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RESEARCH ARTICLE

Determination of C_0–C_9 alkyl phenols in produced-water-exposed fish eggs using gas chromatography/tandem mass spectrometry

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Rationale: Produced water (PW) discharge from the oil and gas industry represents the largest intentional marine waste volume. Alkyl phenols (APs) are one of the main toxic component groups found in PW, with concentration of APs in discharged PW from the Norwegian Sector of the North Sea up to >16 mg/L. Several species of fish spawn in direct proximity to offshore production platforms and may be at risk of AP exposure. Therefore, a sensitive method to determine the potential for bioaccumulation of APs in fish eggs is needed.

Methods: Fish eggs were extracted using liquid–solid extraction followed by gel permeation chromatography cleanup. Analysis was performed by gas chromatography coupled to triple quadrupole mass spectrometry. Extraction and analytical conditions were optimized for analysis of phenol and 30 APs (C_1–C_9) with different degrees of branching in the alkyl chain. The method was verified and applied to analyze the body residue of APs in PW-exposed marine fish (Atlantic cod, *Gadus morhua*) eggs.

Results: A comprehensive and sensitive method for the determination of C_0–C_9 APs was developed. Detection limits were in the range 0.03–8 ng. Apart from a few compounds with poor recovery, the method generally provided reliable results with good precision (<15%).

Conclusions: We demonstrate the successful application of an optimized extraction method for APs in fish eggs and show first results of AP accumulation in cod embryos exposed to PW in the laboratory.

1 | INTRODUCTION

Discharge of produced water (PW) from the oil and gas industry supplies the marine environment with the largest waste stream worldwide and is estimated to represent on average 1700 tons of crude oil/year.[1] Even though it is known that PW is toxic to marine organisms, there is limited knowledge of which compounds in PW elicit the observed toxic effects, and how these compounds work in combination.[1]

Alkyl phenols (APs) are one of the component groups typically found in relatively large concentrations in PW.[2] One of their primary sources is degradation of alkylbenzenes present in crude oil.[3] In addition, some offshore production facilities use alkylphenol ethoxylates (APEs) as either detergents or surfactants to enable the pumping of very viscous and/or waxy crude oils.[4,5] APEs may degrade into long-chained APs such as octyl- and nonylphenols. The concentration of APs in discharged PW from the Norwegian Sector of

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the North Sea ranges from 0.36 to 16.8 mg/L and the PW consists predominantly of the less alkylated phenols (C_1–C_3).[6]

Earlier studies have investigated the accumulation and effects of PW-derived APs in fish with habitats close to oil production platforms.[6–8] APs exhibit estrogenic properties and have been proven to disrupt the natural endocrine hormone system in both juvenile and adult fish.[9,10] Recent studies indicate that some APs can disturb the reproductive capability and delay maturation in juvenile fish.[6,8] To the authors' knowledge, no targeted studies have investigated the effects of APs on early life stages (ELS) of fish, but recent studies have proved that exposure to low concentrations of PW causes developmental defects in fish ELS, such as changes in the cranial and spine morphology, cardiac failure and DNA damage.[7,11] While polycyclic aromatic hydrocarbons (PAHs) present in PW will contribute to some of the observed toxicity in fish ELS, recent studies have emphasized that PAHs do not explain the full picture of toxicity from crude oil and PW.[1,12–14]

Although PW dilutes rapidly at a distance from the point-of-release, it has been observed that Atlantic cod (*Gadus morhua*) and lumpsucker (*Cyclopterus lumpus*) spawn in close proximity to North Sea oil production platforms and thus are at potential risk of effects from AP exposure through PW.[15] A recent field survey demonstrated levels of C_1–C_4 APs up to 350 ng/L at stations in the vicinity of an operational North Sea platform.[16] Rather than use of external concentrations (water, sediments), a more realistic approach for estimating the risk of operational discharge is to investigate effects based on actual accumulated body burden over time. Internal body concentrations can be used to predict a critical body residue of PW compounds, rather than using external water or sediment concentrations as toxicity threshold values. By observing accumulation in relation to exposure it is possible to predict a more appropriate dose-effect relationship that will help promote more precise regulations and management of operational discharges to the marine environment.[17] A more comprehensive understanding of PW compound accumulation in biota is necessary to better evaluate the long-term ecological effects of discharges from oil and gas production.

