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Report

WAF and toxicity testing of diesel and hybrid oils

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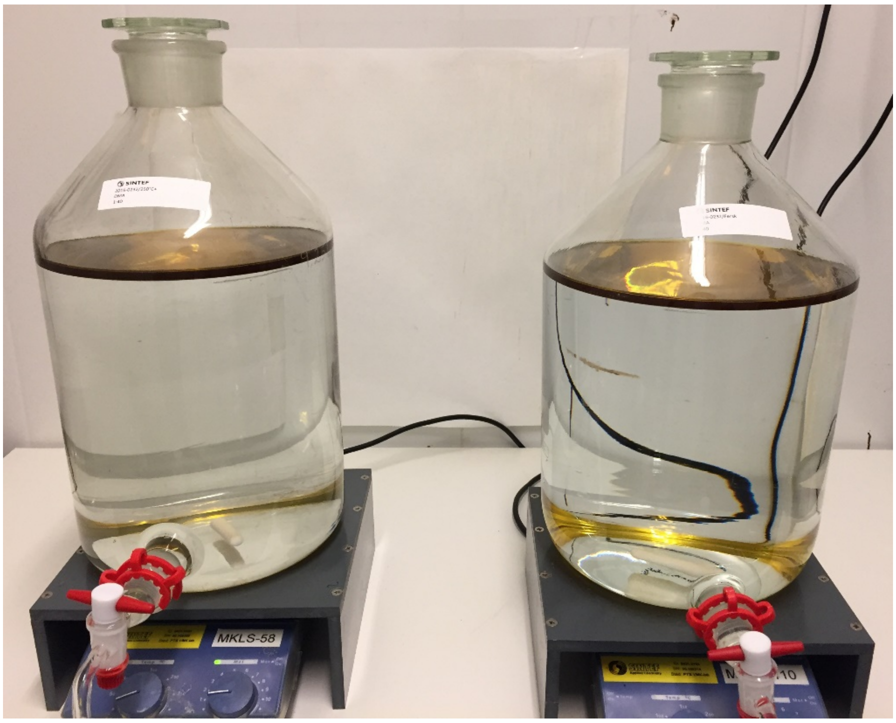

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Report

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ABSTRACT

The WAFs (22 systems) of six oil products, mainly different diesel qualities and so-called hybrid oils, have been studied with special emphasis on chemistry and acute toxicity. The total WAF concentration varied and in WAFs with an oil to water ratio of 1 to 40, it varied from 0.14 ppm (HDME 50) to 9.4 ppm (weathered GO).

The level of toxic effect observed in several of the tested WAFs was not sufficient to calculate conclusive values for EC_{50} and LC_{50} as the effect of undiluted WAF was less than 50% for one or both organisms, especially with oil-to-water loading of 1 to 10 000. WAFs prepared with Rotterdam Diesel fresh and 250°C+ with an oil-to-water loading of 1 to 40 were most toxic for both organisms, WAFs of MGO 250°C+ and GO 250°C+ had also a relatively high toxicity compared to the other WAFs.

Toxic Unit (TU) has been calculated and the results indicate that the WAF system prepared of fresh Rotterdam diesel, where the 2-3 ring PAHs seem to contribute to nearly 80% of the TU, was more toxic than the other oil products, with a TU of 3.8. WAFs prepared of the weathered residues of MGO and Rotterdam diesel had a TU of approximately 2. All other WAFs had a TU below 1, including the more heavier oil products HDME 50 and ULSFO 250°C+, which were the least toxic WAFs. This was in accordance with the observed relative toxicity.

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1 Introduction

The results in the present report is a summary of WAF and toxicity testing of different diesel qualities performed by SINTEF in 2015 and of two hybrid oils and a DMA-diesel (Rotterdam Diesel) in 2016. The oils received in 2015 are produced on Norway, while the hybrid oils and Rotterdam Diesel are produced in the Netherlands. All oils were provided by NCA. The oil products weathering, dispersibility, ignitability, and other properties are discussed and reported in Hellstrøm *et al*. (2017) and Hellstrøm and Daling (2017).

2 Materials and methods

2.1 Tested oil products

Sample description and SINTEF ID are given in Table 2.1. More detailed description of the oil products and their properties are given in Hellstrøm et al. (2016). Rotterdam diesel is referred to as DMA or DMA diesel in the figures and texts.

Table 2.1 Sample description, SINTEF ID and label used in tables and figures

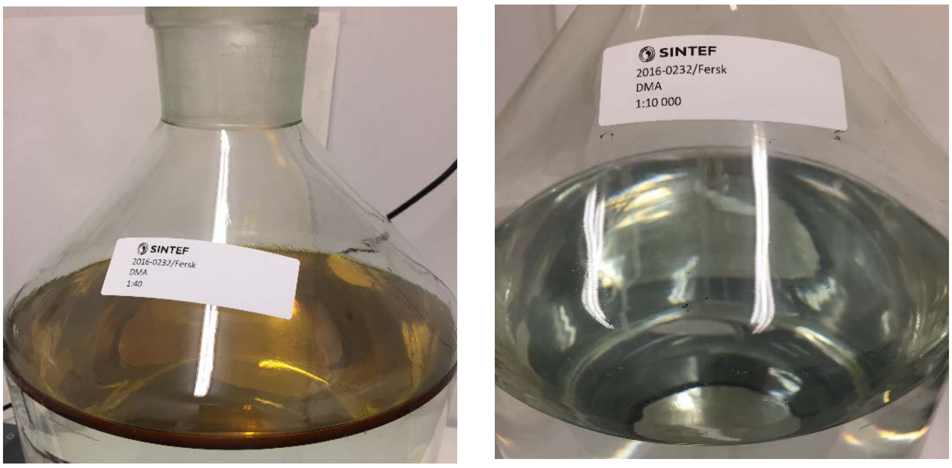
SINTEF ID	Oil product	Description	Source	Labelled
2014-0551	MGO 500 ppm S	Marine gas oil with maximum 500 ppm sulfur, DMA-quality	Esso Slagen	MGO
2014-0552	GO 10 ppm S	Gas oil (dyed) with max 10 ppm sulfur, DMA-quality	Mongstad	GO
2014-0553	WRG	Wide range gas oil, DMZ-RMA quality	Mongstad	WRG
2016-0231	HDME 50	Heavy Distillate Marine ECA 50 (Emission Commission Control Area)	ExxonMobil	HDME
2016-0232	Rotterdam diesel	Marine gas oil (dyed) from Rotterdam, DMA-quality	Shell	DMA
2016-0233	ULSFO	Ultra Low Sulfur Fuel Oil	Shell	ULSFO

2.2 WAF preparation

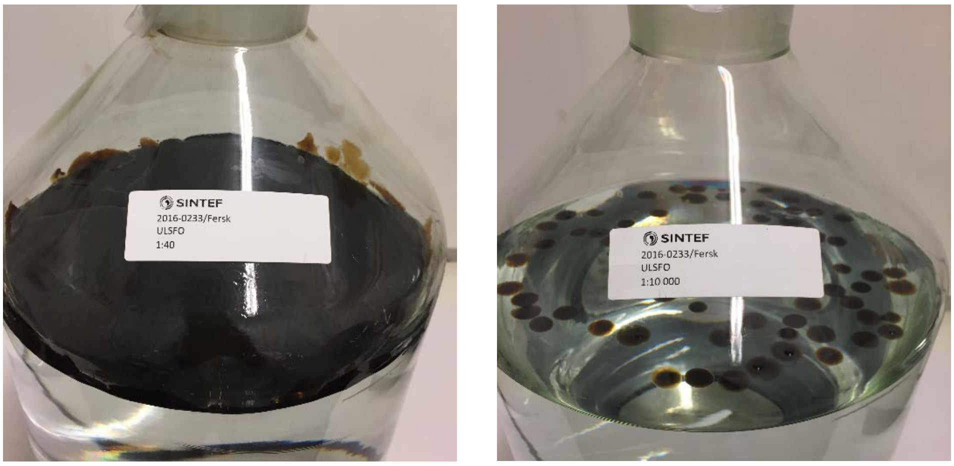
Preparation of low energy WAF (LE-WAF) has been performed under controlled conditions following the guidelines established by the Chemical Response to Oil Spills: Ecological Research Forum (CROSERF). These guidelines were developed to standardize WAF preparation, laboratory exposures to aquatic organisms, and analytical chemistry measurements used to determine the acute toxicity of the water soluble components in the oil (Aurand and Coelho, 2005). LE-WAF can be defined as a water solution of dissolved oil components prepared in closed vessels, as shown in the picture on the front page and Figure 2.1, with calm mixing of oil and water without the formation of any vortex. LE-WAFs were chosen in order to avoid generation of oil droplets.

The WAFs were prepared with the oil-to-water loadings of 1 to 40 (25 g oil/L water) and 1 to 10000 (100 mg oil/L water), and are described in Table 2.2. Photos of some of the systems are shown Figure 2.1. These different WAFs illustrate "snapshots" in the dynamic process of dissolution occurring during a spill situation. The oil-to-water ratio of 1:40 is assumed to be "saturated" and therefore represents a "conservative" estimate of the concentrations foreseeable during an oil spill. An oil-to-water ratio of 1 to 10000 is considered to be a more realistic concentration found in the upper surface layer a short time after treatment with chemical dispersants. The WAFs were generated with a contact time between water and oil for four days before the water was collected for chemical characterization and toxicity tests.

HDME 50 fresh 1:40 HDME 50 fresh 1:10 000



DMA fresh 1:40 DMA fresh 1:10 000



ULSFO fresh 1:40 ULSFO fresh 1:10 000

Figure 2.1 WAF systems of fresh oil products with oil to water ratios of 1 to 40 (left photos) and 1 to 10 000 (right photos).

Table 2.2 WAF systems with exposure temperatures, oil-to-water ratio, and analyses.

Oil	Temperature (°C)	Oil-to-water	Chemistry	*C. finmarchicus*	*Skeletonema* sp.
MGO fresh	13	1:40	X	X	X
MGO fresh	13	1:10 000	X	X	X
MGO 250°C+	13	1:40	X	X	X
MGO 250°C+	13	1:10 000	X	X	X
GO fresh	13	1:40	X	X	X
GO fresh	13	1:10 000	X	X	X
GO 250°C+	13	1:40	X	X	X
GO 250°C+	13	1:10 000	X	X	X
WRG fresh	13	1:40	X	X	X
WRG fresh	13	1:10 000	X	X	X
WRG fresh	2	1:40	X	X	X
WRG fresh	2	1:10 000	X	X	X
HDME fresh	13	1:40	X	X	X
HDME fresh	13	1:10 000	X	X	X
DMA fresh	13	1:40	X	X	X
DMA fresh*	13	1:40	X		X
DMA fresh	13	1:10 000	X	X	X
DMA 250°C+	13	1:40	X	X	X
DMA 250°C+	13	1:10 000	X	X	X
ULSFO fresh	13	1:40	X	X	X
ULSFO fresh	13	1:10 000	X	X	X
USLFO 250°C+	13	1:40	X	X	X
ULSFO 250°C+	13	1:10 000	X	X	X

** New system prepared for re-testing of* Skeletonema *sp. (DMA-2)*

2.3 Sample preparation

Surrogate internal standards (SIS, *o*-terphenyl, naphthalene-d_8, phenanthrene-d_{10}, chrysene-d_{12}, phenol-d_6, 4-methylphenol-d_8) were added to the water samples prior to processing, and recovery internal standards (RIS, 5α-androstane, fluorene-d_{10}, and acenaphthene-d_{10}) were added prior to analysis on GC/FID (gas chromatography/flame ionization detection) and GC/MS (gas chromatography/mass spectrometry).

For analyses of semi-volatile organic compounds (SVOC) and total petroleum hydrocarbons (TPH), the water samples were spiked with the appropriate surrogate internal standards and serially extracted with dichloromethane (DCM), thereby following a modification of EPA method 3510C (US EPA, 1996). The combined extracts were dried with sodium sulphate and concentrated to approximately 1 mL using a Zymark Turbovap® 500 Concentrator. The final extract was spiked with the appropriate recovery internal standards and analyzed on GC/FID and GC/MS. The components quantified are given in Appendix C (Table C 1).

2.4 Chemical analyses

The samples were analyzed for SVOC (decalins, PAHs and phenols) using GC/MS, for TPH using GC/FID, and for volatile organic compounds (VOC, C_5-C_9), including BTEX (benzene, toluene, ethylbenzene, and xylenes), by use of P&T GC/MS (Purge and Trap Gas Chromatography Mass Spectrometry). A list of all target analytes is shown_in Appendix C (Table C 1). This list includes the recommended analytes given by Singer et al. (2000), and is a typical standard list for the target compounds used during post-oil spill damage assessments.

The GC/FID analyses were performed according to a modification of EPA Method 8015D (US EPA, 2003). TPH (resolved plus unresolved TPH) was quantified by the method of internal standards using the baseline corrected total area of the chromatogram and the average response factor for the individual C_{10} to C_{36} n-alkanes.

The semi-volatiles were quantified by modifications of EPA Method 8270D (US EPA, 2007). The mass spectrometer was operated in the selective ion monitoring mode to achieve optimum sensitivity and specificity. The quantification of target compounds was performed by the method of internal standards, using average response factors (RF) for the parent compounds. The PAH and phenol alkyl homologues were quantified using the straight baseline integration of each level of alkylation and the RF for the respective parent PAH compound. The response factors were generated for all targets and surrogates versus fluorene-d_{10}.

A total of 35 target volatile analytes in the C_5 to C_{10} range were determined by P&T GC/MS using a modification of EPA method 8260C (US EPA, 2006). The samples were spiked with SIS (toluene-d_8 and ethylbenzene-d_8) and RIS (chlorobenzene-d_5). The quantification of individual compounds was performed by using the RFs of the individual compounds relative to the internal standards. All standards and samples were analysed in a full scan mode.

2.5 Acute toxicity of the WAFs

Crude oil is a complex, highly variable mixture of hydrocarbons and other trace components. Exposure may cause a variety of adverse effects, including narcosis, slowed growth, reduced reproduction, and death in marine organisms. Toxicity is the “inherent potential or capacity of a material (e.g. oil) to cause adverse effects in living organisms” (US EPA, 2002). In order to be toxic, oil components must be bio-available to the organisms being exposed. Many of the components in oil are considered toxic to marine organisms, but have limited bioavailability in the environment due to their low solubility in water. Toxic effects depend on the duration of exposure and the concentration of the chemicals involved. Toxic effects can be lethal (causing death) and sub-lethal (e.g. disorientation, reduced growth and reproduction). Toxic effects can also be acute (caused by short-term exposure, such as following an acute oil spill) or chronic (caused by long-term exposure, such as release of produced water). The toxicity of a chemical is normally quantified as an LC_{50} or EC_{50} value, defined as the concentration causing 50% of the organisms in a test population to die or to show a significant negative effect when they are exposed to a fixed concentration of the chemical for a defined time period (Rand et al., 1995).

2.5.1 Growth inhibition to the algae *Skeletonema sp.*

The unicellular marine algae *Skeletonema* sp. (previously known as *S. costatum*, clone NIVA BAC-1) was chosen as a relevant representative of marine producers. The bioassays were performed as a modification of ISO 10253 (2006a) with inhibition of growth rate and biomass production as endpoints (EC_{50}). The original protocol is not designed for testing of solutions containing volatiles, and has been 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 (2006b).

As an experimental design, a fixed dilution scheme with a spacing factor of 1.6 between concentrations (from undiluted 100% WAF to 3% WAF in sea water) was used covering a total of eight different dilutions with six replicate tubes in each dilution. A re-test of Rotterdam diesel (DMA) fresh (1:40) was performed, using a dilution from 5.04% WAF to 0.15% WAF in sea water. All tubes were inoculated with the same volume of exponentially growing *Skeletonema sp.* (clone NIVA BAC-1) and nutrient mix. All tubes were filled to a headspace of 0.5 mL to ensure mixing in the tube during incubation. As negative control, 12 tubes in each bioassay were filled with autoclaved seawater and inoculated with the algae/nutrient mix in the same manner. *In vivo* fluorescence was measured by a Turner TD700 fluorometer (Turner Systems, Sunnyvale, CA, USA) after preparation. The tubes were then 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 under constant light.

