendations for the handling and storage of water samples after collection, and before analyses should also be followed. Specific details are discussed below.

2.1.1. Source oil

2.1.1.1. Compositional characteristics. Since oil toxicity is driven by the chemical composition of the source oil, it is important to understand the complexity of the oil. Petroleum products are mixtures of organic compounds that can range from dissolved gasses containing one to four carbon atoms (C1 to C4) to molecular compounds greater than C120 in size. For example, the high temperature simulated distillation (HTSD) data shown in Fig. 1 illustrates how the distributions of the different sized compounds vary across oils. The red horizontal lines marking C10 and C40 in the graph delineate three oil fractions: 1) the <C11 for alkanes and <C10 for aromatics are present in the volatile organic compounds (VOCs) fraction; 2) the intermediate size (C11 to C40) are the semi-volatile organic compounds (SVOCs) fraction; and 3) the >C40 are referred to as vacuum residue. Table 6 shows a summary of the relative contents of the three fractions in the different fresh and weathered oil products shown in Fig. 1. The relative size of the "X"s in the table indicates the relative contents of the fractions in the oil. For example, gasoline consists primarily of VOCs so has a font 85 "X" in the VOCs row and a font 15 "X" in the SVOC row. More information for the use of HTSD data is given in SI – Analytical Methods.

From the toxicity perspective, VOCs are important because this fraction includes the one-ring aromatic compounds, BTEX and substituted benzenes, as well as low molecular weight aliphatic compounds. These compounds are responsible for acute but transient toxicity effects due to their high evaporation and biodegradation rates. If toxicity tests are being designed to include study of acute toxicity impacts, sample handling will be important to prevent loss of volatiles from oil samples during storage (see SI – Storage and Handling Procedures). The individual compounds in VOC fractions of oils can be quantified using the gas chromatography technique called detailed hydrocarbon analysis (DHA) where ~1200 individual compounds can be resolved. VOCs can also be analyzed using headspace gas chromatography-mass spectrometry (GC–MS) by spiking an appropriate internal standard into the sample. However, Table 1B shows the updated minimum list of VOCs to be reported.

The second distillation fraction, SVOCs can also contribute to oil toxicity. This fraction can contain polycyclic aromatic compounds (PACs) including heteroaromatics, i.e., those containing sulfur, nitrogen and oxygen (Achten and Andersson, 2015). Polar resins and asphaltenes compounds can also be found in this fraction. Individual analytes such as those recommended in Table 1B can be quantified in this fraction using GC techniques, but the polar resins and asphaltenes need to be removed prior to analyses. Depending on the instrumentation used for SVOCs analysis, oils are usually diluted with appropriate solvents, and spiked with appropriate surrogates for quality control and quantification. As an option, the oil can be fractionated into different fractions for different target analysis by one-dimensional chromatographic techniques equipped with conventional mass spectrometry (MS) (Yang et al., 2017). However, fractionation is not necessary for quantification of Table 1B SVOC analytes using more advanced instrumentation, such as comprehensive two-dimensional GC equipped with a high-resolution MS detection system (e.g., time-of-flight mass spectrometry, TOFMS). In this scenario, the diluted oil can be analyzed directly without any prior liquid chromatography and/or molecular sieving steps (Ventura et al., 2010; Kumar and Dutta, 2021), if guard columns are used. Beyond the

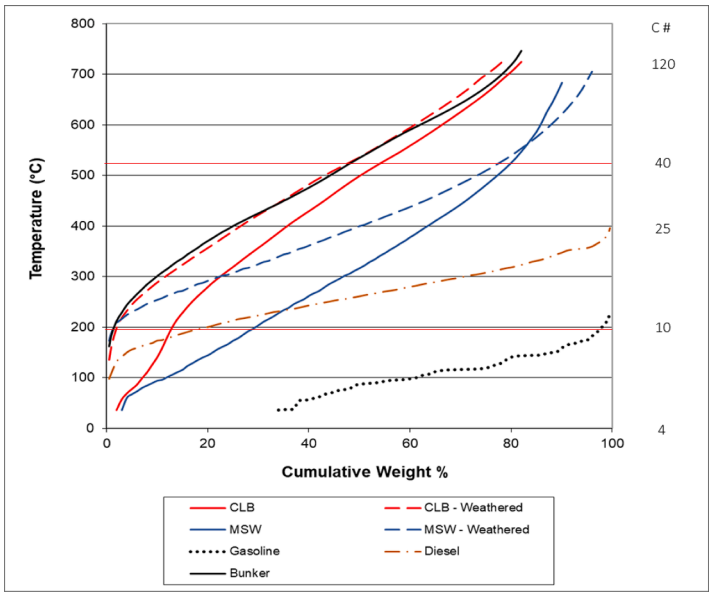

Fig. 1. Boiling point distribution for Cold Lake Blend (CLB) diluted bitumen (fresh and weathered 8 days), Mixed Sweet Blend (MSW) conventional crude (fresh and weathered 8 days), gasoline, diesel and bunker fuel[1]. The representative carbon numbers (C#) compounds that boil at selected temperatures are shown on the y-axis on the right side of the graph. The lower and upper horizontal red lines indicate C10 (decalin) and C40 alkane sizes, respectively.
[1] Data collected at CanmetENERGY Devon, Natural Resources Canada.