There is a need for highly sensitive analytical methods to evaluate the fate and effect of PW compounds in marine organisms.[18] Gas chromatography coupled with mass spectrometry (GC/MS) has been widely used to determine the concentration of polycyclic aromatic hydrocarbons (PAHs) and APs in PW and environmental samples. Increasing demand for detection of these contaminants at trace levels combined with challenging sample matrices, such as small biotic samples, has resulted in the use of more sophisticated detection methods such as tandem mass spectrometry (MS/MS).[18] Methods for extraction and analysis of PAHs accumulating in fish embryos following crude oil exposure have previously been reported.[18,19] In the current study, we demonstrate a methodology for extraction and analysis of APs from PW-exposed fish eggs, using liquid–solid extraction followed by gel permeation chromatography (GPC) cleanup and GC/MS/MS analysis. The developed method is applied to demonstrate the potential for accumulation of APs and PAHs in cod eggs exposed to PW in the laboratory.

2 | EXPERIMENTAL

2.1 | Chemicals and materials

Certified standard solutions of alkyl phenols, deuterated phenols and deuterated PAHs were purchased from Chiron AS (Trondheim, Norway). Spike and calibration standards were prepared by dilution in dichloromethane (DCM). A PW sample (~200 L) was taken at the point of release of a North Sea offshore facility during a period of normal operation, immediately acidified (HCl, pH ~1.6) and shipped to the onshore laboratory, where it was kept cold (5°C) and in the dark until extraction (<14 days). A total of 195 L of the sample was extracted using DCM, and the extract combined and reduced by gentle evaporation to 250 mL for use in exposure experiments. The extract was kept frozen (−20°C).

2.2 | PW characterization

For quantification of APs, a 0.8 L sub-sample of the PW was extracted using DCM with addition of surrogate internal standards (2508 ng naphthalene-*d*8, 500 ng phenanthrene-*d*10, 486 ng chrysene-*d*12, 508 ng perylene-*d*12, 25,334 ng phenol-*d6*, 1042 ng *p*-cresol-*d*8 and 1374 ng 4-*n*-propylphenol-*d*12) to account for target analyte losses of the non-deuterated analogues during the extraction step. The extract was dried (anhydrous sodium sulfate) and the volume of DCM adjusted to ~1 mL by gentle evaporation. Prior to analysis, a recovery internal standard was added (1 μg fluorene-*d*10). For GC/MS analysis of APs in the PW extract, a model 7890B gas chromatograph coupled with a model 5977A quadrupole mass spectrometer (both from Agilent Technologies, Santa Clara, CA, USA) was used. The GC-column was a HP-5MS UI (Agilent Technologies; 60 m × 0.25 mm × 0.25 μm) and the carrier gas was helium at a constant flow rate of 1 mL/min. Samples (1 μL) were injected at 325°C by pulsed splitless injection. The oven was programmed to 40°C for 1 min (1 min hold), ramped to 220°C at 6°C/min and then to 325°C at 4°C/min (15 min hold). The transfer line temperature was 300°C, the ion source temperature was 300°C and the quadrupole temperature was 165°C. The electron ionization (EI) source was operated at 70 eV. Analysis was performed in selected ion monitoring (SIM) mode using the molecular ion and the most abundant fragment ion for qualification and quantification, respectively.[1] Quantification of target compounds was performed using average response factors (RFs).

2.3 | Extraction of fish egg samples

In lieu of a standard reference material for APs in fish eggs, a spiked laboratory reference was made to validate the method. For this purpose, three cod and one lumpsucker egg samples (respectively ~25 mg and 200 mg wet weight) were spiked with increasing levels of APs (1–100 ng/sample). To study the effect of the matrix on

extraction and analysis results, a set of spiked samples without matrix was included.

PW-exposed cod eggs were collected after exposure to PW whole effluent (10%, 33%, 100%, dilutions made in sterile filtered seawater) for a duration of 4 days (3–7 days post-fertilization) at 8.5°C. At the end of exposure, approximately 25 eggs from each beaker were pooled for body burden analysis of PAHs and APs and 25 eggs were pooled for body burden analysis of metals. Three replicates at each exposure concentration was included for analysis.

Extractions of clean, spiked and exposed egg samples were performed as described in Sørensen et al.[19] Briefly, samples were accurately weighed and transferred to glass vials. After addition of *n*-hexane/DCM (1:1 v/v, 4 mL) and surrogate standards (25.08 ng naphthalene-*d*8, 5.00 ng phenanthrene-*d*10, 4.86 ng chrysene-*d*12, 5.08 ng perylene-*d*12, 2533.4 ng phenol-*d*6, 104.2 ng *p*-cresol-*d*8, 137.4 ng 4-*n*-propylphenol-*d*12), the samples were homogenized using a disperser (IKA 10 basic ULTRA-TURRAX®; IKA-Werke, Staufen, Germany), sodium sulfate was added followed by a brief vortex and centrifuged (2000 rpm (720 *g*), 2 min). The supernatant was collected and the extraction repeated two additional times. Extracts were subjected to cleanup by gel permeation chromatography (GPC). Samples (500 μL) were injected with DCM as a mobile phase (5 mL/min) and separated using an Envirogel column (19 × 300 mm, 15 μm; Waters Milford. MA, USA). Chromatograms were monitored at 210, 254 and 280 nm UV. After initial optimization, analyte fractions were collected from 10.1–14.5 min with pre-added *n*-hexane in the collection vials as a keeper. The sample volume was adjusted to 0.4 mL by solvent evaporation (40°C under a gentle flow of N_2) and a recovery internal standard (100 ng fluorene-*d*10) was added prior to analysis.