During the test period of 72 hours, *in vivo* fluorescence was measured daily by the Turner TD700 fluorometer. At the end of exposure, pH was measured in a pooled sample from three tubes from the control series as well as from the different dilutions in the exposure series. The calculated values are normalized by setting the response in the control series to 100% for growth rate and then calculating the effect within the span 0 to 100% relative to the control series. The top and bottom of the concentration-effect curve are constrained to 100 and 0, thus eliminating any stimulatory effects.

2.5.2 Acute toxicity to *Calanus finmarchicus*

Potential effects on primary consumers were assessed with the marine copepod *Calanus finmarchicus*, which is one of the key ecological species in northern boreal to arctic oceans. The acute toxicity testing was performed according to ISO 14669:1999 (ISO, 1999) with lethal immobilization (LC_{50}) as the endpoint. The original ISO protocol is not designed for testing of solutions containing volatiles with *C. finmarchicus*, and was modified by using borosilicate glass bottles (0.5 L) with Teflon lined screw caps to preserve volatiles and to accommodate for the larger body mass of *C. finmarchicus* compared to the listed species in the ISO protocol (ISO, 1999).

To ensure that the observed effects were approaching the incipient toxicity level of LC_{50} for the species, the exposure time was increased to 96 hours at a set temperature of 10±2°C. The WAF samples were diluted in a series of seven concentrations with a spacing factor of 1.7 between dilutions with each exposure concentration made in triplicate. Six bottles were used as negative controls containing seawater only and three bottles with 0.8 mg L^{-1} of 3,5-dichlorophenol were used as positive control. The exposure vessels were filled close to the rim to keep potential evaporative loss to a minimum during exposure, and each vessel was stocked with seven copepodites V of *C. finmarchicus* at onset exposure. Mortality was monitored at 24, 48, 72 and 96 hours. The test animals were not fed during exposure. The calculated values are not corrected for any mortality in the control series and the effect is calculated within the span 0-100% effect by constraining the top and bottom of the concentration-effect curve to 100 and 0.

2.5.3 Predicted toxicity using toxic units

In the WAFs from petrogenic products, the compounds of concern for toxicity assessment are typically limited to the VOCs and SVOCs, which are structurally classified as Type I narcotics. The target lipid model of narcotic toxicity demonstrates that the acute toxicities of these chemicals vary and are correlated with the octanol-water partition coefficient (K_{ow}) such that LC_{50} decreases with increasing K_{ow} (DiToro et al., 2007). A toxic unit (TU) for the individual compounds is computed by dividing the measured concentration in the WAF by the compound's water effect concentration using regression models. It appears to be a linear negative relation between log LC_{50} of the marine organisms and log Kow of the components that may cause toxic effects (McCarty et al. (1992; 1993) and Di Toro et al. (2007)):

$$\log LC_{50} = m \log (K_{ow}) + b \qquad (1)$$

The slope (m), log K_{ow}, and the intercept (b) for different component groups (e.g MAH, PAH and phenols) are given in McCarty (1993) and Neff et al. (2000). The LC_{50} (mg/L) is calculated for each component by use of equation (1). Different regression coefficients can be used to calculate TU relative to different species (McGrath and DiToro, 2009), resulting in other values of the TU than calculated here. However, all oils used for comparison in this report are calculated using the same equation.

In the WAFs, the TUs of the individual components are summed up to compute the total TUs of the WAF. If the sum of the TUs is less than 1 (TU<1), observed effects should be lower than as defined by the water effect concentration (e.g. 50% lethality if using the LC_{50}). If the sum of the TUs in the WAF is greater than 1 (TU>1), adverse effects could potentially be observed.

3 Results and discussion

Six oil products, including oil residues artificially weathered to 250^{o}C+[1] for four of them, were studied, and the chemistry and toxicity of their WAFs have been characterized. Marine gas oil (MGO), Gas oil (GO), and wide range gas oil (WRG) were studied in 2015, and HDME 50, Rotterdam diesel (DMA), and ULSFO were studied in 2016. Detailed results are provided in the Attachments (Chemistry in Attachment A and Toxicity in Attachment B).

3.1 Chemical composition of the oils and the WAFs

GC chromatograms of all oils are provided Figure A 1 to Figure A 10 and of the WAFs in Figure A 11 to Figure A 33 (Attachment A). There are no indications of the presence of oil droplets in any of the WAFs according to the GC chromatograms; solely the water soluble fractions are detected.

Figure 3.1 summarizes the composition of the main groups of the aromatics in the oil products and their artificially weathered residues. The data are also provided in Table A 1 and Table A 2. The contribution of the different oil component groups of the fresh oils and the weathered residues reflects the effect of evaporative weathering with the reduction of volatiles in the weathered residues. The non-weathered gas oils (MGO and GO) contain approximately 50 % volatiles, and GO has a relatively high content of decalins. The composition of WRG and Rotterdam diesel (DMA) are dominated of naphthalenes and 2-3 ring PAH. HDME 50, which is a heavy distillate cut (see GC chromatogram in Figure A 6), contains low concentrations of volatiles and naphthalenes, and mostly 2-6 ring PAHs.

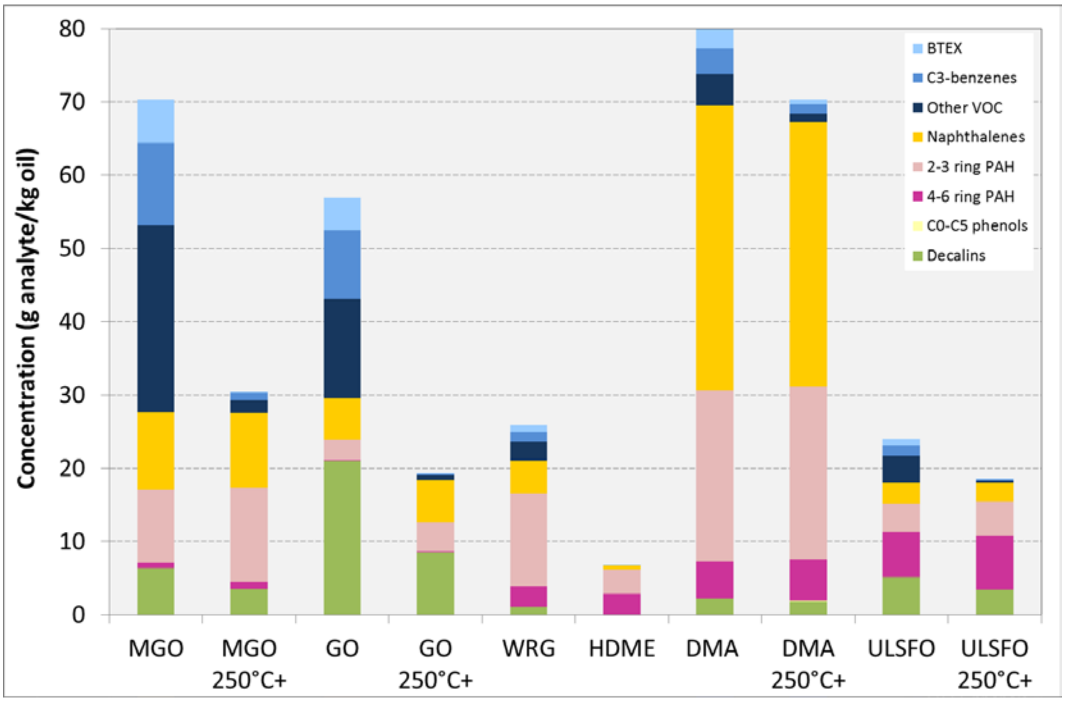


Figure 3.1 Chemical composition of selected component groups (aromatics) in all oil products studied.

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[1] Components with boiling point < 250 °C have been removed through evaporation

The "chemical profile" of a WAF is unlike that of it's parent oil due to the different water solubilities of the various oil components. Figure 3.2 and Figure 3.3 (data are also provided in Table A 3 to Table A 5) show the concentrations of the water soluble components of the WAFs prepared, including unresolved complex materials (UCM). The UCM is calculated by subtraction the SVOC concentration from the TPH (total petroleum hydrocarbon) concentration. Total WAF concentrations are based on the sum of TPH and volatiles (C5-C9), including BTEX.

The VOCs (especially BTEX and C3-benzenes) constitute a major part of the WAFs from fresh crude oils, and the naphthalenes are generally dominating SVOC components, as they have a relatively high solubility in water. This can be seen the WAFs from the fresh gas oils (MGO and GO) and in ULSFO.

However, as seen in Figure 3.2, the WAFs prepared of the weathered MGO and GO have higher WAF concentrations than the WAFs from fresh oils, even though the most volatile components had gone. The increase is due to a higher content of UCM in the weathered WAFs.

The WAFs of Rotterdam diesel (DMA) with oil to water loading of 1 to 40 were also dominated by the UCM, but the concentration in the weathered WAF were lower than in the WAF of fresh Rotterdam diesel (DMA) (Figure 3.3). WAF prepared of HDME 50, was the lowest of the WAFs tested here (0.14 ppm for 1 to 40 and 0.03 ppm for 1 to 10000). WAF of fresh ULSFO, the ultra-low sulphur fuel oil, had a lower content of naphthalenes and 2-3 PAHs than WAFs from other oils (except HDME 50), which reflect that the concentration of sulphur containing components (e.g. dibenzothiophenes, one of the 3-ring PAHs) have been reduced during the oil producing process.

No weathered residue of WRG was prepared, but WAFs were prepared at both 2 and 13 °C in order to reveal any significant temperature dependent variations. The WAF concentration in the WAF prepared at low temperature was lower than at 13 °C, which is due to that WAFs prepared of oils with low pour point need longer time to reach equilibrium (Faksness et al., 2008).

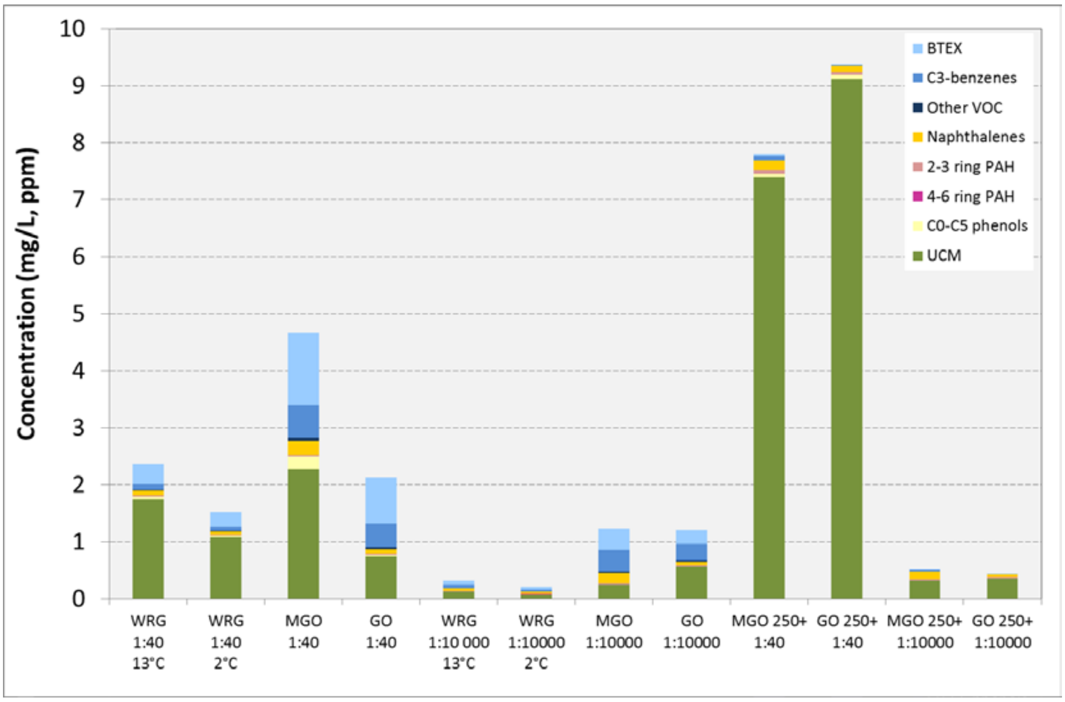

Figure 3.2 *Chemical composition of selected component groups in the WAF systems studied in 2015. No artificially weathered residue of WRG was prepared.*

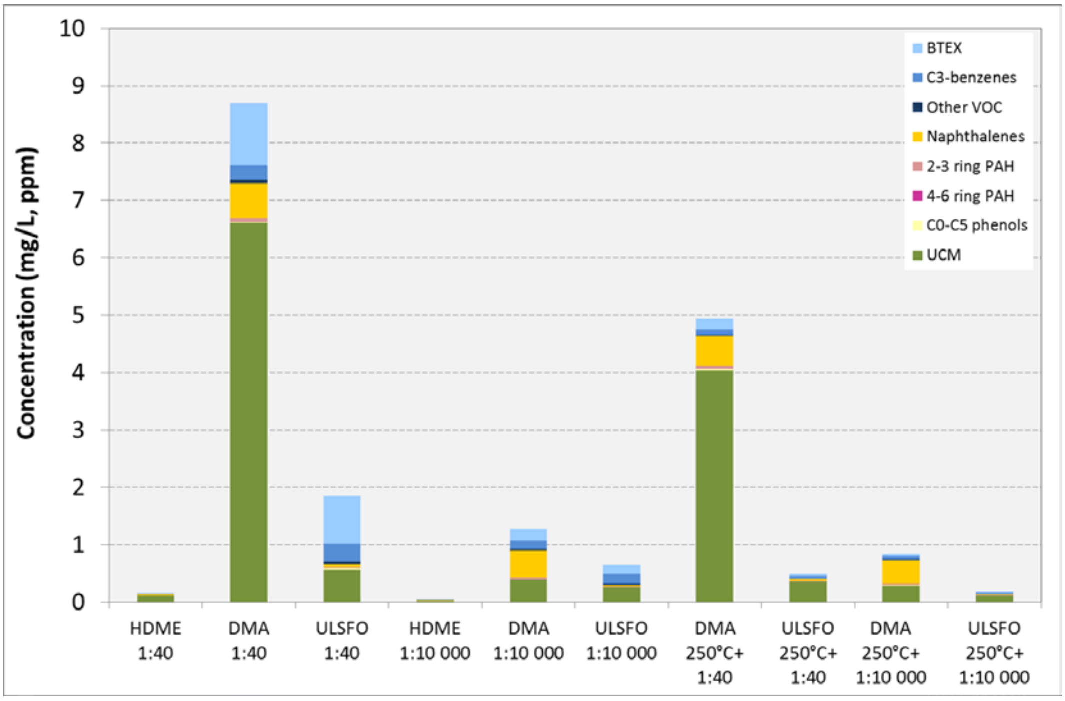

Figure 3.3 *Chemical composition of selected component groups in the WAF systems studied in 2016. No artificially weathered residue of HDME 50 250°C+ was prepared.*

3.2 Toxicity

3.2.1 Acute toxicity of WAFs to marine organisms

There are several ways to present the toxicity results. The toxicity of a chemical is normally quantified as an LC_{50} or EC_{50} value, defined as the concentration causing 50% of the organisms in a test population to die or to show a significant negative effect when they are exposed to a fixed concentration of the chemical for a defined time period (Rand et al., 1995). LC_{10} and EC_{10} values reflect the concentrations causing 10% of the organisms to die or to show a significant negative effect, and can be used if the toxicity is too low to be expressed as EC_{50} or LC_{50}. The EC_{50} and LC_{50} can be given in percent dilution of the undiluted (or 100 %) WAF (relative toxicity, EC_{50} or LC_{50} (%)), or as normalized to the total WAF concentration (specific toxicity, EC_{50} or LC_{50} (mg/L or ppm)). Low values for LC_{50} or EC_{50} indicates a high toxicity, while a high value for LC_{50} or EC_{50} corresponds to a lower toxicity. A summary of the toxicity results is given in Table B 1 to Table B 3 (Appendix B), both EC_{50} or LC_{50} and EC_{10} or LC_{10} are calculated for all systems. In addition, predicted acute toxicity expressed as toxic units is provided in Table B 4.

Toxicity can also be expressed as percent effect on the organisms at test endpoint in 100 % WAF (undiluted). This means toxicity results are presented as percent reduction in growth rate for *Skeletonema* sp. after 72 hours and in percent mortality for *C. finmarchicus* after 96 hours (Figure 3.4). In WAFs with an oil to water loading of 1 to 40, HDME 50 had lowest toxicity for both organisms. In WAFs with an oil to water loading of 1 to 10000, no effects were observed in HDME 50, and relatively low effects for ULSFO fresh and 250°C+, WRG, and MGO. In addition, percent reduction in growth for *Skeletonema* sp. was low for GO 250°C+. If the percent effect is lower than 50%, relative toxicity for EC_{50} and LC_{50} cannot be calculated (e.g. as shown in Figure 3.5 for MGO and ULSO with an oil to water loading of 1 to 10000).