Table 6
Summary of the distillation fraction contents of different oil products including the key attributes of the fractions in terms of toxicity, size of compounds, and length of time in the environment.

Distillation Fraction	Scaled Content							Attributes
	Diluted Bitumen	Weathered Diluted Bitumen	Crude Oil	Weathered Crude Oil	Gasoline	Diesel	Bunker	
VOCs	x		X		X	x		• Primarily responsible for acute toxicity • X <C11 saturates • X <C10 aromatics • Readily evaporates/biodegrades
SVOCs	X	X	X	X	x	X	X	• Can contribute to acute toxicity • Responsible for chronic toxicity • C11 ≤ X ≤ C40 saturates • C10 ≤ X ≤ C40 aromatics • Can include resins and asphaltenes • Partially evaporates/biodegrades
Vacuum Residue	X	X	x	x			X	• Undefined contributions to chronic toxicity (with and without degradation) • X > C40 saturates • X > C40 aromatics • Includes resins and asphaltenes • Minimal evaporation; undefined conditions for degradation

Table 1B analytes, a significant portion of SVOC compounds elute in the "unresolved complex mixture" (UCM) of chromatograms.

The third distillation fraction called "vacuum residue" (name derived for how it is prepared in refineries) consists of saturates, aromatics, resins and asphaltenes. These large compounds have negligible solubility in water and are not generally considered toxic to aquatic organisms but are responsible for the higher viscosity and density of weathered oil compared to fresh oil. The high densities of asphaltenes and resins can cause portions of weathered oil to settle over time to the bottom of aquatic environments, where sunken oil can impact benthic organisms by restricting oxygen, smothering, and/or releasing SVOCs that are trapped within the oil mass.

Some crude oils have high contents of aliphatic compounds called waxes. These hydrocarbons are primarily alkane (paraffin) chains having carbon numbers C18 and higher and so are found in the SVOC and vacuum residue fractions of the oils (Bridié et al., 1980; Chen et al.,

2004; Jokuty et al., 1996). Larger wax compounds can also include branched-chains and cycloalkanes (naphthenes) in their structures (Suaria et al., 2018). Waxes and asphaltenes can also be co-precipitated during analyses (Hoff and Dettman, 2012). Because of the relationship between high wax content and high viscosity, waxy oils spilled into water at temperatures below their pour point can become semi-solid fragments on the sea surface (ITOPF, 2014). Furthermore, waxes can interfere with oil biodegradation (Lee and Levy, 1991).

In addition to evaluating the inherent toxicity of fresh oil, it is important to include weathered oil when designing toxicity studies. Typical response times after an oil spill range from 6 to 72 h depending on the location and size of the spill. Since the physical properties of oil change rapidly within the first 48 h after a release (King et al., 2014b, 2017, 2019; Overton et al., 2016; Stiver and Mackay, 1984), aquatic biota are likely to have the greatest exposure to weathered oil for prolonged periods. With weathering, the VOCs and the lowest boiling portion of the SVOCs are lost from the oil within hours to weeks after the spill primarily due to evaporation (Faksness et al., 2020) (see, for example, the changes in composition between fresh and weathered diluted bitumen and crude oil in Table 6). Consequently, if oil like bunker fuel or weathered oils are going to be tested, Barron & Ka'aihue (2003) suggested that quantification of VOCs may not be needed as part of the test design.

2.1.1.2. Source oil analyses. With respect to oil composition, the approach to oil analyses for toxicity testing has been to focus on the quantification of specific compounds in the VOC and SVOC fractions listed in Table 1B. However, this approach greatly limits the usefulness of the toxicity data generated. As the contents of BTEX and PACs quantified in Table 1B describe only a few wt% of the oil composition, their contents indicate little about the compositional quality of the oil being tested. As well, without measuring at least the minimum boiling point distribution information illustrated in Table 5, the toxicity results cannot be used to improve oil fate and effects models. For example, SIMAP and PETROTOX (see Tables 2 and 4; and French-McCay et al., 2023) are hazard models that estimate dissolved exposure concentrations of oil components based on knowledge of their chemical compositions (i.e., pseudo-components or hydrocarbon blocks), thus allowing estimations of both acute and chronic toxicities. Without information about the properties of the oil being used to generate the WAF, the toxicity data cannot be linked to oil spill behavior models. The models SIMAP and OSCAR (Tables 2 and 3) incorporate oil properties such as viscosity, density, and distillation fraction contents from simulated distillation for prediction of the environmental fate of spilled oil (see French-McCay et al., 2023 and references therein).