2.4 | GC/MS/MS analysis

Fish egg extracts were analyzed using a model 7890 gas chromatograph coupled with a model 7010B triple quadrupole mass spectrometer fitted with an EI source (Agilent Technologies). Two HP-5MS UI GC-columns (30 m × 0.25 mm × 0.25 μm; Agilent J&W) were coupled in series through a purged ultimate union (PUU). The carrier gas was high-purity helium at a constant flow rate of 1.2 mL/min. Samples (1 μL) were injected at 310°C in splitless mode. The oven temperature was kept at 40°C for 1.5 min, then ramped to 110°C at 40°C/min and finally to 325°C at 20°C/min. The temperature was then held at 330°C for 5 min, while the first column was backflushed. The transfer line temperature was 300°C, the ion source temperature was 230°C and the quadrupole temperatures were each 150°C. The EI source was operated at 70 eV. N_2 was used as the MS/MS collision gas at a flow rate of 1.5 mL/min and helium was used as a quench gas at a flow rate of 2.25 mL/min. APs were quantified by quadratic regression after normalizing to fluorene-*d*10 applied as an internal standard.

For the purpose of GC/MS/MS method optimization, standards of 0.1–10 μg/mL APs were prepared. Precursor ions were selected based on the most intense peaks in the EI mass spectrum. The multiple reaction monitoring (MRM) transitions and the collision energy (CE, 2–40 eV) were optimized.

To determine the overall method (extraction and analytical) performance, non-exposed cod and lumpsucker eggs were spiked with a standard solution of APs and PAHs (1 ng, 10 ng and 100 ng of each analyte). For comparison, spiked samples without matrix were also extracted. For determination of the limits of detection and quantification (LODs/LOQs) of the method, six laboratory blank samples were extracted and limits determined as LOD = average + 3 * standard deviation, LOQ = 3*LOD. Where no signal was obtained in the blank samples, the LOD was set to 1/3 of the lowest calibration level (0.01 ng).

3 | RESULTS AND DISCUSSION

3.1 | Method performance

The optimized MRM transitions and collision energies for each target AP are shown in Table 1. The most abundant transition was chosen for quantification. Where possible, an MRM transition based on fragmentation from the base peak in the EI mass spectrum was chosen as a qualifier (where the relative abundance was a minimum of 20% of the quantification MRM).

Extraction of APs from biotic tissue using DCM has previously been demonstrated.[20] For maximum recovery of PAHs, herein we used a combination of DCM and *n*-hexane to suit the combined extraction of APs and PAHs.[19] The suitability of the GPC cleanup for PAHs and alkyl PAHs has previously been demonstrated.[14] The most challenging compounds to recover were higher mass, long-chain and branched APs, which had retention times (RTs) close to the desired cut-off (Table S1, supporting information). To retain these compounds while minimizing matrix compounds (Figures S1 and S2, supporting information), the cut-off for fraction collection was set at 10.1 min.

An overview of the method validation parameters at the highest level of spike (100 ng) is given in Table 2. All the method validation parameters are given in Tables S2, S3 and S4 (supporting information). The LODs for the selected APs used in this study were in the range 0.03–8 ng, which closely match those previously reported for APs.[20] The high LODs and LOQs for some compounds (e.g. phenol) can be explained by the compound's high volatility and general presence as a laboratory contaminant. Other APs with high LODs (such as nonylphenol) are compounds typically found in plastics, which are ubiquitously present in the laboratory.[20] Three analyte spike levels were tested for all compounds (1, 10, 100 ng). Approximately half of the APs had LODs in the range 1–10 ng, while all other compounds were theoretically detectable at all spike levels. One AP (2-methyl-4-*tert*-octylphenol) was not recovered using the described methodology (recovery <10% at all spike levels). This was the case for samples both with and without matrix. At the two lowest spike levels, most APs were poorly recovered (<50%), while at the highest spike level (100 ng), 2/3 of the compounds were recovered satisfactorily (>50%) and only three compounds were recovered below 25%:

TABLE 1 Optimized GC/MS/MS method for alkyl phenols

Analyte	MW (g/mol)	RT (min)	Quantifier MRM (*m/z*) (CE eV)	Qualifier MRM (*m/z*) (CE eV)
Phenol	94	8.1	94 → 66 (10)	94 → 65 (25)
2-Methylphenol	108	9.1	107 → 77 (25)	108 → 77 (25)
4-Methylphenol	108	9.3	107 → 77 (25)	108 → 77 (25)
4-Ethylphenol	122	10.5	107 → 77 (20)	122 → 77 (30)
2,4-Dimethylphenol	122	10.8	107 → 77 (25)	122 → 107 (25)
3,5-Dimethylphenol	122	10.8	107 → 77 (25)	122 → 107 (25)
2,4,6-Trimethylphenol	136	11.6	121 → 77 (25)	136 → 77 (25)
2,3,5-Trimethylphenol	136	11.8	136 → 77 (30)	121 → 77 (25)
4-*n*-Propylphenol	136	12.8	136 → 77 (30)	136 → 121 (5)
4-*tert*-Butylphenol	150	13.1	135 → 77 (25)	150 → 107 (25)
4-Isopropyl-3-methylphenol	150	13.9	135 → 91 (15)	150 → 135 (5)
4-*n*-Butylphenol	150	14.3	107 → 77 (25)	150 → 107 (25)
2-*tert*-Butyl-4-methylphenol	164	14.2	149 → 121 (5)	164 → 149 (5)
4-*tert*-Butyl-2-methylphenol	164	14.4	149 → 121 (5)	164 → 149 (5)
4-*n*-Pentylphenol	164	16.3	107 → 77 (25)	164 → 107 (25)
2,6-Diisopropylphenol	178	14.4	163 → 91 (30)	178 → 163 (5)
2,5-Diisopropylphenol	178	15.0	178 → 163 (10)	163 → 91 (5)
6-*tert*-Butyl-2,4-dimethylphenol	178	15.6	163 → 135 (10)	178 → 163 (10)
2-*tert*-Butyl-4-ethylphenol	178	16.1	178 → 163 (10)	163 → 121 (10)
4-(1-Ethyl-1-methylpropyl)-2-methylphenol	178	18.2	163 → 121 (10)	163 → 77 (35)
4-*n*-Hexylphenol	178	18.3	107 → 77 (10)	178 → 107 (10)
2,6-Dimethyl-4-(1,1-dimethylpropyl)phenol	192	17.3	163 → 135 (5)	192 → 163 (5)
4-*n*-Heptylphenol	192	20.2	107 → 77 (15)	107 → 51 (35)
2,6-Di-*tert*-butylphenol	206	16.1	206 → 191 (10)	191 → 163 (10)
2,4-Di-*sec*-butylphenol	206	18.1	206 → 177 (5)	177 → 57 (5)
4-*tert*-Octylphenol	206	19.3	135 → 107 (10)	135 → 77 (35)
4-*n*-Octylphenol	206	22.1	107 → 77 (20)	206 → 107 (25)
2,6-Di-*tert*-butyl-4-methylphenol	220	17.3	220 → 205 (10)	205 → 57 (10)
4,6-Di-*tert*-butyl-2-methylphenol	220	17.9	205 → 57 (10)	220 → 205 (10)
2-Methyl-4-*tert*-octylphenol	220	20.1	149 → 121 (15)	149 → 77 (30)
4-*n*-Nonylphenol	220	23.9	107 → 77 (20)	220 → 107 (20)
Phenol-*d6*	100	8.0	99 → 71 (40)	71 → 60 (30)
p-Cresol-*d8*	116	9.3	115 → 113 (45)	113 → 81 (45)
4-*n*-Propylphenol-*d12*	148	12.3	113 → 81 (45)	147 → 113 (45)

2,5-diisopropylphenol, 2,6-di-*tert*-butyl-4-methylphenol and 4,6-di-*tert*-butyl-2-methylphenol. Lower recoveries were typically observed for more alkylated APs with highly branched side groups, while lower mass (C_0–C_5) and straighter chain APs in general were better recovered. Better recoveries for para- and meta-substituted and less branched APs have previously been described by Meier et al,[20] which support the findings in this study. Some of the lower molecular weight APs on the other hand gave a higher than expected recovery (150–350%). The non-optimal recovery could partly be accounted for by subtraction of laboratory blank values and mainly by use of recovery correction to a surrogate internal standard spiked before sample extraction. Calculated recoveries of spiked surrogates, phenol-*d6*, *p*-cresol-*d8*, 4-*n*-nonylphenol-*d12*, naphthalene-*d8* and phenanthrene-*d10*, were used for this purpose. The standard with the most similar recovery from spiked samples was selected and applied to the individual analytes (Table S5, supporting information). In some cases, a combination of volatility, polarity and branching had an influence on the observed recovery. Due to the limited selection of surrogate standards, for a few analytes, the recovery was estimated as the average recovery of two of the surrogates. Only recovery-corrected values are discussed further. The precision (relative standard deviation (RSD)) was acceptable (<25%) for most recovered analytes at the