The relative toxicity (Figure 3.5) is presented as EC_{50} and LC_{50} given in percent of the diluted WAF. WAFs prepared with DMA fresh and 250°C+ with an oil-to-water loading of 1 to 40 were most toxic for both organisms, WAFs of MGO 250°C+ and GO 250°C+ had also a relatively high toxicity compared to the other WAFs. The level of toxic effect observed in several of the tested WAFs was not sufficient to calculate conclusive values for EC_{50} and LC_{50} as the effect was less than 50% for one or both organisms, especially with oil-to-water loading of 1 to 10 000. Therefore, EC_{10} and LC_{10} are provided in tables in Appendix B.

Specific toxicity is normalized to the total WAF concentration, and has been the traditional approach for expressing toxicity (Figure B 1). The mass-based analysis neglects the fact that the composition of the WAFs varies both between oil types and with oil-to-water loading. WAF of HDME 50 (1 to 40) and WRG (1 to 10 000) are examples of this. The relative toxicities to *Skeletonema* sp. are just below 100% (99.4% and 98.4, respective), but when normalized to the WAF concentration, which is low (0.14 and 0.32 ppm, correspondingly), the specific toxicities indicated that these WAFs are among the most toxic of the tested oils for *Skeletonema* sp. Based on the mass-based approach, the so-called hybrid oils (tested in phase 2 of the project in 2016) were more toxic than the oil products tested in 2015. Fresh Rotterdam diesel (DMA) and ULSFO seem to be the most toxic of the WAFs with an oil-to-water loading of 1 to 40 to both test organisms, while DMA fresh and DMA 250°C+ were most toxic of the WAFs with oil-to-water loading of 1 to 10000.

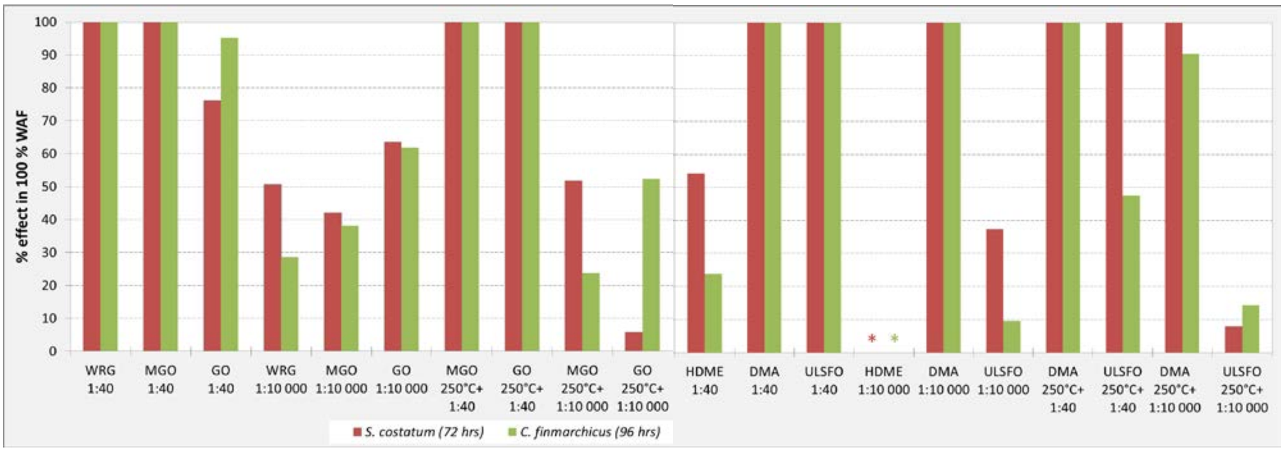

Figure 3.4 *Acute toxicity expressed as percent effect on the test organisms in 100% WAF (undiluted): Percent reduction in growth for* Skeletonema sp. (*red bars) at test endpoint (72 hrs) and percent mortality for* C. finmarchicus (*green bars) at test endpoint (96 hrs). High bars indicate higher toxicity and no bars indicate absence of an observed effect on the test organisms (*). Left part of the figure gives WAFs studied in 2015, and right part WAFs studied in 2016.*

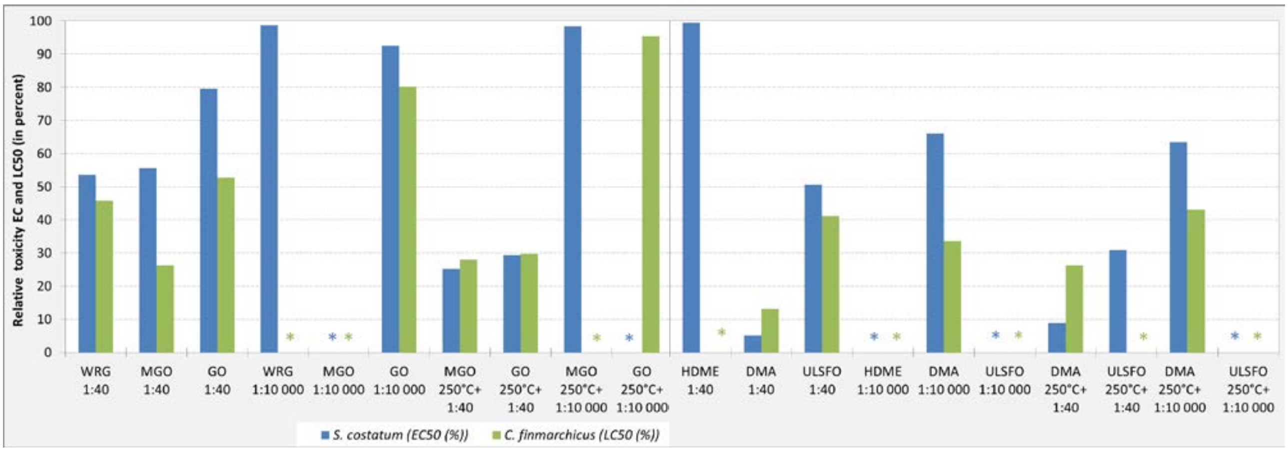

Figure 3.5 *Relative toxicity of the WAFs shown as* EC_{50} *and* LC_{50} *given in percent of dilution of 100% WAF (undiluted). Low bars indicate higher toxicity, and high bars indicate lower toxicity, but no bars indicate absence of an observed effect on the test organisms (*). Left part of the figure gives WAFs studied in 2015, and right part WAFs studied in 2016.*

3.2.2 Calculation of toxic units

The acute toxicity, expressed as TU (Toxic Unit), can be predicted based on the chemical composition of the WAFs and the K_{ow} for the individual components. A TU > 1 for the total WAF implies that it is expected to cause more than 50% mortality in the test organisms. TU for all WAFs are computed and provided in Table B 4, and TU for WAFs with an oil-to-water loading of 1 to 40 are shown in Figure 3.6. Although the UCM is not included in the calculated TU, the results indicate that the WAF system prepared of fresh Rotterdam diesel (DMA), where the 2-3 ring PAHs seem to contribute to nearly 80% of the TU, was more toxic than the other oil products, with a TU of 3.8. WAFs prepared of the weathered residues of MGO and Rotterdam diesel had a TU of approximately 2. These three WAFs were the only WAFs that had TU > 1. All WAFs with an oil-to-water loading of 1 to 10000 had a TU below 1.

Expressing the toxicity as TU, Rotterdam diesel was the most toxic WAF, and the heavier oil products HDME 50 and ULSFO 250°C+ were among the less toxic WAFs. This is in accordance with the observed relative toxicity presented in Figure 3.5, and reflects that the chemical composition of a WAF is an important factor when observed toxicity should be evaluated.

Specific toxicity is commonly used as input to spreading and trajectory models used for risk assessment, and the calculated specific toxicities from the performed tests are presented in Table B 3. This method of expressing toxicity is not suited for comparing the toxicities of oils in an easily comprehensive way.

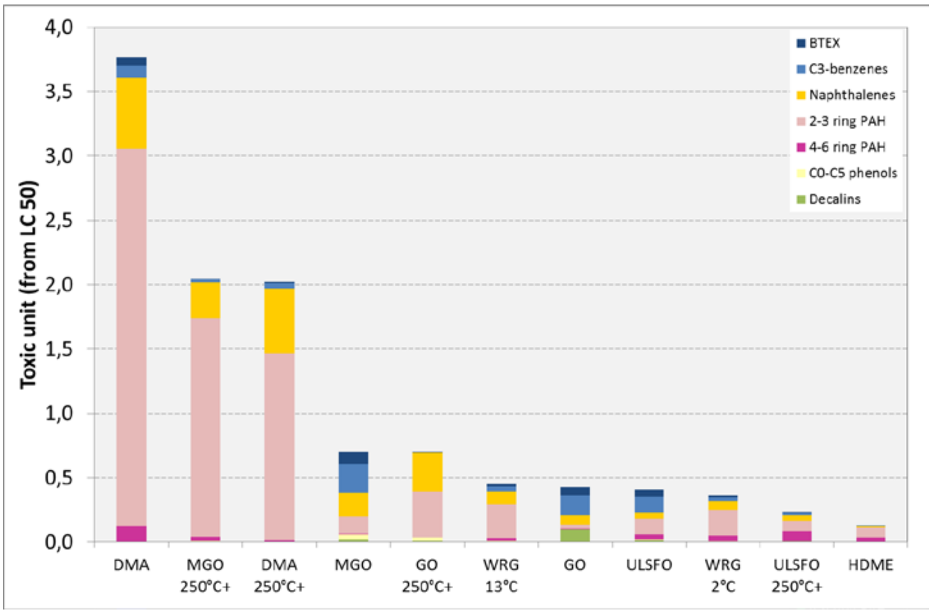

Figure 3.6 *Predicted acute toxicity expressed as toxic unit (TU) for WAFs with an oil-to-water loading of 1 to 40. A TU > 1 indicates a 50% mortality in the tested organisms. TU calculated for WAFs with and oil-to-water ratio of 1 to 10000 are provided in Table B 4.*

3.3 Comparison with other oils and oil products

WAF concentration and TU for the WAFs with an oil-to-water loading of 1 to 40 were computed and are compared with other oils in Figure 3.7 and Figure 3.8, respective.

The computed TU for Rotterdam diesel was similar to the condensate Sleipner (3.7 vs 3.8). Even though, as seen in Figure 3.7, the total WAF concentration of Rotterdam diesel (DMA) (8.7 ppm) was much lower than of Sleipner (77 ppm). The total WAF concentration in condensates are dominated by the mono aromatic hydrocarbon, MAH (> 95 %), while more than 75% of the WAF from Rotterdam diesel consisted of UCM, which is not included in the TU. However, the contribution of the component groups to the TUs were very different, with Sleipner being dominated by the MAH (> 80%) and Rotterdam diesel by the 2-3 ring PAHs (>80%).

The WAF concentration of MGO was similar to a previous studied marine diesel and the British wreck oils from Boardale and Bittern, while WRG, GO, and ULSFO were in the same range as a previous studied bunker oil (IFO 180). TU of these WAFs were well below 1, and were in the range of the British wreck oils and the IFO180. HDME 50 had the lowest WAF concentrations, and the lowest TU (0.13).

This reflects once more that the chemical composition of a WAF is an important factor when observed toxicity should be evaluated, and indicates that a combination of relative toxicity and calculations of TU could be the approach that should be used when evaluating acute toxicity of WAFs.

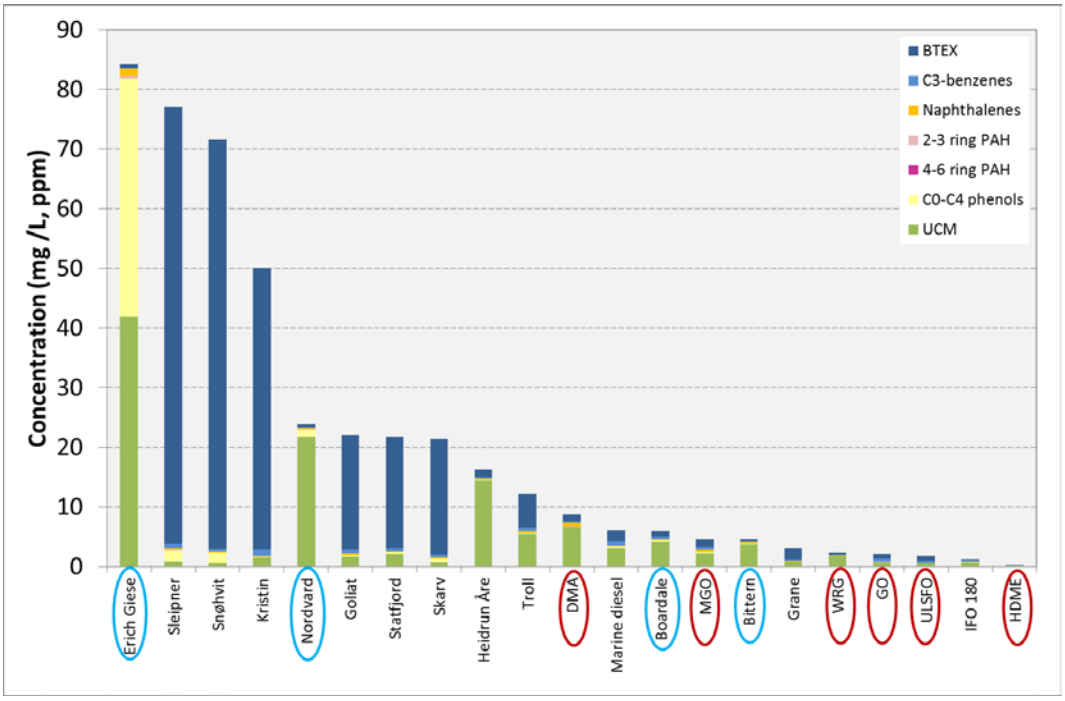

Figure 3.7 *WAF composition of fresh oil products: The oil products tested (red circles) are compared with North Sea crudes, refined products, and shipwreck oils from WW2 (blue circles, from Faksness et al., 2015). Oil-to-water loading of 1 to 40 in all systems.*

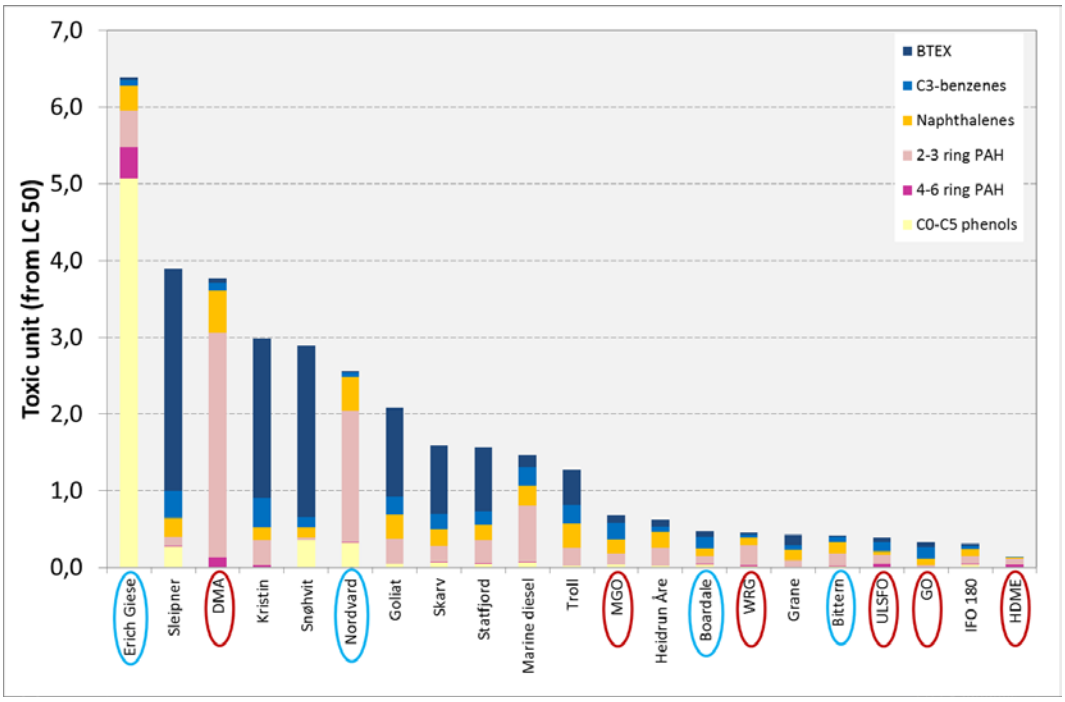

Figure 3.8 *Predicted acute toxicity expressed as toxic unit (TU) for WAFs with an oil-to-water loading of 1 to 40. The oil products tested (red circles) are compared with North Sea crudes, refined products and shipwreck oils from WW2 (blue circles, from Faksness et al., 2015). A TU > 1 indicates a mortality of 50 % for the tested organisms.*

4 Summary and conclusions

The WAFs of six oil products, mainly different diesel qualities and so-called hybrid oils, have been studied with special emphasis on chemistry and biological effects (algae growth (*Skeletonema* sp., clone NIVA BAC-1) and copepod lethality (*Calanus finmarchicus*)). Four of the oil products were artificially weathered to 250^{o}C+ residues. In total, 22 WAF systems were studied.

