



Comparison of artificially weathered Macondo oil with field samples and evidence that weathering does not increase environmental acute toxicity

Liv-Guri Faksness^{a,*}, Dag Altin^b, Trond R. Størseth^a, Trond Nordtug^a, Bjørn Henrik Hansen^a

^a SINTEF Ocean AS, Environment and New Resources, NO-7465, Trondheim, Norway

^b BioTriX, NO-7022, Trondheim, Norway

ARTICLE INFO

Keywords:

Petroleum hydrocarbons
Toxicity
Bioavailability
Water accommodated fractions
Weathered oil
Deepwater horizon
Macondo

ABSTRACT

Macondo source oils and artificially weathered oil residues from 150 °C+ to 300 °C+, including artificially photo oxidized oils, were prepared and used for generating low energy water accommodated fractions (LE-WAFs) in order to assess the impact of oil weathering on WAF chemistry composition and toxicity to marine organisms. Two pelagic species representing primary producers (the marine algae *Skeletonema pseudocostatum*) and invertebrates (the marine copepod *Acartia tonsa*) were tested. Obtained acute toxicity levels, expressed as EC/LC50 values, were in the same range or above the obtained maximum WAF concentrations for WAFs from most weathering degrees. Based on % WAF dilutions, reduced toxicity was determined as a function of weathering. The chemical compositions of all WAFs were compared to compositions obtained from water samples reported in the GRIIDC database using multivariate analysis, indicating that WAFs of photo oxidized and two field weathered oils resembled the field data the most.

1. Introduction

Assessing the environmental effects of accidental releases of crude oil to the marine environment is a complex process. The crude oil is compositionally complex and reservoir-specific, and the influence of environmental factors on the fate and weathering of the released oil adds to the complexity. As oil weathers, the exposure scenarios for marine organisms become highly dynamic. Many components in crude oils are considered toxic and their environmental effects dependent on environmental concentration as well as on their bioavailability. Dissolved components are considered more bioavailable and more acutely toxic than components residing in dispersed oil droplets (Hansen et al., 2009, 2018b). Oil exposure can cause a variety of adverse effects in marine copepods such as narcosis (Hansen et al., 2012, 2016), altered growth and development (Toxværd et al., 2018) and reduced fecundity (Hansen et al., 2015; Olsen et al., 2013) in marine copepods.

In terms of acute toxicity, low molecular weight aromatic compounds (mono aromatics (MAHs)) and polycyclic aromatic hydrocarbons (PAHs) are considered the main toxicity drivers due to their solubility in water. The weathering process is expected to reduce the toxicity of spilled crude oils due to evaporative loss of volatiles and microbial degradation of aromatic hydrocarbons (Faksness et al., 2015;

Neff et al., 2000), however, some studies have shown that crude oil may be more toxic during weathering (Hansen et al., 2018a), particularly shown after photo-oxidation and photo-toxicity (Barron et al., 2003; Duesterloh et al., 2002). The impact of weathering on oil toxicity is a very important aspect to consider when performing environmental risk and damage assessments of acute oil spills, like the Macondo spill in the Gulf of Mexico (GoM) in 2010.

To increase the understanding of the weathering processes during the Macondo oil spill, a comprehensive laboratory study of the physical and chemical properties of fresh and field collected Macondo oils was performed by Daling et al. (2014). The mass of the Macondo oil, a light paraffinic crude oil, was reduced by 50–55 wt % through evaporation within 3–5 days on the sea surface (Daling et al., 2014). Artificially weathered source oil residues from 150 °C+ to 300 °C+ were prepared, representing the range from newly surfaced oil (150 °C+) to oil found after approximately 5 days on the sea surface (300 °C+). Corresponding evaporated oils additionally subjected to photo oxidation by artificial sunlight for 18 and 40 h were also included prepared. The results were used as inputs to the SINTEF Oil Weathering Model (OWM) to predict the change in properties due to weathering on sea surface and the effective “time window” for application of dispersants under various sea surface weather conditions (Daling et al., 2014).

* Corresponding author.

E-mail address: livgurif@sintef.no (L.-G. Faksness).

<https://doi.org/10.1016/j.marenvres.2020.104928>

Received 2 October 2019; Received in revised form 10 February 2020; Accepted 23 February 2020

Available online 24 February 2020

0141-1136/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

In the present work we utilized Macondo source oils and all artificially weathered crude oils generated by Daling et al. (2014) in order to assess changes in chemical composition and acute toxicity of these artificially weathered residues of the Macondo oil. Alaska North Slope (ANS) crude was also included as a reference oil, as ANS has been studied extensively in laboratory toxicity assessments to evaluate the acute and chronic aquatic toxicity to different species (Carls et al., 1999; Heintz et al., 1999).

Low energy water accommodated fractions (LE-WAFs) were prepared, chemically characterized, and acute toxicity tests on the marine algae *Skeletonema pseudocostatum* (clone: NIVA BAC1) and the herbivorous copepod *Acartia tonsa* were performed to evaluate the weathering mediated changes in toxicity of the oils. The CROSERF group (Chemical Response to Oil Spills: Ecological Research Forum) has specified the technical definition of WAF as “a laboratory-prepared medium derived from low-energy (no vortex) mixing of e.g. an oil or a petroleum product that is essentially free of particles of bulk material” (Aurand and Coelho, 2005). The mixing is maintained for e.g. 2 days for the water-soluble components to reach an approximate equilibrium between water and oil and thus representing the highest achievable dissolved concentration at a defined oil-water ratio. The term WAF, as opposed to “water soluble fraction” (WSF), is considered to be technically more accurate because solutions do not undergo steps to ensure that particulate bulk oil has been removed. Such steps would require physical handling (i.e. filtration or centrifugation) of the medium and are likely to introduce uncontrolled chemical loss or change in composition. As such, WAFs only illustrate “snapshots” in the dynamic process of weathering and dissolution occurring during a spill situation. We investigated the environmental relevance of our laboratory prepared WAFs by comparing compositional data with field collected water samples provided in the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC (<https://data.gulfresearchinitiative.org/data-discovery>)) using principal component analysis (PCA). The GRIIDC data base covers water chemistry data describing over 24000 samples by up to 321 analytes.