A minimum set of oil analyses are recommended where descriptions of the methods are given in SI – Analytical Methods. Beyond the analytes listed in Table 1B, researchers should also have HTSD (or another more suitable simulated distillation analysis if the oil does not contain vacuum residue) and Total Petroleum Hydrocarbons Fractionated (TPHF) (such as Zemo, 2016) analyses run on the oil used to make the WAF. Finally, density and viscosity measurement at three temperatures such as 5, 15 and 25 °C, and oil pour point will make the toxicity data highly useful to the spill response community. These analyses would not have to performed before every toxicity test if the recommended procedures in SI – Storage and Handling Procedures for oil handling are being followed. However, the simulated distillation analyses should be run periodically to confirm that oil composition is not changing. And the complete set of analyses should be repeated if the oil is weathered, photo-oxidized, biodegraded, or otherwise pretreated before WAF preparation.

The tests already mentioned and other possible oil analyses are shown in Table 7. The need for SARA fractionation, elemental (CHNSO) analyses and carbonyl content determinations could arise if the design of toxicity tests begin to focus on the toxicity of compounds that are not measurable by GC. For example, these techniques could be used to explore the toxicity of heteroaromatic compounds in oil, or oxidized compounds resulting from oil photo-oxidization or biodegradation), or specific subfractions such as the aromatics isolated from the vacuum residue fraction. Liquid chromatography techniques could also be developed to help resolve polar compounds (Arboleda et al., 2015).

In Table 7, considerations for the suitability of the techniques for use during toxicity testing in terms of the relative amount of time to run the analysis, its complexity to perform and interpret the results, and its expense and availability are suggested with rankings from low to very high. With regards to time for running analyses, rankings of low, moderate, high, or very high indicate that the analysis can be run in minutes, hours, days, or more than a week. For complexity, rankings from low to very high reflect the number of steps needed to perform the analysis as well as the degree of specialization needed for the analyst to process the resulting data, from simple to run and easy to prepare the data for interpretation to the analytical procedure requiring specialized analytical training to both perform the analysis and process the data. For expense, rankings of low, moderate, high, or very high reflect the cost per sample where "very high" costs can be 40 times higher than the lowest charge rates. Very low availability indicates that only highly specialized, primarily academic analytical laboratories would be able to conduct the analysis while a ranking of very high availability suggests that the analytical capability is available in most academic and commercial laboratories.

2.1.2. Exposure media analyses

The compositions of exposure media change with time (are unstable)

Table 7
Summary of analytical techniques to characterize fresh and weathered source oils. Explanation of "Suitability of Technique" evaluations are given in the text. See SI – Analytical Methods section for method descriptions.

Purpose	Property Measured	Technique	Considerations for Suitability of the Technique (Low, Moderate, High, Very High)			
			Time	Complexity	Expense	Availability
Quality Control	TPHF	Wet chemistry/GC-FID	Low	Low	Low	Moderate
	Total boiling point distribution profile	Simulated distillation	Moderate	Moderate	Moderate	Low
Bulk physical properties and rheology	Density (ideally at 3 temperatures)	Digital density meter following ASTM 5002	Low	Low	Low	Moderate
	Pour point	ASTM D97	Low	Low	Low	Low
	Viscosity (ideally at 3 temperatures)	Viscometer/Rheometer following ASTM or in house methods	Low	Low	Low	Moderate
Fractionation by polarity	SARA[1]	Wet chemistry/GC hybrid (ASTM)/Thin layer chromatography (TLC)	Moderate	Moderate	High	Low
Bulk composition for biodegradation or photo-oxidation oil weathering conditions	Elemental (CHNSO)	ASTM D5291 and/or equipment manufacturer recommendations	Low	Low	Low	Low
	Carbonyl content	Fourier transform infrared spectroscopy	Moderate	Moderate	Moderate	Moderate

[1] SARA represents saturates, aromatics, resins and asphaltenes.

due to ongoing processes such as evaporation and biodegradation. To capture the composition information that is relevant to the toxicity tests conducted, both timing and handling of the samples are important (see Stubblefield et al., [2023] for additional discussion). Recommended procedures for handling and storage of exposure media samples are given in SI – Storage and Handling Procedures. Briefly, because the collected samples are unstable, they should be stored in the dark at 4 ± 2 °C with no head space and left unopened before analysis. If not acid preserved where polar compounds are not the targets, the samples need to be extracted and analyzed within 7 days to avoid further biodegradation/evaporation loss (Rodriguez-Gil et al., 2021). If acid preserved (pH<2), the samples must be extracted within 14 days (NOAA, 2014).