TABLE 2 Method optimization parameters, results from 100 ng spike of cod eggs. (*results from 10 ng spike)

Analyte	LOD (ng)	LOQ (ng)	Precision (%RSD)	Recovery (%)	Matrix effects (%)	Bias (% deviation)
Phenol	7.5	22	8*	312* ± 77	74* ± 6	51* ± 12
2-Methylphenol	0.03	0.09	7	343 ± 39	90 ± 6	−7 ± 6
4-Methylphenol	0.64	1.9	6	269 ± 20	91 ± 6	−29 ± 5
4-Ethylphenol	0.03	0.09	14	132 ± 15	79 ± 11	−29 ± 10
2,4-Dimethylphenol	0.19	0.56	6	145 ± 6	93 ± 6	−22 ± 5
3,5-Dimethylphenol	0.23	0.69	7	190 ± 20	99 ± 7	1 ± 7
4-*n*-Propylphenol	0.95	2.8	15	80 ± 6	102 ± 15	6 ± 16
2,4,6-Trimethylphenol	0.60	1.8	2	134 ± 4	93 ± 1	2 ± 2
2,3,5-Trimethylphenol	0.96	2.9	15	80 ± 6	102 ± 15	6 ± 16
4-*n*-Butylphenol	2.0	6.1	23	94 ± 14	110 ± 25	25 ± 29
4-*tert*-Butylphenol	1.4	4.2	12	103 ± 4	105 ± 12	32 ± 15
4-Isopropyl-3-methylphenol	0.15	0.45	9	91 ± 3	109 ± 10	20 ± 11
2-*tert*-Butyl-4-methylphenol	1.0	3.1	21	29 ± 5	53 ± 11	−30 ± 14
4-*n*-Pentylphenol	1.1	3.3	5	146 ± 7	101 ± 5	11 ± 6
4-*tert*-Butyl-2-Methylphenol	1.0	3.1	25	48 ± 10	75 ± 19	−18 ± 21
4-*n*-Hexylphenol	2.5	7.6	4	119 ± 5	105 ± 4	−9 ± 4
2,5-Diisopropylphenol	0.51	1.5	26	15 ± 3	37 ± 9	−65 ± 9
2,6-Diisopropylphenol	0.97	2.9	29	44 ± 11	87 ± 25	−42 ± 17
2-*tert*-Butyl-4-ethylphenol	1.1	3.2	9	57 ± 2	93 ± 8	−3 ± 9
6-*tert*-Butyl-2,4-dimethylphenol	0.57	1.7	3	52 ± 2	109 ± 3	−11 ± 3
4-*n*-Heptylphenol	1.0	3.0	9	110 ± 1	124 ± 11	45 ± 13
2,6-Dimethyl-4-(1,1-dimethylpropyl)phenol	2.1	6.2	43	28 ± 12	55 ± 24	−32 ± 29
4-(1-Ethyl-1-methylpropyl)-2-methylphenol	1.0	3.1	13	53 ± 5	100 ± 13	−9 ± 12
4-*n*-Octylphenol	0.14	0.41	5	145 ± 6	117 ± 6	11 ± 5
4-*tert*-Octylphenol	0.93	2.9	11	86 ± 2	121 ± 13	14 ± 12
2,4-Di-*sec*-butylphenol	0.15	0.45	15	43 ± 4	100 ± 15	−27 ± 11
2,6-Di-*tert*-butylphenol	2.0	6.1	7	27 ± 2	53 ± 3	−36 ± 4
4-*n*-Nonylphenol	1.9	5.7	5	144 ± 7	124 ± 6	10 ± 5
2-Methyl-4-*tert*-octylphenol	0.39	1.2	111	0 ± 0	12 ± 13	−99 ± 1
2,6-Di-*tert*-butyl-4-methylphenol	0.15	0.45	76	8 ± 7	31 ± 23	−80 ± 15
4,6-Di-*tert*-butyl-2-methylphenol	0.43	1.3	48	14 ± 7	39 ± 19	−65 ± 17

highest spike level (100 ng). Matrix effects were low for smaller APs, straight-chain APs and all PAHs. Severe matrix effects (<75%) were observed mainly for highly branched APs. Furthermore, more severe matrix effects were observed for lumpsucker than for cod eggs, probably due to the higher content of lipids that may not have been sufficiently removed using the GPC cleanup. Overall, for cod eggs, the method provided reliable results given the low concentrations. Only five APs had a measurement bias of >50% deviation.