The objectives of this study were to provide a better understanding of how changes in water soluble components caused by weathering influence acute toxicity to the marine organisms, and to provide comparisons between laboratory-based studies utilizing artificial weathering of oils and field data from the Deepwater Horizon oil spill and with regard to weathering degree, water depth and sampling time.

2. Materials and methods

2.1. Crude oil and weathering procedures

Source oil B was collected aboard the Enterprise Discover on May 22, 2010, directly from the MC252 well via the riser insertion tube downstream of the separators, and has been used as a basis for the bench scale toxicity testing and artificial weathering (Echols et al., 2015; Faksness et al., 2015).

The artificial weathering of Source oil B was performed to mimic the natural weathering of the oil at sea; the residues and their estimated times at sea are given in Supporting Information (SI6, Table S4). The distillation procedure used to simulate evaporation is described in Stiver and Mackay (1984). A simple one-stage distillation to vapor temperatures of 150 °C (all compounds with a boiling point lower than 150 °C are evaporated off) to 300 °C resulted in oil residues with increasing evaporative loss. These distillation residues are referred to as 150 °C+, 200 °C+, 250 °C + 275 °C+, and 300 °C+, respectively. Photo-oxidation was performed by irradiating a layer of oil with a sunlight simulator (4 kW lamp; K.H. Steuernagel Lichttechnik GmbH, Waldorf, Germany) for a defined period. The solar simulator was calibrated for mid-summer North Sea conditions in a wavelength area of 200–3000 nm (CIE, 1989). The oil was spread to a 2 mm thick slick on seawater in a temperature-controlled vessel. As the oil was exposed, a fan created a gentle wind which caused the oil to flow around in the vessel. Two

photo-oxidized samples were prepared; irradiated for 18 and 40 h respectively.

In addition, an artificially weathered Macondo oil, submitted to SINTEF from AECOM, and an Alaska North Slope (ANS) crude oil, submitted to SINTEF from Oregon State University, were tested. The Macondo oil was artificially weathered by B & B Laboratories following the procedure developed by NOAA (NOAA, 2012), which is also based on evaporative loss. This oil is called “NOAA weathered” in tables and figures. GC chromatograms of all oils are shown in Supporting Information (SI5, Fig. S1).

2.2. Preparation of water accommodated fraction (WAF)

LE-WAFs were prepared under controlled conditions following the guidelines established by Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) (Aurand and Coelho, 2005). Briefly, a volume (9.25 L) of sterile filtered (0.2 µm) natural seawater collected from 90 m depth in the Trondheimsfjord was added to 10 L bottles giving water to air headspace ratio of 4 to 1. An oil-to-water loading of 1–10000 (100 mg oil/L seawater) was chosen and the oil was carefully applied to the water surface. The water was stirred gently with a magnetic stirrer (<200 rpm) assuring that the oil film rested on the water surface without creating a vortex and without dispersing oil droplets into the water. The preparation was carried out in darkness in room temperature (approximately 22 °C) using a mixing time of 48 h. Samples for chemical analysis and toxicity testing were collected in glass vials and bottles with Teflon lined caps, without headspace in order to minimize the loss of volatiles. Samples for chemical analysis were acidified (hydrochloric acid to pH < 2) immediately after sampling to avoid biodegradation during storage.

2.3. Chemical composition of the WAFs

The samples were analyzed for semi-volatile organic compound, SVOC (decalins, PAHs and phenols) using gas chromatography-mass spectrometry (GC-MS), for total petroleum hydrocarbons (TPH) using gas chromatograph with flame ionization detector (GC-FID), and for volatile organic compounds (VOC, C₅-C₉), including BTEX (benzene, toluene, ethylbenzene, and xylenes), by use of Purge and Trap GC-MS (P&T GC-MS). Extraction and analyses procedures have previously been published (Faksness et al., 2015), but are also supplied in Supporting Information (SI1) including a list of all target analytes (SI2, Table S1). This list includes the recommended analytes given by Singer et al. (2000) as a typical standard list for the target compounds used during post-oil spill damage assessments. For the toxicity testing, the individual dilutions were not analyzed, but concentrations were calculated from the analysis of the undiluted WAF, representing the highest exposure concentration. To avoid accumulation of error due to serial dilution, each tested concentration was prepared as a dilution from the 100% WAF.

2.4. Growth inhibition experiments with marine algae

The unicellular marine algae *Skeletonema pseudocostatum* was chosen to represent primary producers. The bioassays were performed as a modification of ISO 10253 (ISO, 1991) with growth rate and biomass production as endpoints (EC50 or EC10). The original protocol is not designed for testing of solutions containing volatiles and was adapted for testing of WAFs by exchanging the recommended semi open Erlenmeyer flasks (250 mL) with closed culture tubes (15 mL) in borosilicate glass following recommendations in ISO 14442 (ISO, 2006).

To maintain the chemical composition of the WAF solution during preparation of the exposure solution, the eight dilutions were performed within the individual culture tubes from undiluted (100%) to 3% LE-WAF in autoclaved water as previously described (Faksness et al., 2012). Briefly, 6 replicates were used for each treatment and 12 tubes

with sea water were used as negative controls. All tubes were inoculated with the algae/nutrient mix and filled to a headspace of 0.5 mL. In vivo fluorescence was measured after preparation and daily for 72 h during which the tubes were placed horizontally on a rocking shaker in a temperature-controlled room at nominally 20 ± 2 °C under a mixture of white (Philips TLD 965 18W) and pale yellow (Philips TL20W/33RS) fluorescent tubes.

The calculated effect concentration values (EC50/EC10) were normalized by setting the response in the control series to 100% for both growth rate and biomass production and then calculating the effect within the span 0–100%. The top and bottom of the concentration-effect curve were constrained to 100 and 0, eliminating any stimulatory effects.