Table 8 outlines various techniques to obtain compositional information from both exposure media and source oil samples. The explanations of the rankings for the time, complexity, expense, and availability of the analyses are the same as described for Table 7. The analytical methods needed to characterize exposure media used in toxicity tests varies according to the design of the toxicity test as well as the method used to prepare the media (Parkerton et al., 2023a). In addition to source oil analyses mentioned above, interpretation of toxicity results requires measurement of the composition of dissolved oil compounds in the exposure media as well as the quantification of any oil droplets, if present (see Section 2.1.3). Consequently, the same analyses

Table 8
Summary of analytical techniques to characterize exposure media and source oils by purpose and consideration of data resolution needs. Explanation of "Suitability of Technique" evaluations are given in the text. See Table 1B for recommended analytes and SI – Analytical Methods section for method descriptions.

Purpose	Sample Type and Measurement Approach	Category of Analysis	Specific Analytes	Technique	Considerations for Suitability of the Technique (Low, Moderate, High, Very High)			
					Time	Complexity	Expense	Availability
Minimum	Grouped analytes, inexpensive measure-ments	Oil fractions differentiated by chemical identity/ functionality	TPHF	Wet chemistry /GC-FID	Moderate	Moderate	Low	High
			CCME[1] F1 to F4	Wet chemistry /GC-FID	Moderate	Moderate	Low	High
		Aromatic hydrocarbons	PACs	Fluorescence Spectroscopy, Multispectral Fluorescence	Low	Low	Low	High
Low Resolution Analyses	Basic analyte sets	Chemical functional fractions	Saturates (e.g., TSH[2]) Aromatics (e.g., TPAH[3])	Wet Chemistry fractionation + GC-FID	Moderate	Moderate	Low	High
			TOC[4]	Combustion-Infrared spectroscopy	Low	Low	Low	Low
			TSEM[5]	Extraction/ Gravimetric	Moderate	Low	Low	High
		Aromatic hydrocarbons (total and dissolved phases*)	VOCs/BTEX	Headspace/Purge & Trap + GC–MS	High	High	High	Low
			PACs, including alkylated PAHs and EPA 16 PAHs	Wet chemistry fractionation & GC–MS Fractionation & GC–MS	High	High	High	Low
Higher Resolution and Comprehensive Analyses	Larger analyte sets selected by experimental design needs	Chemical functional fractions	Aliphatic compounds to ~C40	Wet Chemistry fractionation + GC-FID	High	High	High	Low
		Aromatic hydrocarbons	VOCs/BTEX	Headspace /Purge & Tap GC–MS	High	High	High	Low
			PACs, including alkylated PAHs and EPA 16 PAHs	Fractionation & GC–MS	High	High	High	Low
			PIANO/PIONA[6]	GC-FID	High	High	Moderate	Low
New Directions/ Highest Resolution Analyses	New analyses under development	Additional chemical groups	Polar compounds	Fractionation & LC–HRMS	Very High	Very High	Very High	Very Low
			Oxidized products	Fractionation & GC–MS	High	High	High	Low
				Fractionation & GCxGC-TOF/GC-QTOF/LC–HRMS	Very High	Very High	Very High	Very Low
			Unresolved complex mixture	GCxGC-TOF/GC-QTOF/LC–HRMS	Very High	Very High	Very High	Very Low
		Dissolved oxygenated PACs and dissolved polar components (*Dissolved phase)	Oxygenated PACs, naphthenic acidic fraction components	Filtration/SPME extraction & LC–HRMS	Very high	Very high	Very high	Very Low

Notes:.
[1] CCME fractions are four TPH fractions based on carbon ranges regulated by Canadian Council of Ministers of the Environment.
[2] Total saturated hydrocarbons (TSH).
[3] Total aromatic hydrocarbons (TAH), all TPHF, TSH and TAH are measured by gas chromatography-flame ionization detector (GC-FID) using a modified EPA Method 8015B.
[4] Total organic carbon (TOC).
[5] Total solvent extracted material (TSEM).
[6] Paraffin-isoparaffin-aromatic-naphthene-olefin (PIANO) and Paraffin-isoparaffin-olefin-naphthene-aromatic (PIONA).
[*] Dissolved phase prepared by filtration or non-depletive SPME extraction.

chosen to characterize the exposure media must also be used to analyze the source oil.