The total WAF concentration varied and in WAFs with an oil to water ratio of 1 to 40, the highest concentrations were quantified in the WAFs of weathered residues of GO (9.4 ppm) and MGO (7.8 ppm), and in WAF of fresh Rotterdam diesel (DMA; 8.7 ppm). The heaviest product, HDME 50, had the lowest WAF concentration of 0.14 ppm.

The level of toxic effect observed in several of the tested WAFs was not sufficient to calculate conclusive values for EC_{50} and LC_{50} as the effect was less than 50% for one or both organisms, especially with oil-to-water loading of 1 to 10 000. The relative toxicity was presented as EC_{50} and LC_{50} given in percent of the diluted WAF. WAFs prepared with fresh Rotterdam diesel and weathered 250°C+ residue with an oil-to-water loading of 1 to 40 were most toxic for both organisms, WAFs of MGO 250°C+ residue and GO 250°C+ residue had also a relatively high toxicity compared to the other WAFs.

The acute toxicity, expressed as TU, can be predicted based on the chemical composition of the WAFs and the K_{ow} for the individual components. A TU > 1 for the total WAF implies that it is expected to cause more than 50 % mortality in the test organisms. Although the UCM is not included in the calculated TU, the results indicate that the WAF system prepared of fresh Rotterdam diesel (DMA), where the 2-3 ring PAHs seem to contribute to nearly 80 % of the TU, was more toxic than the other oil products, with a TU of 3.8. WAFs prepared of the weathered 250°C+ residues of MGO and Rotterdam diesel (DMA) had a TU of approximately 2. All other WAFs had a TU below 1, including the more heavier oil products HDME 50 and ULSFO 250°C+, which were the least toxic WAFs. This is in accordance with the observed relative toxicity.

5 References

Aurand, D., and G. Coelho. 2005. Cooperative Aquatic Toxicity Testing of Dispersed Oil and the "Chemical Response to Oil Spills: Ecological Research Forum (CROSERF)". Ecosystem Management & Associates, Inc., Lusby, MD. Technical report 07-03, 105 pages + Appendices.

DiToro, D.M., J.A: McGrath, and W.A. Stubblefield. (2007). Predicting the toxicity of neat and weathered crude oil: Toxic potential and the toxicity of saturated mixtures. Environmental Toxicity and Chemistry 26: 24-36.

Faksness, L.G., Brandvik, P.J., and Sydnes, L.K. (2008). Chemical composition of water accommodated fractions as a function of mixing times and temperatures. Marine Pollution Bulletin 56: 1746-1754.

Faksness, L.G., P. Daling, D. Altin, H. Dolva, B. Fosbæk, and R. Bergstrøm (2015). Relative bioavailability and toxicity for fuel oils leaking from World War II shipwrecks. Marine Pollution Bulleting 94: 120-130.

Hellstrøm, K.C. and Daling, P.S., 2017. Summary report: marine fuel oils. Work in progress, OC2017-A123

Hellstrøm, K. C., Daling, P. S., Brönner, U., Sørheim, K. S., Johnsen, M., Leirvik, F. and Altin, D., 2017. Memo report: Collection of memo reports with detailed results from performed experiments, work in progress. Report nr.: OC2017-A124

ISO [International Organization for Standardization] 1999. Water quality - Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea). ISO 14669:1999.

ISO [International Organization for Standardization] 2006a.Water quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. ISO 10253:2006.

ISO [International Organization for Standardization] 2006b. Water quality – Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and wastewater. ISO 14442:2006.

McCarty, L.S., Mackay, D., Smith, A.D., Ozburn, G.W., and Dixon, D.G. (1992). Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: Neutral narcotic organics. Environmental Toxicology and Chemistry 11, 917-930.

McCarty, L.S., Mackay, D., Smith, A.D., Ozburn, G.W., and Dixon, D.G. (1993). Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: Polar narcotic organics. Ecotoxicology and Environmental Safety 25, 253-270.

McGrath, J.A., and D.M. Di Toro (2009). Validation of the target lipid model for toxicity assessment of residual petroleum constituents: monocyclic and polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 28: 1130-1148.

Neff, J.M., S. Ostazeski, W. Gardiner and I. Stejskal (2000). Effects of weathering on the toxicity of three Australian crude oils and a diesel fuel to marine animals. Environmental Toxicology and Chemistry 19, 1809-1821.

Rand, G.M., Wells, P.G., and McCarty, L.S. 1995. Introduction to aquatic toxicology. In: Fundamentals of aquatic toxicology 2nd edition (Rand, G.M (ed)), Taylor & Francis Publishers, Washington DC, pp. 3-67.

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 Quantification of Water-Accommodated Fractions of Petroleum for Toxicity Testing. Marine Pollution Bulletin, 40 (11): 1007-1016

US EPA. 2002. Stormwater effect handbook: A toolbox for watershed managers, scientists, and engineers. United States Environmental Protection Agency, Washington DC, USA.
www.epa.gov/ednnrmrl/publications/books/

US EPA, Method 3510C (1996). Separatory Funnel Liquid-Liquid Extraction.
http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/3510c.pdf

US EPA, Method 8015D (2003). Non-halogenated organics using GC/FID.
http://www.epa.gov/epawaste/hazard/testmethods/sw846/new_meth.htm#8015D

US EPA, Method 8260C (2006). Volatile organic compounds by gas chromatography/mass spectrometry (GC/MS). http://www.epa.gov/osw/hazard/testmethods/sw846/new_meth.htm#8260C

US EPA, Method 8270D (2007). Semivolatile Organic Compounds by GC/MS.
http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/8270d.pdf

A Appendix A Additional results from the chemical analyses

Table A 1 *Summary of the chemical composition of the oil products studied in 2015, given in g analyte/kg oil (ppm). Total SVOC concentration is the sum of decalins, naphthalenes, 2-6 ring PAHs, phenols. Total VOC concentration is the sum of BTEX, C3-benzenes, and other volatiles from C5-C10.*

	2014-0551 MGO g/kg	2014-0551/250°C+ MGO 250°C+ g/kg	2014-0552/fersk GO g/kg	2014-0552/250°C+ GO 250°C+ g/kg	2014-0553 WRG g/kg
Sum SVOC	27,6	27,6	29,6	18,4	21,1
Sum VOC	42,6	2,91	27,4	0,89	4,83
Decalins	6,41	3,52	21,1	8,59	1,03
Naphthalenes	10,6	10,2	5,71	5,76	4,41
2-3 ring PAH	9,96	12,9	2,75	3,96	12,7
4-6 ring PAH	0,71	0,93	0,02	0,05	2,89
C0-C5 phenols	ND	ND	ND	ND	ND
BTEX	5,83	0,14	4,49	0,02	0,95
C3-benzenes	11,3	1,06	9,41	0,17	1,31
Other VOC	25,5	1,71	13,5	0,70	2,58

ND: Not detected

Table A 2 *Summary of the chemical composition of the oil products studied in 2016, given in g analyte/kg oil (ppm). Total SVOC concentration is the sum of decalins, naphthalenes, 2-6 ring PAHs, phenols. Total VOC concentration is the sum of BTEX, C3-benzenes, and other volatiles from C5-C10.*

	2016-231 HDME g/kg	2016-232 DMA g/kg	2016-232/250°C+ DMA 250°C+ g/kg	2016-233 USLFO g/kg	2016-233/250°C+ ULSFO 250°C+ g/kg
Sum SVOC	6,74	69,5	67,3	18,0	18,1
Sum VOC	ND	10,5	3,05	5,96	0,44
Decalins	0,07	2,19	1,83	5,10	3,43
Naphthalenes	0,59	38,8	36,1	2,79	2,56
2-3 ring PAH	3,29	23,3	23,7	3,96	4,72
4-6 ring PAH	2,80	5,14	5,52	6,16	7,36
C0-C5 phenols	ND	ND	0,11	ND	ND
BTEX	ND	2,70	0,57	0,83	0,04
C3-benzenes	ND	3,44	1,40	1,39	0,15
Other VOC	ND	4,34	1,09	3,74	0,26

ND: Not detected

Table A 3 *Summary of the chemical composition of the WAFs of oil products studied in 2015, given in µg/L (ppb). Total WAF concentration is the sum of TPH and total VOC, total SVOC is the sum of decalins, naphthalenes, 2-6 ring PAHs, and phenols, total VOC concentration is the sum of BTEX, C3-benzenes, and other volatiles from C5 to C9. All WAFs were generated at 13 ^{o}C.*

	MGO Fresh 1:40 µg/L	MGO Fresh 1:10000 µg/L	MGO 250°C+ 1:40 µg/L	MGO 250°C+ 1:10000 µg/L	GO Fresh 1:40 µg/L	GO Fresh 1:10000 µg/L	GO 250°C+ 1:40 µg/L	GO 250°C+ 1:10000 µg/L
Tot WAF	4661	1227	7799	525	2133	1211	9359	431
TPH	2764	456	7690	476	871	654	9343	425
Sum SVOC	483	207	290	160	121	92,0	224	73,8
Sum VOC	1897	771	108	49,0	1262	557	16,4	5,99
UCM	2281	249	7401	316	750	562	9119	351
Decalins	0,60	0,47	0,13	0,16	1,81	1,42	0,33	0,31
Naphthalenes	236	186	173	132	76,1	67,1	108	47,5
2-3 ring PAH	23,9	18,1	68,8	25,7	25,3	22,7	41,8	25,2
4-6 ring PAH	0,30	0,20	1,57	0,59	0,02	0,01	ND	0,02
C0-C5 phenols	222	2,50	45,9	0,93	18,2	0,74	73,6	0,73
BTEX	1265	368	34,2	6,99	818	247	6,19	0,77
C3-benzenes	568	380	69,0	37,9	408	291	8,21	4,35
Other VOC	64,1	23,5	5,13	4,15	36,5	18,5	2,01	0,88

ND: Not detected

Table A 4 *Summary of the chemical composition of the WAFs of oil products studied in 2015, given in µg/L (ppb). Total WAF concentration is the sum of TPH and total VOC, total SVOC is the sum of decalins, naphthalenes, 2-6 ring PAHs, and phenols, total VOC concentration is the sum of BTEX, C3-benzenes, and other volatiles from C5 to C9.*

	WRG 13 °C Fresh 1:40 µg/L	WRG 13 °C Fresh 1:10000 µg/L	WRG 2 °C Fresh 1:40 µg/L	WRG 2 °C Fresh 1:10000 µg/L
Tot WAF	2359	318	1529	215
TPH	1901	187	1184	120
Sum SVOC	154	63,8	95,3	37,6
Sum VOC	459	131	345	95,3
UCM	1747	123	1089	82,2
Decalins	0,10	0,06	0,10	0,07
Naphthalenes	82,4	45,5	55,6	28,6
2-3 ring PAH	24,8	16,8	15,0	8,05
4-6 ring PAH	0,81	0,58	0,56	0,20
C0-C5 phenols	45,6	0,90	24,0	0,61
BTEX	343	67,7	260	54,0
C3-benzenes	104	58,3	75,7	39,3
Other VOC	10,8	4,99	8,56	2,05

Table A 5 *Summary of the chemical composition of the WAFs studied in 2016, given in µg/L (ppb). Total WAF concentration is the sum of TPH and total VOC, total SVOC is the sum of decalins, naphthalenes, 2-6 ring PAHs, and phenols, total VOC concentration is the sum of BTEX, C3-benzenes, and other volatiles*

	HDME	HDME	DMA	DMA-2*	DMA	DMA	DMA	ULSFO	ULSFO	ULSFO	ULSFO
	Fresh	Fresh	fresh	fresh	fresh	250°C+	250°C+	fresh	fresh	250°C+	250°C+
	1:40	1:10000	1:40	1:40	1:10000	1:40	1:10000	1:40	1:10000	1:40	1:10000
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Tot WAF	142	33,2	8695	7293	1278	4941	845	1850	653	491	177
TPH	136	30,4	7298	5719	901	4642	737	660	301	407	141
Sum SVOC	21,4	8,58	691	620	505	596	447	92,8	38,8	51,8	23,7
Sum VOC	6,76	2,85	1397	1574	378	300	107	1190	352	84,1	36,1
UCM	114	21,8	6606	5099	396	4045	291	568	262	355	118
Decalins	0,01	ND	ND	0,14	0,05	ND	0,04	0,45	0,50	0,18	0,22
Naphthalenes	16,0	6,10	597	532	469	533	410	51,9	30,8	36,6	18,7
2-3 ring PAH	4,28	1,96	71,9	65,4	33,1	48,8	34,6	10,4	5,33	9,01	3,67
4-6 ring PAH	0,77	0,26	1,24	1,75	0,54	0,75	0,86	0,96	0,82	2,07	0,70
C0-C5 phenols	0,35	0,26	21,2	20,4	1,82	13,8	1,09	29,2	1,28	3,94	0,43
BTEX	ND	ND	1086	1263	213	186	42,3	831	158	31,5	7,45
C3-benzenes	6,19	2,57	248	243	144	105	59,8	308	177	49,2	26,2
Other VOC	0,57	0,28	62,7	68,5	20,8	8,43	5,29	50,3	17,6	3,33	2,47

*ND: Not detected; * New system prepared for re-test of* Skeletonema *sp..*

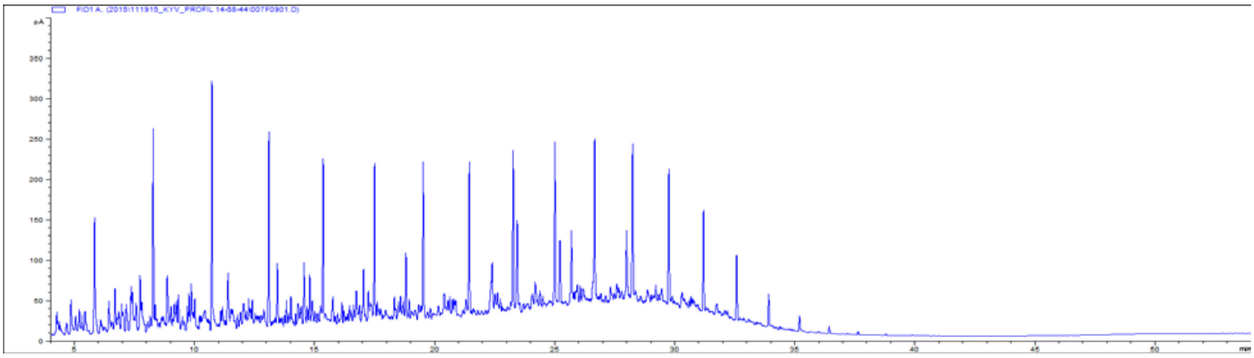

Figure A 1 GC chromatogram of fresh MGO (SINTEF ID 2014-0551)