2.5. Acute toxicity to *Acartia tonsa*

Potential effects on primary consumers were assessed with the marine pelagic copepod *Acartia tonsa*. The bioassays were performed as a modification of ISO/DIS 14669 (ISO, 1999) with lethal immobilization (LC50 or LC10) as endpoint. The original protocol was not designed for testing of solutions containing volatiles and was adapted as described in Faksness et al. (2015). All bioassays were performed with a fixed number of vessels and a dilution series of WAF ranging from undiluted (100%) to 4% WAF in sea water with 4 parallel vessels for each dilution. Eight vessels filled with seawater were used as negative controls. After adding exposure media, the vessels were added 10–15 sub-adult (copepodite V) or adult *A. tonsa* and incubated in a temperature-controlled room at nominal 20 ± 2 °C under a light regime comparable to culturing conditions. Vessels were inspected daily for the next 2 days (48 h) for immobilized animals and recording of temperature. At the end of exposure, the pH and saturation of oxygen were measured in one vessel in each of the exposure dilutions and in two control vessels. The calculated effect values (LC50/LC10) were corrected for mortality in the control series and the effect is calculated within the span 0–100% by constraining the top and bottom of the concentration-effect curve to 100 and 0.

2.6. Comparison to BP's Gulf Science database

When data used in this study was downloaded, the water chemistry data in the GRIIDC Database contained 2.4 million lines of data describing 24072 samples by up to 321 analytes. The data had been collected from 6243 different geographical sampling points (i.e. different latitude/longitude). This comprised a data file counting 1.4 GB. To enable comparison of chemical composition data from the WAFs (components displayed in Supporting Information, SI2, Table S1) with the database, nine data sets covering different depth ranges were constructed where concentrations for 77 compounds from the AQAP (NOAA, 2012) SVOC and VOC lists (Supporting Information, SI3, Table S2) were collected for the samples in the database. The raw text files of the GRIIDC were processed using Python (version 2.7) and the csv module. The data were transformed from the original format to a format with one line per sample ID. This process reduced the size of the database from 1.4 GB to about 30 MB, while keeping all chemical information intact. After dividing the datasets into the desired depth intervals, R (R-Core-Team, 2014) was used to sort and filter data using the plyr library filter (Wickham, 2011). Principal component analysis (PCA) was done using the command “prcomp” (Mevik and Wehrens, 2007). Two analyses were done for each comparison, where the data were used with and without normalization to mean = 0 and variance = 1.

An overview of the number of samples for each depth is given in Supporting Information (SI4, Table S3). To obtain the final dataset to be compared with the WAF data, the data from 2010 (comprising 23284 of the 24072 records in the database) were extracted from the database, and subsequently, the data with a TPH of zero were removed together with samples with no concentrations for the compounds found in the

SVOC and VOC lists.

Comparing the chemical composition of the 13 prepared WAFs (Table 1) with those of the field samples (Supporting Information, SI4, Table S3) involved comparing 77 components in between 32 and 248 samples. To do this in a practical way, multivariate analysis (PCA) was required. This reduced the complexity of the comparison significantly. PCA separates the data into scores, which were new variables describing the samples, and loadings which described differences in the original variables. The relatively few score values of the PCA have the potential of showing similarities and dissimilarities between samples where the differences are dependent of the largest variations in the dataset. The corresponding PCA loadings still retained the information on differences in compounds so that no information was lost in the data reduction. For this study, when described by their scores, WAF samples closely clustered with field samples would be expected to have a similar chemical composition, whereas samples with larger differences in their scores would be expected to have larger differences in their composition.

3. Results and discussion

3.1. Chemical characterization of the oils and their WAFs

The artificial weathering procedure causes a loss of light saturated hydrocarbons which increases with distillation temperature and photo oxidation (Supporting Information, SI5, Fig. S1). Based on the evaporative loss, they can be used as representatives for oil with increasing estimated time at sea. The residue after weathering to 150 °C+ is representative of surfacing oil, while the estimated time at sea for the 300 °C+ is 3–5 days. The evaporative loss after artificial weathering to 250 °C+ and the artificially weathered oil received from NOAA are similar, representing approximately 0.5–2 days on sea. Details on the physical properties of the oil residues from the artificial weathering are discussed in detail in Daling et al. (2014), and some properties are given in Supporting Information (SI6, Table S4).

The composition of the main hydrocarbon groups measured using GC-MS are summarized for all oil residues in Fig. 1A. The fractions of the different hydrocarbons of the source and weathered oils reflect the effects of evaporative weathering. The naphthalenes and PAHs become relatively more important contributors to the total oil concentration compared to the source oils as the volatiles are lost during evaporative weathering. The slight increase of the relative content of naphthalenes in the 150 °C+ and 200 °C+ residues is due to the total mass of evaporative loss (mainly the BTEX) which is larger relative to the total loss of the naphthalenes. In the heavily weathered oils, the relative content of naphthalenes and PAHs is reduced.

The chemical profile of a WAF (Fig. 1B) deviates from the parent oil (Fig. 1A) due to the different water solubilities of the various oil compounds. The artificially weathered oil submitted to SINTEF from NOAA, appeared to be slightly more evaporated than the 250 °C+ residue, but less evaporated than the 275 °C+ residue. The content of SVOC in Source oil B and ANS comparable, but ANS contained less volatiles than Source oil B. Concentrations of the dissolved components of the undiluted (or 100%) WAFs prepared with an oil-to-water ratio of 1–10000 (100 mg oil/L water) are given in Fig. 1B. Total WAF concentrations are given as the sum of TPH (sum of SVOC and the unresolved complex materials (UCM)) and volatiles (C5–C9, including BTEX). The VOCs (especially BTEX and C3-benzenes) constitute a major part of the WAF from the source oil and the less weathered oils. Parent and alkylated naphthalenes were the dominating SVOC components because they have a relatively high solubility in water. The total WAF concentration of the dissolved fraction decreased dramatically as a function of increased weathering degree, mostly due to reduction in the volatiles through evaporation. As observed for the oil residues, a higher content of SVOC was observed in the WAFs from 150 °C+ and 200 °C+ than from source oil. No volatiles were detected in the WAF from 300 °C+ and the photo oxidized residues. The remaining components consisted mainly of

Table 1

Summary of chemistry and acute toxicity results in WAFs from Macondo and ANS oils. Algae growth rate inhibition (EC10) and copepod lethality (LC10) are given as concentrations relative to undiluted WAFs, and as % effect at 100% WAF concentration. (Skel: *Skeletonema pseudocostatum*, *Acartia*: *Acartia tonsa*). For comparison data on Source oil MASS, CTC, and Juniper are included (Faksness et al., 2015).