Chemical characterization of exposure media used in toxicity testing (e.g., WAF, CEWAF) has long been recognized as an essential element for generating reliable information and for the correct interpretation of test results (Aurand and Coelho, 2005; National Research Council, 2005; Bejarano et al., 2014; Hodson et al., 2019; NASEM, 2020). While oil loading may be the recommended reporting metric for environmental hazard classification and labeling of chemical substances (OECD, 2001, 2019), this metric is inappropriate for the assessment of oil toxicity as it does not account for the bioavailability of hydrocarbons in the exposure media, resulting in erroneous interpretation of test results (Aurand and Coelho, 2005; National Research Council, 2005; Bejarano et al., 2014; Coelho et al., 2013; NASEM, 2020). Similarly, an understanding of the relative contributions of single hydrocarbons in the exposure media is also important (Di Toro et al., 2007; Bejarano et al., 2014; Redman and Parkerton, 2015), and thus quantification of individual compounds is preferred over simply reporting constituent summation e.g., total PACs (TPAC) or total PAHs (TPAH) or total petroleum hydrocarbon (TPH). A key challenge for using simple summations for reporting oil toxicity is that chemical composition is influenced by media preparation methods (e.g., oil type, weathering stage, mixing energy). Study designs and the methods used to prepare exposure media alter the relative composition of dissolved compounds and whole oil droplets in the exposure media, confounding test results and limiting across-study comparability. Summations, however, could prove useful for confirming the stability of test solutions via fluorometry (see section above) (Forth et al., 2017; Hodson et al., 2019) as well as to indirectly confirm the presence of oil droplets (i.e., saturated hydrocarbons [n-C9 to n-C40]). See below for a discussion of media droplet content (Section 2.1.3) (Parkerton et al., 2023a).

In Table 8, different types of compositional analyses are listed, organized from the minimum (simplest) to the most comprehensive (complex) to new directions for analytical development focused on identifying new types of analytes of interest.

2.1.2.1. Minimum – grouped analyte sets and semi-quantitative monitoring. When released into the aquatic environment, polar compounds in the oil as well as oxidized compounds produced by biodegradation and photo-oxidation can move from the oil phase into the water. Just as a minimum analytical dataset for the test oil was recommended, similarly a minimum analytical dataset for oil compounds extracted from the water phase is needed. As for the source oil, TPHF analysis that quantifies distributions of both aromatic and aliphatic compounds should be performed on samples collected at multiple time points throughout toxicity tests. This includes at least at the start and the end of the test, even if semi-quantitative methods are used to monitor experimental progress over time. See "Total Petroleum Hydrocarbons Fractionated" in SI – Analytical Methods for a description of the TPHF method. In Canada, CCME fractions, four total petroleum hydrocarbon fractions based on carbon ranges recommended by Canadian Council of Ministers of the Environment (CCME) (2016) may also be required depending on the design purposes of the toxicity tests.

Semi-quantitative methods, including fluorescence spectroscopy and ultraviolet spectrophotometry, are relatively low-resolution analyses that can be cost-effective and particularly useful when confirming the stability of test solutions and consistency of exposures. These methods take advantage of the optical properties of PACs in the oil. Specifically their ability to absorb ultraviolet-visible wavelength (UV–Vis) radiation and generate fluorescence emissions, provides a means to detect the presence and relative concentrations of oil in test solutions (Østgaard and Jensen, 1983; Wade et al., 2011). Fluorescence has been used to detect the presence of petroleum directly in the water column or in water samples during oil spills (Diercks et al., 2010; Wade et al., 2011). This technique can also be used to estimate oil in WAF and CEWAF (Wade et al., 2017) and can be correlated with more sophisticated GC–MS analyses of PACs. Several aquatic toxicity studies have taken advantage of the inexpensive and rapid features of these tools for the semi-quantitative estimation of TPH and total concentrations of 2–4 ring PAHs in exposure media (e.g., Singer et al., 1990; Adams et al., 2014) as well as for measurements of single hydrocarbon compounds (e.g., Knap et al., 2017). This technique is amenable to many fluorescent platforms when used with the detailed analyses of the test petroleum (Bera et al., 2019).

The primary limitation of using fluorescence spectroscopy is that these methods do not quantify speciated constituents, and therefore cannot resolve individual compounds present in the exposure media, resulting in limited information on the drivers of toxicity. While not recommended by the original CROSERF protocol, the use of semi-quantitative methods, together with the recommended TPHF method, could be used if no other options are possible.

2.1.2.2. Low resolution analyses. The analytes quantified using the GC methods listed for low resolution analyses are the basic datasets such as the list provided in Table 1A. However, they appear to be just as difficult to perform as the higher resolution tests, once the expanded methods have been setup. In contrast, total organic carbon (TOC) and total solvent extractable material (TSEM) methods do not identify analytes but report the total mass% content of organic carbon or extracted material, respectively in the water where the sensitivity is in the parts per million range. These tests do not have the limitation of compound sizes having to be < C40 like the GC techniques. However, careful selection of control samples is needed to identify possible contributions from biomass that could also be present in the water. In meso-scale studies, the measurement of TOC can track the presence of carbon in the water even as the concentrations of measured analytes have seemingly decreased to minimal levels, supporting research into new types of toxic compounds (Lara-Jacobo et al., 2021; Heshka et al., 2022).