3.2 | Determination of AP body residue in PW-exposed cod eggs

The cod egg sample size was generally in the range 20–40 mg. Detection limits of APs in these samples would thus be 1–250 μg/kg. Apart from phenol, that could not be measured in the eggs due to the relatively high LOD for this compound, all APs that were present in exposures in levels above those in the seawater control samples (Figure 1) were detectable in the cod eggs. These were mainly the C_1–C_4 APs. A few other compounds with longer chain length substitution were detected in samples, but the presence of these also in control samples indicates that their main source was contamination of samples during exposure or storage. The relative accumulated body burden (C_{BB}/C_W) was compared with the octanol–water partition coefficient (K_{OW}) of the corresponding compounds, and in general a good correspondence was observed (Figure 2). This suggests that, while C_4 APs constituted only a minor fraction of the APs in PW, they accumulated in equal amounts to C_2 and C_3 APs. While the potential for bioaccumulation and toxicity

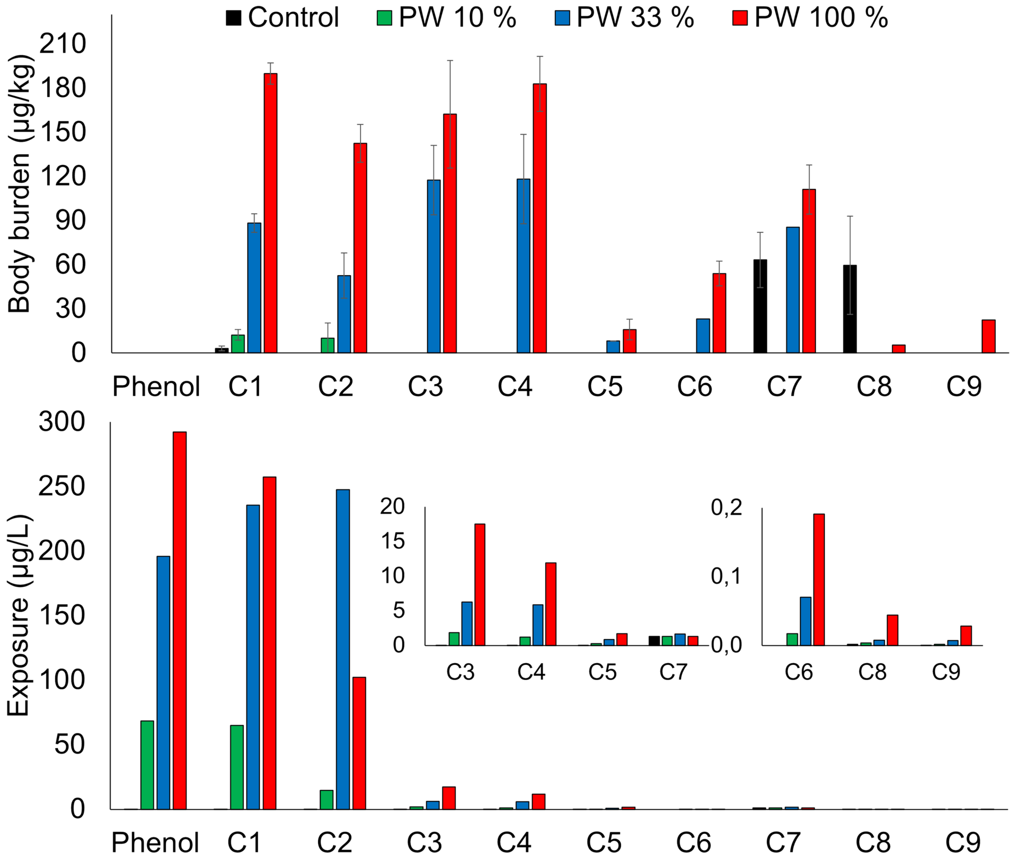

FIGURE 1 Alkyl phenols accumulating in PW-exposed cod eggs (μg/kg) compared with exposure concentrations (μg/L) [Color figure can be viewed at wileyonlinelibrary.com]