WAF systems	Concentration			Relative toxicity		Observed tox at test endpoint	
	Tot WAF	TPAH	VOC	Skel	Acartia	Skel 72 h	Acartia 48 h
						Growth reduction	Mortality
	mg/L	mg/L	mg/L	EC10 (% WAF)	LC10 (% WAF)	% effect	% effect
Source oil B	4.04	0.14	3.37	63	43	37	89
150 °C+	2.10	0.17	1.68	98	28	14	52
200 °C+	1.04	0.17	0.66	67	74	23	49
250 °C+	0.43	0.14	0.08	>100	30	7	45
NOAA weathered	0.36	0.13	0.02	>100	61	9	41
275 °C+	0.29	0.10	0.01	>100	59	4	27
300 °C+	0.16	0.05	ND	>100	83	None	21
Phox 18 h	0.25	0.06	ND	87	38	13	34
Phox 40 h	0.27	0.03	ND	71	65	34	21
ANS crude	2.45	0.10	2.05	>100	64	2	50
Source oil MASS ^a	2.59	0.156	2.18	95	65	32	74
Field weathered CTC ^a	0.14	0.012	ND	>100	>100	None	9
Field weathered Juniper ^a	0.10	0.004	ND	>100	>100	None	None

ND: Not detected.

^a Data from Faksness et al. (2015).

unresolved complex materials (UCM), which was estimated by subtracting the SVOC concentration from the TPH concentration.

Fig. 1C displays more clearly how the relative composition (shown as percentage distribution) of the different component groups change as a function of weathering degree. In line with Fig. 1B, the relative contribution from the UCM increases from approximately 5% in WAF from source oil B (total WAF concentration was 4 mg/L) to 90% in WAF from the residue that had been photo oxidized for 40 h (total WAF concentration was less than 0.3 mg/L). Concentration levels in the Gulf of Mexico (GoM) following the Deepwater Horizon oil spill have often been reported as total PAH (TPAH), and Table 1 provides TPAH concentrations in the LE-WAFs prepared in the current study. In the LE-WAFs from Source oil B and the residues from 150 to 200 °C+, the TPAH concentrations were from 143 to 176 µg/L, which are in the same range as the highest concentrations reported in a small number of samples from the GoM by e.g. Boehm et al. (2016) and Diercks et al. (2010). The TPAH concentrations in LE-WAFs prepared of the field collected oils CTC (12 µg/L) and Juniper (4.5 µg/L), reported in Faksness et al. (2015), were also in the higher range of the larger group of water samples from the GoM collected during the release (e.g. Boehm et al., 2016), as well as for the field data collected as part of the Natural Resource Damage Assessment (NRDA) program (Echols et al., 2015).

3.2. Acute toxicity of LE-WAFs to algae and copepods

The test organisms were exposed to a dilution series of WAFs in order to determine median effect concentrations (EC50 and LC50). However, the toxic effects observed for the algae were not sufficient in any of the tested WAFs to calculate conclusive EC50 values after 72 h exposure. Overall, the effects were less than 50% at 100% WAF for all oils. Also, for the copepods, less than 50% mortality was observed at the highest concentration for most of the WAFs. Hence, for comparison, values for EC10 and LC10 were calculated for the two species and are included in Table 1. There was no clear pattern of differences in toxicity as a function of weathering based on % WAF dilution. However, when comparing the effect in the 100% WAFs between oils, a clear reduction in toxicity is observed as a function of weathering for both species (Table 1).

As the oil become more weathered, the total concentration of the WAFs also decreased (Fig. 1B and Table 1). However, traditional mass-based toxicity analysis relating the effect concentrations to mg/L total WAF concentration suggests that the acute toxicity of WAFs increased with weathering degree, as the EC10 and LC10 decreased, suggesting

that the residues with the highest degree of weathering (artificially weathered to 250 °C+ or more) are more toxic than WAF from Source oil. To relate the toxicity to the solubility of the WAF, the maximum concentrations obtained after the 48 h WAF generation is indicated as a diagonal line in Fig. 2. We observed that the 10% effect concentrations (in mg/L total WAF) decreases with the solubility of the WAF. The mass-based toxicity for *A. tonsa* (slope 0.98 ± 0.10 , $r^2 = 0.91$) decreased in proportion to the reduction in solubility (slope = 1) and the average concentration required to reach 10% mortality was 52% (\pm sd = 18%) of the maximum WAF concentrations. A similar pattern was seen for *S. pseudocostatum* growth rate inhibition, but here the effect was somewhat less than for *A. tonsa* and did not reach 10% inhibition in undiluted WAF for four of the artificially weathered oil residues (Fig. 2, vertical arrows).

The copepod *A. tonsa* was more sensitive to exposure than *S. pseudocostatum*, and on average the LC10s were a factor of two lower for *A. tonsa*. In the current experiments, a mixing time of 48 h was used to create equilibrium WAFs. Thus, given the expected rate of dilution in a field situation, the maximum WAF concentration used in the current tests most likely overestimates the actual concentrations reached under field conditions.