2.1.2.3. Higher resolution and comprehensive analyses. In Table 8, continuing improvements of GC techniques to identify increasing numbers of analytes are illustrated upon comparing the analyte list in Table 1A to that in Table 1B. Advances in analytical chemistry techniques have also both reduced analyte detection limits and identified new analytes of concern. Enhanced data analyses allows inclusion of analytes whose concentrations are below detection limits (Shoari and Dubé, 2018). These improvements allow toxicologists greater flexibility in the choice of analytes of most relevance to their experimental objectives. Assistance for selection of target analytes can be found by using data from oil chemistry web-based databases such as the "Crude Oil and Petroleum Product Database" (Environment and Climate Change Canada, 2021) or in the oil database associated with ADIOS (NOAA, 2021) or from crude oil assay tables available on petroleum company websites.

2.1.2.4. Highest resolution analyses and new directions. Despite the general observation that oil toxicity correlates with its aromatic hydrocarbon contents, the correlation is not perfect. For example, discrepancies between the abundance of the earlier recommended sets of petroleum hydrocarbons (e.g., PAHs) and biological responses, suggest that in addition to PAHs quantified, there are likely unidentified compounds contributing to adverse biological responses (Hatlen et al., 2010; Incardona et al., 2013; Lara-Jacobo et al., 2021). Consequently, other types of organic compounds are involved and need to be identified and quantified to fully understand the toxicity response. New types of compounds require development of new types of analytical techniques. In the bottom section of Table 8, a list of potential types of analytes that are not currently quantified but are likely to be involved in toxicity responses are given. These include polar compounds that are either present in the source oil or are created during weathering in the water environment. For example, sunlight rapidly photo-oxidizes oil resulting in the formation of oxidized compounds that are more water-soluble (S.

M. King et al., 2014; Ray et al., 2014; Z. Yang et al., 2017) and so are more bioavailable than the parent compounds (see Alloy et al., 2023). Photo-oxidized oils have been shown to negatively impact algae, bacteria, and marine invertebrates and fish (Hatlen et al., 2010) more than the original oils, indicating that oxidized intermediates may contribute to toxicity. These intermediates usually contain more oxidized PACs than the parent oil (e.g., ketones and quinones) (Mallakin et al., 1999).

Oxidized compounds generated by photo-oxidation or biodegradation include organic acids that are also referred to as "naphthenic acid fraction compounds (NAFCs)" (see SI – Terminology). Characterization and identification of organic acids in exposure media and weathered oils can help elucidate their contribution to toxicity of spilled oil in the environment (Bartlett et al., 2017; Lara-Jacobo et al., 2021). Ultrahigh resolution mass spectrometry, such as Orbitrap MS (Orbitrap-MS), Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), and time of flight mass spectrometry (TOF-MS), either coupled with liquid chromatography or working alone, are suitable techniques for characterizing organic acids (Mohler et al., 2020). Studies have been conducted to determine their fate and behavior in both water and oil matrices (Headley et al., 2011; Huang et al., 2018; Yang et al., 2021; Heshka et al., 2022). Despite challenges for quantification of oxidized compounds, high resolution tools continue to be developed which will improve understanding of their chemical properties (see Alloy et al., 2023).

The aromatic compounds shown in Table 1B include only unsubstituted and low alkyl-substituted aromatic compounds. However, oils can contain all possible variations of types of substituted aromatic and hydroaromatic compounds, particularly as the carbon number increases. These compounds are not resolved by GC–MS techniques but are detected as the broad, unresolved complex mixture (UCM) peak in the chromatogram and include compounds in the range of C25 in size and larger (Gough et al., 1990). These larger compounds are more resistant to degradation processes and can become toxicologically important in weathered oil (e.g., Barron et al., 1999; Smith et al., 2001; Faksness et al., 2015). However, efforts are seldom made to characterize the toxicity of UCMs. One of the most advanced techniques is two dimensional GC (GC × GC) coupled with high resolution mass spectrometry, such as TOF-MS. This technique has produced high-resolution information of exact molecular constitution for the lower UCM (Weng et al., 2015).

GC × GC techniques can give higher resolution of aromatic compounds from C10 to C40 in size (McKenna et al., 2013). However, other approaches are needed to identify contributions from aromatics larger than C40 as well as from the more polar, heteratom-containing resins and asphaltenes. An alternate approach could be to design the toxicity experiment in a way where not all compounds contributing to toxicity need to be quantified by traditional methods. For example, toxicity has been shown to be inversely related to the octanol-water partition coefficient (K_{ow}) of the compound (McCarty and Mackay 1993; Verhaar et al., 1992; Swartz et al., 1995). Consequently, the UCM could be characterized by considering K_{ow} (Verhaar et al., 1992; McCarty and Mackay, 1993; French-McCay, 2002; McGrath et al., 2018). Quantifying fractions of the UCM by $\log_{10}(K_{ow})$ range increments by analyzing the exposure media using passive samplers and calculating the UCM contribution by difference from the speciated analytes contributions, would be an indirect method to assess the toxicity of the unresolved species.