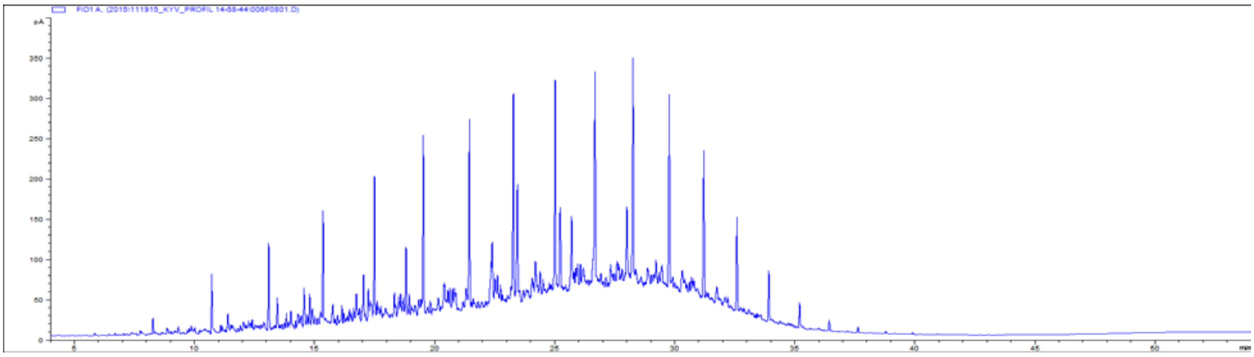

Figure A 2 GC chromatogram of MGO 250°C+ (SINTEF ID 2014-0551)

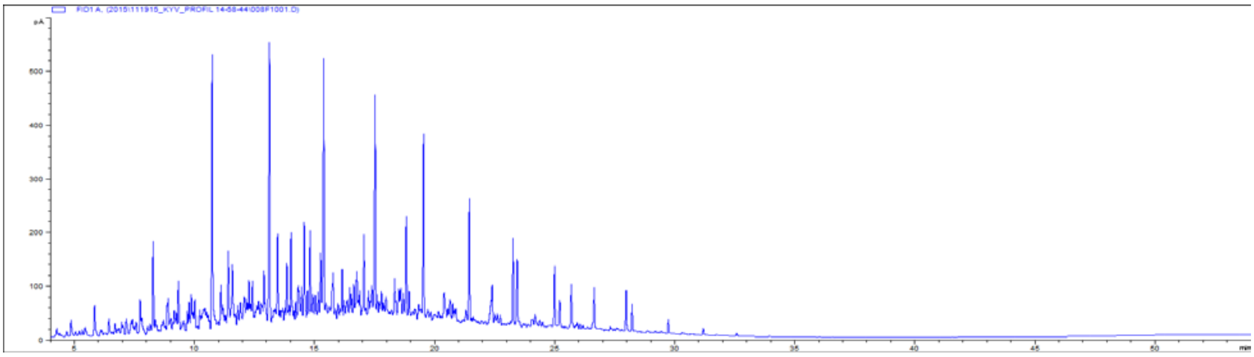

Figure A 3 GC chromatogram of fresh GO (SINTEF ID 2014-0552)

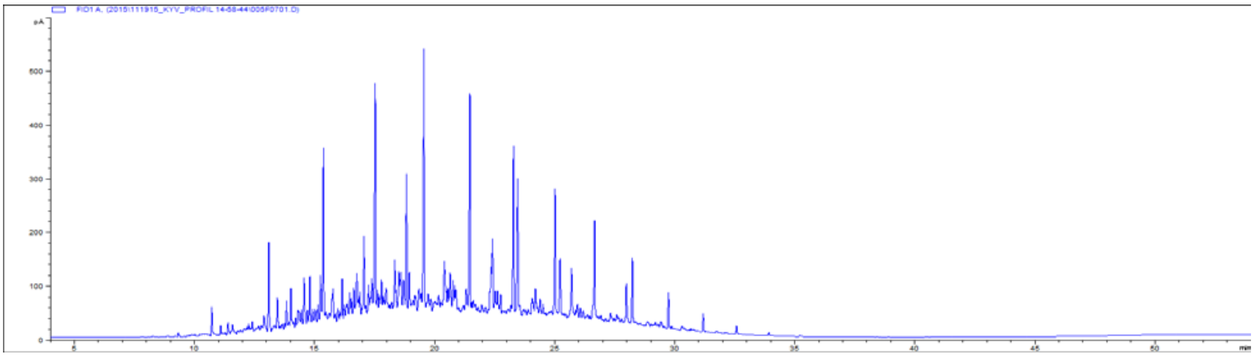

Figure A 4 GC chromatogram of GO 250°C+ (SINTEF ID 2014-0552)

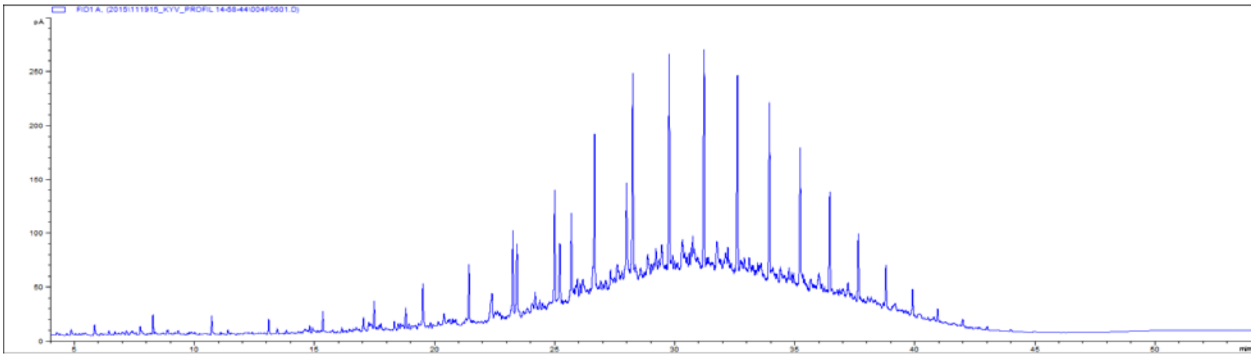

Figure A 5 GC chromatogram of fresh wide range gas oil (WRG) (SINTEF ID 2014-0553).WRG not weathered.

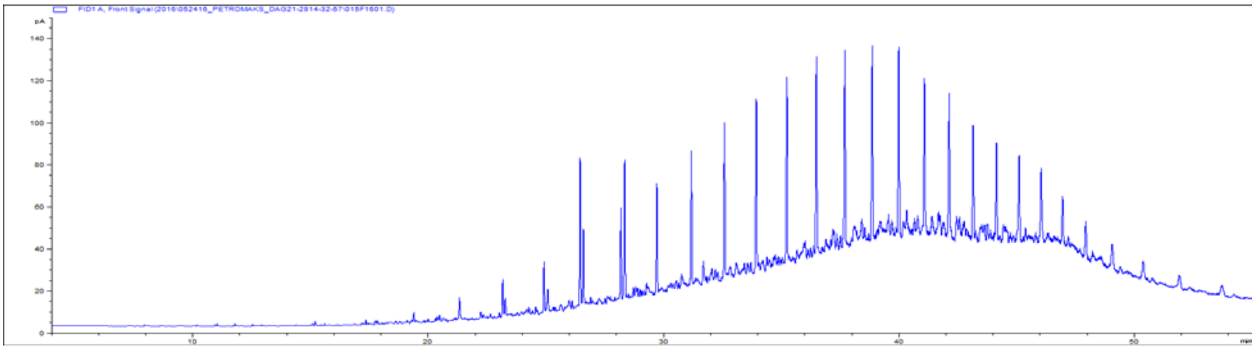

Figure A 6 GC chromatogram of fresh HDME 50 (SINTEF ID 2016-0231). HDME 50 was not weathered

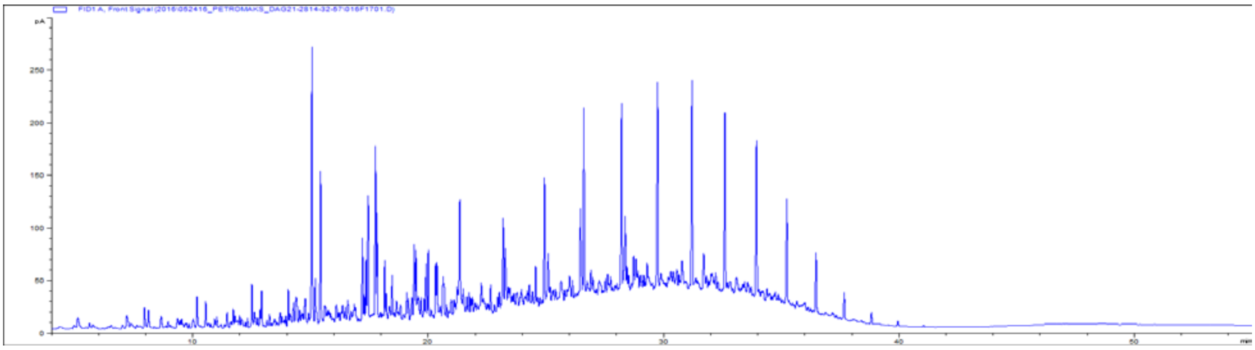

Figure A 7 GC chromatogram of fresh DMA (SINTEF ID 2016-0232)

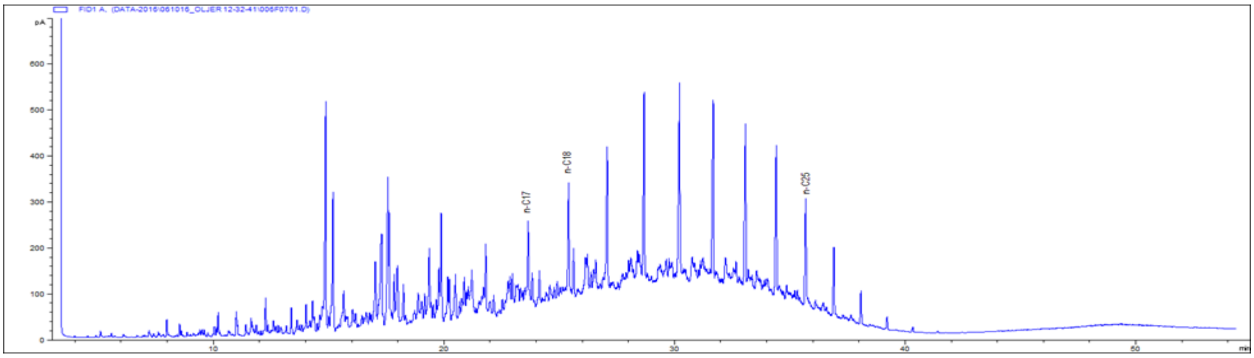

Figure A 8 GC chromatogram of DMA 250°C+ (SINTEF ID 2016-0232)

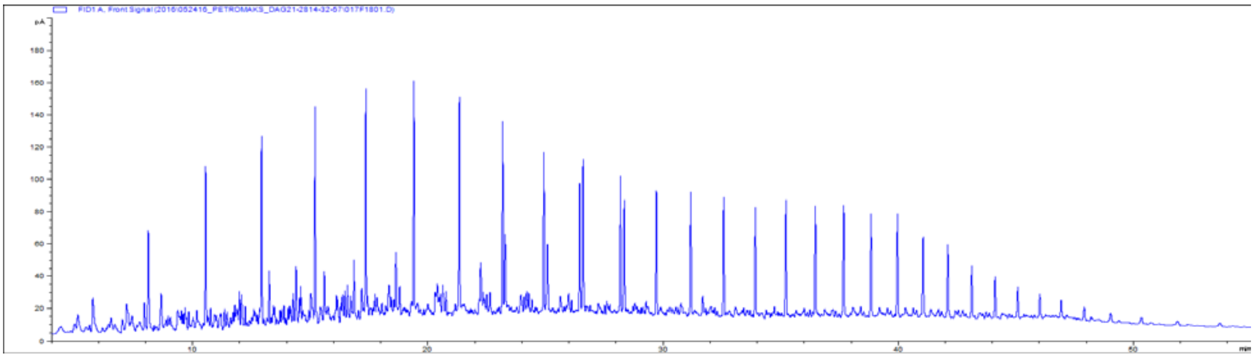

Figure A 9 *GC chromatogram of fresh ULSFO (SINTEF ID 2016-0233)*

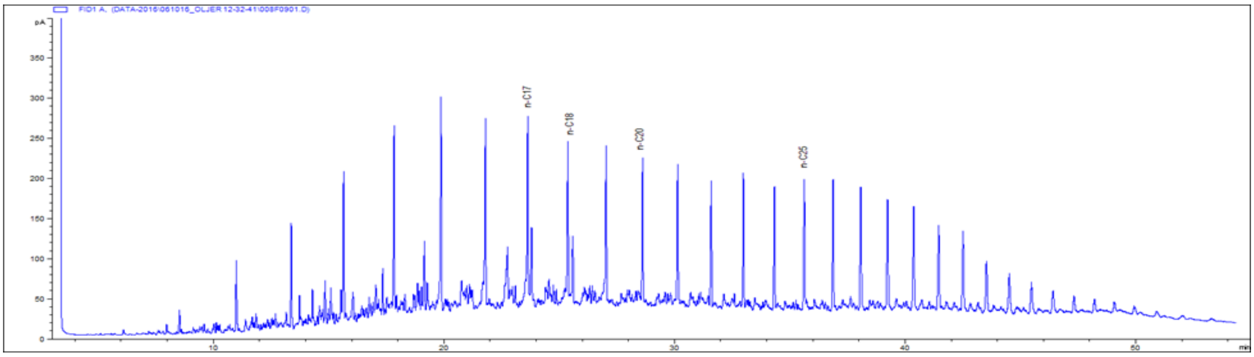

Figure A 10 *GC chromatogram of ULSFO 250°C+ (SINTEF ID 2016-0233)*

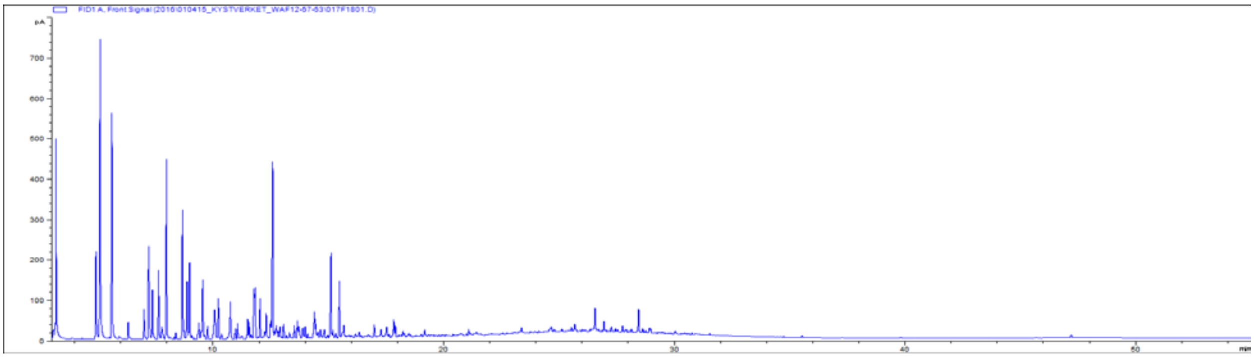

Figure A 11 *GC chromatogram of WAF of fresh MGO, oil to water loading of 1 to 40 (13°C).*

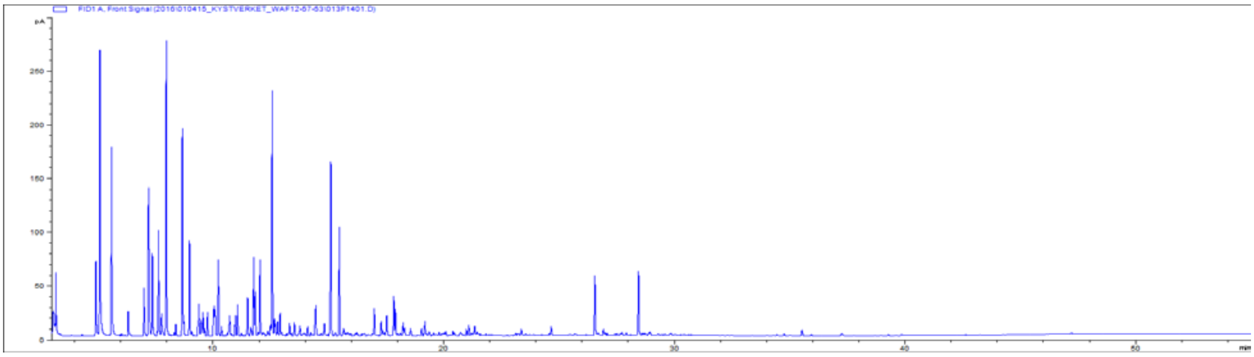

Figure A 12 *GC chromatogram of WAF of fresh MGO, oil to water loading of 1 to 10 000 (13°C).*

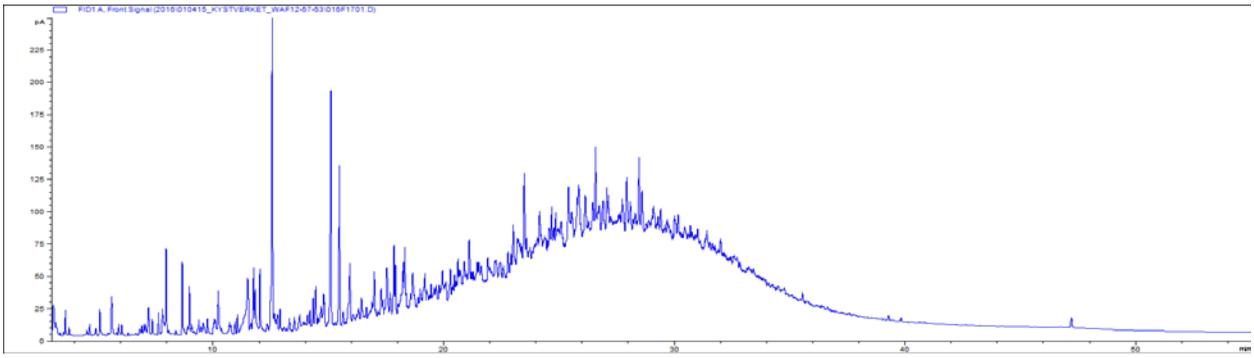

Figure A 13 GC chromatogram of WAF of MGO 250°C+, oil to water loading of 1 to 40 (13°C).