In a previous study, we found that the maximum concentration in WAFs (100% WAF) generated from the field collected CTC and Juniper weathered oils in the range 0.10–0.14 mg/L (Faksness et al., 2015), and these were lower than the most weathered residue (300 °C+) used in the current study (Fig. 1 and Table 1). Furthermore, the exposure of *S. pseudocostatum* and *A. tonsa* to WAFs from the field collected oils caused <10% effect for both species when tested with the same procedure as used for the artificially weathered oils. The most probable explanation for this may be that the field collected oils were subject to dissolution of components into the water column as the oils rose to the surface and by evaporative loss after reaching the surface. This caused a reduction in the potential for acute toxicity and is supported by observations showing that the fraction of naphthalenes is significantly reduced in the field collected oils compared to the oils artificially weathered by evaporative loss (Faksness et al., 2015). A large number of tests have been performed on field collected samples from the water column, but only a small fraction of these have shown significant acute toxicity (Echols et al., 2015; OSAT, 2011) indicating that the potential for acute mortality during the incident was limited.

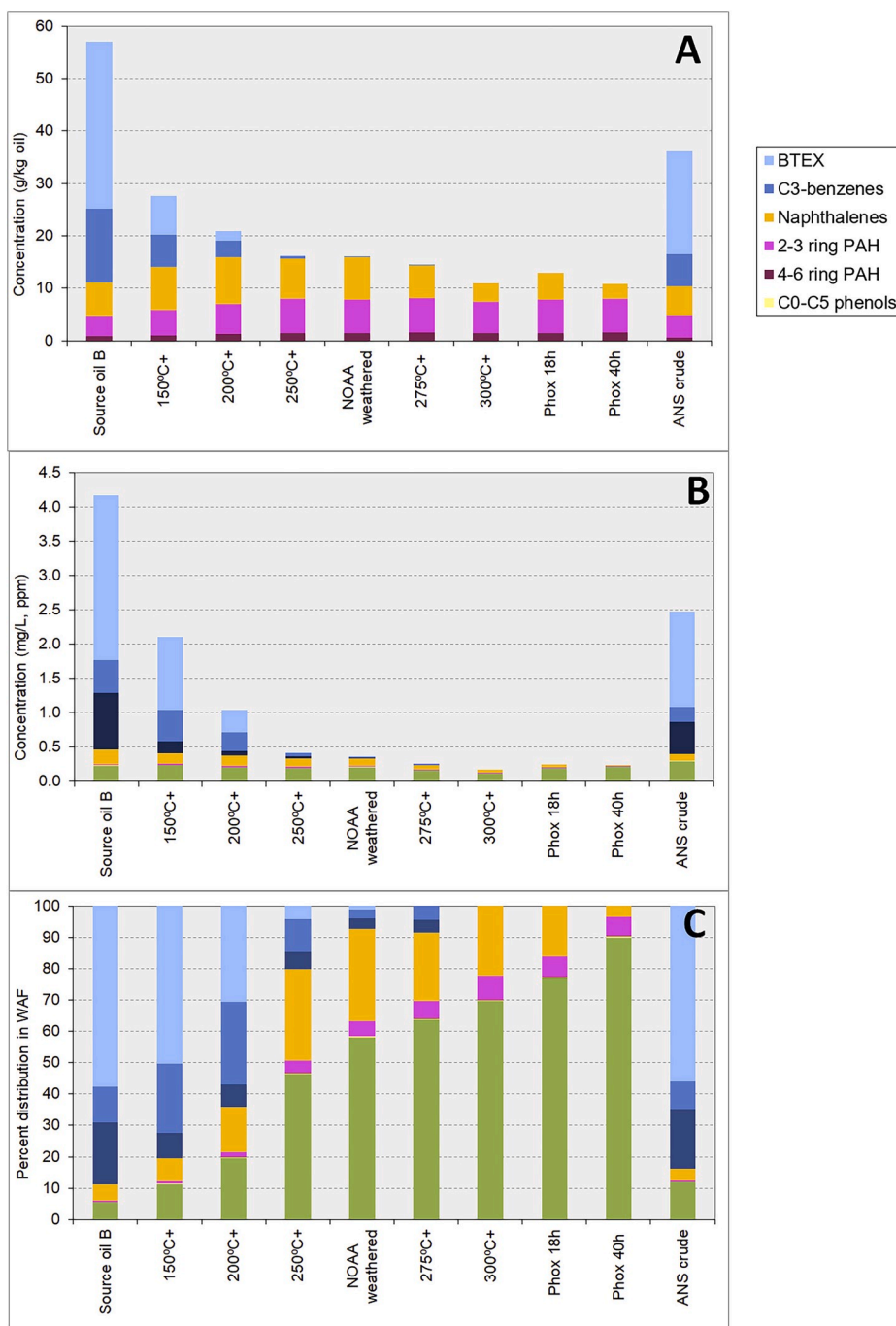


Fig. 1. A: Composition of aromatic compounds in the oils used for WAF preparation given in g analyte/kg oil. No phenols were detected. B: Chemical composition of the WAFs, all prepared with an oil-to-water ratio of 1–10000 at room temperature. Chemical compositions shown in mg/L seawater. C: Chemical composition of the WAFs based on percentage of total concentration.

3.3. Comparison with the chemistry data in the GRIIDC database

The WAF samples were first subjected to PCA analysis separate from the GRIIDC data to obtain an overview of the clustering behavior of the samples. The initial PCA of all WAF samples revealed a cluster of all samples except WAFs prepared with the two source oils and the artificially weathered residues 150 °C+ and 200 °C+ (as illustrated in Supporting Information S17, Fig. S2A). The loadings of this analysis revealed the differences between these four samples and the others to be largely attributed to more and differing ratios of VOC compounds and naphthalenes. This was expected from the compositional data in Fig. 1 where these compounds were easily identifiable as the largest difference

between WAFs. These four samples were removed from the dataset in a subsequent PCA analysis, resulting in a better description of the differences between the weathered samples (Supporting Information S17, Fig. S2B). It was then possible to rank WAF samples with respect to their similarities with the two field-collected weathered oils, CTC and Juniper. The WAF of the 40 h photo-oxidation residue oil (Phox 40h) was closest and the NOAA weathered oil was the least similar to field-collected weathered oil. As with the preceding analysis, differences in the naphthalenes and VOC composition were revealed by the loadings to be responsible for the major differences (Supporting Information, S18, Fig. S3).