Oil complexity makes the identification of causal toxic compounds, as well as compounds causing synergistic effects, difficult. Effects driven chemical fractionation (EDCF) is a common and efficient means for identifying the toxic components in a complex mixture (Bornstein et al., 2014). EDCF involves a series of stepwise physical and chemical separations to isolate groups of compounds with similar properties (e.g., polarity). After separation, each fraction may be utilized in toxicity testing, with the most toxic fraction(s) subjected to further separation and testing (Tian et al., 2021).

This approach is particularly useful for toxicity studies of oil that has been exposed to oxidation processes such as photo-oxidation and biodegradation. An EDCF approach has previously been used to demonstrate that naphthenic acids are responsible for most of the observed acute and chronic toxicity of dissolved organic fractions of oil sands process-affected waters (Morandi et al., 2015). This approach relied first on fractionation along a pH gradient (pH 2, 7, and 11), followed by separation of the nonionizable neutral substances from acids, and lastly by separating the isolated naphthenic acids from all other compounds by chromatography.

Effects-driven analysis also has great potential for identifying the significance of photo-modified products towards overall toxicity. This technique can be applied on whole oils/WAFs, and also on single PACs (e.g., Anthracene, Brack et al., 2003), where dissolved concentrations were irradiated by UV light (natural or artificial, see Alloy et al., 2023 for guidance) and then subjected to an effect-directed fractionation scheme with accompanying chemical and toxicological analyses. More applications of EDCF will help to fully explore the depth of information that can be discerned from this approach.

2.1.3. Oil droplets

To be able to quantify toxic effects levels based on bioassay tests that can be used in biological effects assessments in the environment, the chemical composition and partitioning of oil constituents in dissolved versus droplet phases of the exposure media need to be measured. Ideally, all compounds present in the exposure media contributing to the effects are quantified. However, as this is not yet possible, pseudo-components have been identified for modeling purposes where the concentrations of pseudo-components in the dissolved and droplet phases are determined (Hansen et al., 2019b) (see for examples, Tables 2 to 5). However, those tables also illustrate that pseudo-component definitions vary from model to model and by the level of detail used for the model application.

Another approach rather than using pseudo-components is to use modeling of the partitioning of soluble and semi-soluble compounds in the exposure media between the dissolved and particulate (droplet) phases (Parkerton et al., 2023a; Stubblefield et al., 2023). Dissolved concentrations for a system at equilibrium can be predicted based on solubility using Raoult's law. To facilitate such an analysis, the PETROTOX model (Redman et al., 2017) includes the required physical-chemical property library (Hilal et al., 2007) to estimate the dissolved oil pseudo-components exposures in a closed test system at steady state (equilibrium) (French-McCay et al., 2023). An example application of this method is in Hansen et al. (2019).

Oil droplets sizes may be measured in solution using a variety of instruments (e.g., LISST, Coulter counter, zeta-sizer, fluorescence microscopy) where the important metrics to report are the droplet size distribution (DSD), the volume mean droplet diameter (VMD, for a log-normal distribution, i.e., the geometric mean), and the droplet concentration (by mass). Extensive droplet size measurements of physically and chemically dispersed oil using different instruments show that most chemically dispersed oils yield a VMD of 10 μm (Nordtug et al., 2011) to 18 μm (Fingas and Kyle, 1995; Li et al., 2011). VMD decreases with increasing dispersant/oil ratios (DOR) and mixing energy (Li et al., 2011a), with physically dispersed oils often having a VMD $>$ 200 μm. Physical dispersion usually produces droplets with diameters ranging from 1 to $>$500 μm, depending on oil viscosity and mixing energy (Li et al., 2009, 2011). Droplets with sizes above 50–70 μm tend to coalesce and resurface, while droplets smaller than 1 μm are difficult to measure. The oil droplet size distribution obtained at the end of mixing can be used to estimate the required settling time needed for oil droplets to resurface to allow their removal from the exposure media (Parkerton et al., 2023a).

Dissolved-phase PACs are more toxicologically important than undissolved-phase PACs because only the dissolved phase is bioavailable. Most analytical methods currently combine the contributions from

these groups. However, the presence of oil droplets in the exposure media could result in the misinterpretation of toxicity test results. One approach to remove contributions from oil microdroplets is to filter the sample where PAC concentrations are measured before and after filtration. The amount of toxicity lost by removal of the droplets during the filtration process is then calculated using toxic unit calculations (see Parkerton et al., 2023b). Another approach that directly measures the dissolved fraction is solid-phase microextraction (BE-SPME) (passive sampling). BE-SPME allows the quantification of dissolved PACs based on their partitioning through membranes, thus serving as a surrogate for the target lipid (Letinski et al., 2014; Redman et al., 2018). GC-FID analyses of compounds collected by passive samplers could then be taken to represent bioavailable fractions that could facilitate interpretation of toxicity test results, and provide a potential improvement over current exposure metrics (Letinski et al., 2014; Redman and Parkerton, 2015). Concerns about fouling of SPME fibers are generally limited to solutions where there are significant concentrations of undissolved oil (e.g., high energy WAF [HEWAF]). This fouling is readily apparent as the results are highly variable and non-reproducible. Despite their increased use, standardization of test procedures using passive samplers is still needed (NASEM, 2020; Letinski et al., 2022).