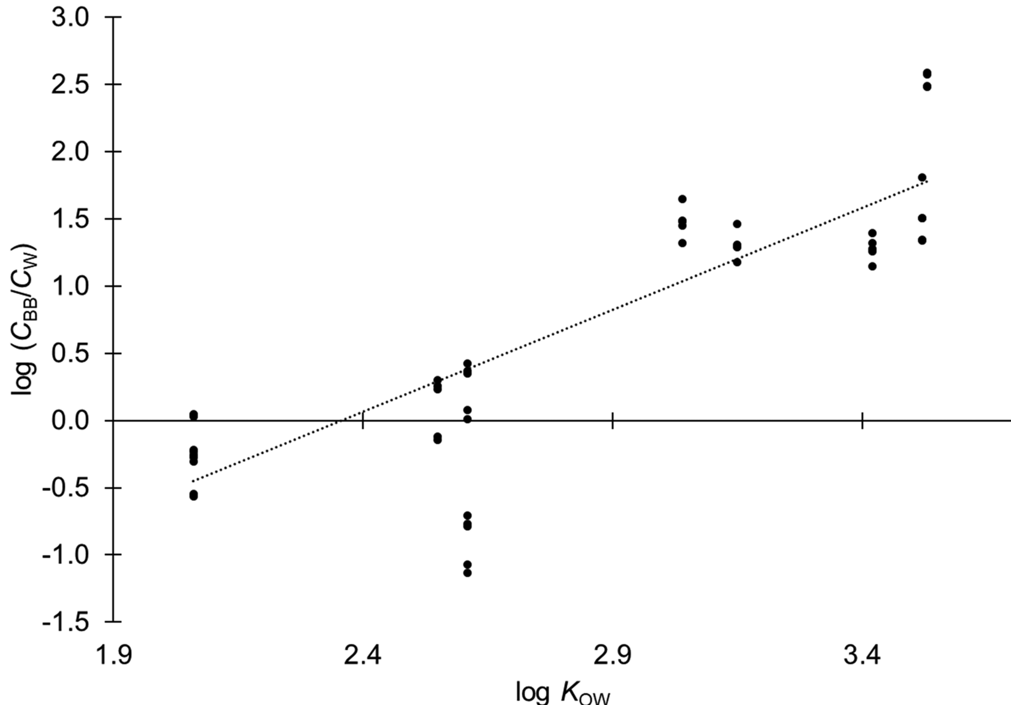

FIGURE 2 Relationship between AP log K_{OW} and accumulation in cod eggs

of larger (C_8 and C_9) APs is well established,[21] less work has been done to characterize the effects of smaller APs in the marine environment. C_4–C_7 APs from PW have been demonstrated to cause effects on cellular redox status, elevated CYP1A and CYP3A expression, and negative reproductive fitness effects in cod.[6,10,22] C_3–C_4 APs have also proven acutely toxic to several marine species, including fish, at concentrations similar to or below what is found in PW effluents (1 μg-12 mg/L).[23] While most APs are expected to biodegrade rapidly in seawater, highly branched APs (e.g. C_4 AP 4-*tert*-butylphenol) have been demonstrated to be persistent – with longer half-lives than the naphthalenes and 3–4-ring PAHs typically expected to dominate PW toxicity.[24] Future work should focus on elucidating the combined effects of several PW compound groups on their toxicity towards fish ELS.

4 | CONCLUSIONS

In the current study, application of GC/MS/MS MRM analysis of a wide range of APs was demonstrated. Biota sample preparation using solid–liquid extraction followed by gel permeation chromatography allows co-extraction of APs and hydrophobic contaminants (e.g. PAHs), which is relevant for instance for PW effect studies and environmental monitoring. AP detection limits were in the same range as those obtained using previous, more time-consuming, methods which are suitable for APs only. The potential for bioaccumulation of even low molecular weight APs was demonstrated, which needs to be considered in future PW risk management processes.