To compare the WAFs with field data from the GSD, the dataset for

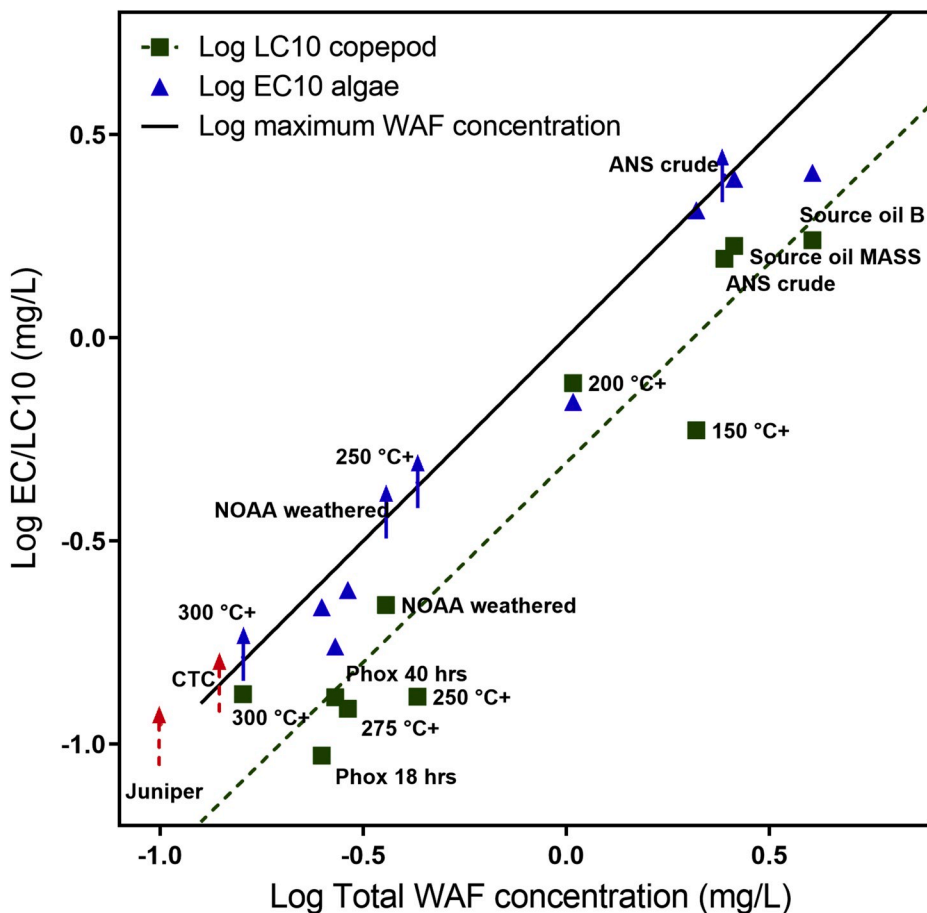


Fig. 2. Specific toxicity for *A. tonsa* mortality (LC10, squares) and *S. pseudocostatum* biomass growth reduction (EC10, triangles) when exposed to WAFs from different oils at oil:water ratio of 1:10 000 related to maximum total WAF concentration. Solid diagonal line indicates the maximum WAF concentration obtained (maximum solubility) at oil: water ratio of 1:10 000 (100 mg/L). Four vertical arrows with solid line indicate tests with less than 10% effect for *S. pseudocostatum* at the highest (undiluted) WAF concentration. Broken line indicates linear regression for *A. tonsa* (Log_{10} LC10 vs Log_{10} total WAF concentration). For comparison corresponding data for the field collected oils Juniper and CTC is (arrows with broken line in lower left corner) indicating less than 10% effect in undiluted WAF for *A. tonsa* and *S. pseudocostatum* (from Faksness et al., 2015). Note that for algae reduction in biomass production is used as endpoint, whereas the corresponding growth rate inhibition is presented in Table 1.

each depth interval (Supporting Information, S14, Table S3) was combined with WAF compositions. Concentrations and normalized concentrations (mean = 0 and variance = 1) were used in separate analyses. In both cases, the datasets showed that the WAFs prepared from the most highly weathered oil and field collected oils clustered together with the field data, whereas the Source oils and the less artificially weathered oils differ to a varying extent. Fig. S4 (Supporting Information S19) shows analysis of depth intervals 1–200 m and 1000–1300 m. When the

normalized data were used, it was observed that the Phox 40h WAF clustered together with Juniper and CTC and that there was a varying degree of separation from the other artificially weathered oil WAFs. The PCA analysis (Fig. 3) showed that the field weathered CTC and Juniper WAFs and the Phox 40h WAF clustered well with the majority of samples in most depth regions when the concentrations were used. The Source oils, B and MASS, and the 150 °C+ and 200 °C+ WAFs appeared to have the most dissimilar composition from the field sample chemistry. This