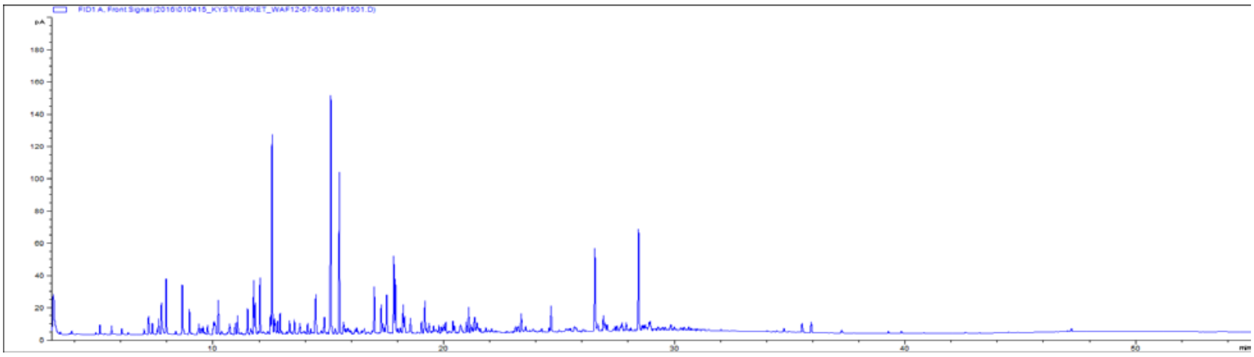

Figure A 14 GC chromatogram of WAF of MGO 250°C+, oil to water loading of 1 to 10 000 (13°C).

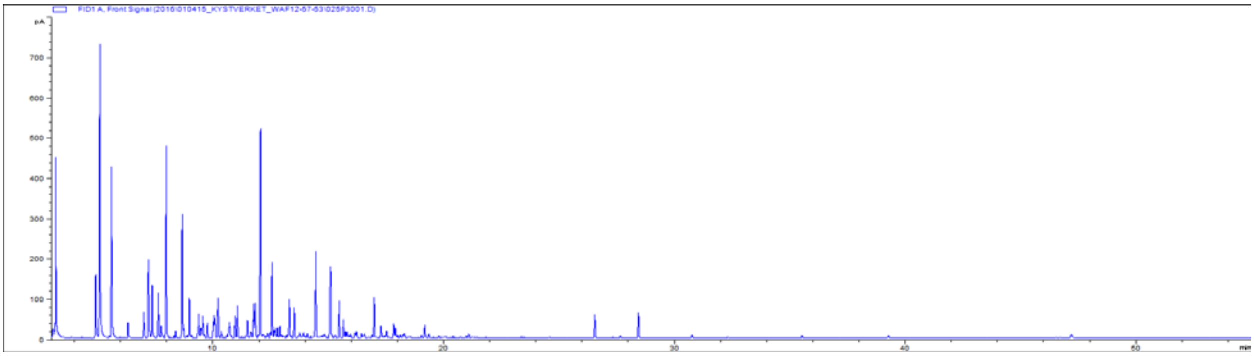

Figure A 15 GC chromatogram of WAF of fresh GO, oil to water loading of 1 to 40 (13°C).

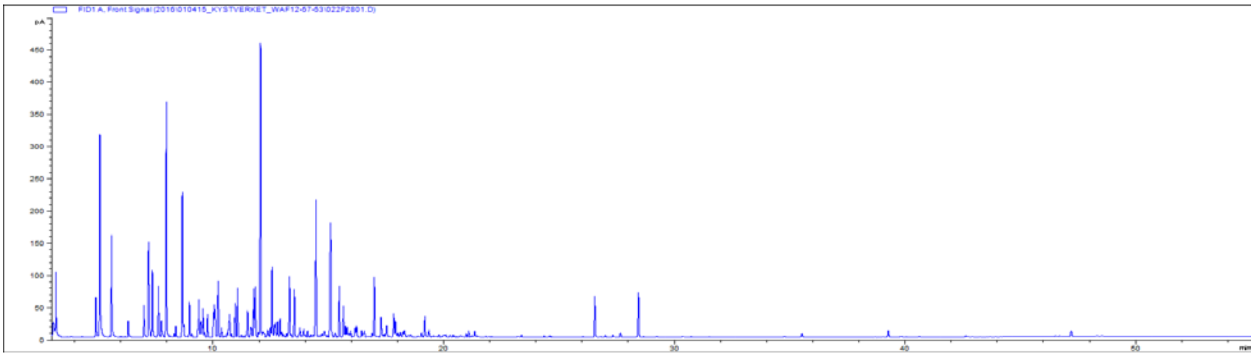

Figure A 16 GC chromatogram of WAF of fresh GO, oil to water loading of 1 to 10 000 (13°C).

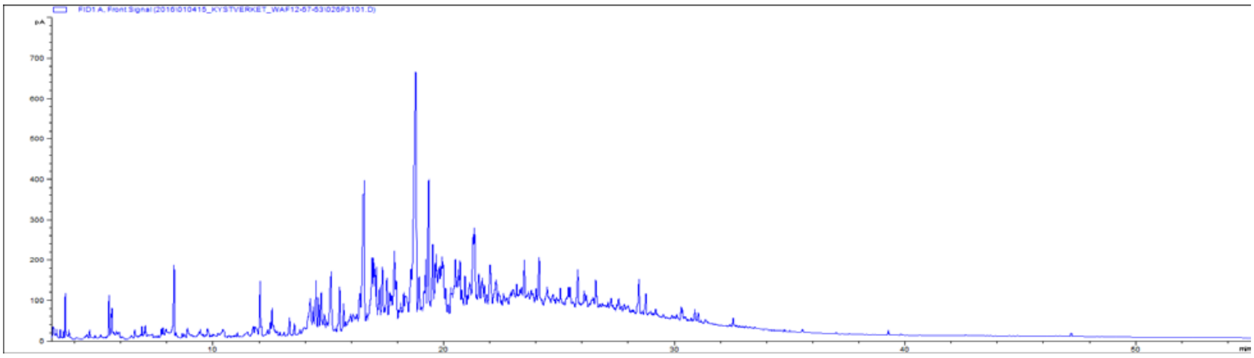

Figure A 17 *GC chromatogram of WAF of GO 250°C+, oil to water loading of 1 to 40 (13°C).*

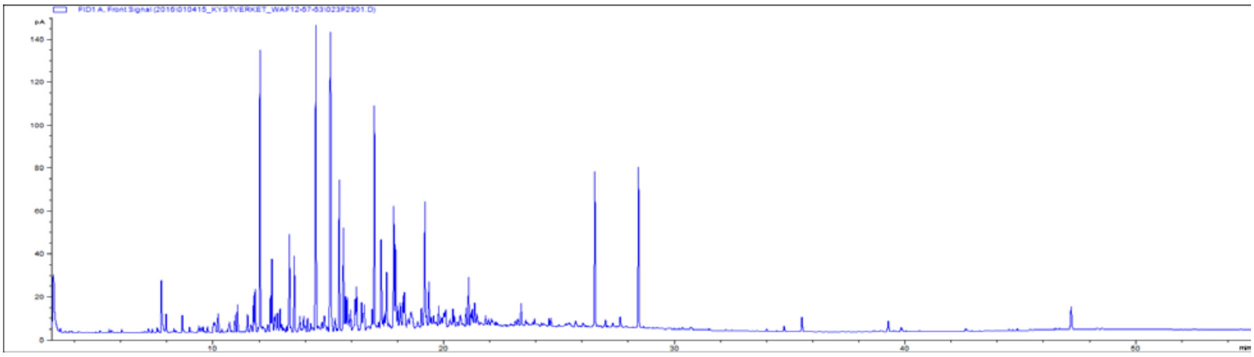

Figure A 18 *GC chromatogram of WAF of GO 250°C+, oil to water loading of 1 to 10 000 (13°C).*

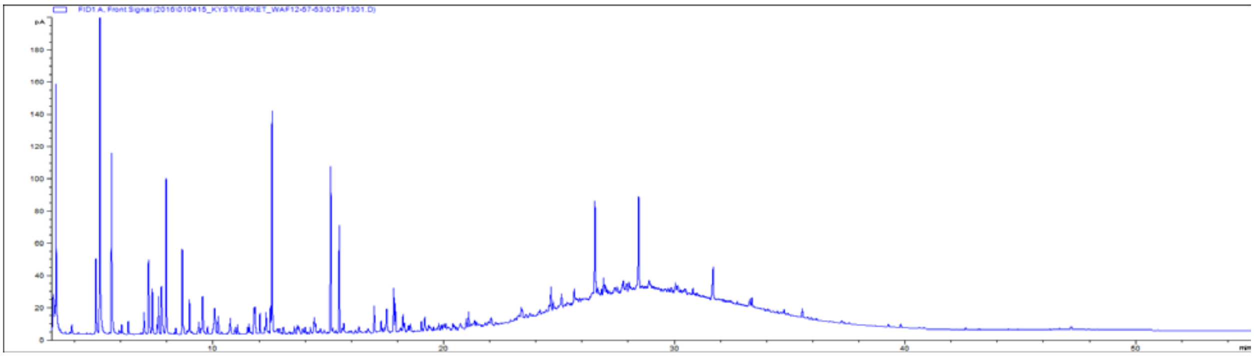

Figure A 19 *GC chromatogram of WAF of fresh WRG, oil to water loading of 1 to 40 (13°C).*

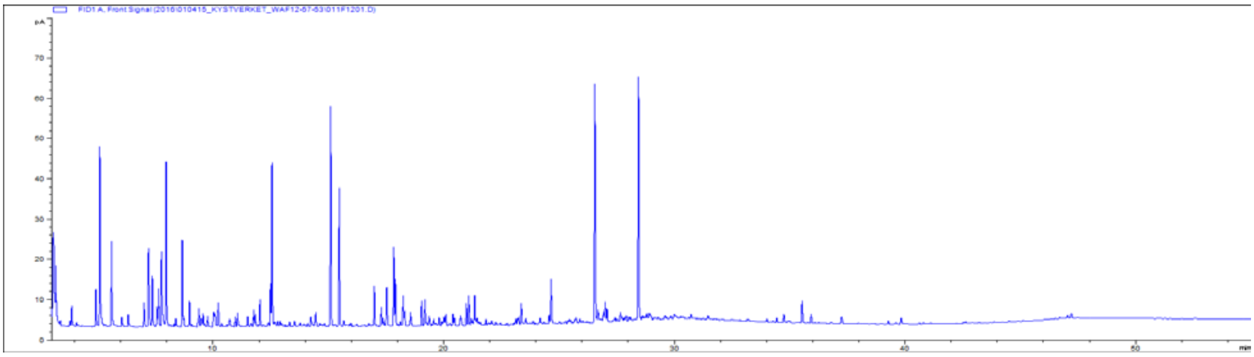

Figure A 20 *GC chromatogram of WAF of fresh WRG, oil to water loading of 1 to 10 000 (13°C).*

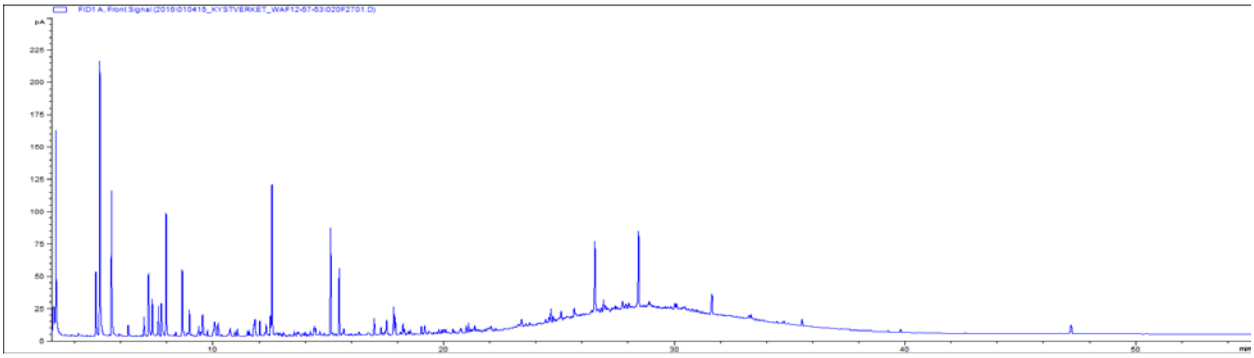

Figure A 21 GC chromatogram of WAF of fresh WRG, oil to water loading of 1 to 40 (2°C).

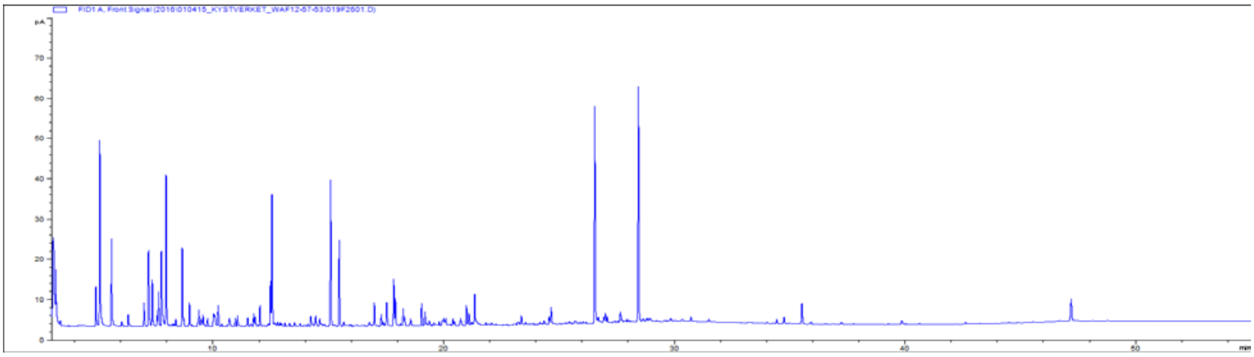

Figure A 22 GC chromatogram of WAF of fresh WRG, oil to water loading of 1 to 10 000 (2°C).

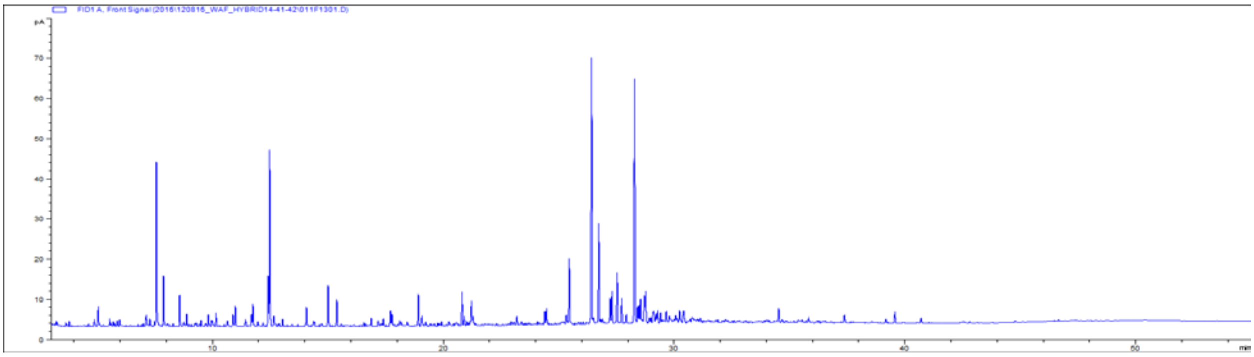

Figure A 23 GC chromatogram of WAF of fresh HDME 50, oil to water loading of 1 to 40 (13°C).