2.2. Analytical reporting needs for toxicity testing

For the source and weathered oils, it is important to describe how the oils were handled, stored, and prepared. Providing simulated distillation data such as high temperature simulated distillation analyses of crude oils, as well as the TPHF and detailed hydrocarbon data chosen for the study will demonstrate that the compositions of the source oils were maintained throughout the toxicity test program. As well, toxicologists from different labs can verify that their oils are similar to each other which will assist during interpretation of toxicity results both within labs and between labs over time.

Detailed reporting of analytical chemistry results for the exposure media is essential to maximize the utility of toxicity tests, and facilitate repeatability (Bejarano et al., 2023). Regardless of the sample chemically characterized (e.g., fresh or weathered source oil, exposure media) all analytical results should provide sufficient details on the analytical methods, including reference to the guidance or methodology used, as well as any deviations from standard methods. It is essential that analytical chemistry efforts follow and report data quality objectives (i.e., precision, bias, sensitivity, completeness, and comparability), and quality assurance and quality controls (QA/QC). Reporting should include all relevant QA/QC undertaken during media sampling and analysis (e.g., method blanks, sample duplicates, reference materials, internal standards, recovery efficiency of spiked standard solutions, and specific storage preservation techniques). For each analyte or groups of analytes, limits of detection (LOD), quantification (LOQ) and reporting (LOR), along with their variance, should be reported. In the case of exposure media, the recommended minimum required analytes (Table 1B) should be reported. Some analytes may not be present in some test oils and thus, there is flexibility in the recommendations presented here. However, to increase access to analytical chemistry results by external users (e.g., modelers), all relevant raw chemistry data over the duration of the study should be reported (Bejarano et al., 2023).

3. Conclusions

Chemical analysis is a significant cost component of oil toxicity studies. Judicious selection of methods, type and number of samples will ensure that resources (financial, human, and experimental animals) are effectively used. The choice of analytical methods is dictated by the toxicity study objective and should meet minimum reporting elements to facilitate comparisons between studies and maximize data utility for other end users, such as biological effects modellers and spill response decision makers. Failure to appropriately characterize the source oil and especially the exposure media, greatly reduces the utility of the data, and may represent a significant waste of effort. In this paper we provide guidance to help in selection of analytical methods that are fit for purpose based on the study objective and will advance the links between oil toxicity data and oil spill effect models through compositional analysis. Key recommendations have been identified which will improve the comparability and utility of data:

- A minimum set of oil analyses are recommended for source oil and exposure media (Table 1B).
- Beyond the minimum analytes listed researchers should perform suitable simulated distillation analysis and Total Petroleum Hydrocarbons Fractionated (TPHF) analyses on the source oil to document its composition.
- Inclusion of the density and viscosity, ideally at three temperatures (e.g., 5, 15 and 25 °C), and oil pour point of the source oil will make the toxicity data highly useful to the spill response community.
- Source oil characterization needs to be repeated if the oil is weathered, photo-oxidized, biodegraded, or otherwise pretreated before WAF preparation.
- Additional analyses (Section 2.1.2.4) can be chosen based the experimental designs of the toxicity tests and provide valuable insight into drivers of toxicity especially in cases of photomodification or biodegradation.
- New analytical approaches and capabilities are needed to determine the toxicity of organic compounds found in the unresolved complex mixture detected by gas chromatography, as well as those containing heteroatoms such as sulfur and nitrogen, and after photo-oxidation or biodegradation processes, oxygen.

CRediT authorship contribution statement

Heather D. Dettman: Conceptualization, Writing – original draft, Writing – review & editing. **Terry L. Wade:** Writing – review & editing. **Deborah P. French-McCay:** Writing – original draft, Writing – review & editing. **Adriana C. Bejarano:** Writing – original draft, Writing – review & editing. **Bruce P. Hollebone:** Writing – original draft. **Liv-Guri Faksness:** Writing – original draft. **Fatemeh S. Mirnaghi:** Writing – original draft. **Zeyu Yang:** Writing – original draft, Writing – review & editing. **Jennifer Loughery:** Project administration. **Travers Pretorius:** Writing – review & editing. **Benjamin de Jourdan:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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