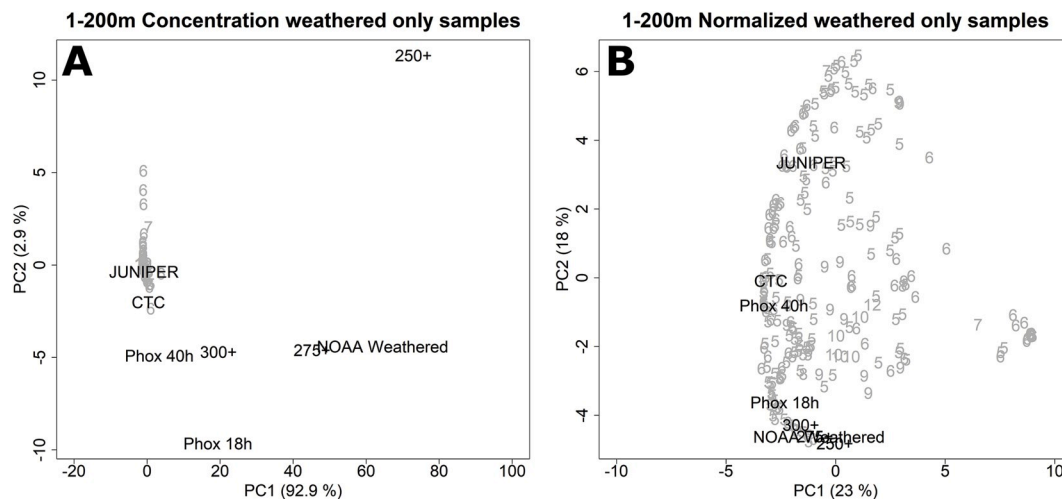


Fig. 3. PCA analysis performed with the GSD samples and WAFs (without the outlier WAFs (Source oils B and MASS, and 150 °C+ and 200 °C+)). Here the GSD samples from depth range 1–200 m are shown (concentrations (A) normalized data (B)). The samples from GSD are shown as numbers identifying the sampling month (e.g. 5 indicates sampling in May 2010).

could indicate that for a sub-surface release, artificial oil weathering with photo-oxidation on the water surface will be more realistic than evaporative loss by one-step distillation. The sampling month also had an influence on the clustering of field data, and the WAF samples clustered mostly in the proximity of samples from May and June, i.e. before capping of the well. It is important to emphasize that the likely occurrence of oil droplets in some of the field collected water samples (Li et al., 2011) will significantly affect the compositional profiles and this will contribute to the differences observed between the current WAFs and the field samples. Field samples, in contrast to the laboratory prepared WAFs, have been subjected to both continuous evaporation and dilution. Hence, the concentration in the water under field conditions at comparable oil-water ratios will be limited by dissolution rates. This will reduce the environmental concentration and affect the composition compared to laboratory generated WAFs.

4. Conclusions

The contribution of the different oil chemical component groups of the Source oils and weathered oils reflects the effects of evaporative weathering in the laboratory. With the loss of the volatiles during evaporative weathering, the naphthalenes and PAHs usually become relatively more important contributors to the total oil concentration. For the more heavily weathered residues, the semi-volatile compounds will be reduced due to the evaporative loss (e.g. the residues from photo oxidation). The maximum total concentration in WAFs decreased with increased artificial weathering of the oil, ranging from 4 mg/L in WAF from fresh Source oil to 0.16 mg/L in WAF of the 300 °C+ residue.

Analysis of the acute aquatic toxicity (decrease in EC_x and LC_x) related to total WAF concentration may lead to the conclusion that environmental toxicity of crude oil increases with increasing degree of weathering. This conclusion is incorrect because the solubility of the remaining oil components and thus maximum achievable WAF concentration decreases during the weathering process. The current results show that all EC₅₀ and LC₅₀s were at or above the maximum total WAF concentration tested, and that the EC₁₀ and LC₁₀ values decreased with increased weathering of the oil. Consequently, the toxic potential of the equilibrium WAFs for the tested residues (up to 300 °C+) is approximately the same because the solubility of the oil residues decreased in proportion to their effect limit (LC/EC₁₀).

The PCA analysis showed that the chemistry of the WAFs prepared with the field collected CTC and Juniper oils were more similar to the field samples in the Gulf Science Database than the artificially weathered oils. Among the WAFs prepared using artificially weathered oil residues, the PCA analyses indicated that the oil artificially photo oxidized for 40 h was the most similar to the field collected oil samples based on chemical composition.

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.

CRedit authorship contribution statement

Liv-Guri Faksness: Conceptualization, Methodology, Investigation, Writing - original draft, Supervision, Project administration. **Dag Altin:** Methodology, Investigation, Writing - review & editing. **Trond R. Størseth:** Methodology, Validation, Formal analysis, Data curation, Writing - review & editing. **Trond Nordtug:** Validation, Formal analysis, Data curation, Writing - review & editing. **Bjørn Henrik Hansen:** Validation, Formal analysis, Writing - original draft.

Acknowledgements

This project was funded through NRDA of BP GCRO Science, Technology, Environment & Regulatory Affairs, and was a part of BP's NRDA Aquatic Toxicology Testing Program. The authors acknowledge Kjersti Almås, Kristin Bonaunet, Per S. Daling, Tor Nordam, Inger Steinsvik, and Marianne U. Rønsberg for their valued assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2020.104928>.