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REFERENCES

1. Sørensen L, McCormack P, Altin D, et al. Establishing a link between composition and toxicity of offshore produced waters using comprehensive analysis techniques – A way forward for discharge monitoring? *Sci Total Environ*. 2019;694:133682.
2. Strømgren T, Sørstrøm SE, Schou L, et al. Acute toxic effects of produced water in relation to chemical composition and dispersion. *Mar Environ Res*. 1995;40(2):147-169.
3. Lofthus S, Almås IK, Evans P, Pelz O, Brakstad OG. Biotransformation of potentially persistent alkylphenols in natural seawater. *Chemosphere*. 2016;156:191-194.
4. Neff J, Lee K, DeBlois EM. Produced water: Overview of composition, fates, and effects. In: Lee K, Neff J, eds. *Produced Water: Environmental Risks and Advances in Mitigation Technologies*. New York, NY: Springer; 2011:3-54.
5. Henderson SB, Grigson SJW, Johnson P, Roddie BD. Potential impact of production chemicals on the toxicity of produced water discharges from North Sea oil platforms. *Mar Pollut Bull*. 1999; 38(12):1141-1151.
6. Meier S, Andersen TE, Norberg B, et al. Effects of alkylphenols on the reproductive system of Atlantic cod (*Gadus morhua*). *Aquat Toxicol*. 2007;81(2):207-218.
7. Holth TF, Thorsen A, Olsvik PA, Hylland K. Long-term exposure of Atlantic cod (*Gadus morhua*) to components of produced water: Condition, gonad maturation, and gene expression. *Can J Fish Aquat Sci*. 2010;67(10):1685-1698.
8. Hasselberg L, Meier S, Svardal A. Effects of alkylphenols on redox status in first spawning Atlantic cod (*Gadus morhua*). *Aquat Toxicol*. 2004;69(1):95-105.
9. Meier S, Morton HC, Andersson E, et al. Low-dose exposure to alkylphenols adversely affects the sexual development of Atlantic cod (*Gadus morhua*): Acceleration of the onset of puberty and delayed seasonal gonad development in mature female cod. *Aquat Toxicol*. 2011;105(1):136-150.
10. Meier S, Morton CH, Nyhammer G, et al. Development of Atlantic cod (*Gadus morhua*) exposed to produced water during early life stages: Effects on embryos, larvae, and juvenile fish. *Mar Environ Res*. 2010;70(5):383-394.
11. Hansen BH, Sørensen L, Størseth TR, et al. Embryonic exposure to produced water can cause cardiac toxicity and deformations in Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) larvae. *Mar Environ Res*. 2019;148:81-86.
12. Hansen BH, Farkas J, Nordtug T, Altin D, Brakstad OG. Does microbial biodegradation of water-soluble components of oil reduce the toxicity to early life stages of fish? *Environ Sci Technol*. 2018; 52(7):4358-4366.
13. Meador JP, Nahrgang J. Characterizing crude oil toxicity to early-life stage fish based on a complex mixture: Are we making unsupported assumptions? *Environ Sci Technol*. 2019;53(19):11080-11092.
14. Sørensen L, Hansen BH, Farkas J, et al. Accumulation and toxicity of monoaromatic petroleum hydrocarbons in early life stages of cod and haddock. *Environ Pollut*. 2019;251:212-220.
15. Todd VLG, Lavallin EW, Macreadie PI. Quantitative analysis of fish and invertebrate assemblage dynamics in association with a North Sea oil and gas installation complex. *Mar Environ Res*. 2018;142: 69-79.
16. Harman C, Thomas KV, Tollefsen KE, Meier S, Bøyum O, Grung M. Monitoring the freely dissolved concentrations of polycyclic aromatic hydrocarbons (PAH) and alkylphenols (AP) around a Norwegian oil platform by holistic passive sampling. *Mar Pollut Bull*. 2009;58(11): 1671-1679.
17. Baussant T, Sanni S, Skadsheim A, Jonsson G, Børseth JF, Gaudebert B. Bioaccumulation of polycyclic aromatic compounds: 2. Modeling bioaccumulation in marine organisms chronically exposed to dispersed oil. *Environ Toxicol Chem*. 2001;20(6):1185-1195.
18. Sørensen L, Meier S, Mjøs SA. Application of gas chromatography/tandem mass spectrometry to determine a wide range of petrogenic alkylated polycyclic aromatic hydrocarbons in biotic samples. *Rapid Commun Mass Spectrom*. 2016;30(18):2052-2058.
19. Sørensen L, Silva MS, Booth AM, Meier S. Optimization and comparison of miniaturized extraction techniques for PAHs from crude oil exposed Atlantic cod and haddock eggs. *Anal Bioanal Chem*. 2016;408(4):1023-1032.
20. Meier S, Klungsøyr J, Boitsov S, Eide T, Svardal A. Gas chromatography–mass spectrometry analysis of alkylphenols in cod (*Gadus morhua*) tissues as pentafluorobenzoate derivatives. *J Chromatogr A*. 2005;1062(2):255-268.

21. Servos MR. Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. *Water Qual Res J*. 1999;34(1):123-178.
22. Hasselberg L, Meier S, Svardal A, Hegelund T, Celander MC. Effects of alkylphenols on CYP1A and CYP3A expression in first spawning Atlantic cod (*Gadus morhua*). *Aquat Toxicol*. 2004;67(4): 303-313.
23. Choi K, Sweet LI, Meier PG, Kim P-G. Aquatic toxicity of four alkylphenols (3-*tert*-butylphenol, 2-isopropylphenol, 3-isopropylphenol, and 4-isopropylphenol) and their binary mixtures to microbes, invertebrates, and fish. *Environ Toxicol*. 2004;19(1):45-50.
24. Lofthus S, Almås IK, Evans P, Pelz O, Brakstad OG. Biodegradation in seawater of PAH and alkylphenols from produced water of a North Sea platform. *Chemosphere*. 2018;206:465-473.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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