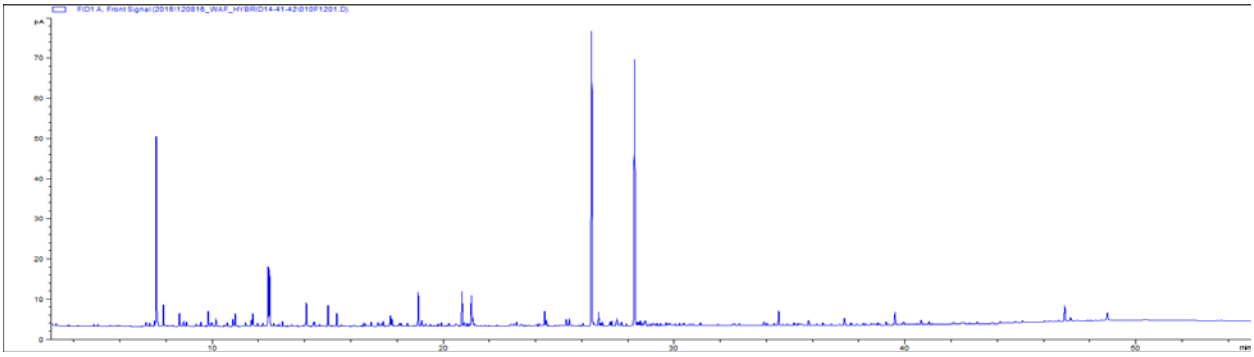

Figure A 24 GC chromatogram of WAF of fresh HDME 50, oil to water loading of 1 to 10 000 (13°C).

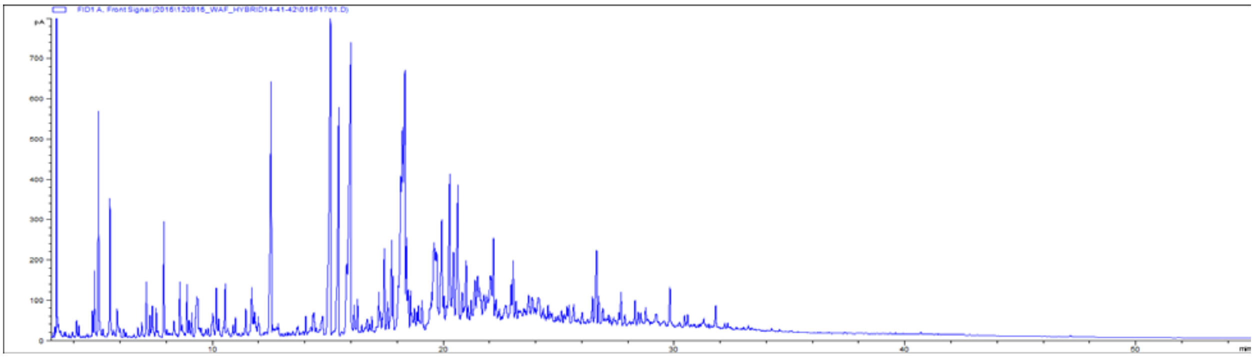

Figure A 25 GC chromatogram of WAF of fresh DMA, oil to water loading of 1 to 40 (13°C).

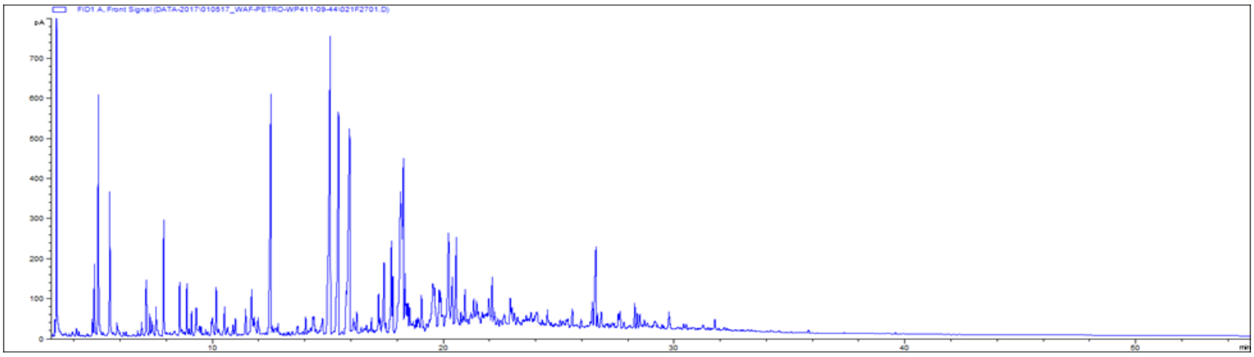

Figure A 26 GC chromatogram of WAF of fresh DMA (2.system), oil to water loading of 1 to 40 (13°C).

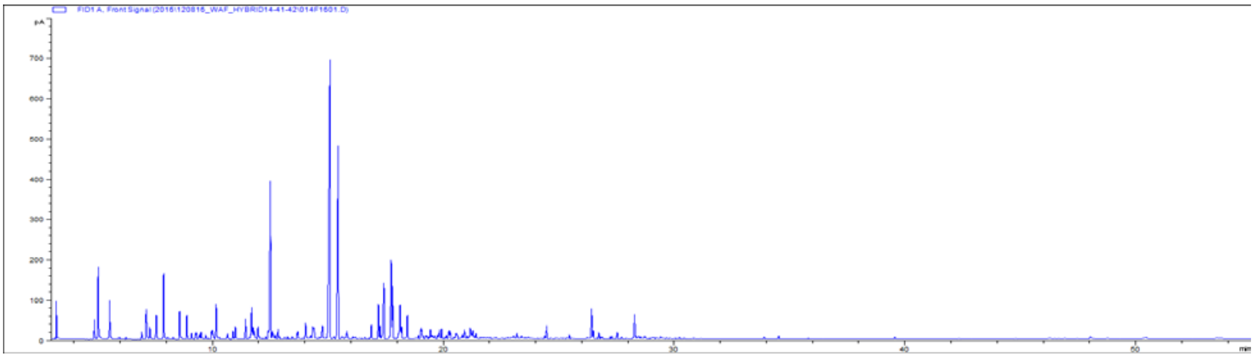

Figure A 27 GC chromatogram of WAF of fresh DMA, oil to water loading of 1 to 10 000 (13°C).

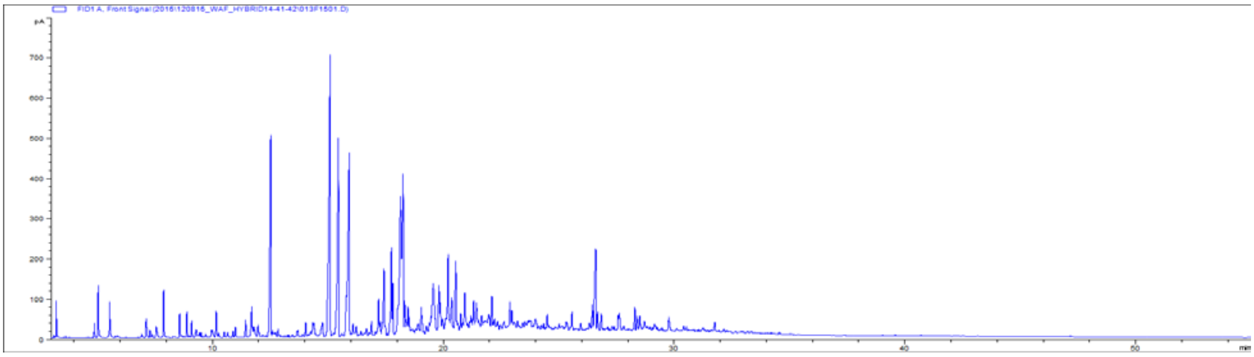

Figure A 28 GC chromatogram of WAF of DMA 250°C+, oil to water loading of 1 to 40 (13°C).

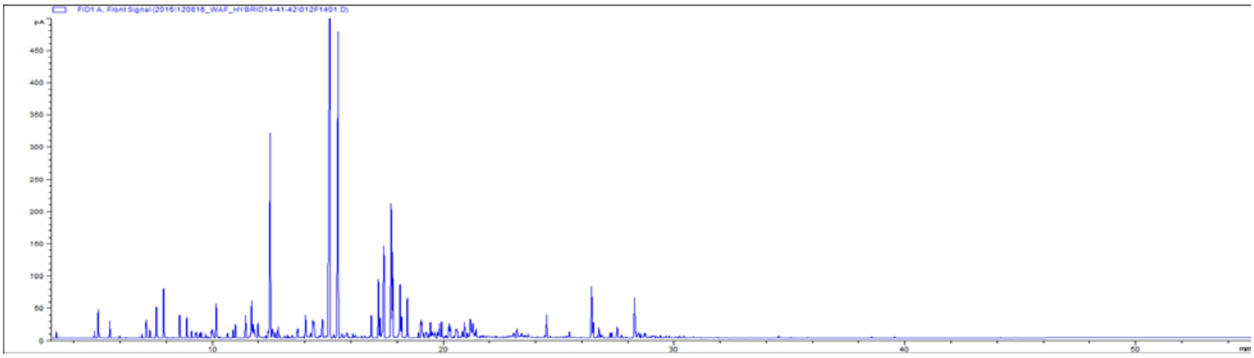

Figure A 29 *GC chromatogram of WAF of DMA 250°C+, oil to water loading of 1 to 10 000 (13°C).*

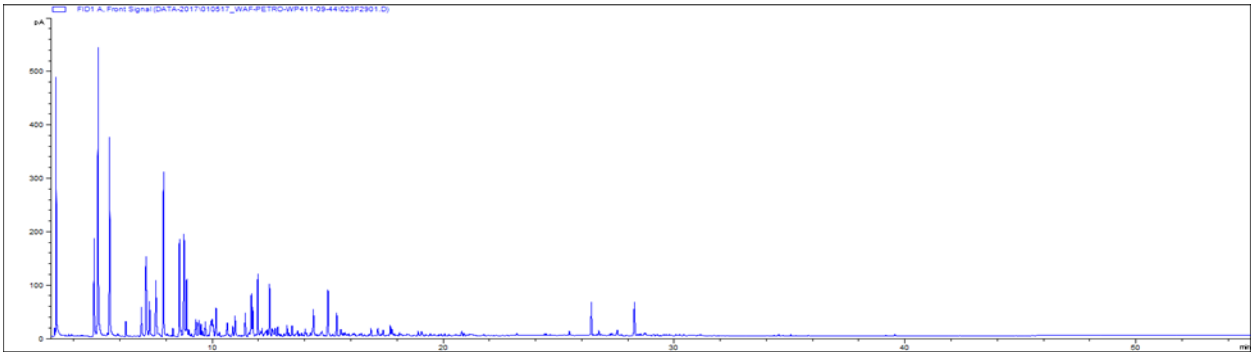

Figure A 30 *GC chromatogram of WAF of fresh ULSFO, oil to water loading of 1 to 40 (13°C).*

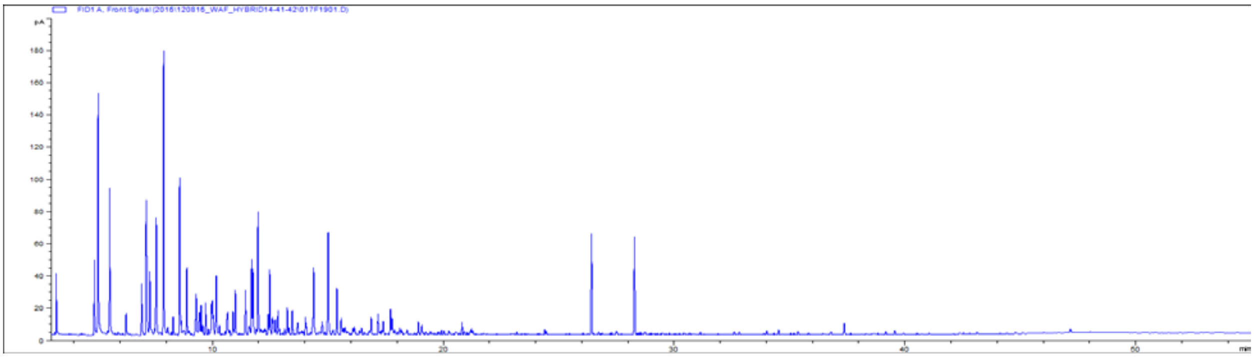

Figure A 31 *GC chromatogram of WAF of fresh ULSFO, oil to water loading of 1 to 10 000 (13°C).*

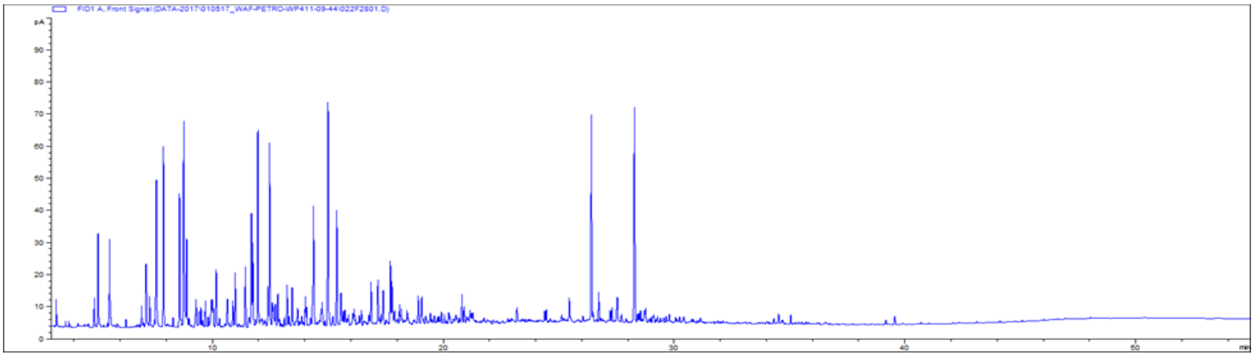

Figure A 32 *GC chromatogram of WAF of ULSFO 250°C+, oil, oil to water loading of 1 to 40 (13°C).*

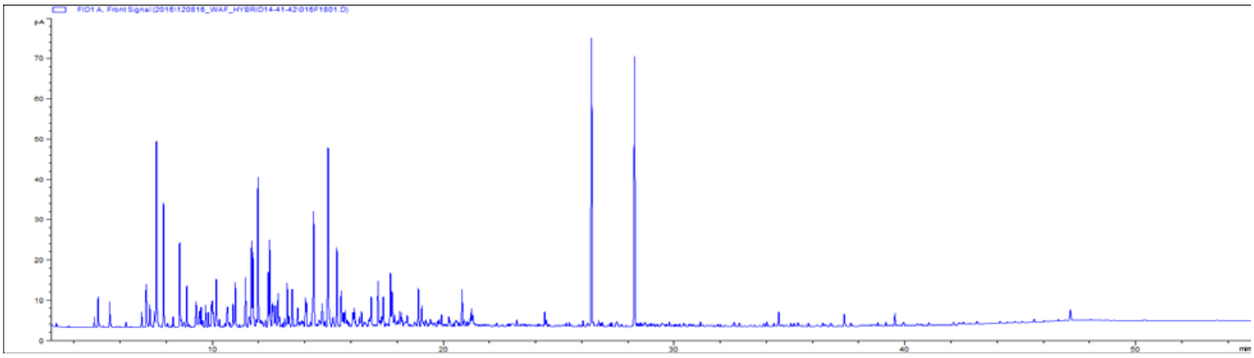

Figure A 33 *GC chromatogram of WAF of ULSFO 250°C+, oil to water loading of 1 to 10 000 (13°C).*

B Appendix B Additional results from the toxicity studies

Table B 1 *Summary of toxicity results from WAFs of oil products studied in 2015. All WAFs are prepared at 13 ⁰C. Acute specific toxicities are normalized to total WAF concentration, and are given as both LC_{50} and EC_{50}, and LC_{10} and EC_{10}. In addition, percentage effect in 100% WAF (non-diluted) at test endpoint, meaning % reduction in growth rate and biomass for the algae, and % mortality for the copepod.*