References

- Aurand, D., Coelho, G., 2005. Cooperative Aquatic Toxicity Testing of Dispersed Oil and the Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF). Inc. Lusby, MD. Tech. Report, 07-03.
- Barron, M.G., Carls, M.G., Short, J.W., Rice, S.D., 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. *Environ. Toxicol. Chem.* 22, 650–660.
- Boehm, P., Murray, K.J., Cook, L.L., 2016. Distribution and attenuation of polycyclic aromatic hydrocarbons in Gulf of Mexico seawater from the Deepwater Horizon oil accident. *Environ. Sci. Technol.* 50, 584–592.
- Carls, M.G., Rice, S.D., Hose, J.E., 1999. Sensitivity of fish embryos to weathered crude oil: Part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasii*). *Environ. Toxicol. Chem.* 18, 481–493.
- CIE, 1989. Solar Spectrum Irradiance.
- Daling, P.S., Leirvik, F., Almås, I.K., Brandvik, P.J., Hansen, B.H., Lewis, A., Reed, M., 2014. Surface weathering and dispersibility of MC252 crude oil. *Mar. Pollut. Bull.* 87, 300–310.
- Diercks, A.R., Highsmith, R.C., Asper, V.L., Joung, D., Zhou, Z., Guo, L., Shiller, A.M., Joye, S.B., Teske, A.P., Guinasso, N., Wade, T.L., Lohrenz, S.E., 2010. Characterization of subsurface polycyclic aromatic hydrocarbons at the Deepwater Horizon site. *Geophys. Res. Lett.* 37, 1–6.
- Duesterloh, S., Short, J.W., Barron, M.G., 2002. Photoenhanced toxicity of weathered Alaska north slope crude oil to the calanoid copepods *Calanus marshallae* and *Metridia okhotskensis*. *Environ. Sci. Technol.* 36, 3953–3959.
- Echols, B., Smith, A., Gardinali, P., Rand, G., 2015. Acute aquatic toxicity studies of Gulf of Mexico water samples collected following the Deepwater Horizon incident (May 12, 2010 to December 11, 2010). *Chemosphere* 120, 131–137.
- Faksness, L.-G., Altin, D., Nordtug, T., Daling, P.S., Hansen, B.H., 2015. Chemical comparison and acute toxicity of water accommodated fraction (WAF) of source and field collected Macondo oils from the Deepwater Horizon spill. *Mar. Pollut. Bull.* 91, 222–229.
- Faksness, L.-G., Hansen, B.H., Altin, D., Brandvik, P.J., 2012. Chemical composition and acute toxicity in the water after in situ burning – a laboratory experiment. *Mar. Pollut. Bull.* 64, 49–55.
- Hansen, B.H., Altin, D., Olsen, A.J., Nordtug, T., 2012. Acute toxicity of naturally and chemically dispersed oil on the filter-feeding copepod *Calanus finmarchicus*. *Ecotoxicol. Environ. Saf.* 86, 38–46.
- Hansen, B.H., Farkas, J., Nordtug, T., Altin, D., Brakstad, O.G., 2018a. Does microbial biodegradation of water-soluble components of oil reduce the toxicity to early life stages of fish? *Environ. Sci. Technol.* 52, 4358–4366.
- Hansen, B.H., Jager, T., Altin, D., Øverjordet, I.B., Olsen, A.J., Salaberria, I., Nordtug, T., 2016. Acute toxicity of dispersed crude oil on the cold-water copepod *Calanus finmarchicus*: elusive implications of lipid content. *J. Toxicol. Environ. Health, Part A* 79, 549–557.
- Hansen, B.H., Nordtug, T., Altin, D., Booth, A., Hessen, K.M., Olsen, A.J., 2009. Gene expression of GST and CYP330A1 in lipid-rich and lipid-poor female *Calanus finmarchicus* (Copepoda: Crustacea) exposed to dispersed oil. *J. Toxicol. Environ. Health* 72, 131–139.
- Hansen, B.H., Olsen, A.J., Salaberria, I., Altin, D., Øverjordet, I.B., Gardinali, P., Booth, A., Nordtug, T., 2018b. Partitioning of PAHs between crude oil microdroplets, water, and copepod biomass in oil-in-seawater dispersions of different crude oils. *Environ. Sci. Technol.* 52, 14436–14444.
- Hansen, B.H., Salaberria, I., Olsen, A.J., Read, K.E., Øverjordet, I.B., Hammer, K., Altin, D., Nordtug, T., 2015. Reproduction dynamics in copepods following exposure to chemically and mechanically dispersed crude oil. *Environ. Sci. Technol.* 49, 3822–3829.
- Heintz, R.A., Short, J.W., Rice, S.D., 1999. Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered Exxon valdez crude oil. *Environ. Toxicol. Chem.* 18, 494–503.
- ISO, 1991. Water Quality - Marine Algae Growth Inhibition Test *Skeletonema Costatum* and *Phaeodactylum Tricornutum*.
- ISO, 1999. Water Quality - Determination of Acute Lethal Toxicity to Marine Copepods (Copepoda, Crustacea). International Organization for Standardization, Geneva.
- ISO, 2006. ISO 14442:2006: Water Quality – Guidelines for Algal Growth Inhibition Tests with Poorly Soluble Materials, Volatile Compounds, Metals and Waste Water. International Organization for Standardization, Geneva.

- Li, Z., Lee, K., Kepkey, P.E., Mikkelsen, O., Pottsmith, C., 2011. Monitoring dispersed oil droplet size distribution at the Gulf of Mexico Deepwater Horizon spill site. In: International Oil Spill Conference Proceedings (IOSC). American Petroleum Institute, p. abs377.
- Mevik, B.-H., Wehrens, R., 2007. The pls package: principal component and partial least squares regression in R. *J. Stat. Software* 18, 1–24.
- Neff, J.M., Ostazeski, S., Gardiner, W., Stejskal, I., 2000. Effects of weathering on the toxicity of three offshore australian crude oils and a diesel fuel to marine animals. *Environ. Toxicol. Chem.* 19, 1809–1821.
- NOAA, 2012. Analytical Quality Assurance Plan (AQAP). Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment. Version 3.1. Prepared for US Department of Commerce. NOAA.
- Olsen, A.J., Nordtug, T., Altin, D., Lervik, M., Hansen, B.H., 2013. Effects of dispersed oil on reproduction in the cold water copepod *Calanus finmarchicus* (Gunnerus). *Environ. Toxicol. Chem.* 32, 2045–2055.
- OSAT, 2011. Summary Report for Sub-sea and Sub-surface Oil and Dispersant Detection: Ecotoxicity Addendum. Operational Science Advisory Team, Unified Area Command, Deepwater Horizon MC252, pp. 1–36.
- R-Core-Team, 2014. In: Computing, R.F.f.S. (Ed.), R: A Language and Environment for Statistical Computing. Vienna, Austria.
- Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., Tjeerdema, R.S., 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Mar. Pollut. Bull.* 40, 1007–1016.
- Stiver, W., Mackay, D., 1984. Evaporation rate of spills of hydrocarbons and petroleum mixtures. *Environ. Sci. Technol.* 18, 834–840.
- Toxværd, K., Van Dinh, K., Henriksen, O., Hjorth, M., Nielsen, T.G., 2018. Impact of pyrene exposure during overwintering of the Arctic copepod *Calanus glacialis*. *Environ. Sci. Technol.* 52, 10328–10336.
- Wickham, H., 2011. The split-apply-combine strategy for data analysis. *J. Stat. Software* 40, 1–29.