	MGO Fresh 1:40	MGO Fresh 1:10000	MGO 250°C+ 1:40	MGO 250°C+ 1:10000	GO Fresh 1:40	GO Fresh 1:10000	GO 250°C+ 1:40	GO 250°C+ 1:10000
Total WAF concentration (ppm)	4,66	1,23	7,80	0,53	2,13	1,21	9,36	0,43
Skeletonema sp. EC_{50} (%) (growth rate)	55,6	>100	25,2	98,4	79,5	92,5	29,4	>100
Skeletonema sp. EC_{50} (%) (biomass prod)	40,2	61,0	18,1	58,9	59,6*	64,7	18,3	>100
C. finmarchicus LC_{50} (%)	26,3	>100	28,0	>100	52,7	80,2	29,7	95,4
Skeletonema sp. EC_{10} (%) (growth rate)	36,0	58,2	21,0	56,2	51,9	68,1	19,5	>100
Skeletonema sp. EC_{10} (%) (biomass prod)	26,5	41,3	10,9	36,7	53,5*	48,0	11,6	80,5
C. finmarchicus LC_{10} (%)	14,6	44,3	18,8	52,2	25,4	29,4	14,5	41,3
Skeletonema sp. EC_{50} (ppm) (growth rate)	2,59	NC	1,97	0,52	1,70	1,12	2,75	NC
Skeletonema sp. EC_{50} (ppm) (biomass prod)	1,87	0,75	1,41	0,31	1,27	0,78	1,71	NC
C. finmarchicus LC_{50} (ppm)	1,23	NC	2,18	NC	1,12	0,97	2,78	0,41
Skeletonema sp. EC_{10} (ppm) (growth rate)	1,68	0,71	1,64	0,30	1,11	0,82	1,83	NC
Skeletonema sp. EC_{10} (ppm) (biomass prod)	1,24	0,51	0,85	0,19	1,14	0,58	1,09	0,35
C. finmarchicus LC_{10} (ppm)	0,68	0,54	1,47	0,27	0,54	0,36	1,36	0,18
Skeletonema sp. (%) (red in growth rate)	100	42	100	52	76	64	100	5,8
Skeletonema sp. (%) (red in biomass prod)	99	92	100	94	98	98	100	41
C. finmarchicus (%) (mortality)	100	38	100	24	95	62	100	52

*NC: Not calculated; * Ambiguous data.*

Table B 2 *Summary of toxicity results from WAFs of WRG studied in 2015, prepared at 2 and 13 ^oC. Acute specific toxicities are normalized to total WAF concentration, and are given as both LC_{50} and EC_{50}, and LC_{10} and EC_{10}. In addition, percentage effect in 100% WAF (non-diluted) at test endpoint, meaning % reduction in growth rate and biomass for the algae, and % mortality for the copepod.* Skeletonema sp. *was not tested in WAF prepared at 2 ^oC.*

	WRG 13°C	WRG 13°C	WRG 2°C	WRG 2°C
	Fresh	Fresh	Fresh	Fresh
	1:40	1:10000	1:40	1:10000
Total WAF concentration (ppm)	2,36	0,32	1,53	0,22
Skeletonema sp. EC_{50}% (growth rate)	53,5	98,6		
Skeletonema sp. EC_{50} (%) (biomass prod)	31,4	45,0		
C. finmarchicus LC_{50} (%)	45,8	>100	82,1	>100
Skeletonema sp. EC_{10}% (growth rate)	38,1	56,4		
Skeletonema sp. EC_{10} (%) (biomass prod)	16,0	22,7		
C. finmarchicus LC_{10} (%)	23,8	97,0**	43,9	>100
Skeletonema sp. EC_{50} (ppm) (growth rate)	1,26	0,31		
Skeletonema sp. EC_{50} (ppm) (biomass prod)	0,74	0,14		
C. finmarchicus LC_{50} (ppm)	1,08	NC	1,26	NC
Skeletonema sp. EC_{10} (ppm) (growth rate)	0,90	0,18		
Skeletonema sp. EC_{10} (ppm) (biomass prod)	0,38	0,07		
C. finmarchicus LC_{10} (ppm)	0,56	0,31	0,67	NC
Skeletonema sp. (%) (red in growth rate)	100	51		
Skeletonema sp. (%) (red in biomass prod)	100	96		
C. finmarchicus (%) (mortality)	100	29	67	9,5

*NC: Not calculated; **Interrupted*

Table B 3 *Summary of toxicity results from WAFs of oil products studied in 2015. All WAFs are prepared at 13 ^{0}C. Acute specific toxicities are normalized to total WAF concentration, and are given as both LC$_{50}$ and EC$_{50}$, and LC$_{10}$ and EC$_{10}$. In addition, percentage effect in 100% WAF (non-diluted at test endpoint), meaning % reduction in growth rate and biomass for the algae, and % mortality for the copepod.*

	HDME Fresh 1:40	DMA Fresh 1:40	ULSFO Fresh 1:40	HDME Fresh 1:10000	DMA Fresh 1:10000	ULSFO Fresh 1:10000	DMA 250°C+ 1:40	ULSFO 250°C+ 1:40	DMA 250°C+ 1:10000	ULSFO 250°C+ 1:10000
Total WAF concentration (ppm)	0,14	8,70	1,85	0,03	1,28	0,65	4,94	0,49	0,85	0,18
Skeletonema sp. EC$_{50}$ (%) **	99,4	5,05*	50,6	>100	66,0	>100	8,85	30,9	63,5	>100
Skeletonema sp. EC$_{50}$ (%) ***	95,3	3,78*	40,8	>100	42,1	66,8	7,80	15,3	51,4	>100
C. finmarchicus LC$_{50}$ (%)	>100	13,2	41,1	>100	33,6	>100	26,3	>100	43,1	>100
Skeletonema sp. EC$_{10}$ (%) **	90,1	3,03*	40,4	>100	52,4	60,9	7,00	9,1	52,1	>100
Skeletonema sp. EC$_{10}$ (%) ***	89,5	2,23*	31,8	15,3	16,5	12,4	5,88	6,8	36,7	32,1
C. finmarchicus LC$_{10}$ (%)	74,8	7,64	17,9	>100	17,0	>100	15,5	40,6	22,5	85,1
Skeletonema sp. EC$_{50}$ (ppm) **	0,14	0,37*	0,94	NC	0,84	NC	0,44	0,15	0,54	NC
Skeletonema sp. EC$_{50}$ (ppm) ***	0,14	0,28*	0,75	NC	0,54	0,44	0,39	0,08	0,43	NC
C. finmarchicus LC$_{50}$ (ppm*)*	NC	1,15	0,76	NC	0,43	NC	1,30	NC	0,36	NC
Skeletonema sp. EC$_{10}$ (ppm) **	0,13	0,22*	0,75	NC	0,67	0,40	0,35	0,04	0,44	NC
Skeletonema sp. EC$_{10}$ (ppm) ***	0,13	0,16*	0,59	0,01	0,21	0,08	0,29	0,03	0,31	0,06
C. finmarchicus LC$_{10}$ (ppm)	0,11	0,66	0,33	NC	0,22	NC	0,77	0,20	0,19	0,15
Skeletonema sp. (%) **	54	100	100	0	100	37	100	100	100	8,0
Skeletonema sp. (%) ***	87	100	100	17	99	81	100	100	98	27
C. finmarchicus (% mortality)	24	100	100	0	100	9,5	100	48	91	14

Re-test in new system, chemistry from Exp. 2 (Tot WAF conc. of 7,3 ppm); ** Reduction in growth rate; * Reduction in biomass production; NC: Not calculated*

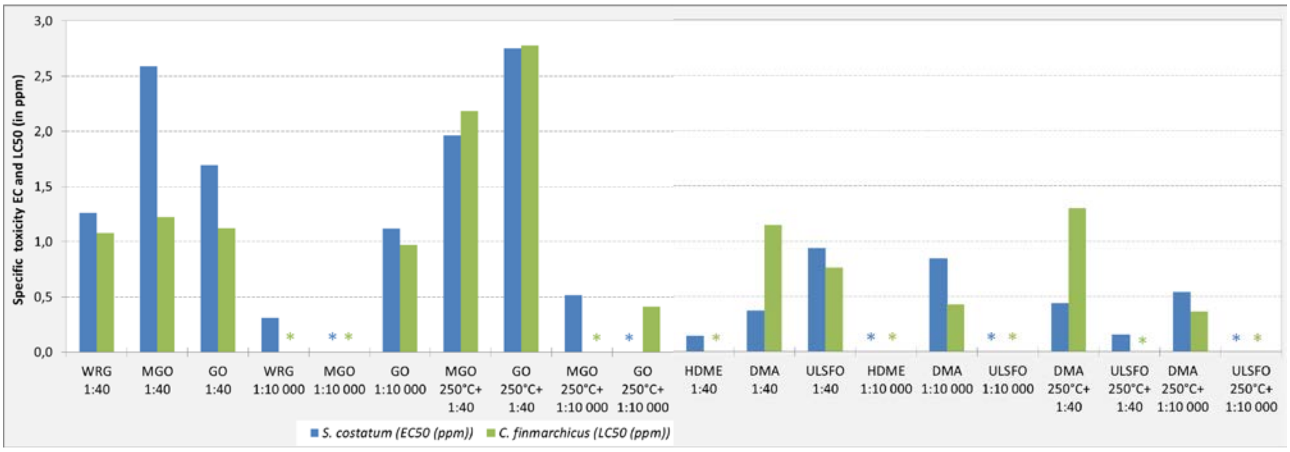

Figure B 1 *Specific toxicity of WAFs given as EC$_{50}$ (Skeletonema sp., blue bars) and LC$_{50}$ (*C. finmarchicus*, green bars) normalized to total WAF concentration. Low bars indicate higher toxicity, and no bars indicate absence of an observed effect or that the effect was less than 50 % for the test organisms (*).*

Table B 4 Predicted acute toxicity expressed as toxic unit (TU) for all prepared WAF systems. A TU > 1 indicates a 50% mortality for 50% of the tested organisms.

		BTEX	C3-benzenes	Naphthalenes	2-3 ring PAH	4-6 ring PAH	C0-C5 phenols	Decalins	**Total HI**
GO	1:40	0,060	0,157	0,076	0,029	0,0003	0,0031	0,101	**0,43**
MGO	1:40	0,095	0,218	0,185	0,137	0,0051	0,0378	0,021	**0,70**
WRG 13°C	1:40	0,024	0,040	0,098	0,261	0,0227	0,0073	0,004	**0,46**
WRG 2°C	1:40	0,018	0,029	0,068	0,200	0,0411	0,0040	0,003	**0,36**
DMA	1:40	0,063	0,095	0,550	2,931	0,1240	0,0053	0,000	**3,77**
ULSFO	1:40	0,056	0,118	0,049	0,120	0,0403	0,0041	0,017	**0,41**
HDME	1:40	0,000	0,002	0,012	0,077	0,0362	0,0001	0,000	**0,13**
GO	1:10000	0,022	0,112	0,067	0,028	0,0001	0,0002	0,078	**0,31**
MGO	1:10000	0,032	0,146	0,150	0,100	0,0046	0,0006	0,030	**0,46**
WRG 13°C	1:10000	0,006	0,022	0,061	0,224	0,0338	0,0002	0,002	**0,35**
WRG 2°C	1:10000	0,005	0,015	0,039	0,113	0,0101	0,0001	0,002	**0,18**
DMA	1:10000	0,016	0,055	0,438	0,185	0,0088	0,0005	0,001	**0,70**
ULSFO	1:10000	0,013	0,068	0,026	0,048	0,0289	0,0001	0,023	**0,21**
HDME	1:10000	0,000	0,001	0,007	0,033	0,0137	0,0000	0,000	**0,06**
GO 250°C+	1:40	0,000	0,003	0,299	0,356	0,0000	0,0237	0,015	**0,70**
MGO 250°C+	1:40	0,003	0,027	0,276	1,699	0,0269	0,0112	0,002	**2,04**
DMA 250°C+	1:40	0,013	0,040	0,503	1,451	0,0127	0,0031	0,000	**2,02**
ULSFO 250°C+	1:40	0,003	0,019	0,046	0,079	0,0760	0,0009	0,008	**0,23**
GO 250°C+	1:10000	0,000	0,002	0,087	0,058	0,0004	0,0002	0,033	**0,18**
MGO 250°C+	1:10000	0,001	0,015	0,162	0,242	0,0224	0,0002	0,006	**0,45**
DMA 250°C+	1:10000	0,004	0,023	0,423	0,195	0,0202	0,0004	0,001	**0,67**
ULSFO 250°C+	1:10000	0,001	0,010	0,026	0,048	0,0289	0,0001	0,023	**0,14**
DMA *	1:40	0,070	0,093	0,534	1,976	0,1460	0,0040	0,002	**2,82**

**System prepared for re-testing of* Skeletonema *sp.*

C Appendix C Overview of component groups used in tables and figures

Table C 1 Target organic analytes (SVOC: Semi volatile organic compounds, VOC: Volatile organic compounds, TPH: Total petroleum hydrocarbons, UCM. Unresolved organic materials).

	Compound	Abb	Group	Compound	Abb
SVOC	Decalin	DE	C0-C5 phenols	Phenol	PH
	C1-decalins	DE1		C1-phenols	PH1
	C2-decalins	DE2		C2-phenols	PH2
	C3-decalins	DE3		C3-phenols	PH3
	C4-decalins	DE4		C4-phenols	PH4
Naphthalenes	Naphthalene	N		C5-phenols	PH5
	C1-naphthalenes	N1	Other VOC	Isopentane	
	C2-naphthalenes	N2		n-C5 (Pentane)	
	C3-naphthalenes	N3		Cyclopentane	
	C4-naphthalenes	N4		2-methylpentane	
2-3 ring PAHs	Benzo(b)thiophene	BT		3-methylpentane	
	Biphenyl	B		n-C6 (Hexane)	
	Acenaphthylene	ANY		Methylcyclopentane	
	Acenaphthene	ANA		Cyclohexane	
	Dibenzofuran	DBF		2,3-dimethylpentane	
	Fluorene	F		3-methylhexane	
	C1-fluorenes	F1		n-C7 (Heptane)	
	C2-fluorenes	F2		Methylcyclohexane	
	C3-fluorenes	F3		2,4-dimethylhexane	
	Phenanthrene	P		2-methylheptane	
	Anthracene	A		n-C8 (Octane)	
	C1-phenanthrenes/anthracenes	P1		n-C9 (Nonane)	
	C2-phenanthrenes/anthracenes	P2		n-C10 (Decane)	
	C3-phenanthrenes/anthracenes	P3		n-Butylbenzene	
	C4-phenanthrenes/anthracenes	P4		1,2,4,5-tetramethylbenzene	
	Dibenzothiophene	D		n-pentylbenzene	
	C1-dibenzothiophenes	D1	BTEX	Benzene	
	C2-dibenzothiophenes	D2		Toluene	
	C3-dibenzothiophenes	D3		Ethylbenzene	
	C4-dibenzothiophenes	D4		*m*-xylene	
4-6 ring PAHs	Fluoranthene	FL		*p*-xylene	
	Pyrene	PY		*o*-xylene	
	C1-fluoranthrenes/pyrenes	FL1	C3-benzenes	Propylbenzene	
	C2-fluoranthenes/pyrenes	FL2		1-methyl-3-ethylbenzene	
	C3-fluoranthenes/pyrenes	FL3		1-methyl-4-ethylbenzene	
	Benz[*a*]anthracene	BA		1,3,5-Trimethylbenzene	
	Chrysene	C		1-methyl-2-ethylbenzene	
	C1-chrysenes	C1		1,2,4-trimethylbenzene	
	C2-chrysenes	C2		1,2,3-trimethylbenzene	
	C3-chrysenes	C3			
	C4-chrysenes	C4			
	Benzo[*b*]fluoranthene	BBF			
	Benzo[*k*]fluoranthene	BKF	TPH	C10-C36	
	Benzo[*e*]pyrene	BEP	WAF	Sum of VOC and TPH	
	Benzo[*a*]pyrene	BAP	UCM	TPH - SVOC	
	Perylene	PE			
	Indeno[*1,2,3-c,d*]pyrene	IN			
	Dibenz[*a,h*]anthracene	DBA			
	Benzo(g,h,i)perylene	BPE			

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